NEUROENDOCRINE MECHANISMS THAT CONNECT FEEDING BEHAVIOR AND STRESS

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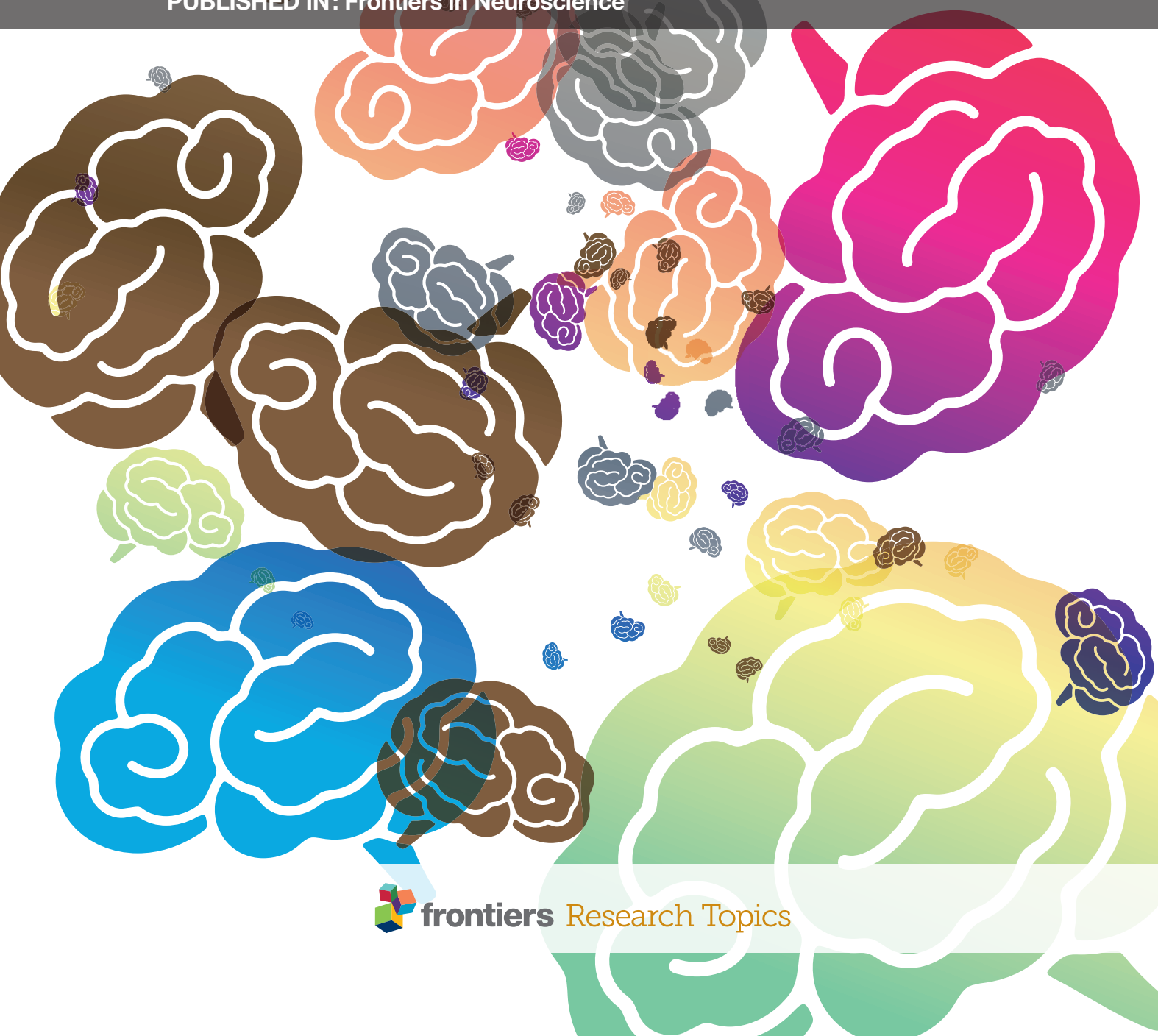

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NEUROENDOCRINE MECHANISMS THAT CONNECT FEEDING BEHAVIOR AND STRESS

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Neuroendocrine mechanisms that connect feeding behavior and stress

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Research during the past decade highlights the strong link between appetitive feeding behavior, reward, and motivation. Interestingly, stress levels can affect feeding behavior by manipulating hypothalamic circuits and brain dopaminergic reward pathways. Indeed, animals and people will increase or decrease their feeding responses when stressed. In many cases, acute stress leads to a decrease in food intake, yet chronic social stressors are associated to increases in caloric intake and adiposity. Interestingly, mood disorders and the treatments used to manage these disorders are also associated with changes in appetite and body weight. These data suggest a strong interaction between the systems that regulate feeding and metabolism and those that regulate stress and ultimately mood. This Research Topic compiles a number of review and research articles that focus how hormonal mechanisms regulate the nexus between feeding behavior and stress. It highlights the hormonal regulation of hypothalamic circuits and/or brain dopaminergic systems, as the potential sites controlling the converging pathways between feeding behavior and stress.

The regulation of energy balance is controlled by hypothalamic nuclei that include the arcuate nucleus (ARC), a mediobasal hypothalamic region that has access to circulating peripheral signals including those that regulate metabolism and stress. Neuropeptide Y (NPY) neurons within the ARC were identified over three decades ago as potent stimulators of food intake and adiposity, and later on as important regulators of the hypothalamic-pituitary-adrenal (HPA) axis, sympathetic activity, and anxiety related behaviors. In this issue, Sundstrom et al. (2013) use a Zebrafish model to illustrate the specific affinities for NPY peptides with a number of specific Y-family receptors. Their results clearly suggest that NPY and its related peptides, all of which are released in response to stress, bind preferentially to the brain and other tissues like heart and kidney that are affected by stressors. Given that NPY cells in the ARC are extremely sensitive to circulating hormones, it is not surprising that these hormones modulate appetitive behaviors. The review by Keen-Rhinehart (Keen-Rhinehart et al., 2013) focuses on the neuroendocrine roles of ghrelin and leptin in appetitive behavior, and provides an excellent overview of the neuroendocrine regulation of appetitive behavior, which includes not only food intake but also foraging, hoarding, chewing, and swallowing. Patton and Mistlberger (2013) provide an excellent review on how neuroendocrine mechanisms influence circadian meal timing. They show how food availability entrains food anticipation and critically examine the role of peripheral metabolic hormones as potential internal stimuli entraining behavioral and physiological rhythms. Key hormones discussed are corticosterone, ghrelin, leptin, insulin, glucagon, and glucagon-like peptide (GLP1). The influence of metabolic hormones on metabolism in relation to timing events is also highlighted by Cahill et al. (2013) showing that circannual changes in stress and feeding hormones affect food seeking behaviors. This review also highlights the importance of circulating levels of glucocorticoids, ghrelin, and leptin to the fluctuations in feeding-related behaviors associated with seasonality, and discuss in detail how metabolic stressors like restricted food availability influence appetitive and cosummatory eating behavior. In addition to nutritional stressors, psychological stressors can have both organizational and activational effects on the brain systems controlling food intake and energy balance. For instance, Spencer (2013) addresses how early-life nutrition, stress, and hormonal profiles influence the development of the HPA axis, as well as the development of the hypothalamic circuitry that regulates feeding behavior. The paper by Patterson and Abizaid (2013) discusses how psychological stress, and in particular social stress, represents also a metabolic challenge that is associated with hormonal changes akin to those observed in animals that are calorically restricted. Among these, ghrelin, a gut peptide that stimulates appetite and promotes adiposity, is released during stress and is a factor that contributes to stress induced caloric intake and weight gain. Furthermore, Patterson et al. (2013) provide data indicating the intricacy of the PVN in mediating the effects of ghrelin on stress-induced caloric intake and weight gain. Uchida et al. (2013) provide an excellent description detailing the use of genetically-modified mouse models to understand the effects of ghrelin on feeding behavior. Specifically, they show that ghrelin receptor signaling on catecholaminergic neurons influences chronic stress-induced food reward. This is followed by Schellekens, Dinan, and Cryan's review illustrating how ghrelin receptor dimerization influences stress and reward (Schellekens et al., 2013). This new dimension in ghrelin receptor signaling shows that independent from ghrelin binding, ghrelin receptors interact with serotonin and dopamine receptors to influence aminergic tone and modulate stress and reward processes. In support of this, Wellman

et al. (2013) discuss the importance of ghrelin receptor signaling on modulating psychostimulant action, including the action of addictive drugs like nicotine, cocaine, and amphetamine. This review shows that ghrelin universally enhances sensitivity to psychostimulants and that ghrelin receptor antagonism may be an important therapeutic target to help quit smoking and curb addictive behaviors. Ghrelin may also be implicated in the narcotic drug seeking following stress, given that, as shown by Sedki et al. (2013), blocking CRF receptors or adrenalectomy do not attenuate the effects of food restriction on heroin seeking behavior.

In addition to ghrelin, other peptide hormones have been implicated in stress induced feeding and psychopathology. For instance, Merali and colleagues draw attention to the bombesin family of peptides and provide intriguing evidence that they regulate stress-induced anorexia or obesity depending on subtle differences in neural circuitry and neurochemical phenotypes (Merali et al., 2013). Skibicka reviews the central actions of GLP1 of food and drug reward. In this review, we learn that GLP1 acts in the mesolimbic dopamine system to reduce hunger driven food intake, the hedonic value of food and food motivation (Skibicka, 2013). Emerging evidence shows that GLP1 will reduce not only food-based reward behavior but also cocaine, amphetamine, and alcohol rewards. Finally, a paper by McQuaid et al. and a commentary by Tabak both highlight the role of oxytocin receptor function and how it links early-life stress and vulnerability to depression (McQuaid et al., 2013; Tabak, 2013). They show that human gene polymorphisms in the oxytocin receptor affect depressive symptoms when exposed to early life maltreatment. Not surprisingly, this collection of manuscripts provide for new links between unconventional metabolic hormones and pathological conditions associated with continued exposure to stress. This is further highlighted Hryhorczuk and colleagues, who examine how metabolic disturbances connect obesity and depression. In particular, these authors highlight evidence showing that the metabolic and hormonal changes that accompany obesity have a direct and indirect impact on mood and emotion (Hryhorczuk et al., 2013).

Collectively, the articles published in this research topic offer an in depth analysis of how neuroendocrine mechanisms connect feeding behavior and stress. All articles offer unique and novel elements on this topic and we hope you enjoy.

REFERENCES

Cahill, S., Tuplin, E., and Holahan, M. R. (2013). Circannual changes in stress and feeding hormones and their effect on food-seeking behaviors. *Front. Neurosci.* 7:140. doi: 10.3389/fnins.2013.00140

Hryhorczuk, C., Sharma, S., and Fulton, S. E. (2013). Metabolic disturbances connecting obesity and depression. *Front. Neurosci.* 7:177. doi: 10.3389/fnins.2013.00177

Keen-Rhinehart, E., Ondek, K., and Schneider, J. E. (2013). Neuroendocrine regulation of appetitive ingestive behavior. *Front. Neurosci.* 7:213. doi: 10.3389/fnins.2013.00213

McQuaid, R. J., McInnis, O. A., Stead, J. D., Matheson, K., and Anisman, H. (2013). A paradoxical association of an oxytocin receptor gene polymorphism: early-life adversity and vulnerability to depression. *Front. Neurosci.* 7:128. doi: 10.3389/fnins.2013.00128

Merali, Z., Graitson, S., Mackay, J. C., and Kent, P. (2013). Stress and eating: a dual role for bombesin-like peptides. *Front. Neurosci.* 7:193. doi: 10.3389/fnins.2013.00193

Patterson, Z. R., and Abizaid, A. (2013). Stress induced obesity: lessons from rodent models of stress. *Front. Neurosci.* 7:130. doi: 10.3389/fnins.2013.00130

Patterson, Z. R., Parno, T., Isaacs, A. M., and Abizaid, A. (2013). Interruption of ghrelin signaling in the PVN increases high-fat diet intake and body weight in stressed and non-stressed C57BL6J male mice. *Front. Neurosci.* 7:167. doi: 10.3389/fnins.2013.00167

Patton, D. F., and Mistlberger, R. E. (2013). Circadian adaptations to meal timing: neuroendocrine mechanisms. *Front. Neurosci.* 7:185. doi: 10.3389/fnins.2013.00185

Schellekens, H., Dinan, T. G., and Cryan, J. F. (2013). Taking two to tango: a role for ghrelin receptor heterodimerization in stress and reward. *Front. Neurosci.* 7:148. doi: 10.3389/fnins.2013.00148

Sedki, F., Abbas, Z., Angelis, S., Martin, J., D'Cunha, T., and Shalev, U. (2013). Is it stress? The role of stress related systems in chronic food restriction-induced augmentation of heroin seeking in the rat. *Front. Neurosci.* 7:98. doi: 10.3389/fnins.2013.00098

Skibicka, K. P. (2013). The central GLP-1: implications for food and drug reward. *Front. Neurosci.* 7:181. doi: 10.3389/fnins.2013.00181

Spencer, S. J. (2013). Perinatal programming of neuroendocrine mechanisms connecting feeding behavior and stress. *Front. Neurosci.* 7:109. doi: 10.3389/fnins.2013.00109

Sundstrom, G., Larsson, T. A., Xu, B., Heldin, J., and Larhammar, D. (2013). Interactions of zebrafish peptide YYb with the neuropeptide Y-family receptors Y4, Y7, Y8a, and Y8b. *Front. Neurosci.* 7:29. doi: 10.3389/fnins.2013.00029

Tabak, B. A. (2013). Oxytocin and social salience: a call for gene-environment interaction research. *Front. Neurosci.* 7:199. doi: 10.3389/fnins.2013.00199

Uchida, A., Zigman, J. M., and Perello, M. (2013). Ghrelin and eating behavior: evidence and insights from genetically-modified mouse models. *Front. Neurosci.* 7:121. doi: 10.3389/fnins.2013.00121

Wellman, P. J., Clifford, P. S., and Rodriguez, J. A. (2013). Ghrelin and ghrelin receptor modulation of psychostimulant action. *Front. Neurosci.* 7:171. doi: 10.3389/fnins.2013.00171

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Interactions of zebrafish peptide YYb with the neuropeptide Y-family receptors Y4, Y7, Y8a, and Y8b

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The neuropeptide Y (NPY) system influences numerous physiological functions including feeding behavior, endocrine regulation, and cardiovascular regulation. In jawed vertebrates it consists of 3–4 peptides and 4–7 receptors. Teleost fishes have unique duplicates of NPY and PYY as well as the Y8 receptor. In the zebrafish, the NPY system consists of the peptides NPYa, PYYa, and PYYb (NPYb appears to have been lost) and at least seven NPY receptors: Y1, Y2, Y2-2, Y4, Y7, Y8a, and Y8b. Previously PYYb binding has been reported for Y2 and Y2-2. To search for peptide-receptor preferences, we have investigated PYYb binding to four of the remaining receptors and compared with NPYa and PYYa. Taken together, the most striking observations are that PYYa displays reduced affinity for Y2 (3 nM) compared to the other peptides and receptors and that all three peptides have higher affinity for Y4 (0.028–0.034 nM) than for the other five receptors. The strongest peptide preference by any receptor selectivity is the one previously reported for PYYb by the Y2 receptor, as compared to NPY and PYYa. These affinity differences may be helpful to elucidate specific details of peptide-receptor interactions. Also, we have investigated the level of mRNA expression in different organs using qPCR. All peptides and receptors have higher expression in heart, kidney, and brain. These quantitative aspects on receptor affinities and mRNA distribution help provide a more complete picture of the NPY system.

Keywords: NPY, PYY, G-protein-coupled receptor, zebrafish, evolution

INTRODUCTION

The neuropeptide Y (NPY) system of receptors and peptides in vertebrates is complex with 4–7 G-protein coupled receptors and 3–4 peptide ligands depending on the species. Members of both the receptor and peptide families have been found in invertebrates as well as vertebrates, suggesting an ancient origin of the system (Tensen et al., 1998; Garczynski et al., 2002; Hill et al., 2002; Larhammar and Salaneck, 2004). The receptor family expanded in the two whole genome duplications (tetraploidizations) early in vertebrate evolution, resulting in a repertoire of seven receptors named Y1 through Y8 in a hypothetical ancestral gnathostome (Fredriksson et al., 2004). (Receptor Y3 is missing; this receptor was postulated from pharmacological studies but remains unidentified as a separate gene.) The full repertoire is still present in a cartilaginous fish, the elephant shark, *Callorhinchus milii* (Larsson et al., 2009). At the time of the teleost-specific whole genome duplication the teleost ancestor seems to have had a repertoire of five receptors (Salaneck et al., 2008). After the teleost-specific tetraploidization only the new copy of Y8 was retained, all other duplicates seem to have been lost.

The receptors can be divided into three subfamilies, the Y1, Y2, and Y5 subfamilies. Teleost fishes have 3–4 members in the Y1 subfamily depending on species, Y1, Y4 (formerly called Ya), Y8a (Yc), and Y8b (Yb). Teleosts have the same repertoire as amphibians and birds in the Y2 subfamily, Y2 and Y7, whereas mammals have lost the latter. No teleost has yet been found to possess a Y5 receptor, suggesting that this was lost in the common ancestor of the teleost lineage (Salaneck et al., 2008; Larsson et al., 2009). We recently described a local duplicate of the Y2 receptor in zebrafish and medaka, called Y2-2 (Fallmar et al., 2011). Bioinformatic studies have shown that the teleost tetraploidization resulted in four peptides, NPYa, NPYb, PYYa, and PYYb (formerly called PY). Medaka (*Oryzias latipes*) seems to have lost PYYb and zebrafish (*Danio rerio*) lacks NPYb (unless they have escaped sequencing in the genome projects), while other species with sequenced genomes have the full repertoire of four peptides: green spotted pufferfish (*Tetraodon nigroviridis*), fugu (*Takifugu rubripes*), and three-spined stickleback (*Gasterosteus aculeatus*) (Larsson et al., 2009).

The NPY system has attracted considerable attention during the past several years due to its function on appetite regulation. In mammals the system is able to both enhance and decrease food intake depending on which receptor-peptide combination is activated (Suzuki et al., 2010; Zhang et al., 2011).

Other physiological processes that can be influenced by NPY-family peptides, as mostly studied in mammals, are blood pressure, anxiety, regulation of pituitary release of hormones, bone formation, and pain (Pedrazzini et al., 2003; Lee and Herzog, 2009; Zhang et al., 2011). Until recently, neither of the receptors that stimulate appetite in mammals, Y1 and Y5, had been found in fishes. This was puzzling because feeding studies in goldfish have shown that administration of NPY enhances feeding (Narnaware et al., 2000; Narnaware and Peter, 2001). The zebrafish Y1 receptor has been identified using whole genome data (Salaneck et al., 2008; Larsson et al., 2009). Its single intron has expanded to approximately 40 kb which partially explains why the receptor was not found by PCR. Interestingly, the receptor was not present in any of the other four fully sequenced teleost genomes available (Salaneck et al., 2008; Larsson et al., 2009), but it has been cloned in more basal teleosts such as bowfin (*Amia calva*) and Atlantic herring (*Clupea harengus*) (Salaneck et al., 2008).

Functional and evolutionary studies in mammals have shown that their Y4 receptor has evolved a partnership with pancreatic polypeptide (PP). It should be stressed that PYYb is not the teleost fish ortholog of PP, instead the latter is a tandem duplicate of PYY that probably took place early in the tetrapod lineage. The selectivity of PP for Y4 is particularly strong in rat and mouse. In contrast, chicken PP, PYY, and NPY bind to Y4 with equal affinities. Thus, the PP-Y4 preference seems to have evolved in mammals (Lundell et al., 1995, 2002). One aim of the present study was to investigate if similar partnerships have arisen between NPY-family peptides and receptors in zebrafish.

Pharmacological characterization of the Y2, Y2-2, Y4, Y7, Y8a, and Y8b receptors in zebrafish, using the endogenous ligands NPY (i.e., NPYa) and PYYa, has previously been reported from our laboratory (Lundell et al., 1997; Ringvall et al., 1997; Starback et al., 1999; Berglund et al., 2000; Fredriksson et al., 2004, 2006). After PYYb was discovered, it was included in the characterization of Y2 and Y2-2 (Fredriksson et al., 2006; Fallmar et al., 2011; Zhang et al., 2011). The zebrafish Y1 receptor will be described in detail separately (Larson and Larhammar, in preparation). What is missing is a description of PYYb binding to receptors Y4, Y7, Y8a, and Y8b which is therefore the focus of the present study. We also report the mRNA distribution of the NPY-family peptides and receptors as determined by qPCR.

MATERIALS AND METHODS

CLONING AND TRANSFECTION

The zebrafish receptors have previously been cloned and expressed in cell lines by our laboratory. The zebrafish Y8a and Y8b receptors were inserted in a pTEJ-8 vector and stably expressed in CHO-cells (Ringvall et al., 1997; Berglund et al., 2000). Y7 has been inserted in a pCEP4 vector and semi-stably expressed in HEK 293-EBNA cells (Fredriksson et al., 2004). The zebrafish Y4 receptor coding region was inserted in a pTEJ-8 vector (Starback et al., 1999) and transient expressed in HEK cells. The cell-lines were grown according to the recommendations of the manufacturer. Ninety percent of the confluent cells were harvested and mixed with 100 µl 25 mM HEPES buffer (pH 7.4 and 2.5 mM $CaCl_2$ and 1 mM $MgCl_2$) and stored at −80°C.

BINDING ASSAY

Thawed aliquots of transfected cells were mixed with 1 ml 25 mM HEPES buffer (pH 7.4, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.2 g/l bacitracin). The samples were homogenized and another 1 ml buffer was added. ^{125}I-pPYY was used as radioligand in all experiments, radioligand, and unlabeled ligands (serial concentrations of zfNPY, zfPYYa, and zfPYYb respectively) and cells were mixed at the proportion 2:1:1 in 100 µl reactions followed by 2 h incubation. The zebrafish NPY (zfNPY) and PYYa (zfPYYa) were synthesized at Eli Lilly and Company. The zebrafish PYYb (zfPYYb) was supplied in a crude form by GL Biochem Shanghai and purified as previously described (Fredriksson et al., 2006). The incubation was terminated by rapid filtration through GF/C filters pre-soaked in 0.3% polyethyleneimin, using a TOMTEC cell harvester (Orange, CT, USA). The filters were washed with 50 mM Tris-HCl (pH 7.4) and dried at 50°C for 30 min. Filters were treated with MeltiLex A melt on scintillator sheets (Perkin Elmer, Boston, MA, USA). The radioactivity was measured using a Wallac 1450 Betaplate counter and the results were analyzed using Prism 4.0 software package (GraphPad, San Diego, CA, USA). The pK_i values were compared to each other using One-Way ANOVA followed by Tukey-Kramer Multiple Comparison Test (GraphPad, San Diego, CA, USA).

RNA ISOLATION AND cDNA SYNTHESIS

Four adult zebrafish were purchased from Akvarie Hobby (Uppsala, Sweden) and all procedures involving animals were conducted in accordance with approval #C264/6 from the local ethics committee. The animals had been kept on a regular daily feeding scheme. The following tissues were obtained by dissection: eye, muscle, heart, spleen, GI, liver, kidney, and brain. The tissues were frozen at −80°C until the preparation of total RNA. Total RNA was prepared using the Trizol Reagent (Invitrogen, Sweden) following the manufacturer's instructions. cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen, Sweden) and random hexamers as primers following the manufacturer's recommendations. Absence of genomic DNA in the cDNA was controlled for by PCR.

PRIMER DESIGN AND QUANTITATIVE REAL-TIME PCR

Primers for the quantitative PCR were designed using the Beacon Designer v4.0 (Premier Biosoft, USA). The primer sequences are listed in **Table 1**. The optimal annealing temperatures for each primer combination were determined by PCR on genomic DNA. All real-time PCR reaction had a total volume of 20 µl and contained cDNA synthesized from 5 ng of total RNA, 5 pM of each primer, 0.64 mM $MgCl_2$, 0.2 mM dNTP, SYBR Green (1:50 000), 0.02 u/µl Taq DNA polymerase and buffer (without $MgCl_2$) (Invitrogen, Sweden). The reactions were run on a MyiQ thermal cycler (Bio-Rad Laboratories, Sweden) under following conditions: 95°C for 4 min, followed by 50 cycles at 95°C for 15 s, 59°C for 30 s, and 72°C for 30 s. To confirm that only one product was formed, a melt curve analysis with 84 cycles at 55°C for 10 s, increased by 0.5°C per cycle were conducted.

DATA ANALYSES AND CALCULATION OF RELATIVE EXPRESSION LEVELS

The data were analyzed and threshold cycle (Ct) values derived using MyIQ software v 1.04 (Bio-Rad Laboratories, Sweden). Differences in the primer efficiency were corrected for using LinRegPCR (Ramakers et al., 2003), Grubbs test for outliers (GraphPad, USA) was used to calculate the mean primer efficiency for each primer pair and detection and exclusion of outliers. The Ct values were transformed into quantities using the delta Ct method (Livak and Schmittgen, 2001) and the highest expression was set to 1. geNorm (Vandesompele et al., 2002) was used to identify the most stable housekeeping gene which was thereafter used to normalize expression due to differences in cDNA concentration between samples.

RESULTS

BINDING ASSAY

The zebrafish receptors were expressed in mammalian cells and the membrane fraction was used for binding experiments. Competition binding experiments were performed using the three endogenous ligands NPY, PYYa and PYYb in competition with iodinated porcine PYY. The mature 36 amino acid PYYb peptide has fourteen differences compared to NPY and seven when compared with PYYa (see **Figure 1**). PYYa differs from NPY at nine positions. Thus, PYYb has clearly changed more than PYYa after duplication of their common ancestral gene.

In **Table 2**, the equilibrium inhibition constants (K_i) for Y4, Y7, Y8a, and Y8b are listed and representative inhibition curves are shown in **Figure 2**. The affinity of PYYb for the Y7 receptor was almost identical to that of NPY (0.59 and 0.57 nM) whereas PYYa has a significantly lower affinity, 1.82 nM. No difference in affinity was detected between ligands tested on the Y4, Y8a, and Y8b receptor.

Table 1 | The primer sequences used for the quantitative PCR.

	Sense primer	Anti-sense primer
zfNPY	CTTGTTCGTCTGCTTGG	GTGCTGAATAATACTTGGC
zfPYYa	TCTGCGTGCTTCTGTGTC	CGTGATGAGGTTGATGTAGTG
zfPYYb	TCTGTGCGTTATAGTGTG	TGATGTAGTGTCTTAGAGC
zfY2	TAGTTGTCATCGCCATC	CCATCTGTGCTACTTCC
zfY4	TGCTGTCCTCTGCTCCTC	CGCCTGTGTTCATCCTCTC
zfY7	GCAGTATGTGGTCCCTTTG	CATCTTGGTGGTCTTCTTCC
zfY8a	TCTCATTGGTGCTCATTGC	GGATGTTGAAGGATAGGAAGG
zfY8b	GCGGAGGACGACAGAAG	GGGAGCCAACACAAAGC

EXPRESSION LEVELS FOR mRNA

We investigated the mRNA expression level in a panel of eight organs (**Figure 3**). Ef1-α was used to normalize expression due to differences in cDNA concentration between samples for all tissues. All three peptides show a similar expression pattern with high expression in kidney, heart, and brain. PYYb was the only peptide with clear expression in the gastrointestinal tract (GI). Note that the scales on the Y axes differ. The receptors too have expression patterns that resemble one another with the main mRNA expression in heart, kidney, and brain (**Figure 4**). The Y7 receptor also has expression in liver.

DISCUSSION

The main objective of this study was to examine the affinity of PYYb for the Y4, Y7, Y8a, and Y8b receptors, and to compare it to the other two endogenous peptides, NPY and PYYa. Before this study, PYYb had only been analyzed with regard to its binding to the Y2 and Y2-2 receptors with the result that Y2 displayed a clear preference for PYYb, the difference in affinity in comparison with PYYa being as great as 50-fold (Fredriksson et al., 2006).

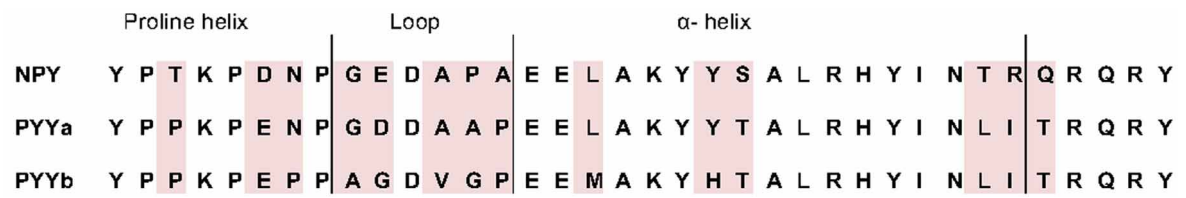

FIGURE 1 | Alignment of the mature peptides in the NPY family in zebrafish. Amino acid differences are shadowed. Proposed tertiary structures are described at the top.

Table 2 | The K_i values and standard error of the mean for four zebrafish receptors and their endogenous ligands.

	Y4(Ya)		Y7		Y8a (Yc)		Y8b (Yb)	
Ligand	K_i (nM) ± *SEM*	*n*	K_i (nM) ± *SEM*	*n*	K_i (nM) ± *SEM*	*n*	K_i (nM) ± *SEM*	*n*
zfNPY	0.033 ± 0.005	3	0.57 ± 0.16	5	0.21 ± 0.07	7	0.13 ± 0.04	4
zfPYYa	0.034 ± 0.009	3	1.8 ± 0.14	3	0.51 ± 0.001	6	0.25 ± 0.12	3
zfPYYb	0.028 ± 0.013	3	0.59 ± 0.17	5	0.32 ± 0.15	4	0.22 ± 0.14	4

n, number of experiments done. The Kd value of pPYY for the Y7 receptor is from Fredriksson et al. (2004), for Y4 from Starback et al. (1999) and for Y8a and Y8b receptor from Berglund et al. (2000).

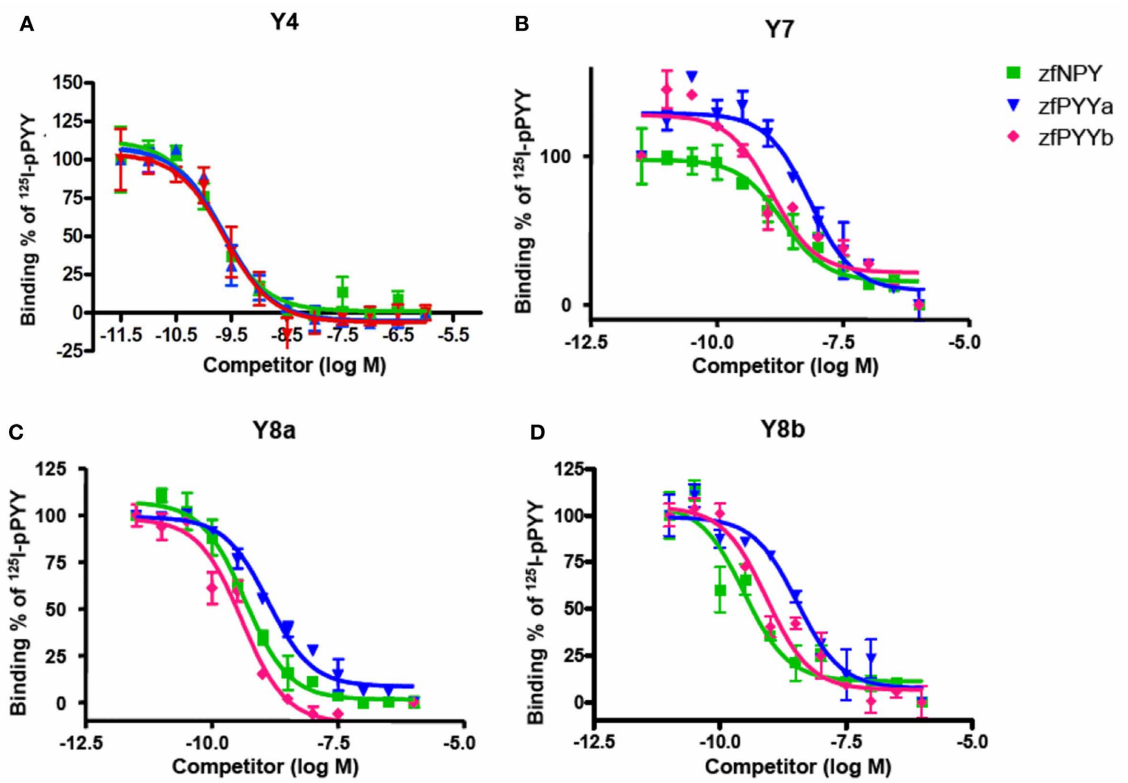

FIGURE 2 | Binding curves for each receptor. Panels **(A–D)** are the representative binding curves for Y4, Y7, Y8a and Y8b, respectively. The *x*-axis is the logarithmic concentration of unlabeled ligand. All experiments were done in duplicate. Note that the curve shown for the binding of zfPYYa to Y8b displays greater difference to the other peptides than if all experiments are considered (see **Table 2**).

In our present study, PYYb performs most similar to NPY despite the larger number of sequence differences between these two peptides (14) than between PYYb and PYYa (7), as well as PYYa and NPY (9). The most deviating binding constants are noted for PYYa which has a 3-fold lower affinity than NPY and PYYb for the Y7 receptor and slightly lower than the other two peptides for Y8a. The zebrafish Y7 receptor is 50% identical to zebrafish Y2 and PYYa has low affinity for both of these. This observation will be interesting to correlate with the physiological roles of the peptides and receptors in zebrafish. No differences in affinities were detected for the three zebrafish peptides in previous studies of the rainbow trout (*Onchorhynchus mykiss*) Y7 receptor. Zebrafish Y7 and rainbow trout Y7 have an amino acid identity of approximately 73% (Larsson et al., 2006). None of the receptors studied here showed the same kind of ligand preference as the Y2 receptor (Fredriksson et al., 2006). This indicates that Y2 may have a unique ligand partnership with PYYb in zebrafish, just as in the case for Y4 and PP in mammals. The evolutionary distance between zebrafish and trout has been estimated to be more than 300 million years (Hedges et al., 2006 http://www.timetree.org/), so there has been ample time to evolve differences in binding preference. The differences in affinity, comparing both within and between species, will be useful for precise mapping of the points of interaction between peptide ligands and receptor in three-dimensional modeling.

PYYa and PYYb are the result of a whole genome duplication that took place early in teleost evolution more the 300 million years ago (Sundstrom et al., 2008). All peptides in the family are believed to have a tertiary structure called the PP-fold or hairpin structure. The first eight amino acids are involved in a proline-helix followed by a β-turn constructed of position number 9–14 (see **Figure 1**). The remaining positions form an alpha helix, except for the last five (Darbon et al., 1992; Keire et al., 2000). Only two amino acids differ between PYYa and PYYb in the alpha-helix but four out of six positions have changes in the β-turn region (**Figure 1**). Only one amino acid has been changed in the proline helix but the change is from an aspargine to a proline which might have implications for the tertiary structure. These changes could possibly influence the binding pattern, even if the highly conserved amidated C-terminal of the peptide has been found to be the part most involved in receptor binding. The loop region has not previously been shown to have any effect on binding to the different Y receptors (Berglund et al., 2000).

Studies of how NPY affects appetite have been conducted in several fish species (Narnaware et al., 2000; Narnaware and Peter, 2001) and the NPY system seems to influence food intake, although the orexigenic receptor subtypes have not been identified in the fish species studied. The receptors that in mammals are involved in inhibition of appetite have been known in zebrafish

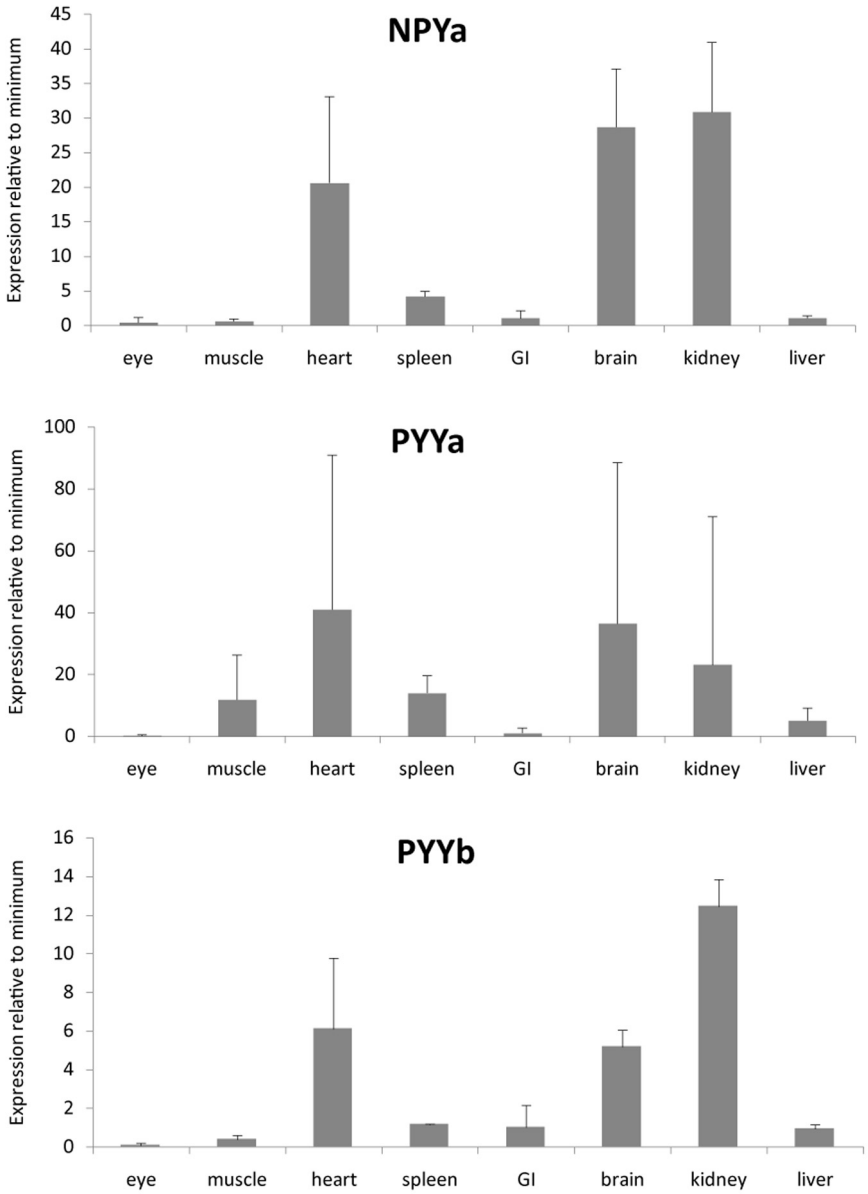

FIGURE 3 | Expression data for neuropeptide Y peptides in a panel of eight zebrafish organs. Error bars display the standard error of the mean. Normalized Ct values were used to calculate the relative expression values. For each transcript the tissue with the lowest expression was used to calculate relative expression. For all genes, the eye had lowest expression. The analysis was performed twice, each time with duplicate samples.

for several years and there have been some studies investigating how PYY can inhibit feeding. In mammals it seems like the main inhibitory action on appetite is performed by PYY3-36. This peptide is the result of cleavage of the two first amino acids by dipeptidyl peptidase IV (Mentlein et al., 1993). This processing probably turned out to be favorable because it results in selectivity for Y2 over the other receptor subtypes, of which especially Y1 is expressed in blood vessels, at least in mammals. However, all known non-mammalian PYY peptides start with the sequence Tyr-Pro-Pro (Cerda-Reverter and Larhammar, 2000; Sundstrom et al., 2008) and the peptidase is unable to cleave between two proline residues. Thus, non-mammalian PYY cannot be processed to the Y2-preferring PYY3-36 in non-mammals. However, the non-selectivity of intact PYY may have no biological consequences if no other receptor subtypes can be targeted by PYY circulating in the blood.

Measurements of the changes of PYYa expression in fed and fasted fishes have been conducted for several species. No expression difference was detected in either brain or GI tract for salmon (Murashita et al., 2009), while fed goldfish showed a higher expression of PYYa in the brain compared to the unfed (Gonzalez and Unniappan, 2010). One study has also investigated the expression pattern of PYYb in yellowtail (*Seriola quinqueradiata*) showing a higher expression in the

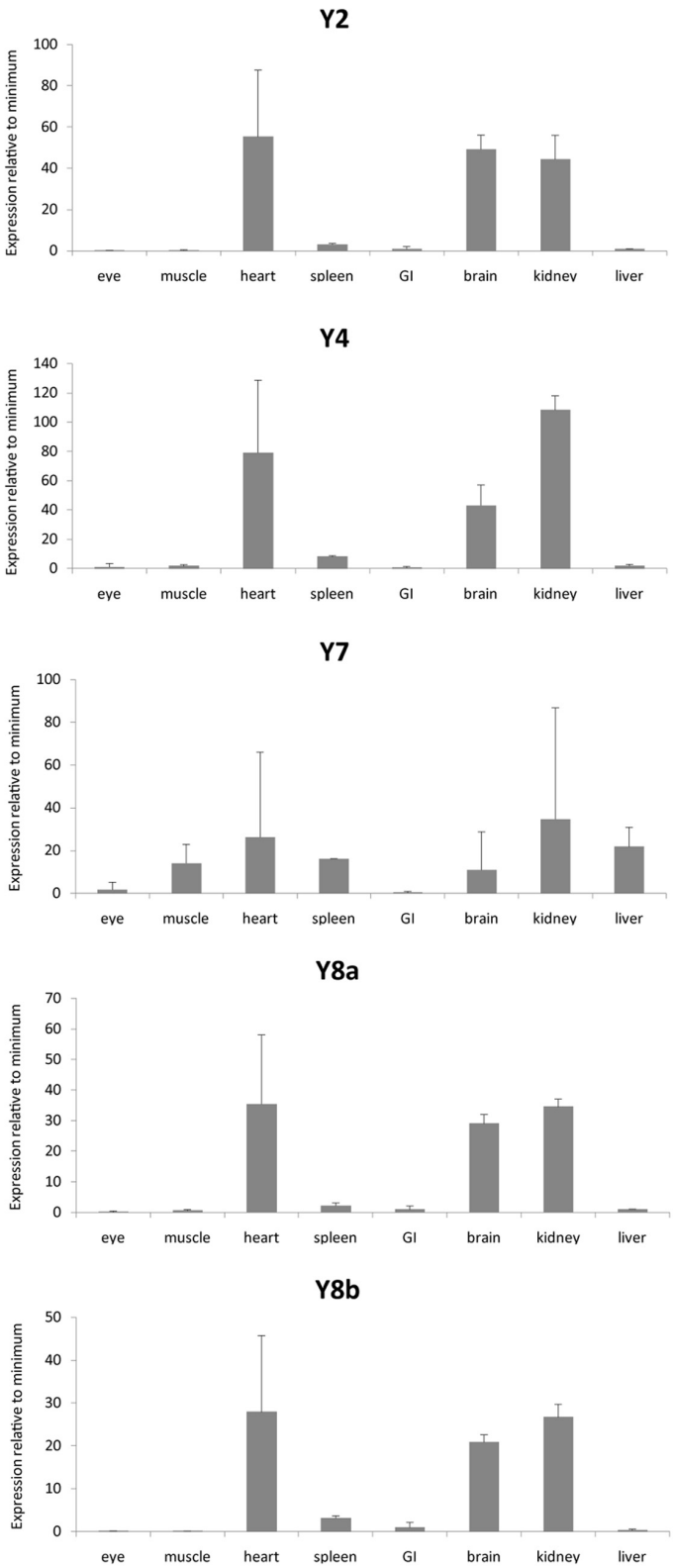

FIGURE 4 | Expression data for neuropeptide Y receptors in a panel of eight zebrafish organs. Error bars display standard error of the mean. Normalized Ct values were used to calculate the relative expression values. For each transcript the tissue with the lowest expression was used to calculate relative expression. For the different genes, the following tissues had lower expression: Y2, eye; Y4, GI; Y7, GI; Y8a, eye; Y8b, muscle. The analysis was performed twice, each time with duplicate samples.

intestine in fasted fishes compared to fed fishes (Murashita et al., 2006). Goldfish have been injected with full-length PYYa either intraperitoneally or intracerebroventricularly. Independent of administration method, food intake was reduced by approximately 30% (Gonzalez and Unniappan, 2010). Both the salmon lineage and the goldfish/carp lineage have however undergone additional separate whole genome duplications (i.e., a fourth tetraploidization) which might have resulted in extra receptors (and peptides) in the NPY system.

PYY and PP are mainly expressed in endocrine cells in the GI in mammals (Larhammar and Salaneck, 2004). Zebrafish has two different PYY peptides and in our quantitative PCR analysis it was possible to detect some difference in the tissue distribution between PYYa and PYYb. PYYb has some expression in the GI (**Figure 3**) which indicates that subfunctionalization has occurred since the duplication. Neither the peptides nor the receptors showed any expression in the eye. Among the receptors, Y7 was the only one investigated that showed expression in liver. The NPY system seems to be prominent in the kidney because all three peptides and all five receptors were abundant there, suggesting perhaps a role in electrolyte regulation. These findings encourage detailed studies with *in situ* hybridization to see which cell types express the peptides and the receptors.

Zebrafish diverged early in teleost evolution and it is the only fully sequenced teleost where a Y1 receptor has been described. Studies of the binding to the Y1 receptor show similar affinities for all three peptides (Larson and Larhammar, in preparation). Taken together, these results show that some lineage specific peptide-receptor affinity differences have evolved in the teleost lineage after duplication of the NPY system genes. Y2 may have a unique ligand partnership with PYYb and the PYYa peptide has low affinity for both Y7 and Y2. Functional studies are required to see if the affinity differences have physiological importance. Some subfunctionalization regarding tissue distribution seems also to have occurred both among the peptides and receptors. These comprise important aspects to take into consideration to understand the complex NPY system of teleost fish.

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REFERENCES

Berglund, M. M., Lundell, I., Cabrele, C., Serradeil-Le Gal, C., Beck-Sickinger, A. G., and Larhammar, D. (2000). Binding properties of three neuropeptide Y receptor subtypes from zebrafish: comparison with mammalian Y1 receptors. *Biochem. Pharmacol.* 60, 1815–1822.

Cerda-Reverter, J. M., and Larhammar, D. (2000). Neuropeptide Y family of peptides: structure, anatomical expression, function, and molecular evolution. *Biochem. Cell Biol.* 78, 371–392.

Darbon, H., Bernassau, J. M., Deleuze, C., Chenu, J., Roussel, A., and Cambillau, C. (1992). Solution conformation of human neuropeptide Y by 1H nuclear magnetic resonance and restrained molecular dynamics. *Eur. J. Biochem.* 209, 765–771.

Fallmar, H., Sundstrom, G., Lundell, I., Mohell, N., and Larhammar, D. (2011). Neuropeptide Y/peptide YY receptor Y2 duplicate in zebrafish with unique introns displays distinct peptide binding properties. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 160, 166–173.

Fredriksson, R., Larson, E. T., Yan, Y. L., Postlethwait, J. H., and Larhammar, D. (2004). Novel neuropeptide Y Y2-like receptor subtype in zebrafish and frogs supports early vertebrate chromosome duplications. *J. Mol. Evol.* 58, 106–114.

Fredriksson, R., Sjodin, P., Larson, E. T., Conlon, J. M., and Larhammar, D. (2006). Cloning and characterization of a zebrafish Y2 receptor. *Regul. Pept.* 133, 32–40.

Garczynski, S. F., Brown, M. R., Shen, P., Murray, T. F., and Crim, J. W. (2002). Characterization of a functional neuropeptide F receptor from Drosophila melanogaster. *Peptides* 23, 773–780.

Gonzalez, R., and Unniappan, S. (2010). Molecular characterization, appetite regulatory effects and feeding related changes of peptide YY in goldfish. *Gen. Comp. Endocrinol.* 166, 273–279.

Hedges, S. B., Dudley, J., and Kumar, S. (2006). TimeTree: a public knowledge-base of divergence times among organisms. *Bioinformatics* 22, 2971–2972.

Hill, C. A., Fox, A. N., Pitts, R. J., Kent, L. B., Tan, P. L., Chrystal, M. A., et al. (2002). G protein-coupled receptors in Anopheles gambiae. *Science* 298, 176–178.

Keire, D. A., Kobayashi, M., Solomon, T. E., and Reeve, J. R. Jr. (2000). Solution structure of monomeric peptide YY supports the functional significance of the PP-fold. *Biochemistry* 39, 9935–9942.

Larhammar, D., and Salaneck, E. (2004). Molecular evolution of NPY receptor subtypes. *Neuropeptides* 38, 141–151.

Larsson, T. A., Larson, E. T., Fredriksson, R., Conlon, J. M., and Larhammar, D. (2006). Characterization of NPY receptor subtypes Y2 and Y7 in rainbow trout *Oncorhynchus mykiss*. *Peptides* 27, 1320–1327.

Larsson, T. A., Tay, B. H., Sundstrom, G., Fredriksson, R., Brenner, S., Larhammar, D., et al. (2009). Neuropeptide Y-family peptides and receptors in the elephant shark, *Callorhinchus milii* confirm gene duplications before the gnathostome radiation. *Genomics* 93, 254–260.

Lee, N. J., and Herzog, H. (2009). NPY regulation of bone remodelling. *Neuropeptides* 43, 457–463.

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25, 402–408.

Lundell, I., Berglund, M. M., Starback, P., Salaneck, E., Gehlert, D. R., and Larhammar, D. (1997). Cloning and characterization of a novel neuropeptide Y receptor subtype in the zebrafish. *DNA Cell Biol.* 16, 1357–1363.

Lundell, I., Blomqvist, A. G., Berglund, M. M., Schober, D. A., Johnson, D., Statnick, M. A., et al. (1995). Cloning of a human receptor of the NPY receptor family with high affinity for pancreatic polypeptide and peptide YY. *J. Biol. Chem.* 270, 29123–29128.

Lundell, I., Boswell, T., and Larhammar, D. (2002). Chicken neuropeptide Y-family receptor Y4: a receptor with equal affinity for pancreatic polypeptide, neuropeptide Y and peptide YY. *J. Mol. Endocrinol.* 28, 225–235.

Mentlein, R., Dahms, P., Grandt, D., and Kruger, R. (1993). Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. *Regul. Pept.* 49, 133–144.

Murashita, K., Fukada, H., Hosokawa, H., and Masumoto, T. (2006). Cholecystokinin and peptide Y in yellowtail (*Seriola quinqueradiata*): molecular cloning, real-time quantitative RT-PCR, and response to feeding and fasting. *Gen. Comp. Endocrinol.* 145, 287–297.

Murashita, K., Kurokawa, T., Nilsen, T. O., and Ronnestad, I. (2009). Ghrelin, cholecystokinin, and peptide YY in Atlantic salmon (*Salmo salar*): molecular cloning and tissue expression. *Gen. Comp. Endocrinol.* 160, 223–235.

Narnaware, Y. K., and Peter, R. E. (2001). Neuropeptide Y stimulates food consumption through multiple receptors in goldfish. *Physiol. Behav.* 74, 185–190.

Narnaware, Y. K., Peyon, P. P., Lin, X., and Peter, R. E. (2000). Regulation of food intake by neuropeptide Y in goldfish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 279, R1025–R1034.

Pedrazzini, T., Pralong, F., and Grouzmann, E. (2003). Neuropeptide Y: the universal soldier. *Cell. Mol. Life Sci.* 60, 350–377.

Ramakers, C., Ruijter, J. M., Deprez, R. H., and Moorman, A. F. (2003). Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci. Lett.* 339, 62–66.

Ringvall, M., Berglund, M. M., and Larhammar, D. (1997). Multiplicity of neuropeptide Y receptors: cloning of a third distinct subtype in the zebrafish. *Biochem. Biophys. Res. Commun.* 241, 749–755.

Salaneck, E., Larsson, T. A., Larson, E. T., and Larhammar, D. (2008). Birth and death of neuropeptide Y receptor genes in relation to the teleost fish tetraploidization. *Gene* 409, 61–71.

Starback, P., Lundell, I., Fredriksson, R., Berglund, M. M., Yan, Y. L., Wraith, A., et al. (1999). Neuropeptide Y receptor subtype with unique properties cloned in the zebrafish: the zYa receptor. *Brain Res. Mol. Brain Res.* 70, 242–252.

Sundstrom, G., Larsson, T. A., Brenner, S., Venkatesh, B., and Larhammar, D. (2008). Evolution of the neuropeptide Y family: new genes by chromosome duplications in early vertebrates and in teleost fishes. *Gen. Comp. Endocrinol.* 155, 705–716.

Suzuki, K., Simpson, K. A., Minnion, J. S., Shillito, J. C., and Bloom, S. R. (2010). The role of gut hormones and the hypothalamus in appetite regulation. *Endocr. J.* 57, 359–372.

Tensen, C. P., Cox, K. J., Burke, J. F., Leurs, R., Van Der Schors, R. C., Geraerts, W. P., et al. (1998). Molecular cloning and characterization of an invertebrate homologue of a neuropeptide Y receptor. *Eur. J. Neurosci.* 10, 3409–3416.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 3, RESEARCH0034.

Zhang, L., Bijker, M. S., and Herzog, H. (2011). The neuropeptide Y system: pathophysiological and therapeutic implications in obesity and cancer. *Pharmacol. Ther.* 131, 91–113.

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Neuroendocrine regulation of appetitive ingestive behavior

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Food availability in nature is often irregular, and famine is commonplace. Increased motivation to engage in ingestive behaviors increases the chance of survival, providing additional potential opportunities for reproduction. Because of the advantages conferred by entraining ingestive behavior to environmental conditions, neuroendocrine mechanisms regulating the motivation to acquire and ingest food have evolved to be responsive to exogenous (i.e., food stored for future consumption) and endogenous (i.e., body fat stores) fuel availability. Motivated behaviors like eating occur in two phases. The appetitive phase brings animals into contact with food (e.g., foraging, food hoarding), and the more reflexive consummatory phase results in ingestion (e.g., chewing, swallowing). Quantifiable appetitive behaviors are part of the natural ingestive behavioral repertoire of species such as hamsters and humans. This review summarizes current knowledge about neuroendocrine regulators of ingestive behavior, with an emphasis appetitive behavior. We will discuss hormonal regulators of appetitive ingestive behaviors, including the orexigenic hormone ghrelin, which potently stimulates foraging and food hoarding in Siberian hamsters. This section includes a discussion of the hormone leptin, its relation to endogenous fat stores, and its role in food deprivation-induced increases in appetitive ingestive behaviors. Next, we discuss how hormonal regulators interact with neurotransmitters involved in the regulation of ingestive behaviors, such as neuropeptide Y (NPY), agouti-related protein (AgRP) and α-melanocyte stimulating hormone (α-MSH), to regulate ingestive behavior. Finally, we discuss the potential impact that perinatal nutrient availability can have on the neuroendocrine regulation of ingestive behavior. Understanding the hormonal mechanisms that connect metabolic fuel availability to central appetite regulatory circuits should provide a better understanding of the neuroendocrine regulation of the motivation to engage in ingestive behavior.

Keywords: food hoarding, leptin, ghrelin, neuropeptide Y, maternal effects, gestational programming, hypothalamus, obesity

INTRODUCTION

Although obesity was rare in the human population for thousands of years (Haslam, 2007), it is now one of the leading health issues worldwide and has been declared a global epidemic by the World Health Organization (Caballero, 2007). Despite concerted efforts to solve this problem, obesity rates continue to rise worldwide. To understand human ingestive behavior and the origins of this epidemic, it is useful to consider an evolutionary perspective (Bronson, 1989; Neel, 1999; Prentice, 2005; Schneider, 2006). Fluctuating food supply and famine have been a consistent threat to survival for most animals, including humans (Prentice, 2005). Selection pressures resulting from these fluctuations favor the ability to store energy sources on the body as fat and/or the propensity to store food in the home. These abilities provide a buffer against a variable or unpredictable food supply, and thus allow animals to optimize reproductive success (Prentice, 2005; Schneider, 2006). These phenotypic properties are physiologic (turning off unnecessary functions), metabolic (slowing basal metabolic rate), behavioral (binge eating, food hoarding, decreased physical activity), and adipogenic (generation of internal fat stores, (Prentice, 2005). In western, industrialized societies, humans inhabit an environment with an overabundance of highly palatable, easily accessible food sources, transforming these once beneficial adaptations into health liabilities. Therefore, understanding the basic neuroendocrine regulation of food and body fat storage and the mechanisms underlying the adaptations to a food-insecure environment is a central issue to understanding and combating the obesity epidemic (Ulijaszek, 2002).

Human obesity studies typically focus on metabolism, adipogenesis, and meal patterns. Human foraging and hoarding behavior typically is dismissed as irrelevant to understanding the health issues surrounding overeating, obesity, and diabetes. Some data is available, however, on food purchasing behavior, and these studies indicated that 85% of the energy content of the human diet consists of food purchased for consumption at home (Ransley et al., 2003). These types of studies also confirm the well-known anecdote that purchasing food when food deprived or "hungry" increases food purchasing, (Dodd et al., 1977; Tom, 1983). In addition, obese individuals are likely to purchase more food per person and more calorically dense food (Ransley et al., 2003; Epstein et al., 2007). After foraging for food at the grocery store, humans then store the food at home for

future consumption in freezers and pantries (a.k.a. hoarding). Efforts to understand the neuroendocrine differences responsible for these incongruities between the behaviors of lean and obese individuals have employed a variety of animal models.

Food intake is the most studied ingestive behavior, but other ingestive behaviors are likely to be important. In the long history of research in behavioral endocrinology, motivated behavior is thought to be comprised of two main phases: the appetitive and the consummatory phases (Craig, 1918). Appetitive behaviors are flexible, non-stereotyped responses that bring an animal into contact with a goal object, whereas consummatory behaviors are the final reflexive, stereotyped responses after decisions and efforts have been made to reach the goal object. Both appetitive and consummatory behaviors can increase with hunger and the desire immediately to consume food and to store energy. Eaten food can be stored on the body as adipose tissue depots, while hoarded food is stored in the home or burrow for later consumption during energetic deficits. The important distinction lies in the fact that consummatory behaviors also reflect the ability to perform motor actions. Therefore, measures of food intake can be confounded by limits on the ability of the animal to eat or digest food, or by many other postingestional cues. By contrast, appetitive behaviors are a more "pure" reflection of motivation for acquiring fuels either for eating or calorie storage. Some appetitive ingestive behaviors occur at different times than consummatory behaviors, and appetitive behaviors most useful for scientific study are those that can occur even in the absence of changes in food intake (Bartness and Clein, 1994; Bartness, 1997; Buckley and Schneider, 2003). For example, hungrier animals initiate meals sooner, avidly consume an unpalatable substance, make more effort to gain access to food, and hoard more food in their home or burrow (reviewed by Keen-Rhinehart et al., 2010; Bartness et al., 2011; Schneider et al., 2013). This review will focus on appetitive ingestive behaviors that: (1) are easily quantified, (2) occur separate in time and space from consummatory behaviors, (3) are controlled by different chemical messengers, neural substrates, and environmental stimuli than consummatory behaviors, and (4) are able to provide an indication of the animals' internal motivational state. The neuroendocrine mechanisms that control consummatory ingestive behavior (food consumption) are relatively well studied (for reviews see: Kalra et al., 1999; Abizaid and Horvath, 2012; Kageyama et al., 2012). After a brief review of the neuroendocrine regulation of consummatory ingestive behavior, this review will focus mostly on the neuroendocrine basis for appetitive behavior, with a major emphasis on food hoarding as a quintessential appetitive behavior.

NEUROENDOCRINE REGULATION OF CONSUMMATORY INGESTIVE BEHAVIOR

Historically, the majority of ingestive behavior studies have used laboratory rats or mice as model systems, measuring the food consumption of animals housed in isolation. Therefore, it is useful to review these data as a starting point for understanding the neuroendocrine control of ingestive behavior. Many neuroendocrine factors affect food intake in laboratory animals (reviewed by Schneider and Watts, 2002; Schneider et al., 2012). Adrenal steroids (Stevenson and Franklin, 1970), and other proteins and peptides of the hypothalamic-pituitary-adrenal (HPA) axis significantly impact food intake (reviewed by Heinrichs and Richard, 1999; Bergonzelli et al., 2001). In addition, estradiol, testosterone, progesterone, and other neuroendocrine factors of the hypothalamic-pituitary-gonadal axis (HPG) axis affect food intake (reviewed by Wade, 1976; Schneider and Wade, 1999; Schneider et al., 2002). Finally, the metabolic hormones, leptin, insulin, glucagon, ghrelin, and glucagon-like peptide-I (GLP-I), as well as central factors, such as neuropeptide Y (NPY), agouti-related protein (AgRP), cocaine and amphetamine-regulated transcript (CART) and the melanocortins reliably affect consummatory ingestive behavior in laboratory animals (reviewed by Barsh and Schwartz, 2002; Zigman and Elmquist, 2003; Schwartz and Porte, 2005). Most recently, there has also been a push to understand the role of reward circuits and dopamine in the regulation of ingestive behavior (Abizaid et al., 2006b; Hommel et al., 2006; Abizaid, 2009). We shall attempt to review the contributions of each of these three systems to the neuroendocrine regulation of consummatory ingestive behavior.

THE ARCUATE PERSPECTIVE ON THE REGULATION OF CONSUMMATORY INGESTIVE BEHAVIOR

The often co-localized orexigenic neuropeptides AgRP and NPY are the major products of the Arc that stimulate food intake (Kalra et al., 1999; Morton and Schwartz, 2001; Ellacott and Cone, 2004). The anorexigenic neuropeptide α-melanocyte stimulating hormone (α-MSH), a cleavage product of the proopiomelanocortin (POMC) gene, and CART are thought to oppose the effects of NPY and AgRP (reviewed by Zigman and Elmquist, 2003; Schwartz and Porte, 2005). According to the Arc perspective, anorexigenic and orexigenic neuron populations in the Arc respond to changes in the availability of oxidizable metabolic fuels, body fat stores, and/or peripheral hormones such as glucocorticoids, ghrelin, and leptin (Baskin et al., 1999; Cone et al., 2001). Peripheral hormones bind to receptors on Arc NPY/AgRP and POMC/CART neurons, affecting gene transcription (Coppari et al., 2005; Elmquist et al., 2005) and the synaptic inputs to the Arc neurons (reviewed by Zigman and Elmquist, 2003; Gao et al., 2007; Gyengesi et al., 2010; Briggs and Andrews, 2011; Eckel, 2011).

Although the plethora of evidence in support of the "arcuate perspective" seems to provide a simple, well-constructed model for the regulation of food intake at first glance, many other studies indicate that the Arc is neither necessary nor sufficient for the regulation of ingestive behavior (Grill, 2010). For example, neonatal Arc ablation does not disrupt food intake (Coppari et al., 2005), nor does adult Arc ablation have an effect if the animals are given GABA agonists in the parabrachial nucleus [PBN (Wu et al., 2009)]. In addition, food intake increases when the leptin receptor (LepR) is deleted outside the Arc and when orexigenic neuropeptides are injected in extra-arcuate regions of the brain (Faulconbridge et al., 2005; Dhillon et al., 2006; Musatov et al., 2007). The "arcuate perspective" also fails to incorporate extra-Arc populations of NPY, AgRP, and α-MSH neurons in areas such as the dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH), paraventricular nucleus of the

hypothalamus (PVN) and lateral hypothalamus (LHA), as well as how these cells are connected to the PBN, the dorsomotor nucleus of the vagus (DMV) and the nucleus of the solitary tract (NTS) in the hindbrain, that are responsible for the motor movements necessary for food intake (Zheng et al., 2010). It has also been demonstrated that peripheral sensory information reaches the diencephalon via the afferent visceral-central pathway and that vagal afferent NTS neurons connecting directly to pre-oral motor neurons of the reticular formation are both essential and sufficient for consummatory ingestive behavior (reviewed by Grill, 2010). Therefore, examining the role of these extra-Arc areas in the regulation of consummatory ingestive behavior in the future should provide a more complete understanding of the neural circuitry controlling food intake.

ROLE OF THE HPA AXIS IN THE REGULATION OF CONSUMMATORY INGESTIVE BEHAVIOR

It has long been known that stress and adrenal steroids affect food consumption (Stevenson and Franklin, 1970). The HPA axis provides critical input to the neuroendocrine mechanisms that regulate energy intake, storage, and expenditure (reviewed by Heinrichs and Richard, 1999). Within the HPA axis, corticotropin-releasing hormone (CRH) regulates the secretion of adrenocorticotropic hormone (ACTH), which controls the secretion of adrenal steroids. Food deprivation decreases CRH expression in the PVN (Brady et al., 1990). CRH plays an anorexigenic role in the basal regulation of food intake (Karolyi et al., 1999). Although CRH is anorectic, glucocorticoids are orexigenic, and obesity does not occur in their absence. For example, lesion-, diet-, NPY-, and leptin gene mutation-induced obesity are reversed by adrenalectomy or by lack of glucocorticoids caused by shutting down the HPA axis (Bruce et al., 1982; Romsos et al., 1987; Stanley et al., 1989; Bray et al., 1992; Feldkircher et al., 1996).

HPA axis hormones and neuropeptides are highly conserved across a wide array of species, with regard to both chemical structure and the effects on the ingestive behavior (Landys et al., 2006). The CRH gene sequence is highly homologous in organisms from fish to mammals, and central CRH treatment decreases food intake in all classes of vertebrates tested to date (Carr, 2002) without increasing plasma glucocorticoids, unlike peripheral administration that increases both glucocorticoid levels and food consumption (Glowa et al., 1992). Although CRH is anorexigenic, glucocorticoids typically stimulate food consumption (Dallman et al., 1993, 2004) and provide negative feedback to inhibit CRH production and release. Thus, orexigenic effects of glucocorticoids could occur via direct effects on appetite regulatory mechanisms or via indirect effects by inhibiting the synthesis and release of CRH (reviewed by Heinrichs and Richard, 1999). The opposing effects of CRH and glucocorticoids are likely part of an adaptive response to stress (Sapolsky et al., 2000). Stress causes the release of adrenaline and CRH, which inhibit ingestive behaviors and trigger "fight-or-flight" behaviors, increasing the likelihood of survival. Increases in CRH directly stimulate sympathetic tone and the uptake of oxygen and metabolic fuel by the brain and muscles. The resulting rise in glucocorticoids inhibits CRH via action on central receptors to turn off the stress response, and then ingestive behavior resumes once the stressful situation has been resolved (Sapolsky et al., 2000). This sequence of events facilitates the reinstatement of energy balance by replacing the energy expended on the fight-or-flight response (Sapolsky et al., 2000). Stress responses are energetically expensive, and the ability to replenish lost energy is affected by fluctuating variables such as food availability, mate availability, and ambient temperature making the metabolic fuel availability a significant modulator of the effects of CRH and glucocorticoids on behavior.

ROLE OF REWARD CIRCUITS IN THE REGULATION OF CONSUMMATORY INGESTIVE BEHAVIOR

Further complexity in the neuroendocrine regulation of consummatory ingestive behavior is afforded by the involvement of the mesolimbocortical dopaminergic (DA) system, which includes the nucleus accumbens, ventral tegmental area (VTA), and parts of the amygdala. This system is implicated in modulating behaviors such as learning, appetite, motivation and reward, as well as the willingness to work to access highly palatable foods (reviewed by Abizaid, 2009). Partly because of the overabundance of data supporting the hypothesis that obesity can be attributed to an addiction to food (Stice et al., 2012; Volkow et al., 2013), numerous studies have been conducted to investigate the connections between metabolic hormones and central reward circuits (for review see Hirasawa et al., 2007; Pandit et al., 2011; Schellekens et al., 2012; Volkow et al., 2013). For example, leptin is apparently able to reduce appetite by diminishing the rewarding value of food. Studies supporting this notion show that increased leptin signaling specifically in the VTA decreases food intake and firing rates of DA neurons while treatments that impair leptin signaling in the VTA increase DA neuronal firing rates, food intake and the proclivity to ingest highly palatable foods (Fulton et al., 2002, 2006; Hommel et al., 2006; Abizaid, 2009). Ghrelin also appears to modulate food intake via action in the VTA, and peripheral ghrelin treatment increases DA turnover, food intake and synapses connecting with DA cells in the VTA in mice (Abizaid et al., 2006a,b). Therefore, the midbrain has emerged as an important site for the integration of information about peripheral metabolic factors in the service of ingestive behavior regulation.

APPETITIVE INGESTIVE BEHAVIOR

It is typically assumed that if a substance applied to the brain affects food intake, it must function in the service of energy balance. One must also consider the possibility that these factors may have altogether different purposes for an animal in a naturalistic setting. It is unlikely that these other functions will be observed in laboratory experiments studying singly housed animals with unlimited food and few prospects for species-typical behaviors or opportunities for energy expenditure. For example, experimentally induced increases in food consumption in an individually housed animal may actually affect motivation that would stimulate foraging, hoarding, or hunting behavior in a more naturalistic setting. Experimentally induced decreases in hunger might increase mate searching, courtship, mating, nest building, incubation, migration, or hibernation. In nature, ingestive behavior must be balanced with other priorities such as predator avoidance, food availability, and copulation. Regardless of the animal

model, experiments examining only consummatory ingestive behavior in isolated animals might support the idea that 40 or more redundant peptide systems regulate this one variable, food intake. Therefore, manipulations of environmentally relevant variables (e.g., nutrient availability) to determine how these affect the expression of appetitive behaviors (those other than food intake) are necessary to provide a more complete understanding about the neuroendocrine regulation of the motivation to eat.

APPETITIVE BEHAVIORS: INDICATORS OF MOTIVATION

Ingestive behavior is multifaceted and cannot be accurately captured by measuring the amount of food consumed because swallowing is only one step in a long sequence of behaviors necessary for eating. Appetitive ingestive behaviors aim to ensure that sufficient energy will be available for future energetic needs (Craig, 1918; Everitt et al., 1984). Studying appetitive ingestive behaviors is the preeminent way to assess the internal motivation to consume food. Furthermore, the study of appetitive behaviors can provide information about how environmental factors such as nutrient availability impact behavioral priorities.

In certain well-designed experimental paradigms, appetitive behaviors are separable from consummatory behaviors, and the mechanisms underlying these two phases of ingestive behaviors can be studied separately. The significance of the separation of the appetitive and consummatory phases of ingestive behavior lies in the fact that the two phases are initiated by different environmental conditions via various neuroendocrine factors that act on separate neural substrates (reviewed by Ball and Balthazart, 2008). One of the clearest examples of the separate neuroendocrine regulation of these behavioral phases is the control of consummatory but not ingestive behaviors by the brain stem (Grill and Smith, 1988; Grill and Kaplan, 2001, 2002). This is skillfully illustrated in experiments showing that decerebrate rats have intact neuroendocrine regulation of intraoral food intake in the absence of the ability to express any appetitive behaviors (Grill and Kaplan, 1992, 2001, 2002; Grill and Hayes, 2009).

To understand the neuroendocrine mechanisms regulating appetitive ingestive behaviors and thus the motivation to eat, it is necessary to look beyond the commonly studied rats and mice to other animal models where appetitive behaviors such as food hoarding are separable from food consumption. While rats and mice typically increase food intake in response to fasting or food restriction (Ross and Smith, 1953; Hill et al., 1983, 1984), hamsters, like humans, reliably increase food hoarding instead of food intake in response to energetic challenges (Silverman and Zucker, 1976; Rowland, 1982; Billington et al., 1984; Bartness and Clein, 1994; Bartness et al., 1995). In fact, *Homo sapiens*, unlike rats and mice, do not typically overeat in response to food deprivation (reviewed by Bartness et al., 2011). People do, however, increase grocery shopping (foraging) and bring more food home (hoarding) in correlation with the length of time since their last meal (Dodd et al., 1977; Beneke and Davis, 1985; Mela et al., 1996). In addition, recent studies on overweight and obese children indicate that a significant proportion of them exhibit disturbances in eating behaviors that include sneaking, hiding and hoarding large quantities of food (Sonneville et al., 2013). Therefore, using a hamster model that responds to energetic challenges such as food deprivation with prolonged increases in food hoarding but not food intake permits the disentanglement of the mechanisms underlying appetitive and consummatory ingestive behaviors that are inextricably intertwined in other rodent models (for review see: Keen-Rhinehart et al., 2010; Bartness et al., 2011). Over the past two decades, studies using Siberian hamsters have thoroughly and systematically determined how appetitive ingestive behaviors are affected by fasting, diet dilution and lipectomy as well as treatment with orexigenic factors such as NPY, Y1R agonists, AgRP, and ghrelin and anorexigenic factors such as leptin, MT-II, CCK, and Y1R antagonists (Bartness and Clein, 1994; Bartness et al., 2011; Wood and Bartness, 1996a,b; Bartness, 1997; Day and Bartness, 2003, 2004; Day et al., 2005; Keen-Rhinehart and Bartness, 2005, 2007; Keen-Rhinehart et al., 2010; Teubner and Bartness, 2010; Teubner et al., 2012).

APPETITIVE BEHAVIOR REGULATION: BEYOND THE ARCUATE PERSPECTIVE

As mentioned earlier, many investigators in the field of ingestive behavior consider the Arc to be the hub for the regulation of food intake. Therefore, initial experiments regarding the neuroendocrine mechanisms that regulate appetitive ingestive behaviors focused on this area as a starting point. As mentioned previously, food deprivation in Siberian hamsters causes large increases in food hoarding with little to no effects on food intake (Bartness and Clein, 1994; Bartness, 1997). The orexigenic hormone ghrelin is increased in response to food deprivation in hamsters, similarly to other animals (Ariyasu et al., 2001; Asakawa et al., 2001; Keen-Rhinehart and Bartness, 2005). When hamsters are treated with ghrelin at doses that produce circulating ghrelin concentrations similar to a 48 h fast, there is an initial, small increase in food intake followed by a massive, long-term increase in food hoarding behavior (Keen-Rhinehart and Bartness, 2005). We now know that both food deprivation and ghrelin stimulate Arc NPY and AgRP neurons (Hahn et al., 1998; Mercer et al., 2000), triggering the release of these orexigenic neuropeptides (Chen et al., 2004). As one would expect, central treatment with either of these orexigenic factors trigger marked increases in food hoarding with minimal increases in food intake [i.e., NPY (Day et al., 2005; Keen-Rhinehart and Bartness, 2005) and AgRP (Day and Bartness, 2004)]. Conversely, the anorexigenic hormone leptin is postulated to act on LepR-expressing hypothalamic neurons (Baskin et al., 1999; Elmquist, 2001) to reduce appetite by reducing expression of NPY and AgRP, and by increasing expression of anorexigenic neuropeptides like α-MSH (Baskin et al., 2001; Elmquist et al., 2005). In hamsters, central or peripheral treatment with leptin reduces fasting- and ghrelin-induced increases in food hoarding behavior (Keen-Rhinehart and Bartness, 2008).

Because the expression of appetitive and consummatory ingestive behaviors are separable in hamsters, the neural substrates controlling food hoarding should be at least partially distinct from those that govern food intake. Several studies provide data in support of that hypothesis. For example, in Siberian hamsters, Arc lesions diminish NPY-induced increases in food intake but not food hoarding (Dailey and Bartness, 2010), and studies using Y1R- and Y5R-specific agonists indicate that NPY increases food intake via action at the Y5 receptor but affects food hoarding by

acting on the Y1 receptor (Keen-Rhinehart and Bartness, 2007). In addition, recent studies show that the pharmacological blockade of ghrelin activity attenuates, but cannot completely block, food deprivation-induced increases in food hoarding (Teubner and Bartness, 2013; Teubner et al., 2013).

While Siberian hamsters typically exhibit increases in food hoarding rather than food intake in response to energetic challenges, there is initially a small increase in food intake before the large increase in food hoarding behavior. Exploiting the fact that increases in food intake appear more rapidly than increases in food hoarding, Teubner et al., differentiated the brain areas activated concurrently with short-term increases in consummatory behavior from those activated later in conjunction with long-term increases in appetitive behavior (Teubner et al., 2012). Combined central NPY + AgRP injection was associated with increased food intake and neuronal activation of the PVN and perifornical area (pFA) within 1 h post-injection, but increased food hoarding and neuronal activation in the PVN, pFA, central nucleus of the amygdala (CeA) and the subzona incerta (SZI) were not detectible until 4–14 h post-injection. These data pinpoint two novel regulatory brain areas (CeA and SZI) that affect ingestive behavior. Despite the prevailing view that neurons in the Arc are critical for ingestive behavior, NPY + AgRP co-injection did not activate Arc neurons at any time when increased food hoarding was observed.

This study also demonstrated that co-injection of subthreshold doses of NPY and AgRP increases food hoarding in Siberian hamsters, suggesting that there is a significant intercellular interaction between these two neuropeptides occurring as part of the regulation of food hoarding. Because AgRP is known to cause long-term increases in food hoarding, AgRP may be providing synergistic effects on NPY-induced food hoarding in the CeA and SZI (Teubner et al., 2012), brain areas with Y1 as well as MC4 receptors (Lyons and Thiele, 2010).

ROLE OF THE HPA AXIS IN THE REGULATION OF APPETITIVE INGESTIVE BEHAVIORS

A great deal of research has been conducted on the effects of CRH on consummatory ingestive behavior, all suggesting that CRH is anorexigenic. Comparatively little has been done to determine how CRH or other HPA axis factors affect appetitive ingestive behaviors. Food deprivation decreases hypothalamic CRH mRNA expression in several animal species (Brady et al., 1990; Glowa et al., 1992). In studies in which hunger motivation is taken as the body weight loss that produces significant increases in food hoarding, hunger motivation is decreased by adrenalectomy and CRH treatment and increased by low doses of glucocorticoids, fasting, and low temperatures (Fantino and Cabanac, 1984; Cabanac and Richard, 1995; Cabanac et al., 1997; Gosselin and Cabanac, 1997; Michel and Cabanac, 1999). Studies in rats and primates seem to indicate that CRH mediates a shift away from ingestive behaviors, affecting appetitive behaviors at lower doses than those at which food intake is impacted. In addition, studies in anuran amphibians (toads) show that CRH treatment inhibits the appetitive behavior of foraging by impairing the efficiency of visual stimuli to stimulate prey-catching behavior (Carr et al., 2002). This might be adaptive if, in the presence of a predator, the hypothalamic stress response prevents the toads from engaging in appetitive ingestive behaviors that would make them an easy target. There is also some indication that the ability of leptin to prevent fasting-induced increases in ingestive behavior is due in part to activation of central CRH circuits (van Dijk et al., 1997). Although the connection between the HPA axis and appetitive ingestive behaviors has not been studied extensively in the hamster model of food hoarding, such studies are likely to yield a great deal of useful information on the nexus between stress and ingestive behavior.

REWARD CIRCUITS AND THE REGULATION OF APPETITIVE INGESTIVE BEHAVIOR

Food intake is a motivated behavior, and animals find food, especially highly palatable, calorically dense food, rewarding. Therefore, when examining the motivation to engage in ingestive behaviors, it is necessary to consider how reward circuits influence the expression of appetitive ingestive behaviors. Studies in Mongolian gerbils (*Meriones unguiculatus*) show that the act of hoarding food increases the activation of tyrosine hydroxylase (TH)-containing cells in the nucleus accumbens, VTA, and LHA (Yang et al., 2011). TH is the rate-limiting enzyme in the chemical process of dopamine synthesis. In addition, both Mongolian gerbils and Brandt's voles (*Lasiopodomys brandtii*) that are consistent food hoarders exhibit food deprivation-induced increases in food hoarding along with cellular activation in the VTA (Yang et al., 2011; Zhang et al., 2011). The association between the activation of DA neurons in the VTA with food hoarding suggests that animals with the propensity to hoard food or with previous food hoarding experience are likely to find food hoarding especially rewarding or reinforcing. Obviously, this, like the connection between the HPA axis and ingestive behavior regulation, is greatly in need of further investigation.

GESTATIONAL PROGRAMMING OF INGESTIVE BEHAVIOR

The relatively recent, rapid increase in the prevalence of obesity has been attributed by some to environmental factors, including the availability of highly palatable foods and "modern" lifestyles involving less physical work, all of which have created an "obesogenic" environment. It is also possible that characteristics of the prenatal environment initiate organizational effects of hormones that modulate neuroendocrine development to produce optimal adaptation of energy balance and ingestive behavior to the anticipated postnatal environment. Several different synonyms are used to describe the process by which changes in the mother's environment lead to changes in fetal development that have permanent effects in the offspring: gestational, fetal, or maternal programming, or metabolic imprinting. Examples of factors in the mother that influence metabolism in offspring include maternal food restriction, caloric, fat, carbohydrate or protein content of food, non-nutritional stress, and nutritional status of the mother (reviewed by Armitage et al., 2004). In *Homo sapiens*, prenatal undernutrition leads to low birth weight (LBW) babies who are prone to develop metabolic syndrome, insulin resistance, and obesity as adults (Hales and Barker, 2001; Cripps et al., 2005). This relationship between LBW and later development of obesity and metabolic syndrome has been replicated in many diverse populations around the world (Hales and Barker,

2001; Gluckman et al., 2005). In addition, rapid neonatal catch-up growth caused by excessive appetite during the neonatal period acts as an additional risk factor that further increases the risk of obesity (Hales and Barker, 2001; Gluckman et al., 2005; Vickers, 2007; Vickers et al., 2007). Because formula-fed infants typically have an accelerated initial growth rate, formula feeding also increases the risk for becoming obese or overweight (Baird et al., 2005; Harder et al., 2005; Vickers, 2007; Vickers et al., 2007). The relationship between LBW and the later development of obesity and metabolic syndrome has been replicated animal models such as the rat, mouse, pig, sheep and guinea pig (for review see Hales and Barker, 2001; Cripps et al., 2005; Gluckman et al., 2005). Interestingly, maternal obesity during pregnancy and gestational diabetes can also lead to offspring obesity (Muhlhausler, 2007; Shankar et al., 2008).

From an ecological point of view, these maternal programming effects might be adaptive (that is, they might improve offspring survival and reproductive success) or maladaptive. One hypothesis is that changes in maternal energy status generate signals *in utero* to communicate reduced energy availability. These signals purportedly prepare the offspring for a postnatal environment with a low or unpredictable food supply. According to this idea, the maternal environment organizes energy balance and the central appetite regulatory circuits so that the offspring will develop traits that will allow them to cope with that particular environment (Gonzalez-Bulnes and Ovilo, 2012). A commonly reported phenomenon in mice, rats, sheep and primates is that LBW offspring have a propensity toward hyperphagia, efficient metabolism, and an increased inclination to store energy on the body as body fat (Cripps et al., 2005). These same animals might be better adapted to survive in an environment where food supply is low or unpredictable. In an environment of energy abundance, however, they are likely to develop metabolic syndrome and obesity.

GESTATIONAL PROGRAMMING OF CONSUMMATORY INGESTIVE BEHAVIOR

As mentioned previously, impaired prenatal nutrition causes offspring hyperphagia and obesity. Given the pervasive nature of the "Arcuate perspective" on the control of ingestive behavior, most studies on the gestational programming of appetite have focused on the effects of prenatal nutrient availability on orexigenic (i.e., ghrelin, NPY, and AgRP) and anorexigenic (leptin and α-MSH) neuroendocrine factors. For instance, the stomach-derived orexigenic hormone ghrelin is elevated at birth in LBW offspring (Wang et al., 2007; Sahin et al., 2012) and continues to be elevated into adulthood (Sahin et al., 2012). LBW offspring typically exhibit rapid catch-up growth facilitated by enhanced appetite shortly after birth (Coupe et al., 2010; Breton, 2013). Therefore, increased postnatal ghrelin likely plays a role in the rapid catch-up growth observed in most models of gestational programming. In rodents, 50% maternal food restriction during the second half of gestation results in LBW pups that, like human LBW infants, have significantly increased ghrelin expression (Cortelazzi et al., 2003; Nagata et al., 2011). These animals develop hyperphagia and obesity and demonstrate that catch-up growth among LBW offspring results in programmed elevation of plasma ghrelin levels (reviewed by Breton, 2013). Arc NPY and AgRP neurons in hypothalamic slices from adult nutrient-restricted offspring are more sensitive to the stimulatory effects of ghrelin, which could indicate how LBW offspring maintain elevated expression of ingestive behavior throughout their lifespan (Yousheng et al., 2008). Together, these results suggest that appetite regulatory circuits can be gestationally programmed to favor hyperphagia and storage of energy as fat by developmental conditions that produce LBW offspring.

One of the hypothesized mechanisms for long-term changes in the neuroendocrine regulation of ingestive behavior is an alteration in hormonal milieu at critical periods of development. As mentioned above, circulating ghrelin concentrations are elevated in LBW offspring. In addition, leptin functions perinatally as a neurotrophic factor to facilitate the development of the neural circuits that govern metabolism, adipose tissue distribution and ingestive behavior (reviewed by Cottrell and Ozanne, 2007, 2008; Breton, 2013). In rats and mice, there is a surge in plasma leptin concentration during postnatal days (PND) 4–12 (Ahima et al., 1998; Delahaye et al., 2008; Cottrell et al., 2009), the time when connections between hypothalamic neurons are developing and leptin has no effect on appetite (Ahima and Flier, 2000). Rodents exposed to reduced nutrient availability have reduced circulating leptin concentrations and increased hypothalamic LepR protein expression at birth (Cottrell et al., 2010; Coupe et al., 2010), as well as a delayed and/or reduced leptin surge, which results in permanent changes in hypothalamic ingestive behavior regulatory circuits (Gluckman et al., 2005; Bautista et al., 2008). Recent studies provide further convincing evidence that leptin specifically promotes the development of Arc neuronal projections, consistent with a role in brain development. Arc projections in mice are formed primarily during the second week of postnatal life (reviewed by Ahima and Flier, 2000), developmentally similar to human third trimester of pregnancy. In leptin deficient (*ob/ob*) mice, these projection pathways regulating appetite are permanently disrupted, causing Arc axonal densities one third to one fourth that of controls (reviewed by Ahima and Flier, 2000). Reduced gestational nutrient availability permanently impairs the development and connectivity of hypothalamic neurons that produce anorectic neuropeptides such as α-MSH and orexigenic neuropeptides such as NPY and AgRP from weaning through adulthood (Muhlhausler et al., 2006; Delahaye et al., 2008; Coupe et al., 2010). Gestationally-restricted offspring also display resistance to the anorectic effects of peripheral leptin treatment in adulthood (reviewed by Breton, 2013). Therefore, leptin appears to be a critical factor for the gestational programming of appetite regulatory circuits as well.

Alterations in the Arc appetite regulatory network that increase production of and sensitivity to orexigenic agents such as NPY are clearly important for the programmed hyperphagia that occurs in response to perinatal nutritional perturbations. As mentioned above, the complex nature of ingestive behavior regulation makes is highly unlikely that just focusing on the Arc will be sufficient to explain all the effects of gestational nutrient restriction on appetite regulation. Alterations in dopamine expression in

the VTA may also be critically important because the reward system is likely also to have gestationally programmed changes that may influence ingestive behavior regulation. For example, female LBW rats are more likely than non-restricted offspring to binge on sucrose when it is presented intermittently for limited amounts of time (unpublished observation). Therefore, further research into the contribution of reward circuitry to the mechanisms of gestational programming of appetite is clearly warranted.

A plethora of data supports the hypothesis that perinatal nutritional disturbances majorly contribute to the development of adult obesity by altering appetite regulation to favor hyperphagia. This complex system of neuroendocrine factors undergoes a short period of plasticity during development when the nutritional environment programs this system to maximize an organism's chances for survival in its anticipated postnatal environment. Several rodent models have been used to study the mechanisms responsible for perinatal appetite programming, including the maternal food restriction, low protein, lactational overnutrition, gestational diabetes, and high fat diet during pregnancy. Currently, there is not a validated model for the study of gestational programming on appetitive ingestive behaviors.

APPETITIVE INGESTIVE BEHAVIORS

Hamsters have several unique characteristics that make them ideal for studying the gestational programming of the regulation of appetitive ingestive behaviors, including the fact that hamsters utilize food hoarding as a significant part of their natural ingestive behavioral repertoire, and, unlike rats, hamsters do not overeat after energetic challenges such as food deprivation and pregnancy (Silverman and Zucker, 1976; Rowland, 1982; Billington et al., 1984; Bartness and Clein, 1994; Bartness et al., 1995). As mentioned above, the effects of gestational nutrient availability on appetitive ingestive behaviors have yet to be studied. Therefore, we recently endeavored to create a model of gestational programming in the hamster. In this model, pregnant hamsters are provided with daily food rations equal to pre-pregnancy intake, preventing maternal food hoarding, a behavior naturally enhanced during the last half of pregnancy. Interestingly, male offspring of restricted mothers exhibit hyperphagia and increased Arc NPY as well as decreased hoarding behavior (unpublished observations). Further characterization of this exciting new model of gestational programming should provide additional insights into the effects of prenatal environment on the neuroendocrine regulation of appetitive ingestive behaviors.

SUMMARY AND CONCLUSIONS

Obesity rates are on the rise worldwide, and research into the neuroendocrine regulation of appetitive ingestive behavior is likely to provide new information about how animals maintain energy balance in their native settings and how to combat the obesity epidemic. Fluctuating food supply has been a threat to survival and a determinant of reproductive success for most animals, including humans (Prentice, 2005; Schneider, 2006). Selection pressures resulting from frequent bouts of famine in the evolutionary history of most organisms favors a "thrifty," a.k.a., metabolically efficient phenotype to increase the likelihood of reproductive success in an environment with a variable food supply (Prentice, 2005; Schneider, 2006). The overabundance of highly palatable, easily accessible food sources present in the human environment in developed countries has turned these adaptations into health liabilities. Unfortunately, the list of neuropeptides that "regulate" appetite is now overwhelming, and most ingestive behavior research continues to focus on the hypothalamic Arc nucleus as the command center for appetite regulation (reviewed by Barsh and Schwartz, 2002; Zigman and Elmquist, 2003; Schwartz and Porte, 2005).

Ingestive behavior regulation is far more complex than can be explained by studies conducted solely on the Arc nucleus. In addition, the study of the neuroendocrine regulation of appetitive behaviors provides clues to the mechanisms responsible for the motivation to eat. This review seeks to demonstrate that while hypothalamic appetite regulatory circuits are important for the control of both appetitive and consummatory ingestive behaviors, it is necessary to think more broadly than the "Arc perspective" by including systems such as the caudal hindbrain, the HPA axis and central reward circuitry to provide a more complete picture of neuroendocrine regulation of ingestive behavior.

Since the creation of the thrifty phenotype hypothesis by Neel in (1962), the maternal programming hypothesis of Jones et al. in (1984), and it's reiteration by Barker in (1994), understanding how nutrient availability during critical periods of development affects offspring appetite and energy balance has been a major focus of obesity research (Jones et al., 1984; Barker, 1994; Neel, 1999). A great deal of research has been done to characterize the effects of reduced perinatal nutrient availability on offspring consummatory ingestive behavior. Although research has not previously been conducted to determine how appetitive ingestive behaviors are impacted by maternal nutrient availability, future studies using hamsters as an animal model may provide critical information on the gestational programming of neuroendocrine systems that regulate feeding motivation.

REFERENCES

Abizaid, A. (2009). Ghrelin and dopamine: new insights on the peripheral regulation of appetite. *J. Neuroendocrinol.* 21, 787–793. doi: 10.1111/j.1365-2826.2009.01896.x

Abizaid, A., Gao, Q., and Horvath, T. L. (2006a). Thoughts for food: brain mechanisms and peripheral energy balance. *Neuron* 51, 691–702. doi: 10.1016/j.neuron.2006.08.025

Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., et al. (2006b). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J. Clin. Invest.* 116, 3229–3239. doi: 10.1172/JCI29867

Abizaid, A., and Horvath, T. L. (2012). Ghrelin and the central regulation of feeding and energy balance. *Indian J. Endocrinol. Metab.* 16, S617–S626. doi: 10.4103/2230-8210.105580

Ahima, R. S., and Flier, J. S. (2000). Adipose tissue as an endocrine organ. *Trends Endocrinol. Metab.* 11, 327–332. doi: 10.1016/S1043-2760(00)00301-5

Ahima, R. S., Prabakaran, D., and Flier, J. S. (1998). Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. Implications for energy homeostasis and neuroendocrine function. *J. Clin. Invest.* 101, 1020–1027. doi: 10.1172/JCI1176

Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., et al. (2001). Stomach is a major source of circulating ghrelin,

and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J. Clin. Endocrinol. Metab.* 86, 4753–4758. doi: 10.1210/jc.86.10.4753

Armitage, J. A., Khan, I. Y., Taylor, P. D., Nathanielsz, P. W., and Poston, L. (2004). Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? *J. Physiol.* 561, 355–377. doi: 10.1113/jphysiol.2004.072009

Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Ueno, N., et al. (2001). Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 120, 337–345. doi: 10.1053/gast.2001.22158

Baird, J., Fisher, D., Lucas, P., Kleijnen, J., Roberts, H., and Law, C. (2005). Being big or growing fast: systematic review of size and growth in infancy and later obesity. *BMJ* 331, 929. doi: 10.1136/bmj.38586.411273.E0

Ball, G. F., and Balthazart, J. (2008). How useful is the appetitive and consummatory distinction for our understanding of the neuroendocrine control of sexual behavior? *Horm. Behav.* 53, 307–311. doi: 10.1016/j.yhbeh.2007.09.023

Barker, D. J. (1994). Outcome of low birthweight. *Horm. Res.* 42, 223–230. doi: 10.1159/000184197

Barsh, G. S., and Schwartz, M. W. (2002). Genetic approaches to studying energy balance: perception and integration. *Nat. Rev. Genet.* 3, 589–600. doi: 10.1038/nrg862

Bartness, T. J. (1997). Food hoarding is increased by pregnancy, lactation and food deprivation in Siberian hamsters. *Am. J. Physiol.* 272, R118–R125.

Bartness, T. J., and Clein, M. R. (1994). Effects of food deprivation and restriction, and metabolic blockers on food hoarding in Siberian hamsters. *Am. J. Physiol.* 266, R1111–R1117.

Bartness, T. J., Keen-Rhinehart, E., Dailey, M. J., and Teubner, B. J. (2011). Neural and hormonal control of food hoarding. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301, R641–R655. doi: 10.1152/ajpregu.00137.2011

Bartness, T. J., Morley, J. E., and Levine, A. S. (1995). Effects of food deprivation and metabolic fuel utilization on the photoperiodic control of food intake in Siberian hamsters. *Physiol. Behav.* 57, 61–68. doi: 10.1016/0031-9384(94)00203-H

Baskin, D. G., Blevins, J. E., and Schwartz, M. W. (2001). How the brain regulates food intake and body weight: the role of leptin. *J Pediatr. Endocrinol. Metab.* 14(Suppl. 6), 1417–1429.

Baskin, D. G., Hahn, T. M., and Schwartz, M. W. (1999). Leptin sensitive neurons in the hypothalamus. *Horm. Metab. Res.* 31, 345–350. doi: 10.1055/s-2007-978751

Bautista, C. J., Boeck, L., Larrea, F., Nathanielsz, P. W., and Zambrano, E. (2008). Effects of a maternal low protein isocaloric diet on milk leptin and progeny serum leptin concentration and appetitive behavior in the first 21 days of neonatal life in the rat. *Pediatr. Res.* 63, 358–363. doi: 10.1203/01.pdr.0000304938.78998.21

Beneke, W. M., and Davis, C. H. (1985). Relationship of hunger, use of a shopping list and obesity to food purchases. *Int. J. Obes.* 9, 391–399.

Bergonzelli, G. E., Pralong, F. P., Glauser, M., Cavadas, C., Grouzmann, E., and Gaillard, R. C. (2001). Interplay between galanin and leptin in the hypothalamic control of feeding via corticotropin-releasing hormone and neuropeptide Y. *Diabetes* 50, 2666–2672. doi: 10.2337/diabetes.50.12.2666

Billington, C. J., Morley, J. E., Levine, A. S., and Gerritsen, G. C. (1984). Feeding systems in Chinese hamsters. *Am. J. Physiol.* 247, R405–R411.

Brady, L. S., Smith, M. A., Gold, P. W., and Herkenham, M. (1990). Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. *Neuroendocrinology* 52, 441–447. doi: 10.1159/000125626

Bray, G. A., Stern, J. S., and Castonguay, T. W. (1992). Effect of adrenalectomy and high-fat diet on the fatty Zucker rat. *Am. J. Physiol.* 262, E32–E39.

Breton, C. (2013). The hypothalamus-adipose axis is a key target of developmental programming by maternal nutritional manipulation. *J. Endocrinol.* 216, R19–R31. doi: 10.1530/JOE-12-0157

Briggs, D. I., and Andrews, Z. B. (2011). Metabolic status regulates ghrelin function on energy homeostasis. *Neuroendocrinology* 93, 48–57. doi: 10.1159/000322589

Bronson, F. H. (1989). *Mammalian Reproductive Biology*, Chicago: University of Chicago Press.

Bruce, B. K., King, B. M., Phelps, G. R., and Veitia, M. C. (1982). Effects of adrenalectomy and corticosterone administration on hypothalamic obesity in rats. *Am. J. Physiol.* 243, E152–E157.

Buckley, C. A., and Schneider, J. E. (2003). Food hoarding is increased by food deprivation and decreased by leptin treatment in Syrian hamsters. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 285, R1021–R1029. doi: 10.1152/ajpregu.00488.2002

Caballero, B. (2007). The global epidemic of obesity: an overview. *Epidemiol. Rev.* 29, 1–5. doi: 10.1093/epirev/mxm012

Cabanac, M., Dagnault, A., and Richard, D. (1997). The food-hoarding threshold is not raised by acute intraventricular NPY in male rats. *Physiol. Behav.* 61, 131–135. doi: 10.1016/S0031-9384(97)00349-1

Cabanac, M., and Richard, D. (1995). Acute intraventricular CRF lowers the hoarding threshold in male rats. *Physiol. Behav.* 57, 705–710. doi: 10.1016/0031-9384(94)00322-X

Carr, J. A. (2002). Stress, neuropeptides, and feeding behavior: a comparative perspective. *Integr. Comp. Biol.* 42, 582–590. doi: 10.1093/icb/42.3.582

Carr, J. A., Brown, C. L., Mansouri, R., and Venkatesan, S. (2002). Neuropeptides and amphibian prey-catching behavior. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 132, 151–162. doi: 10.1016/S1096-4959(01)00545-0

Chen, H. Y., Trumbauer, M. E., Chen, A. S., Weingarth, D. T., Adams, J. R., Frazier, E. G., et al. (2004). Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology* 145, 2607–2612. doi: 10.1210/en.2003-1596

Cone, R. D., Cowley, M. A., Butler, A. A., Fan, W., Marks, D. L., and Low, M. J. (2001). The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *Int. J. Obes. Relat. Metab. Disord.* 25(Suppl. 5), S63–S67. doi: 10.1038/sj.ijo.0801913

Coppari, R., Ichinose, M., Lee, C. E., Pullen, A. E., Kenny, C. D., McGovern, R. A., et al. (2005). The hypothalamic arcuate nucleus: a key site for mediating leptin's effects on glucose homeostasis and locomotor activity. *Cell Metab.* 1, 63–72. doi: 10.1016/j.cmet.2004.12.004

Cortelazzi, D., Cappiello, V., Morpurgo, P. S., Ronzoni, S., Nobile De Santis, M. S., Cetin, I., et al. (2003). Circulating levels of ghrelin in human fetuses. *Eur. J. Endocrinol.* 149, 111–116. doi: 10.1530/eje.0.1490111

Cottrell, E. C., Cripps, R. L., Duncan, J. S., Barrett, P., Mercer, J. G., Herwig, A., et al. (2009). Developmental changes in hypothalamic leptin receptor: relationship with the postnatal leptin surge and energy balance neuropeptides in the postnatal rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R631–R639. doi: 10.1152/ajpregu.90690.2008

Cottrell, E. C., Mercer, J. G., and Ozanne, S. E. (2010). Postnatal development of hypothalamic leptin receptors. *Vitam. Horm.* 82, 201–217. doi: 10.1016/S0083-6729(10)82011-4

Cottrell, E. C., and Ozanne, S. E. (2007). Developmental programming of energy balance and the metabolic syndrome. *Proc. Nutr. Soc.* 66, 198–206. doi: 10.1017/S0029665107005447

Cottrell, E. C., and Ozanne, S. E. (2008). Early life programming of obesity and metabolic disease. *Physiol. Behav.* 94, 17–28. doi: 10.1016/j.physbeh.2007.11.017

Coupe, B., Amarger, V., Grit, I., Benani, A., and Parnet, P. (2010). Nutritional programming affects hypothalamic organization and early response to leptin. *Endocrinology* 151, 702–713. doi: 10.1210/en.2009-0893

Craig, W. (1918). Appetites and aversions as constituents of instincts. *Biol. Bull.* 34, 91–107. doi: 10.2307/1536346

Cripps, R. L., Martin-Gronert, M. S., and Ozanne, S. E. (2005). Fetal and perinatal programming of appetite. *Clin. Sci. (Lond)* 109, 1–11. doi: 10.1042/CS20040367

Dailey, M. J., and Bartness, T. J. (2010). Arcuate nucleus destruction does not block food deprivation-induced increases in food foraging and hoarding. *Brain Res.* 1323, 94–108. doi: 10.1016/j.brainres.2010.01.078

Dallman, M. F., la Fleur, S. E., Pecoraro, N. C., Gomez, F., Houshyar, H., and Akana, S. F. (2004). Minireivew: glucocorticoids – food intake, abdominal obesity and wealthy nations in 2004. *Endocrinology* 145, 2633–2638. doi: 10.1210/en.2004-0037

Dallman, M. F., Strack, A. M., Akana, S. F., Bradbury, M. J., Hanson, E. S., Scribner, K. A., et al. (1993). Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. *Front. Neuroendocinol.* 14, 303–347. doi: 10.1006/frne.1993.1010

Day, D. E., and Bartness, T. J. (2003). Fasting-induced increases in hoarding are dependent on the foraging effort level. *Physiol. Behav.* 78, 655–668. doi: 10.1016/S0031-9384(03)00052-0

Day, D. E., and Bartness, T. J. (2004). Agouti-related protein increases food hoarding, but not food intake by Siberian hamsters. *Am. J. Physiol.* 286, R38–R45. doi: 10.1152/ajpregu.00284.2003

Day, D. E., Keen-Rhinehart, E., and Bartness, T. J. (2005). Role of NPY and its receptor subtypes in foraging, food hoarding and food intake by Siberian hamsters. *Am. J. Physiol.* 289, R29–R36. doi: 10.1152/ajpregu.00853.2004

Delahaye, F., Breton, C., Risold, P. Y., Enache, M., Dutriez-Casteloot, I., Laborie, C., et al. (2008). Maternal perinatal undernutrition drastically reduces postnatal leptin surge and affects the development of arcuate nucleus proopiomelanocortin neurons in neonatal male rat pups. *Endocrinology* 149, 470–475. doi: 10.1210/en.2007-1263

Dhillon, H., Zigman, J. M., Ye, C., Lee, C. E., McGovern, R. A., Tang, V., et al. (2006). Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron* 49, 191–203. doi: 10.1016/j.neuron.2005.12.021

Dodd, D. K., Stalling, R. B., and Bedell, J. (1977). Grocery purchases as a function of obesity and assumed food deprivation. *Int. J. Obes.* 1, 43–47.

Eckel, L. A. (2011). The ovarian hormone estradiol plays a crucial role in the control of food intake in females. *Physiol. Behav.* 104, 517–524. doi: 10.1016/j.physbeh.2011.04.014

Ellacott, K. L., and Cone, R. D. (2004). The central melanocortin system and the integration of short- and long-term regulators of energy homeostasis. *Recent Prog. Horm. Res.* 59, 395–408. doi: 10.1210/rp.59.1.395

Elmquist, J. K. (2001). Hypothalamic pathways underlying the endocrine, autonomic, and behavioral effects of leptin. *Int. J. Obes. Relat. Metab. Disord.* 25(Suppl. 5), S78–S82. doi: 10.1038/sj.ijo.0801918

Elmquist, J. K., Coppari, R., Balthasar, N., Ichinose, M., and Lowell, B. B. (2005). Identifying hypothalamic pathways controlling food intake, body weight, and glucose homeostasis. *J. Comp. Neurol.* 493, 63–71. doi: 10.1002/cne.20786

Epstein, L. H., Dearing, K. K., Paluch, R. A., Roemmich, J. N., and Cho, D. (2007). Price and maternal obesity influence purchasing of low- and high-energy-dense foods. *Am. J. Clin. Nutr.* 86, 914–922.

Everitt, B. J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., and Goldstein, M. (1984). Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11, 443–462. doi: 10.1016/0306-4522(84)90036-8

Fantino, M., and Cabanac, M. (1984). Effect of a cold ambient temperature on the rat's food hoarding behavior. *Physiol. Behav.* 32, 183–190. doi: 10.1016/0031-9384(84)90127-6

Faulconbridge, L. F., Grill, H. J., and Kaplan, J. M. (2005). Distinct forebrain and caudal brainstem contributions to the neuropeptide Y mediation of ghrelin hyperphagia. *Diabetes* 54, 1985–1993. doi: 10.2337/diabetes.54.7.1985

Feldkircher, K. M., Mistry, A. M., and Romsos, D. R. (1996). Adrenalectomy reverses pre-existing obesity in adult genetically obese (*ob/ob*) mice. *Int. J. Obes. Relat. Metab. Disord.* 20, 232–235.

Fulton, S., Pissios, P., Manchon, R. P., Stiles, L., Frank, L., Pothos, E. N., et al. (2006). Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* 51, 811–822. doi: 10.1016/j.neuron.2006.09.006

Fulton, S., Richard, D., Woodside, B., and Shizgal, P. (2002). Interaction of CRH and energy balance in the modulation of brain stimulation reward. *Behav. Neurosci.* 116, 651–659. doi: 10.1037/0735-7044.116.4.651

Gao, Q., Mezei, G., Nie, Y., Choi, C. S., Bechmann, I., Leranth, C., et al. (2007). Anorectic estrogen mimics leptin's effect on the rewiring of melanocortins cells and Stat3 signaling in obese animals. *Nat. Med.* 13, 89–94. doi: 10.1038/nm1525

Glowa, J. R., Barrett, J. E., Russell, J., and Gold, P. W. (1992). Effects of corticotropin releasing hormone on appetitive behaviors. *Peptides* 13, 609–621. doi: 10.1016/0196-9781(92)90097-M

Gluckman, P. D., Hanson, M. A., and Pinal, C. (2005). The developmental origins of adult disease. *Matern. Child Nutr.* 1, 130–141. doi: 10.1111/j.1740-8709.2005.00020.x

Gonzalez-Bulnes, A., and Ovilo, C. (2012). Genetic basis, nutritional challenges and adaptive responses in the prenatal origin of obesity and type-2 diabetes. *Curr. Diabetes Rev.* 8, 144–154. doi: 10.2174/157339912799424537

Gosselin, C., and Cabanac, M. (1997). Adrenalectomy lowers the body weight setpoint in rats. *Physiol. Behav.* 62, 519–523. doi: 10.1016/S0031-9384(97)00010-3

Grill, H. J. (2010). Leptin and the systems neuroscience of meal size control. *Front. Neuroendocrinol.* 31, 61–78. doi: 10.1016/j.yfrne.2009.10.005

Grill, H. J., and Hayes, M. R. (2009). The nucleus tractus solitarius: a portal for visceral afferent signal processing, energy status assessment and integration of their combined effects on food intake. *Int. J. Obes. (Lond)* 33, S11–S15. doi: 10.1038/ijo.2009.10

Grill, H. J., and Kaplan, J. M. (1992). Sham feeding in intact and chronic decerebrate rats. *Am. J. Physiol.* 262, R1070–R1074.

Grill, H. J., and Kaplan, J. M. (2001). Interoceptive and integrative contributions of forebrain and brainstem to energy balance control. *Int. J. Obes. Relat. Metab. Disord.* 25(Suppl. 5), S73–S77. doi: 10.1038/sj.ijo.0801917

Grill, H. J., and Kaplan, J. M. (2002). The neuroanatomical axis for control of energy balance. *Front. Neuroendocrinol.* 23, 2–40. doi: 10.1006/frne.2001.0224

Grill, H. J., and Smith, G. P. (1988). Cholecystokinin decreases sucrose intake in chronic decerebrate rats. *Am. J. Physiol.* 254, R853–R856.

Gyengesi, E., Liu, W., D'Agostino, G., Gan, G., Horvath, T. L., Gao, X. B., et al. (2010). Corticosterone regulates synaptic input organization of POMC and NPY/AgRP neurons in adult mice. *Endocrinology* 151, 5395–5402. doi: 10.1210/en.2010-0681

Hahn, T. M., Breininger, J. F., Baskin, D. G., and Schwartz, M. W. (1998). Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat. Neurosci.* 1, 271–272. doi: 10.1038/1082

Hales, C. N., and Barker, D. J. (2001). The thrifty phenotype hypothesis. *Br. Med. Bull.* 60, 5–20. doi: 10.1093/bmb/60.1.5

Harder, T., Bergmann, R., Kallischnigg, G., and Plagemann, A. (2005). Duration of breastfeeding and risk of overweight: a meta-analysis. *Am. J. Epidemiol.* 162, 397–403. doi: 10.1093/aje/kwi222

Haslam, D. (2007). Obesity: a medical history. *Obes. Rev.* 8(Suppl. 1), 31–36. doi: 10.1111/j.1467-789X.2007.00314.x

Heinrichs, S. C., and Richard, D. (1999). The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. *Neuropeptides* 33, 350–359. doi: 10.1054/npep.1999.0047

Hill, J. O., Fried, S. K., and DiGirolamo, M. (1983). Effects of a high-fat diet on energy intake and expenditure in rats. *Life Sci.* 33, 141–149. doi: 10.1016/0024-3205(83)90406-X

Hill, J. O., Fried, S. K., and DiGirolamo, M. (1984). Effects of fasting and restricted refeeding on utilization of ingested energy in rats. *Am. J. Physiol.* 247, R318–R327.

Hirasawa, M., Parsons, M. P., and Alberto, C. O. (2007). Interaction between orexins and the mesolimbic system for overriding satiety. *Rev. Neurosci.* 18, 383–393. doi: 10.1515/REVNEURO.2007.18.5.383

Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z. W., Gao, X. B., et al. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 51, 801–810. doi: 10.1016/j.neuron.2006.08.023

Jones, A. P., Simson, E. L., and Friedman, M. I. (1984). Gestational undernutrition and the development of obesity in rats. *J. Nutr.* 114, 1484–1492.

Kageyama, H., Takenoya, F., Hirako, S., Wada, N., Kintaka, Y., Inoue, S., et al. (2012). Neuronal circuits involving neuropeptide Y in hypothalamic arcuate nucleus-mediated feeding regulation. *Neuropeptides* 46, 285–289. doi: 10.1016/j.npep.2012.09.007

Kalra, S. P., Dube, M. G., Shuye, P., Bin, X., Horvath, T. L., and Kalra, P. S. (1999). Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocrine Rev.* 20, 68–100. doi: 10.1210/er.20.1.68

Karolyi, I. J., Burrows, H. L., Ramesh, T. M., Nakajima, M., Lesh, J. S., Seong, E., et al. (1999). Altered anxiety and weight gain in corticotropin-releasing hormone-binding protein-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 96, 11595–11600. doi: 10.1073/pnas.96.20.11595

Keen-Rhinehart, E., and Bartness, T. J. (2005). Peripheral ghrelin injections stimulate food intake, foraging and food hoarding in Siberian hamsters. *Am. J. Physiol.* 288, R716–R722. doi: 10.1152/ajpregu.00705.2004

Keen-Rhinehart, E., and Bartness, T. J. (2007). NPY Y1 receptor is involved in ghrelin- and fasting-induced increases in foraging, food hoarding, and food intake. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R1728–R1737. doi: 10.1152/ajpregu.00597.2006

Keen-Rhinehart, E., and Bartness, T. J. (2008). Leptin inhibits food-deprivation-induced increases in food intake and food hoarding. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295, R1737–R1746. doi: 10.1152/ajpregu.90512.2008

Keen-Rhinehart, E., Dailey, M. J., and Bartness, T. (2010). Physiological mechanisms for food-hoarding motivation in animals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 365, 961–975. doi: 10.1098/rstb.2009.0225

Landys, M. M., Ramenofsky, M., and Wingfield, J. C. (2006). Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the

regulation of periodic life processes. *Gen. Comp. Endocrinol.* 148, 132–149. doi: 10.1016/j.ygcen.2006.02.013

Lyons, A. M., and Thiele, T. E. (2010). Neuropeptide Y conjugated to saporin alters anxiety-like behavior when injected into the central nucleus of the amygdala or basomedial hypothalamus in BALB/cJ mice. *Peptides* 31, 2193–2199. doi: 10.1016/j.peptides.2010.09.009

Mela, D. J., Aaron, J. I., and Gatenby, S. J. (1996). Relationships of consumer characteristics and food deprivation to food purchasing behavior. *Physiol. Behav*. 60, 1331–1335. doi: 10.1016/S0031-9384(96)00241-7

Mercer, J. G., Moar, K. M., Ross, A. W., Hoggard, N., and Morgan, P. J. (2000). Photoperiod regulates arcuate nucleus POMC, AGRP, and leptin receptor mRNA in Siberian hamster hypothalamus. *Am. J. Physiol.* 278, R271–R281. doi: 10.1006/appe.1999.0301

Michel, C., and Cabanac, M. (1999). Lipectomy, body weight, and body weight set point in rats. *Physiol. Behav*. 66, 473–479. doi: 10.1016/S0031-9384(98)00317-5

Morton, G. J., and Schwartz, M. W. (2001). The NPY/AgRP neuron and energy homeostasis. *Int. J. Obes. Relat. Metab. Disord.* 25(Suppl. 5), S56–S62. doi: 10.1038/sj.ijo.0801915

Muhlhausler, B. S. (2007). Programming of the appetite-regulating neural network: a link between maternal overnutrition and the programming of obesity? *J. Neuroendocrinol*. 19, 67–72. doi: 10.1111/j.1365-2826.2006.01505.x

Muhlhausler, B. S., Adam, C. L., Findlay, P. A., Duffield, J. A., and McMillen, I. C. (2006). Increased maternal nutrition alters development of the appetite-regulating network in the brain. *FASEB J*. 20, 1257–1259. doi: 10.1096/fj.05-5241fje

Musatov, S., Chen, W., Pfaff, D. W., Mobbs, C. V., Yang, X. J., Clegg, D. J., et al. (2007). Silencing of estrogen receptor alpha in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2501–2506. doi: 10.1073/pnas.0610787104

Nagata, E., Nakagawa, Y., Yamaguchi, R., Fujisawa, Y., Sano, S., Satake, E., et al. (2011). Altered gene expressions of ghrelin, PYY, and CCK in the gastrointestinal tract of the hyperphagic intrauterine growth restriction rat offspring. *Horm. Metab. Res*. 43, 178–182. doi: 10.1055/s-0030-1270528

Neel, J. V. (1962). Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am. J. Hum. Genet.* 14, 353–362.

Neel, J. V. (1999). Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? 1962. *Bull. World Health Organ*. 77, 694–703.

Pandit, R., de Jong, J. W., Vanderschuren, L. J., and Adan, R. A. (2011). Neurobiology of overeating and obesity: the role of melanocortins and beyond. *Eur. J. Pharmacol*. 660, 28–42. doi: 10.1016/j.ejphar.2011.01.034

Prentice, A. M. (2005). Early influences on human energy regulation: thrifty genotypes and thrifty phenotypes. *Physiol. Behav*. 86, 640–645. doi: 10.1016/j.physbeh.2005.08.055

Ransley, J. K., Donnelly, J. K., Botham, H., Khara, T. N., Greenwood, D. C., and Cade, J. E. (2003). Use of supermarket receipts to estimate energy and fat content of food purchased by lean and overweight families. *Appetite* 41, 141–148. doi: 10.1016/S0195-6663(03)00051-5

Romsos, D. R., Vander Tuig, J. G., Kerner, J., and Grogan, C. K. (1987). Energy balance in rats with obesity-producing hypothalamic knife cuts: effects of adrenalectomy. *J. Nutr*. 117, 1121–1128.

Ross, I., and Smith, W. I. (1953). The hoarding behavior of the mouse II. The role of deprivation, satiation and stress. *J. Gen. Psychol*. 82, 279–297.

Rowland, N. E. (1982). Failure by deprived hamsters to increase food intake: some behavioral and physiological determinants. *J. Comp. Physiol. Psychol*. 96, 591–603. doi: 10.1037/h0077905

Sahin, H., Erener, T., Erginoz, E., Vural, M., Ilikkan, B., Kavuncuoglu, S., et al. (2012). The relationship of active ghrelin levels and intrauterine growth in preterm infants. *Eur. J. Endocrinol*. 166, 399–405. doi: 10.1530/EJE-11-0607

Sapolsky, R. M., Romero, L. M., and Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev*. 21, 55–89. doi: 10.1210/er.21.1.55

Schellekens, H., Finger, B. C., Dinan, T. G., and Cryan, J. F. (2012). Ghrelin signalling and obesity: at the interface of stress, mood and food reward. *Pharmacol. Ther*. 135, 316–326. doi: 10.1016/j.pharmthera.2012.06.004

Schneider, J. E. (2006). Metabolic and hormonal control of the desire for food and sex: implications for obesity and eating disorders. *Horm. Behav*. 50, 562–571. doi: 10.1016/j.yhbeh.2006.06.023

Schneider, J. E., Klingerman, C. M., and Abdulhay, A. (2012). Sense and nonsense in metabolic control of reproduction. *Front. Endocrinol. (Lausanne)* 3:26. doi: 10.3389/fendo.2012.00026

Schneider, J. E., and Wade, G. N. (1999). "Inhibition of reproduction in service of energy balance," in *Reproduction in Context: Environmental and Social Influences on Reproductive Physiology and Behavior*, eds K. Wallen and J. E. Schneider (Cambridge, MIT Press), 35–82.

Schneider, J. E., and Watts, A. G. (2002). "Energy balance, ingestive behavior and reproductive success," in *Hormones Brain and Behavior*, ed D. Pfaff (San Diego, CA: Academic Press), 435–523. doi: 10.1016/B978-012532104-4/50009-3

Schneider, J. E., Buckley, C. A., Blum, R. M., Zhou, D., Szymanski, L., Day, D. E., et al. (2002). Metabolic signals, hormones and neuropeptides involved in control of energy balance and reproductive success in hamsters. *Eur. J. Neurosci.* 16, 377–379. doi: 10.1046/j.1460-9568.2002.02118.x

Schneider, J. E., Wise, J. D., Benton, N. A., Brozek, J. M., and Keen-Rhinehart, E. (2013). When do we eat? Ingestive behavior, survival, and reproductive success. *Horm. Behav*. 64, 702–728. doi: 10.1016/j.yhbeh.2013.07.005

Schwartz, M. W., and Porte, D. Jr. (2005). Diabetes, obesity, and the brain. *Science* 307, 375–379. doi: 10.1126/science.1104344

Shankar, K., Harrell, A., Liu, X., Gilchrist, J. M., Ronis, M. J., and Badger, T. M. (2008). Maternal obesity at conception programs obesity in the offspring. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R528–R538. doi: 10.1152/ajpregu.00316.2007

Silverman, H. J., and Zucker, I. (1976). Absence of post-fast food compensation in the golden hamster (Mesocricetus auratus). *Physiol. Behav*. 17, 271–285. doi: 10.1016/0031-9384(76)90076-7

Sonneville, K. R., Rifas-Shiman, S. L., Haines, J., Gortmaker, S., Mitchell, K. F., Gillman, M. W., et al. (2013). Associations of parental control of feeding with eating in the absence of hunger and food sneaking, hiding, and hoarding. *Child Obes*. 9, 346–349. doi: 10.1089/chi.2012.0149

Stanley, B. G., Lanthier, D., Chin, A. S., and Leibowitz, S. F. (1989). Suppression of neuropeptide Y-elicited eating by adrenalectomy or hypophysectomy: reversal with corticosterone. *Brain Res*. 501, 32–36. doi: 10.1016/0006-8993(89)91023-8

Stevenson, J. A., and Franklin, C. (1970). Effects of ACTH and corticosteroids in the regulation of food and water intake. *Prog. Brain Res*. 32, 141–152. doi: 10.1016/S0079-6123(08)61529-0

Stice, E., Figlewicz, D. P., Gosnell, B. A., Levine, A. S., and Pratt, W. E. (2012). The contribution of brain reward circuits to the obesity epidemic. *Neurosci. Biobehav. Rev.* doi: 10.1016/j.neubiorev.2012.12.001. [Epub ahead of print].

Teubner, B. J., and Bartness, T. J. (2010). Cholecystokinin-33 acutely attenuates food foraging, hoarding and intake in Siberian hamsters. *Peptides* 31, 618–624. doi: 10.1016/j.peptides.2009.12.010

Teubner, B. J., and Bartness, T. J. (2013). Anti-ghrelin spiegelmer inhibits exogenous ghrelin-induced increases in food intake, hoarding and neural activation, but not food deprivation-induced increases. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 305, R323–R333. doi: 10.1152/ajpregu.00097.2013

Teubner, B. J., Garretson, J. T., Hwang, Y., Cole, P. A., and Bartness, T. J. (2013). Inhibition of ghrelin O-acyltransferase attenuates food deprivation-induced increases in ingestive behavior. *Horm. Behav*. 63, 667–673. doi: 10.1016/j.yhbeh.2013.02.001

Teubner, B. J., Keen-Rhinehart, E., and Bartness, T. J. (2012). Third ventricular coinjection of subthreshold doses of NPY and AgRP stimulate food hoarding and intake and neural activation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302, R37–R48. doi: 10.1152/ajpregu.00475.2011

Tom, G. (1983). Effect of deprivation on the grocery shopping behavior of obese and nonobese consumers. *Int. J. Obes*. 7, 307–311.

Ulijaszek, S. J. (2002). Human eating behaviour in an evolutionary ecological context. *Proc. Nutr. Soc*. 61, 517–526. doi: 10.1079/PNS2002180

van Dijk, G., Donahey, J. C. K., Thiele, T. E., Scheurink, A. J. W., Steffens, A. B., Wilkinson, C. W., et al. (1997). Central leptin stimulates corticosterone secretion at the onset of the dark phase. *Diabetes* 46, 1911–1914. doi: 10.2337/diab.46.11.1911

Vickers, M. H. (2007). Developmental programming and adult obesity: the role of leptin. *Curr. Opin. Endocrinol. Diabetes Obes*. 14, 17–22. doi: 10.1097/MED.0b013e328013da48

Vickers, M. H., Krechowec, S. O., and Breier, B. H. (2007). Is later obesity programmed *in utero*? *Curr. Drug Targets* 8, 923–934. doi: 10.2174/138945007781386857

Volkow, N. D., Wang, G. J., Tomasi, D., and Baler, R. D. (2013). Obesity and addiction: neurobiological overlaps. *Obes. Rev*. 14, 2–18. doi: 10.1111/j.1467-789X.2012.01031.x

Wade, G. N. (1976). "Sex hormones, regulatory behaviors, and body weight," in *Advances in the Study of Behavior*, *Vol. 6*, eds J. S. Rosenblatt, R. A. Hinde, E. Shaw, and C. G. Beer (New York, NY: Academic Press), 201–279.

Wang, X., Liang, L., and Du, L. (2007). The effects of intrauterine undernutrition on pancreas ghrelin and insulin expression in neonate rats. *J. Endocrinol*. 194, 121–129. doi: 10.1677/JOE-07-0057

Wood, A. D., and Bartness, T. J. (1996a). Caloric density affects food hoarding and intake by Siberian hamsters. *Physiol. Behav.* 59, 897–903. doi: 10.1016/0031-9384(95)02167-1

Wood, A. D., and Bartness, T. J. (1996b). Food deprivation-induced increases in hoarding by Siberian hamsters are not photoperiod-dependent. *Physiol. Behav*. 60, 1137–1145. doi: 10.1016/0031-9384(96)00173-4

Wu, Q., Boyle, M. P., and Palmiter, R. D. (2009). Loss of GABAergic signaling by AgRP neurons to the parabrachial nucleus leads to starvation. *Cell* 137, 1225–1234. doi: 10.1016/j.cell.2009.04.022

Yang, H. D., Wang, Q., Wang, Z., and Wang, D. H. (2011). Food hoarding and associated neuronal activation in brain reward circuitry in Mongolian gerbils. *Physiol. Behav*. 104, 429–436. doi: 10.1016/j.physbeh.2011.04.062

Yousheng, J., Nguyen, T., Desai, M., and Ross, M. G. (2008). Programmed alterations in hypothalamic neuronal orexigenic responses to ghrelin following gestational nutrient restriction. *Reprod. Sci*. 15, 702–709. doi: 10.1177/1933719108316982

Zhang, X. Y., Yang, H. D., Zhang, Q., Wang, Z., and Wang, D. H. (2011). Increased feeding and food hoarding following food deprivation are associated with activation of dopamine and orexin neurons in male Brandt's voles. *PLoS ONE* 6:e26408. doi: 10.1371/journal.pone.0026408

Zheng, H., Patterson, L. M., Rhodes, C. J., Louis, G. W., Skibicka, K. P., Grill, H. J., et al. (2010). A potential role for hypothalamomedullary POMC projections in leptin-induced suppression of food intake. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 298, R720–R728. doi: 10.1152/ajpregu.00619.2009

Zigman, J. M., and Elmquist, J. K. (2003). Minireview: from anorexia to obesity–the yin and yang of body weight control. *Endocrinology* 144, 3749–3756. doi: 10.1210/en.2003-0241

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Circadian adaptations to meal timing: neuroendocrine mechanisms

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Circadian rhythms of behavior and physiology are generated by central and peripheral circadian oscillators entrained by periodic environmental or physiological stimuli. A master circadian pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) is directly entrained by daily light-dark (LD) cycles, and coordinates the timing of other oscillators by direct and indirect neural, hormonal and behavioral outputs. The daily rhythm of food intake provides stimuli that entrain most peripheral and central oscillators, some of which can drive a daily rhythm of food anticipatory activity if food is restricted to one daily mealtime. The location of food-entrainable oscillators (FEOs) that drive food anticipatory rhythms, and the food-related stimuli that entrain these oscillators, remain to be clarified. Here, we critically examine the role of peripheral metabolic hormones as potential internal entrainment stimuli or outputs for FEOs controlling food anticipatory rhythms in rats and mice. Hormones for which data are available include corticosterone, ghrelin, leptin, insulin, glucagon, and glucagon-like peptide 1. All of these hormones exhibit daily rhythms of synthesis and secretion that are synchronized by meal timing. There is some evidence that ghrelin and leptin modulate the expression of food anticipatory rhythms, but none of the hormones examined so far are necessary for entrainment. Ghrelin and leptin likely modulate food-entrained rhythms by actions in hypothalamic circuits utilizing melanocortin and orexin signaling, although again food-entrained behavioral rhythms can persist in lesion and gene knockout models in which these systems are disabled. Actions of these hormones on circadian oscillators in central reward circuits remain to be evaluated. Food-entrained activity rhythms are likely mediated by a distributed system of circadian oscillators sensitive to multiple feeding related inputs. Metabolic hormones appear to play a modulatory role within this system.

Keywords: circadian, food entrainment, ghrelin, leptin, melanocortin, corticosterone, orexin, insulin

INTRODUCTION

Behavior and physiology are regulated by a hierarchically organized system of circadian oscillators located in the brain and in most peripheral tissues and organs (**Figure 1**). Circadian timekeeping coordinates cellular and physiological processes internally, and synchronizes these with daily cycles in the environment, ensuring that biological activities, from gene expression to foraging behavior, occur in the right sequence and at the right time of day. Although daily light-dark (LD) cycles are considered to be the dominant environmental stimulus for synchronizing circadian oscillators to local time, for many circadian processes, it is the timing of food intake that is most important. In this review, we briefly outline the evidence that feeding patterns regulate circadian clocks in mammals. We then critically examine the role of metabolic and stress-related hormones in this process. An important objective for the purposes of this Frontiers Topic (neuroendocrine mechanisms that connect feeding behavior and stress) is to draw attention to chronobiological concepts that provide a framework for understanding how anything that can affect feeding behavior, including acute or chronic stress stimuli (Maniam and Morris, 2012; Patterson and Abizaid, 2013; Sinha and Jastreboff, 2013), can potentially alter circadian organization within the brain and in peripheral organs and tissues. Whether the consequences for the organism are adaptive or pathological may depend on the temporal parameters of feeding behavior.

MEAL TIMING ENTRAINS CIRCADIAN CLOCKS

The role of food intake in the regulation of circadian clocks was first revealed by studies of circadian activity rhythms in food-restricted rodents. Acute and chronic food restriction elicit well-known behavioral and physiological responses to maintain metabolic homeostasis. Acute deprivation recruits neural circuits that promote arousal and food seeking behavior. Chronic caloric restriction induces physiological adaptations to facilitate the extraction and storage of energy from ingested nutrients and to reduce energy expenditure. If caloric restriction is chronic and food availability is limited to a particular time of day, further adaptations can occur in the temporal regulation of metabolism and food seeking behavior. These adaptations involve entrainment of circadian clocks in the brain and in peripheral organs and tissues by stimuli associated with food intake.

Physiology and behavior are regulated by circadian clocks that induce daily rhythms in synchrony with environmental cycles. In mammals, a master circadian clock is located in the

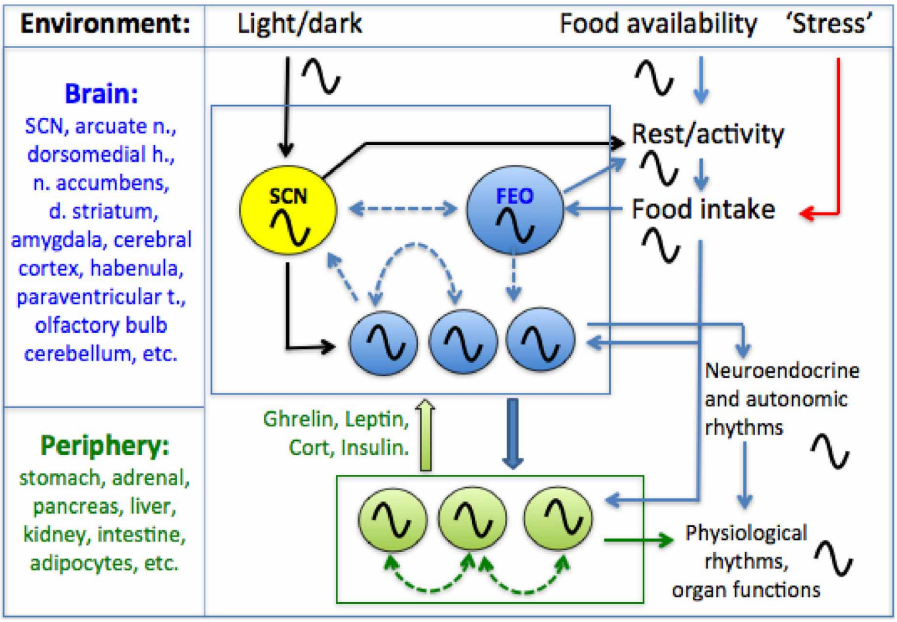

FIGURE 1 | A conceptual model of the mammalian circadian system. A circadian pacemaker located in the retinorecipient suprachiasmatic nucleus (SCN) in the hypothalamus generates circadian rest-activity, feeding, body temperature and other rhythms that entrain to environmental light-dark (LD) cycles. The daily feeding rhythm provides time cues that entrain circadian oscillators elsewhere in the brain and in most peripheral organs and tissues. Limiting food access to a single daily meal entrains central and peripheral oscillators, decoupling these from the SCN pacemaker which remains entrained to LD. Some SCN-independent food-entrainable oscillators (FEOs) drive daily rhythms of food anticipatory activity. The location of these FEOs and the signals that entrain them to mealtime, remain to be determined. Peripheral clocks could entrain central FEOs via hormonal pathways involving ghrelin, leptin, corticosterone and insulin, although none of these alone are required for food-anticipatory activity rhythms. Solid arrows indicated interactions for which empirical evidence exists, while dashed arrows indicate possible interactions (coupling among oscillators in different structures). For simplicity, some other possible interactions among and between central and peripheral clocks and meal-related cues are omitted.

suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Weaver, 1998; Welsh et al., 2010). The SCN contains a large population of circadian oscillator cells that are entrained to daily LD cycles via a direct input from intrinsically photoreceptive retinal ganglion cells. SCN ablation eliminates circadian organization of behavior and physiology if food is freely available. Circadian oscillators also exist in other brain regions and most if not all peripheral organs and tissues (Yamazaki et al., 2000; Guilding and Piggins, 2007; Dibner et al., 2010). Within each tissue, circadian oscillator cells must be coupled with each other for circadian organization to emerge at the tissue level. The SCN plays a special role within this multioscillatory system by providing signals that maintain coupling of oscillators within most tissues. SCN timing signals are conveyed by neural (autonomic), hormonal (hypothalamo-pituitary) and behavioral pathways (Dibner et al., 2010). Of particular importance is SCN control of the daily rhythm of feeding behavior. A dominant role for food intake in the control of peripheral rhythms is readily demonstrated by restricting rats and mice to a 2–6 h mealtime in their usual rest phase (the lights-on period), when they normally eat very little. Scheduled daytime feeding shifts the timing of clock gene rhythms and functional rhythms in most peripheral organs and tissues to realign with expected mealtime (Boulos and Terman, 1980; Dibner et al., 2010). If a daily LD cycle is present, the SCN itself is not shifted by daytime feeding schedules (Damiola et al., 2000; Hara et al., 2001a; Stokkan et al., 2001). Dissociation of peripheral oscillators from the SCN during restricted daytime feeding suggests that the SCN normally coordinates daily rhythms of physiology by its role as the pacemaker for feeding behavior.

Restricted daytime feeding also induces a behavioral rhythm of food seeking behavior that anticipates the daily mealtime (**Figure 2**) (Boulos and Terman, 1980; Mistlberger, 1994; Stephan, 2002). If the SCN are ablated and food is available *ad-libitum*, behavior and physiology lose circadian organization. However, if food is provided once every 24 h in a reduced amount, food anticipatory activity rhythms emerge and circadian rhythms of physiology are restored (Stephan et al., 1979; Boulos et al., 1980). The timing of food intake is thus fundamental to circadian organization of behavior and physiology and involves effects of food on circadian oscillators downstream from the master, LD-entrained circadian pacemaker in the SCN.

Despite the significance of food as a "zeitgeber" (entrainment cue) for circadian regulation of food-anticipatory behavior, the neural and molecular mechanisms of this SCN-independent circadian function are not yet well-understood. The daily rhythm of food anticipatory activity exhibits canonical properties of circadian clock control (Boulos and Terman, 1980; Mistlberger, 1994; Stephan, 2002). Once established, the rhythm persists for several cycles during total food deprivation (**Figure 2C**); the timing mechanism is therefore self-sustaining, unlike simple hourglass or

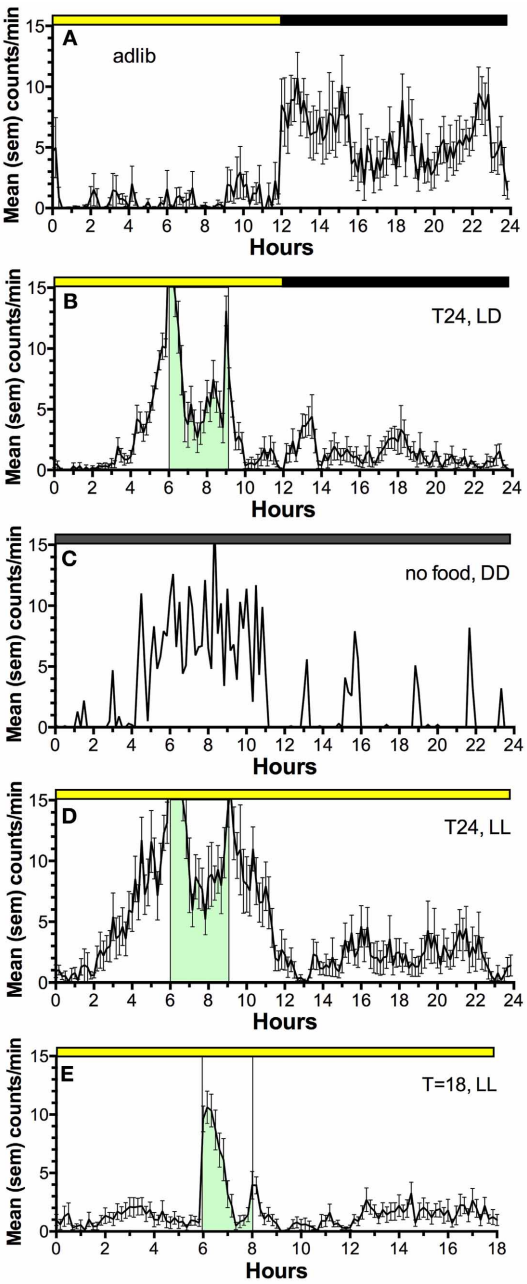

FIGURE 2 | Average waves illustrating the temporal distribution of spontaneous locomotor activity of a representative rat under the following feeding conditions. (A) *ad-lib* access to food (10 day average). **(B)** Food restricted to 3-h/day, beginning 6 h after lights on, for 3 weeks (last 10 day average). **(C)** Day 2 of total food deprivation after 3 weeks of restricted daytime feeding. **(D)** Food restricted to 3-h/day for 3 weeks, after a month in constant light with food *ad-lib*, to eliminate the light-entrainable, free-running rhythm controlled by the SCN (a reversible SCN "lesion" procedure). **(E)** Food restricted to 2-h/day in constant light, delivered every 18 h, illustrating failure of rats to anticipate a daily meal at regular but non-circadian intervals. Mealtime is denoted by green shading and vertical bars. Activity was measured by motion sensors, and plotted as average counts per minute, in 10 min bins.

interval timers (e.g., like a stomach that gradually empties after a large meal). If the feeding schedule differs greatly from 24 h (e.g., is <22 or >30 h) the food anticipation rhythm does not emerge (**Figure 2E**); it has so-called "limits to entrainment" that suggest involvement of an oscillator with an intrinsic periodicity in the circadian (∼24 h) range. If the mealtime is shifted by 6 h or more to a new time of day, the food anticipation rhythm takes several days to realign with mealtime. These properties have been interpreted as evidence that one or more correlates of food intake entrain a circadian oscillator (or population of oscillators) that determines the daily timing of foraging activity. Given that circadian food anticipatory rhythms persist in SCN-ablated rats and mice, food-entrainable oscillators (FEOs) hypothesized to drive anticipatory behavior must be located elsewhere in the brain or body. There are many candidate sites, because, as noted, daily feeding schedules synchronize circadian oscillators in many brain regions and virtually every organ system (Feillet et al., 2006, 2008; Verwey and Amir, 2009; Dibner et al., 2010). Lesion studies have so far failed to confirm a single critical locus in the brain (Mistlberger, 1994, 2011; Davidson, 2009), suggesting that circadian timing of food anticipatory activity involves an anatomically distributed population of FEOs. It is also likely that there are multiple stimuli associated with scheduled feeding that are capable of functioning as zeitgebers for entraining FEOs.

In the following sections, we evaluate a potential role for metabolic hormones as signals by which FEOs are coupled to daily feeding schedules, or by which FEOs broadcast timing information to neural circuits that organize food seeking behavior. Although the primary emphasis is on food entrained circadian regulation of behavior, these hormonal signals may also play a role synchronizing circadian clocks and functions in peripheral organs (Dibner et al., 2010). The light-entrainable circadian pacemaker in the SCN, as noted, remains entrained to LD cycles regardless of the timing of food intake. There are limited circumstances under which its phase can be controlled or modified by mealtime (e.g., in constant dark or light; Abe et al., 1989; Mistlberger, 1993; Challet, 2010), but neural and hormonal signals that mediate these effects are little studied. Consequently, the literature reviewed here concerns circadian oscillators located outside of the SCN pacemaker.

To be considered a candidate as an entrainment signal for food anticipatory circadian rhythms, a hormone (or any stimulus factor) should exhibit a daily rhythm that is induced by or is synchronous with scheduled feeding. The factor should be capable of shifting the phase of one or more food-entrained rhythms (a requirement for co-activation by 2 or more factors is possible). Blocking the factor may prevent shifting of one or more food entrained rhythms (unless there are multiple redundant signals). To be considered a candidate as an output of FEOs responsible for driving food anticipatory activity (or other food entrained rhythms) the factor should exhibit a daily rhythm that correlates strongly with locomotor activity (or other rhythms), under a variety of conditions (e.g., during total food deprivation, when food anticipatory rhythms established by scheduled feeding persist for several cycles despite the absence of a daily meal). Enhanced expression of the factor may increase the amplitude

of food anticipatory activity (or other rhythms), while blocking the factor should attenuate or prevent expression of food anticipatory activity (or other rhythms). Attenuation could be modest or negligible if there are multiple redundant output signals. Food anticipation could also be enhanced or attenuated by factors that are downstream from FEOs, or that converge downstream via other pathways. Therefore, the observation that manipulation of a factor alters the amount of food anticipatory activity does not alone permit a strong conclusion that the factor is either an input or output of FEOs.

PERIPHERAL HORMONAL MECHANISMS

CORTICOSTERONE

The synthesis and secretion of corticosterone (CORT) from the adrenal gland is under control of descending neural (autonomic) and hormonal (adrenocorticotropin) signals, interacting with circadian oscillators intrinsic to cells of the adrenal gland (Kaneko et al., 1981; Oster et al., 2006). Daily restricted feeding markedly alters the circadian profile of circulating CORT levels (Krieger, 1974). In nocturnal rodents under ad lib feeding conditions CORT levels peak close to dark onset. When food is restricted to the middle of the light phase, the daily rhythm of CORT secretion becomes bimodal, with one peak occurring at the expected mealtime and a second at the beginning of the dark period (Krieger, 1974). Treatment with sodium pentobarbital can suppress the pre-feeding peak while sparing the nocturnal peak, suggesting that these two peaks are stimulated by separate neural mechanisms (Honma et al., 1984a).

The correlated rise of CORT release and food seeking activity prior to mealtime raises the possibility that CORT may function as an endogenous entrainment signal for FEOs or as an output from FEOs to behavior. However, preprandial CORT secretion has been ruled out as a signal involved in food anticipatory behavioral rhythms, as adrenalectomized rats display apparently normal food anticipatory activity (Stephan et al., 1979; Boulos et al., 1980; Segall et al., 2008; Sujino et al., 2012). The available evidence reveals no obvious difference in the rate at which anticipation emerges or in the magnitude or duration of steady state anticipatory activity in adrenalectomized rats. This conclusion is supported by studies of food anticipation in rats provided with two daily meals separated by 6 h or more. Under these schedules rat readily anticipate both meals and continue to concentrate activity at both expected mealtimes during total food deprivation tests. The properties of each bout of anticipation suggest involvement of two independently entrained FEOs (Stephan, 1989; Mistlberger et al., 2012). At a 6 h inter-meal interval, CORT secretion rises in anticipation of the first meal, but does not rise again until near the end of the second meal (Honma et al., 1984b). When the meals were omitted only the peak to the first meal was observed, thereby dissociating CORT secretion from behavioral anticipation of the second meal.

Circadian oscillators in peripheral organs, including the adrenal gland, liver, stomach, intestines, pancreas, kidney, heart, and lungs, are entrained by daily feeding schedules (Damiola et al., 2000; Stokkan et al., 2001; Davidson et al., 2003). Circadian clock gene oscillations in many of these tissues can also be shifted by glucocorticoid administration (Balsalobre et al., 2000; Reddy et al., 2007; Pezuk et al., 2012) or by adrenalectomy (Pezuk et al., 2012), suggesting a role for glucocorticoids in phase control of peripheral clocks during adlib food access, and potentially also during restricted feeding. A strong form of the latter hypothesis, that glucocorticoids may be necessary for resetting of peripheral tissues during restricted feeding, is refuted by observations that peripheral clocks shift more rapidly in response to daytime feeding in adrenalectomized mice or knockout mice lacking hepatic glucocorticoid receptors (Balsalobre et al., 2000; LeMinh et al., 2001). This indicates that nocturnal glucocorticoid secretion normally reinforces circadian organization of peripheral clocks in rodents with adlib food access, by opposing rapid phase shifts that might be induced by transient alterations of food access. This role may be more prominent for some tissues. Circadian clock gene rhythms in the liver are not shifted by a daily CORT injection meant to simulate a mid-day feeding schedule (Stokkan et al., 2001) and shifting of the liver clock by mid-day feeding is not impeded by a late-day CORT injection meant to simulate the normal nocturnal rise of CORT. By contrast, shifting of circadian clocks in the kidney and lungs to restricted feeding is blocked by a late day CORT injection. These findings indicate that peripheral oscillators likely respond to multiple feeding-related signals in a tissue specific manner.

GHRELIN

Acyl-ghrelin and des-acyl ghrelin are peptide hormones derived from preproghrelin, which is synthesized by oxyntic cells of the stomach (Kojima et al., 1999) and by neurons in medial and lateral hypothalamic nuclei (Cowley et al., 2003). Systemic and intracerebroventricular (ICV) administration of acyl-ghrelin stimulates feeding in rats and mice (Nakazato et al., 2001; Toshinai et al., 2006). Des-acyl ghrelin may also stimulate feeding if administered (ICV), but not systemically (Toshinai et al., 2006). The orexigenic action of gastric ghrelin is mediated primarily by activation of agouti-related peptide and neuropeptide Y (AgRP/NPY) neurons in the arcuate nucleus (ARC), resulting in release of AgRP and NPY. Ghrelin also exerts an inhibitory action at proopiomelanocortin (POMC) neurons, preventing release of anoxerigenic peptides. The growth hormone secretagogue 1 receptor (GHSR) is the only known ghrelin receptor and is found in high levels in the ARC. GHSR mRNA has also been localized to multiple hypothalamic areas involved in feeding and arousal and extra-hypothalamic regions including the hippocampus, ventral tegmental area (VTA), substantia nigra (SN), tuberomammilary nucleus (TMN), Edinger-Westphal nucleus (EN), dorsal and median raphe nuclei, laterodorsal tegmental nuclei and the facial nucleus (Guan et al., 1997).

Plasma ghrelin increases prior to scheduled meals in food restricted rats and mice (Bodosi et al., 2004; Drazen et al., 2006; LeSauter et al., 2009) and prior to scheduled or habitual mealtimes in humans (Cummings et al., 2001; Frecka and Mattes, 2008). Gastric oxyntic cells exhibit a daily rhythm of circadian clock gene expression that is entrained by restricted feeding, suggesting that food-anticipatory release of gastric ghrelin is a function of gastric FEOs (LeSauter et al., 2009). The food-entrainable rhythmicity and orexigenic properties of gastric ghrelin present an intriguing circumstantial case that gastric ghrelin

may participate in the regulation of circadian food anticipatory rhythms, either by stimulating appetite and activity or by entraining central clocks that drive behavioral rhythms. This hypothesis can be evaluated by the results of correlation, stimulation and gene knockout studies.

Plasma ghrelin and locomotor activity exhibit a correlated rise prior to a scheduled daily meal but become dissociated during subsequent total food deprivation tests. Food entrained activity recurs at the expected mealtime for at least 4 days without food (Boulos et al., 1980; Mistlberger, 1994). This property is critical to the interpretation that anticipation of a daily meal is based on entrainment of a self-sustaining circadian clock. Acyl-ghrelin also rises prior to the first skipped meal, but beyond a day of fasting decreases and remains at low levels. Des-acyl ghrelin, which does not stimulate feeding or activity following systemic administration, is responsible for the rise of total plasma ghrelin evident during extended fasting (Liu et al., 2008; Kirchner et al., 2009). A similar dissociation between acyl-ghrelin and food anticipatory activity during food deprivation tests is evident in rats fed two daily meals separated by 12 h (Patton et al., 2012). During the first 24 h without food, ghrelin levels rise in anticipation of the first mealtime but then remain elevated for the rest of the day, unlike locomotor activity, which decreases after the first meal before rising prior to the second mealtime 10 h later (Patton et al., 2012). A third dissociation can be inferred from studies of clock gene rhythms in the stomach of rats entrained to a midday meal for several weeks, and then allowed ad-lib food access for a week. If the food is then removed for 2–4 days, the rats become active at the previous mid-day mealtime. The gastric circadian clock, by contrast, is shifted by daytime feeding, but reverts to its original phase after a few days of adlib feeding (Davidson et al., 2003). If gastric ghrelin secretion is controlled by gastric circadian oscillators, then plasma ghrelin should rise at night and not in the middle of the day when food anticipatory activity reappears during deprivation tests.

Although systemic administration of acyl-ghrelin stimulates food intake, its effect on locomotor activity is less clear. In mice, central administration of acyl-ghrelin increased eating, activity and wake time, while peripheral ghrelin stimulated food intake but did not alter either wake time or locomotor activity for 1 and 6 h post-injection (Szentirmai, 2012). At orexigenic doses, systemically administered ghrelin was not sufficient to stimulate activity, as predicted if gastric ghrelin were responsible for circadian food anticipatory activity.

The availability of ghrelin ligand and receptor knock-out mice permits a direct assessment of whether ghrelin is necessary for food anticipatory activity. The results of 5 studies are consistent in demonstrating that mice do not require ghrelin signaling to anticipate a daily meal (**Figure 3**). Three of these studies reported food anticipatory activity and body temperature in ghrelin ligand knockout mice (Szentirmai et al., 2010; Gunapala et al., 2011) (**Figures 3C,D**) or ghrelin receptor KO mice (Gooley et al., 2006) that did not differ from anticipatory activity in WT mice. Two other studies also reported significant FAA in ghrelin receptor KO mice, but with alterations relative to WT mice. In one case, the average duration of FAA was decreased (i.e., the onset was closer to mealtime) without a change in the peak level (LeSauter et al., 2009) (**Figure 3A**), while in the other case, the peak level was reduced with little change in FAA duration (Blum et al., 2009) (**Figure 3B**).

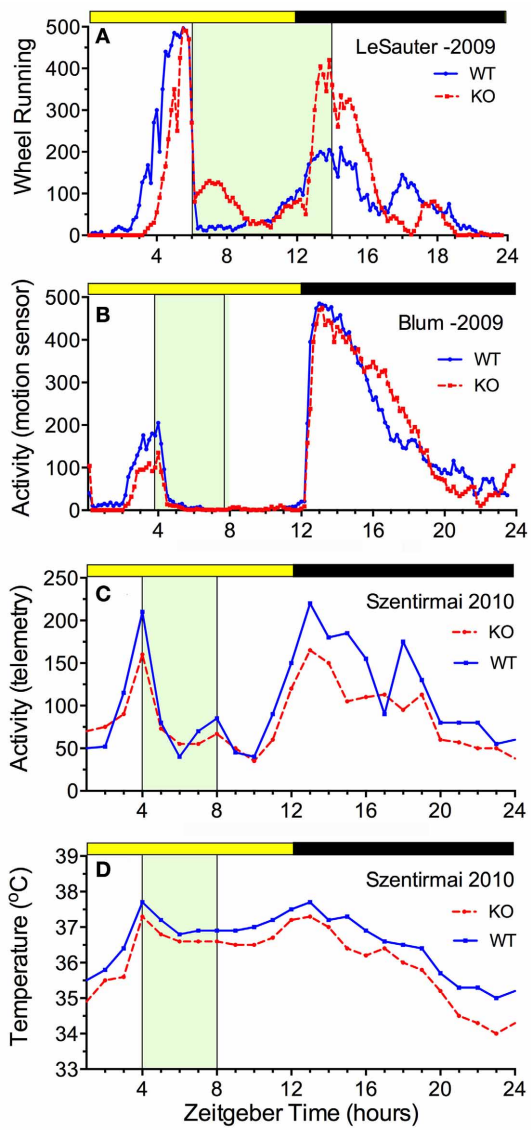

FIGURE 3 | Average waves illustrating the consequences of ghrelin receptor knockout (A,B) and preproghrelin KO (C,D) on food-entrained locomotor activity and body temperature in mice. Knockout groups are represented by the red dashed lines, WT by the solid blue lines. **(A)** The onset of wheel running activity is modestly delayed in ghrelin receptor knockout mice, but rises to the same level (LeSauter et al., 2009). **(B)** The onset of running is not delayed but rises to a lower level in ghrelin receptor knockout mice (Blum et al., 2009). **(C)** Locomotor activity measured by telemetry is slightly reduced at all time points in preproghrelin KO mice compared to WT mice, but increases equivalently prior to a 4-h daily meal in both groups (Szentirmai et al., 2010). **(D)** The same results were obtained for core body temperature measured by telemetry (Szentirmai et al., 2010).

In contrast to these 5 studies, there are two reports that ghrelin receptor knock out mice do not anticipate a daily meal. In one of these studies, food anticipation was quantified as the

total number of beam breaks 2-h prior to a 4-h daily mealtime, during one baseline day and on day 14 of food restriction in a separate recording cage (Davis et al., 2011). Raw data (24 h activity waveforms) were not provided, and without normalizing for total daily activity, comparing counts in a 2-h time bin does not provide information about the presence or absence of anticipation. Also, the mealtime, relative to the LD cycle was not specified. Consequently, the "evidence for absence" in this study is weak. The other study evaluated the effects of ghrelin receptor knockout on hyperactivity in mice induced by severe caloric restriction, in an "activity based anorexia" protocol (a 2-h daily meal provided at lights-off for 3-days; Verhagen et al., 2011a). WT mice exhibited daytime hyperactivity, which was absent in the KO mice. However, activity was maximal in the middle of the light period in both groups and decreased as the nocturnal mealtime approached, indicating that hyperactivity during the first 3 days of extreme caloric restriction in the WT mice was non-specific rather than food-anticipatory. When food is acutely restricted to a few hours/day, mice may exhibit hyperactivity for the first few days, which dissipates as the amount of food consumed during the mealtime increases, and a stable pattern of food anticipatory activity, peaking at expected mealtime time, emerges. If the caloric restriction is too severe, the hyperactivity does not dissipate, appetite is suppressed and metabolic collapse may ensue.

Collectively, the results of ghrelin knockout studies indicate that ghrelin secretion is not required for anticipation of a daily meal. If ghrelin does participate in circadian expression of food anticipatory activity under some experimental conditions, it is likely acting downstream from or in parallel with FEOs hypothesized to drive FAA, or it is redundant with other FEOs or with other signals that can entrain FEOs. The site of action of ghrelin may be both hypothalamic and mesocorticolimbic, given recent observations that ghrelin receptor KO mice exhibit lower levels of food-anticipatory cFos expression (a correlate of neuronal activity) in lateral hypothalamic orexin neurons, the VTA and the nucleus accumbens shell (Lamont et al., 2012). Whether ghrelin is responsible for controlling the phase of circadian oscillators in these areas, or in any peripheral tissues, has not yet been demonstrated.

GHRELIN AND ANTICIPATION OF PALATABLE FOOD

Food anticipation can also be observed in rats and mice without caloric restriction, by providing access to a palatable snack (e.g., chocolate) at a fixed time of day, with regular chow available ad-libitum (Mistlberger and Rusak, 1987; Mendoza et al., 2005; Hsu et al., 2010). Palatable feeding schedules are associated with induction of *cFos* prior to snack time in cortical and limbic structures involved in reward and motivation (n. accumbens core and shell, central nucleus of the amygdala, prefontal cortex). All of these areas express circadian rhythms of clock gene expression, and are innervated by dopaminergic projections from the VTA (Webb et al., 2009a). Neural activity in the VTA exhibits a daily rhythm, but clock gene expression in this area is constitutive rather than circadian, indicating that VTA rhythmicity is driven by inputs (Webb et al., 2009b; Moorman and Aston-Jones, 2010; Baltazar et al., 2013). A candidate rhythmic input is ghrelin, which increases the firing rate of VTA neurons that release DA in the VTA (Abizaid et al., 2006). This raises the possibility that ghrelin, by activating DA neurons in the VTA that innervate circadian oscillators in the limbic forebrain, might play a role as an entraining stimulus for daily rhythms of palatable food anticipation. If so, then ghrelin levels should rise in anticipation of a palatable meal, and ghrelin knockout or receptor antagonists should eliminate or attenuate palatable food anticipation.

Studies of plasma ghrelin levels in rats anticipating a daily palatable meal have yielded mixed results. One study reported that plasma ghrelin, sampled at the expected time of chocolate delivery (6 h after lights-on in LD 12:12), correlated positively with locomotor activity counts during the preceding 3 h (Merkestein et al., 2012). Ghrelin levels in these rats were intermediate between ghrelin levels in adlib fed rats and in rats restricted to a single daily meal at this time of day. A second study reported that plasma ghrelin was significantly increased 30 and 60 min prior to a daily 6 h meal consisting of regular chow or high fat chow, but was not elevated at 30, 60, and 90 min time points prior to 2-h daily access to chocolate (5 h after lights-on), with regular chow available *ad-libitum* (Dailey et al., 2012). Rats in all 3 groups exhibited significant anticipatory activity, although the amount of anticipatory activity in rats receiving chocolate without food restriction was about half of that in the food restricted groups. These results indicate that elevated ghrelin is not necessary for the induction of anticipatory activity, but may promote its expression. An explanation for the different results in the two studies remains to be established, but could be related to the parameters of the feeding schedules (meal time and duration) or small differences in the blood sampling time points.

Anticipation of palatable foods without caloric restriction has not yet been assessed in ghrelin deficient mice. However, the ghrelin receptor antagonist JMV2959, administered i.c.v. 3-h prior to chocolate access, dose-dependently attenuated anticipatory activity by 40–60%, while administration of acyl-ghrelin increased anticipatory activity by about 50% (Merkestein et al., 2012). These findings are consistent with a role for ghrelin as a modulatory factor in palatable meal anticipation.

LEPTIN

Leptin is an anorexigenic hormone released from adipose tissue in proportion to lipid stores (Zhang et al., 1994). Leptin acts in opposition to ghrelin at the ARC, inhibiting NPY/AgRP neurons and stimulating POMC/CART neurons, resulting in decreased food intake and increased metabolic rate (Kageyama et al., 2012). Serum leptin shows a daily rhythm in humans and mice, increasing during the waking/feeding period and decreasing during the sleeping/fasting period (Sinha et al., 1996; Ahima et al., 1998; Ahren, 2000; Shea et al., 2005). In humans, the rhythm is the net result of daily variations in food intake (leptin increases after feeding and decreases during fasting) and an endogenous clock (Shea et al., 2005). In mice, leptin synthesis and secretion are also subject to circadian regulation, at least in part by circadian oscillators intrinsic to adipocytes (Zvonic et al., 2006; Otway et al., 2009). However, expression of the daily rhythm of circulating leptin appears to be dependent on food intake. The rhythms of plasma leptin, and the adipocyte clock (Zvonic et al., 2006), are inverted

if food is restricted to the middle of the light period, but the plasma leptin rhythm is abolished during total food deprivation (Ahima et al., 1998; Elimam and Marcus, 2002; Martinez-Merlos et al., 2004). Food anticipatory activity rhythms, as noted, persist robustly during several days of total food deprivation. This dissociation between circulating leptin levels and food anticipatory rhythms during food deprivation indicates that variations in plasma leptin do not drive the daily rhythm of food anticipatory behavior.

Consistent with this interpretation, leptin insensitive Zucker rats and leptin deficient ob:ob mice are obese and hypoactive when food is available ad-lib, but exhibit strong FAA when restricted to a single mid-day meal and the amount of FAA is increased relative to WT controls (Mistlberger and Marchant, 1999b; Ribeiro et al., 2011). Conversely, systemic administration of leptin reduces pre-meal activity in food-restricted rats/mice (Verhagen et al., 2011b). These results indicate that leptin, like ghrelin, plays a modulatory role in the expression of food anticipatory activity, consistent with its role in modulating food intake under free-feeding conditions.

INSULIN AND GLUCAGON

Homeostasis of plasma glucose is critically dependent on the pancreatic hormones insulin and glucagon. Food ingestion is the major stimulus for insulin secretion, but external stimuli that predict food intake also play an important role stimulating release (Woods et al., 1970). In the scheduled feeding paradigm, time of day (circadian phase) has been conceptualized as a conditioned stimulus for anticipatory insulin secretion (Woods et al., 1977). It is now established that the pancreas contains autonomous circadian oscillators that are reset by restricted daytime feeding, independently of the master clock in the SCN (Damiola et al., 2000). Insulin secreting beta cells are among those thought to contain an intrinsic circadian clock (Marcheva et al., 2010; Sadacca et al., 2011). Therefore, any increase in plasma insulin that occurs in rats at an expected daily mealtime (Wiley and Leveille, 1970; Woods et al., 1977; Drazen et al., 2006; Vahl et al., 2010) presumably reflects entrainment of local pancreatic oscillators, a process distinct from the central neural mechanisms that mediate stimulus-response conditioning of insulin release.

A food anticipatory increase in plasma insulin has not always been reported (e.g., Davidson and Stephan, 1999; Diaz-Munoz et al., 2000; Frecka and Mattes, 2008), This may be due to sampling time and resolution, as significant increases when reported appear to occur very close to mealtime (e.g., within 15 min; Drazen et al., 2006; Vahl et al., 2010). This raises the possibility that preprandial insulin release in some studies is a conditioned response to noises made by research staff arriving in advance to deliver the daily meal (we have observed lever pressing for food conditioned to the sound of a door opening to enter an anteroom outside of an animal recording room, a few minutes prior to a daily mealtime; Peterson and Mistlberger, unpublished observations). Whatever the explanation for the different results across studies, the proximity of preprandial insulin release to mealtime rules out insulin as an initiator of food anticipatory activity, which typically begins 2–3 h before mealtime. It is also appears that prandial insulin release is not required for entrainment of oscillators driving FAA, because streptozotocine-induced diabetes in mice does not prevent induction of food anticipatory activity or resetting of circadian clocks in the liver, heart or kidney by restricted daytime feeding (Davidson et al., 2002; Oishi et al., 2004).

Glucagon, the other major pancreatic hormone, normally varies inversely with insulin. Two studies reported that plasma glucagon levels decrease in the hours preceding a scheduled daytime meal in rats (Davidson and Stephan, 1999; Diaz-Munoz et al., 2000). It has not been established that pancreatic alpha cells that produce glucagon possess an intrinsic circadian clock, and there is no evidence that glucagon participates in food anticipatory behavioral rhythms.

GLUCAGON LIKE PEPTIDE 1

Glucagon like peptide 1 (GLP-1) is synthesized in the periphery and in the brain. In the periphery GLP-1 is derived from preproglucagon synthesized in L cells of the intestine and alpha cells of the pancreas (Mojsov et al., 1990). In the CNS GLP-1 is produced in the nucleus of the solitary tract, the dorsal and central reticular nucleus, the PVN, DMH and to a lesser extent the ARC (Larsen et al., 1997). GLP-1 receptors are widely expressed in the CNS with large numbers in the ARC, PVN, and SON, and fewer in the lateral preoptic area, PVN, LH, bed nucleus of the stria terminalis and DMH (Shughrue et al., 1996). Most regions that express GLP-1 receptors are behind the BBB, and whether peripheral GLP-1 has access to these receptors is under debate (Trapp and Hisadome, 2011). It is also not clear if post-prandial peripherally released GLP-1 signals for the release of central GLP-1.

Two studies have investigated the effects of restricted feeding schedules on GLP-1 secretion. In one study, rats were maintained on a 4-h daily meal beginning 5-h after lights-on for 14 days. Plasma GLP-1 was found to increase 4-fold over an interval from 75 to 60 min before the meal, and then decrease back to baseline levels 30 min before mealtime (Vahl et al., 2010). In a second study, rats anticipating a 6-h daily meal beginning 3-h after lights-on also exhibited increased GLP-1 at 90 and 60 min before mealtime, and no difference at 30 min prior to the meal, by comparison with adlib fed rats. By contrast, a group of rats anticipating 2-h access to chocolate, without caloric restriction, showed no change in GLP-1 at 90, 60, or 30 min time points before feeding (Dailey et al., 2012). These results indicate that a premeal peak of GLP-1 is not necessary for anticipation of a daily palatable meal in the absence of caloric restriction. Whether the onset of GLP-1 secretion prior to a single daily meal in calorically restricted rats correlates with the onset of anticipatory activity has not been assessed. Sustained increments of GLP-1 are clearly not required to produce the continuous food anticipatory activity typical of food-entrained rodents, but it is conceivable that an early GLP-1 spike is part of a cascade of responses that gate the onset of food anticipatory activity or entrain FEOs driving food anticipation.

CENTRAL NEUROPEPTIDES

MELANOCORTINS AND NEUROPEPTIDE Y

Circulating ghrelin and leptin exert opposite effects on the expression of circadian food anticipatory activity, and may do this at least in part by differential effects on the central melanocortin

system. The melanocortin system consists of separate populations of neurons in the arcuate nucleus that release ligands for melanocortin-3 (McR3) and melanocortin-4 (McR4) receptors. One population of arcuate neurons synthesizes POMC, a precursor for α, β, and γ-melanocortin-stimulating hormones (MSH) which function as agonists at Mc3R and Mc4R receptors (Mountjoy, 2010). A second population of arcuate neurons corelease AgRP and NPY. AgRP acts as an inverse agonist at melanocortin receptors (Cone, 2005). Mc3R receptors are predominantly expressed in hypothalamic and limbic structures, with high levels in areas implicated in feeding and reward (VMH and VTA) (Roselli-Rehfuss et al., 1993), while Mc4R are more widely distributed within the brain.

The melanocortin system plays a critical role in the regulation of metabolism and feeding. α-MSH potently inhibits feeding (Fan et al., 1997), whereas AgRP stimulates feeding (Small et al., 2001; Aponte et al., 2011; Krashes et al., 2011). Deletion of POMC and Mc3R and Mc4R receptors induces hyperphagia, obesity, hyperinsulinemia, insulin insensitivity and loss of response to leptin (Barsh and Schwartz, 2002; Sutton et al., 2006), whereas AgRP deletions induce anorexia and weight loss and eliminate the orexigenic response to ghrelin (Chen et al., 2005; Gropp et al., 2005; Luguet et al., 2005; Aponte et al., 2011). Given the effects of ghrelin and leptin deficiency on food anticipatory activity rhythms, a prediction is that melanocortin receptor deficient mice should also exhibit a food anticipation phenotype. $Mc4^{-/-}$ mice exhibit normal FAA, but $Mc3^{-/-}$ mice have been reported to exhibit significantly attenuated food anticipatory rhythms of wheel running activity and waking (Butler et al., 2000; Sutton et al., 2008). However, the data fall short of supporting a strong conclusion that Mc3 receptors are "required" for entrainment of behavioral rhythms to feeding schedules, given that anticipation was evident in KO mice when recordings were made for longer than a few days (Sutton et al., 2008). Also, normal levels of food anticipatory activity have been observed in $Mc3R^{-/-}$ mice assessed by video based analyses (Steele, pers. commun.) and enhanced levels have been reported in mice lacking both Mc3 receptors and leptin assessed (Ribeiro et al., 2011). Whether differences in the amount of anticipatory activity observed in $Mc3R^{-/-}$ mice studied in different labs is related to methodological differences (e.g., recording devices) or to the use of knockout mice obtained from different sources remains to be evaluated.

AgRP neurons co-release the potent orexigen NPY. Synthesis and release of NPY in the rat hypothalamus exhibits a daily rhythm with a peak prior to the nocturnal feeding period in adlib fed rats and prior to a schedule daily meal in food restricted rats (Akabashi et al., 1994; Xu et al., 1999; Yoshihara et al., 1996). Central administration of NPY induces behavioral and endocrine responses characteristic of rats anticipating a daily meal, including secretion of insulin and corticosterone and increased locomotor activity (Stanley and Leibowitz, 1984; Smialowska et al., 1994; Sainsbury et al., 1997). Despite this functional profile, NPY appears to be dispensable for induction of food anticipatory activity rhythms. NPY KO mice show reduced food anticipatory activity on day 7 of restricted daytime feeding but normal food anticipation by day 14 (Gunapala et al., 2011). Treatment of neonatal rats with monosodium glutamate severely reduces NPY immunoreactivity in the arcuate nucleus, but does not attenuate food anticipatory activity in rats (Mistlberger and Antle, 1999a). Radiofrequency lesions of the hypothalamic paraventricular nucleus, a hot spot for orexigenic effects of NPY, also do not impair food anticipatory activity in rats (Mistlberger and Rusak, 1988).

OREXIN/HYPOCRETIN

Orexin A and B (hypocretin 1 and 2) are derived from prepro-orexin synthesized exclusively in a population of neurons distributed within the dorsomedial, perifornical and lateral hypothalamic areas (Date et al., 1999). Orexin neurons project widely throughout the hypothalamus, thalamus, brain stem, and forebrain (Peyron et al., 1998). Orexins act via two G-protein coupled receptors. OX1R is selective for orexin A whereas OX2R binds both orexin A and B with similar affinity. When administered ICV to rats and mice, orexin A acutely stimulates feeding (Lubkin and Stricker-Krongrad, 1998; Sakurai et al., 1998), plasma corticosterone, wakefulness (Hagan et al., 1999), and locomotion (Nakamura et al., 2000). Orexin neurons are activated by a variety of stimuli, including hypoglycemia, caloric restriction, ghrelin administration and non-specific arousal (Sakurai et al., 1998; Cai et al., 1999; Yamanaka et al., 2003; Webb et al., 2008). Conversely, antagonism of ORX1R reduces food intake and body weight (Smart et al., 2002). Restricted feeding has been found to increase FOS staining in orexin neurons in the LHA in a manner similar to glucose-deprivation (Kurose et al., 2002) and in anticipation of a daily meal (Akiyama et al., 2004).

The association of orexin neuron activity with systemic and behavioral variables relevant to food seeking behavior, and the broad distribution of orexinergic efferents within the central nervous system, makes this population of cells an intriguing candidate to mediate the effects of restricted feeding schedules on food anticipatory behavior. The results of ablation studies indicates that orexin neurons do play a role, but not as a driving oscillators responsible for timing anticipatory behavior. An early study using ibotenic acid to ablate lateral hypothalamic neurons nonspecifically in rats reported that while total daily activity was reduced, food anticipatory activity was not eliminated (Mistlberger and Rusak, 1988). Similar results were obtained using an orexin2-saporin toxin to ablate neurons expressing orexin2 receptors, which include neurons producing orexin (Mistlberger et al., 2003). A more selective genetic ablation of orexin neurons occurs developmentally in orexin/ataxin-3 mice. This is associated with behavioral state instability similar to narcolepsy, loss of the increase in consolidated wakefulness that occurs during acute food deprivation, and attenuation of food anticipatory activity (Hara et al., 2001b; Akiyama et al., 2004; Mieda et al., 2004). Orexin ligand knockout mice may also exhibit a reduction in food anticipatory activity compared to WT mice, but nonetheless exhibit a comparable peak of activity at the expected mealtime during a total food deprivation test (Kaur et al., 2008) (**Figures 4D–F**). Circadian timing of food anticipatory activity can thus be described as more accurate and thus

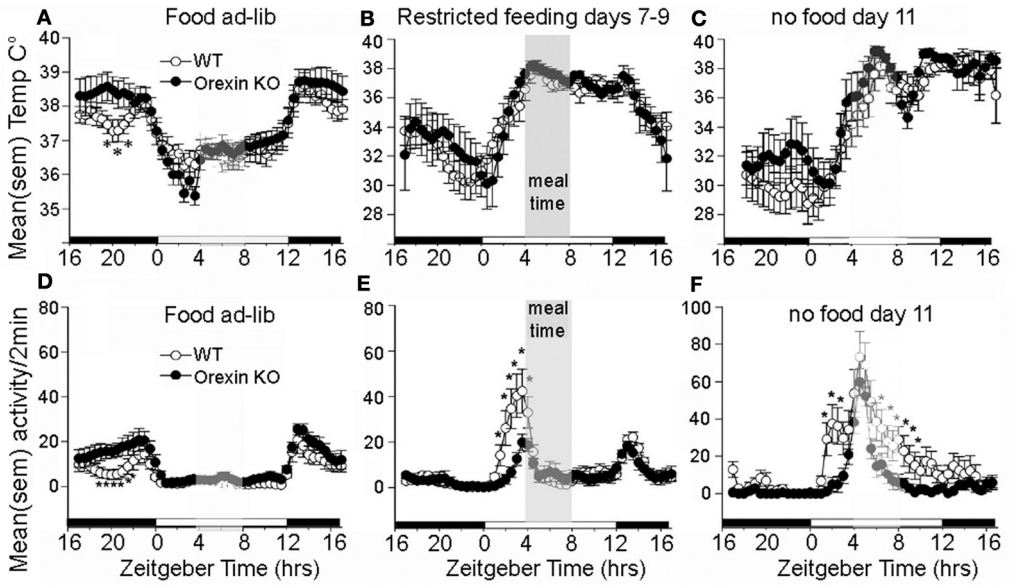

FIGURE 4 | Average waves illustrating daily rhythms of core body temperature (A–C) and locomotor activity (D–F) in WT mice (open circles) and orexin KO mice (closed circles). WT and KO mice show an equivalent anticipatory rise of body temperature prior to a 4-h daily meal **(B)** and this rhythm persists for at least 2 days of total food deprivation. **(C)** By contrast with WT mice, the KO mice exhibit attenuated anticipatory activity during days 7–9 of restricted feeding, but show an equivalent rise of activity at the expected mealtime during the second day without food, indicating that the timing mechanism for food anticipation is present despite the absence of orexin signaling. *significant $p < 0.01$ between WT and KO. Adapted from Kaur et al. (2008).

more efficient in these mice. The same study reported no deficit in the expression of the food anticipatory rise of body temperature (**Figures 4A–C**), and a second study, using a video-based activity recording system detected no reduction in food anticipatory activity in orexin ligand KO mice (Gunapala et al., 2011). These results indicate that the effects of orexin deficiency on food anticipatory activity depend on the variable measured. Orexin neurons appear to play an important role regulating behavioral state stability and promoting wake consolidation during caloric restriction, but orexin and co-localized neuromodulators do not function as FEOs critical for timing food anticipatory activity or body temperature. Orexin neurons do interact with neural substrates mediating reward (Narita et al., 2006; Choi et al., 2010) and are activated in anticipation of reward (Harris et al., 2005), but a role in anticipation of palatable foods or other rewards provided on a circadian schedule has not yet been evaluated.

A portion of the orexinergic neuron population overlaps with the dorsomedial hypothalamus, which contains Mc3 receptors and neurons involved in regulating autonomic and neuroendocrine functions. Some neurons in this area exhibit daily rhythms of clock gene and immediate-early expression in rats and mice anticipating a daily meal (Gooley et al., 2006; Mieda et al., 2006; Verwey and Amir, 2009). Cell body specific lesions of this area may attenuate food anticipatory activity (Gooley et al., 2006; Acosta-Galvan et al., 2011; Landry et al., 2011), but complete removal of the area by radiofrequency ablation does not (Landry et al., 2006, 2007; Moriya et al., 2009). Also, clock gene oscillations in the dorsomedial hypothalamus are not induced in rats anticipating a daily palatable meal without caloric restriction, but are induced in rats maintained on a restricted feeding schedule with a random daily mealtime, which does not entrain anticipatory rhythms (Angeles-Castellanos et al., 2008; Verwey et al., 2009). These results indicate that circadian oscillators in the dorsomedial hypothalamus are neither necessary nor sufficient for the induction of food anticipatory activity rhythms. Dorsomedial hypothalamic neurons are responsive to feeding related signals, and contain neurons projecting to the light-entrainable circadian clock in the SCN that may inhibit its output during the daily rest phase, thereby promoting the expression of food anticipatory activity (Acosta-Galvan et al., 2011; Landry et al., 2011).

SUMMARY AND CONCLUSIONS

Nocturnal animals normally work for food and eat at night. Scheduled feeding can markedly alter the timing of behavioral arousal and locomotor activity, resulting in concentrated periods of waking and food seeking behavior that anticipate expected meals, even if these occur during the usual rest phase of the circadian cycle. Similar changes have been reported in response to time-limited opportunities to obtain other rewards, including water, sex, and addictive drugs (Webb et al., 2009b). These behavioral changes are associated with shifting of circadian oscillators in the brain and in the periphery. However, the endocrine and neural substrates of entrainment of activity rhythms by food (and other rewards) remain to be clarified. Metabolic hormones and the hypothalamic circuits upon which they converge appear to

play modulatory roles, increasing or decreasing the amplitude or duration of food anticipatory rhythms without being required for induction or persistence of these rhythms. These results, in combination with other lesion studies (Mistlberger, 1994; Davidson, 2009), suggest that FEOs critical for behavioral rhythms are either located outside of the hypothalamus, or are widely distributed and substantially redundant within the hypothalamus. Circadian oscillators reset by daily feeding schedules are also found in other brain regions, including mesocorticolimbic and dorsal striatal circuits that participate in reward processing and motivation for natural and drug reward (Verwey and Amir, 2009; Webb et al., 2009b). There is no direct evidence available yet that reward circuits are necessary or sufficient for entraining or driving food anticipatory circadian rhythms, but this remains a testable hypothesis.

Circadian clock adjustments induced by food intake are presumed to be adaptive, by coordinating rest-activity cycles with food availability, and metabolic functions with food ingestion. However, the sensitivity of circadian oscillators to food or other rewards could be maladaptive in at least two ways. First, entrainment to powerful rewards (e.g., palatable food, addictive drugs) could induce daily temporal windows of vulnerability to excessive reward seeking behavior. Second, feeding stimulated at irregular mealtimes could result in disrupted timing of metabolic processes, with negative consequences for metabolic functioning and health (Arble et al., 2010; Golombek et al., 2013). To the extent that stress alters food intake, food sensitive circadian clocks may therefor represent a pathway from stress to disease. This suggests a research agenda exploring effects of stress on temporal parameters of food intake, and associated changes in phase relationships among circadian oscillators that control behavior and physiology. This idea remains to be evaluated experimentally, but would benefit from progress in specifying the neurobiological mechanisms that mediate the effects of food intake on circadian rhythms.

REFERENCES

Abe, H., Kida, M., Tsuji, K., and Mano, T. (1989). Feeding cycles entrain circadian rhythms of locomotor activity in CS mice but not in C57BL/6J mice. *Physiol. Behav.* 45, 397–401. doi: 10.1016/0031-9384(89)90146-7

Abizaid, A., Liu, Z., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., et al. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J. Clin. Invest.* 116, 3229–3239. doi: 10.1172/JCI29867

Acosta-Galvan, G., Yi, C. X., van der Vliet, J., Jhamandas, J. H., Panula, P., Angeles-Castellanos, M., et al. (2011). Interaction between hypothalamic dorsomedial nucleus and the suprachiasmatic nucleus determines intensity of food anticipatory behavior. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5813–5818. doi: 10.1073/pnas.1015551108

Ahima, R. S., Prabakaran, D., and Flier, J. S. (1998). Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. *J. Clin. Invest.* 101, 1020–1027. doi: 10.1172/JCI1176

Ahren, B. (2000). Diurnal variation in circulating leptin is dependent on gender, food intake and circulating insulin in mice. *Acta Physiol. Scand.* 169, 325–331. doi: 10.1046/j.1365-201x.2000.00746.x

Akabashi, A., Levin, N., Paez, X., Alexander, J. T., and Leibowitz, S. F. (1994). Hypothalamic neuropeptide Y and its gene expression: relation to light/dark cycle and circulating corticosterone. *Mol. Cell. Neurosci.* 5, 210–218. doi: 10.1006/mcne.1994.1025

Akiyama, M., Yuasa, T., Hayasaka, N., Horikawa, K., Sakurai, T., and Shibata, S. (2004). Reduced food anticipatory activity in genetically orexin (hypocretin) neuron ablated mice. *Eur. J. Neurosci.* 20, 3054–3062. doi: 10.1111/j.1460-9568.2004.03749.x

Angeles-Castellanos, M., Salgado-Delgado, R., Rodríguez, K., Buijs, R. M., and Escobar, C. (2008). Expectancy for food or expectancy for chocolate reveals timing systems for metabolism and reward. *Neuroscience* 155, 297–307. doi: 10.1016/j.neuroscience.2008.06.001

Aponte, Y., Atasoy, D., and Sternson, S. M. (2011). AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. *Nat. Neurosci.* 14, 351–355. doi: 10.1038/nn.2739

Arble, D. M., Ramsey, K. M., Bass, J., and Turek, F. W. (2010). Circadian disruption and metabolic disease: findings from animal models. *Best Pract. Res. Clin. Endocrinol. Metab.* 24, 785–800. doi: 10.1016/j.beem.2010.08.003

Balsalobre, A., Brown, S. A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H. M., et al. (2000). Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science* 289, 2344–2347. doi: 10.1126/science.289.5488.2344

Baltazar, R. M., Coolen, L. M., and Webb, I. C. (2013). Diurnal rhythms in neural activation in the mesolimbic reward system: critical role of the medial prefrontal cortex. *Eur. J. Neurosci.* 38, 2319–2327. doi: 10.1111/ejn.12224

Barsh, G. S., and Schwartz, M. W. (2002). Genetic approaches to studying energy balance: perception and integration. *Nat. Rev. Gene.* 3, 589–600.

Blum, I. D., Patterson, Z., Khazall, R., Lamont, E. W., Sleeman, M. W., Horvath, T. L., et al. (2009). Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. *Neuroscience* 164, 351–359. doi: 10.1016/j.neuroscience.2009.08.009

Bodosi, B., Gardi, J., Hajdu, I., Szentirmai, E., Obal, F., and Krueger, J. M. (2004). Rhythms of ghrelin, lepton, and sleep in rats: effects of the normal diurnal cycle, restricted feeding, and sleep deprivation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287, R1071–R1079. doi: 10.1152/ajpregu.00294.2004

Boulos, Z., Rosenwasser, A. M., and Terman, M. (1980). Feeding schedule and the circadian organization of behavior in the rat. *Behav. Brain Res.* 1, 39–65. doi: 10.1016/0166-4328(80)90045-5

Boulos, Z., and Terman, M. (1980). Food availability and daily biological rhythms. *Neurosci. Biobehav. Rev.* 4, 119–131. doi: 10.1016/0149-7634(80)90010-X

Butler, A. A., Kesterson, R. A., Khong, K., Cullen, M. J., Pelleymounter, M. A., Dekoning, J., et al. (2000). A unique metabolic syndrome causes obesity in the melanocortin 3 receptor deficient mouse. *Endocrinology* 141, 3518–3521. doi: 10.1210/en.141.9.3518

Cai, X. J., Widdowson, P. S., Harrold, J., Wilson, S., Buckingham, R. E., Arch, J. R., et al. (1999). Hypothalamic orexin expression: modulation by blood glucose and feeding. *Diabetes* 48, 2132–2137. doi: 10.2337/diabetes.48.11.2132

Challet, E. (2010). Interactions between light, mealtime and calorie restriction to control daily timing in mammals. *J. Comp. Physiol. B* 180, 631–644. doi: 10.1007/s00360-010-0451-4

Chen, X., Ge, Y. L., Jiang, Z. Y., Liu, C. Q., Depoortere, I., and Peeters, T. L. (2005). Effects of ghrelin on hypothalamic glucose responding neurons in rats. *Brain Res.* 1055, 131–136. doi: 10.1016/j.brainres.2005.06.080

Choi, D. L., Davis, J. F., Fitgerald, M. E., and Benoit, S. C. (2010). The role of orexin-A in food motivation, reward-based feeding behavioral and food-induced neuronal activation in rats. *Neuroscience* 167, 11–20. doi: 10.1016/j.neuroscience.2010.02.002

Cone, R. D. (2005). Anatomy and regulation of the central melanocortin system. *Nat. Neurosci.* 8, 571–578. doi: 10.1038/nn1455

Cowley, M. A., Smith, R. G., Diano, S., Tschop, M., Pronchuk, N., Grove, K. L., et al. (2003). The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37, 649–661.

Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., and Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50, 1714–1719. doi: 10.2337/diabetes.50.8.1714

Dailey, M. J., Stingl, K. C., and Morgan, T. H. (2012). Dissociation between preprandial gut peptide release

and food anticipatory activity. *Endocrinology* 153, 132–142. doi: 10.1210/en.2011-1464

Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., and Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev*. 14, 2950–2961. doi: 10.1101/gad.183500

Date, Y., Ueta, Y., Yamashita, H., Yamaguchi, H., Maksukura, S., Kangawa, et al. (1999). Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc. Natl. Acad. Sci. U.S.A.* 96, 748–753. doi: 10.1073/pnas.96.2.748

Davidson, A. J., Poole, A. S., Yamazaki, S., and Menaker, M. (2003). Is the food-entrainable circadian oscillator in the digestive system. *Genes Brain Behav*. 2, 32–39. doi: 10.1034/j.1601-183X.2003.00005.x

Davidson, A. J., and Stephan, F. K. (1999). Plasma glucagon, glucose, insulin, and motilin in rats anticipating daily meals. *Physiol. Behav*. 66, 309–315. doi: 10.1016/S0031-9384(98)00308-4

Davidson, A. J., Stokkan, K. A., Yamazaki, S., and Menaker, M. (2002). Food-anticipatory activity and liver per1-luc activity in diabetic transgenic rats. *Physiol. Behav*. 76, 21–26. doi: 10.1016/S0031-9384(02)00680-7

Davidson, A. J. (2009). Lesion studies targeting food-anticipatory activity. *Eur. J. Neurosci*. 30, 1658–1664. doi: 10.1111/j.1460-9568.2009.06961.x

Davis, J. F., Choi, D. L., Clegg, D. J., and Benoit, S. C. (2011). Signaling through the ghrelin receptor modulates hippocampal function and meal anticipation in mice. *Physiol. Behav.* 103, 39–43. doi: 10.1016/j.physbeh.2010.10.017

Diaz-Munoz, M., Vazquez-Martinez, O., Aguilar-Roblero, R., and Escobar, C. (2000). Anticipatory changes in liver metabolism and entrainment of insulin, glucagon, and corticosterone in food-restricted rats. *Am. J. Physiol. Regul. Intergr. Comp. Physiol.* 279, R2048–R2056.

Dibner, C., Schibler, U., and Albrecht, U. (2010). The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annu. Rev. Physiol.* 72, 517–549. doi: 10.1146/annurev-physiol-021909-135821

Drazen, D. L., Vahl, T. P., D'Alessio, D. A., Seeley, R. J., and Woods, S. C. (2006). Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status. *Endocrinology* 147, 23–30. doi: 10.1210/en.2005-0973

Elimam, A., and Marcus, C. (2002). Meal timing, fasting and glucocorticoids interplay in serum leptin concentrations and diurnal profile. *Eur. J. Endocrinol.* 147, 181–188. doi: 10.1530/eje.0.1470181

Fan, W., Boston, B. A., Kesterson, R. A., Hruby, V. J., and Cone, R. D. (1997). Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 385, 165–168. doi: 10.1038/385165a0

Feillet, C. A., Albrecht, U., and Challet, E. (2006). "Feeding time" for the brain: a matter of clocks. *J. Physiol. Paris* 100, 252–260. doi: 10.1016/j.jphysparis.2007.05.002

Feillet, C. A., Mendoza, J., Albrecht, U., Pévet, P., and Challet, E. (2008). Forebrain oscillators ticking with different clock hands. *Mol. Cell. Neurosci*. 37, 209–221. doi: 10.1016/j.mcn.2007.09.010

Frecka, J. M., and Mattes, R. D. (2008). Possible entrainment of ghrelin to habitual meal patterns in humans. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294, G699–G707. doi: 10.1152/ajpgi.00448.2007

Golombek, D. A., Casiraghi, L., Agostino, P. V., Paladino, N., Duhart, J., Plano, S. A., et al. (2013). The times are changing: effects of circadian desynchronization on physiology and disease. *J. Physiol. Paris* 107, 310–322. doi: 10.1016/j.jphysparis.2013.03.007

Gooley, J. J., Schomer, A., and Saper, C. B. (2006). The dorsomedial hypothalamic nucleus is critical for the expression of food-entrainable circadian rhythms. *Proc. Natl. Acad. Sci. U.S.A.* 143, 1413–1422. doi: 10.1038/nn1651

Gropp, E., Shanabrough, M., Borok, E., Xu, A. W., Janoschek, R., Buch, T., Plum, L., et al. (2005). Agouti-related peptide-expressing neurons are mandatory for feeding. *Nat. Neurosci.* 8, 1289–1291. doi: 10.1038/nn1548

Guan, X., Yu, H., Palyha, O., McKee, K. K., Feighner, S. D., Sirinathsinghi, D. J. S., Smith, R. G., et al. (1997). Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Mol. Brain Res.* 48, 23–29. doi: 10.1016/S0169-328X(97)00071-5

Guilding, C., and Piggins, H. D. (2007). Challenging the omnipotence of the suprachiasmatic timekeeper: are circadian oscillators present throughout the mammalian brain. *Eur. J. Neurosci.* 25, 3915–3216. doi: 10.1111/j.1460-9568.2007.05581.x

Gunapala, K. M., Gallardo, C. M., Hsu, C. T., and Steele, A. D. (2011). Single gene deletions of orexin, leptin, neuropeptide y, and ghrelin do not appreciably alter food anticipatory activity in mice. *PLoS ONE* 6:e18377. doi: 10.1371/journal.pone.0018377

Hagan, J. J., Leslie, R. A., Patel, S., Evans, M. L., Wattam, T. A., Homles, S., et al. (1999). Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc. Natl. Acad. Sci. U.S.A.* 96, 10911–10916. doi: 10.1073/pnas.96.19.10911

Hara, R., Wan, K., Wakamatsu, H., Aida, R., Moriya, T., Akiyama, M., et al. (2001a). Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes Cells* 6, 269–278. doi: 10.1046/j.1365-2443.2001.00419.x

Hara, J., Beuckmann, C. T., Nambu, T., Willie, J. T., Chemelli, R. M., Sinton, C. M., et al. (2001b). Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron* 30, 345–354. doi: 10.1016/S0896-6273(01)00293-8

Harris, G. C., Wimmer, M., and Aston-Jones, G. (2005). A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* 437, 556–559. doi: 10.1038/nature04071

Honma, K., Honma, S., and Hiroshige, T. (1984a). Selective depression of prefeeding corticosterone peak in the rat by sodium pentobarbital injection. *Neuroendocrinology* 38, 232–236. doi: 10.1159/000123896

Honma, K., Honma, S., and Hiroshige, T. (1984b). Feeding-associated corticosterone peak in rats under various feeding cycles. *Am. J. Physiol.* 246, R721–R726.

Hsu, C. T., Patton, D. F., Mistlberger, R. E., and Steele, A. D. (2010). Palatable meal anticipation in mice. *PLoS ONE* 5:e11802. doi: 10.1371/journal.pone.0011802

Kageyama, H., Takenoya, F., Hirako, S., Wada, N., Kintaka, Y., Inoue, S., et al. (2012). Neuronal circuits involving neuropeptide Y in hypothalamic arcuate nucleus-mediated feeding regulation. *Neuropeptides* 46, 285–289. doi: 10.1016/j.npep.2012.09.007

Kaneko, M., Kaneko, K., Shinsako, J., and Dallman, M. (1981). Adrenal sensitivity to adrenocorticotropin varies diurnally. *Endocrinology* 109, 70–75. doi: 10.1210/endo-109-1-70

Kaur, S., Thankachan, S., Begum, S., Blanco-Centurion, C., Sakurai, T., Yanagisawa, M., et al. (2008). Entrainment of temperature and activity rhythms to restricted feeding in orexin knock out mice. *Brain Res*. 1205, 47–54. doi: 10.1016/j.brainres.2008.02.026

Kirchner, H., Gutierrez, J. A., Solenberg, P. J., Pfluger, P. T., Czyzyk, T. A., Willency, J. A., et al. (2009). GOAT links dietary lipids with the endocrine control of energy balance. *Nat. Med.* 15, 741–745. doi: 10.1038/nm.1997

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402, 656–660. doi: 10.1038/45230

Krashes, M. J., Koda, S., Ye, C., Rogan, S. C., Adams, A. C., Cusher, D. S., et al. (2011). Rapid, reversible activation of AgRP neruons drives feeding behavior in mice. *J. Clin. Invest*. 121, 1424–1428. doi: 10.1172/JCI46229

Krieger, D. T. (1974). Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. *Endocrinology* 95, 1195–1201. doi: 10.1210/endo-95-5-1195

Kurose, T., Ueta, Y., Yamamoto, Y., Serino, R., Ozaki, Y., Saito, J., et al. (2002). Effects of restricted feeding on the activity of hypothalamic orexin (OX)-A containing neurons and OX2 receptor mRNA level in the paraventricular nucleus of rats. *Regul. Pept.* 104, 145–151. doi: 10.1016/S0167-0115(01)00340-8

Lamont, E. W., Patterson, Z., Rodrigues, T., Vallejos, O., Blum, I. D., and Abizaid, A. (2012). Ghrelin-deficient mice have fewer orexin cells and reduced cFOS expression in the mesolimbic dopamine pathway under a restricted feeding paradigm. *Neuroscience* 218, 12–19. doi: 10.1016/j.neuroscience.2012.05.046

Landry, G. J., Kent, B. A., Patton, D. F., Jaholkowski, M., Marchant, E. G., and Mistlberger, R. E. (2011). Evidence for time-of-day dependent effect of neurotoxic dorsomedial hypothalamic lesions on food anticipatory circadian rhythms in rats. *PLoS ONE* 6:e24187. doi: 10.1371/journal.pone.0024187

Landry, G. J., Simon, M., Webb, I. C., and Mistlberger, R. E. (2006). Persistence of a behavioral food anticipatory circadian rhythm following dorsomedial hypothalamic ablation in rats. *Am. J.*

Physiol. 290, R1527–R1534. doi: 10.1152/ajpregu.00874.2005

Landry, G. J., Yamakawa, G. R., Webb, I. C., Mear, R. J., and Mistlberger, R. E. (2007). The dorsomedial hypothalamic nucleus is not necessary for the expression of circadian food-anticipatory activity in rats. *J. Biol. Rhythms* 22, 467–478. doi: 10.1177/0748730407307804

Larsen, P. J., Tang-Christensen, M., Hoist, J. J., and Orskov, C. (1997). Distribuition of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience* 77, 257–270. doi: 10.1016/S0306-4522(96)00434-4

LeMinh, N., Damiola, F., Tronche, F., Schutz, G., and Schibler, U. (2001). Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. *EMBO J.* 20, 7128–7136. doi: 10.1093/emboj/20.24.7128

LeSauter, J., Hoque, N., Weintraub, M., Pfaff, D., and Silver, R. (2009). Stomach ghrelin-secreting cells as food entrainable circadian clocks. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13582–13587. doi: 10.1073/pnas.0906426106

Liu, J., Prudom, C. E., Nass, R., Pezzoli, S. S., Oliveri, M. C., Johnson, M. L., et al. (2008). Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men. *J. Clin. Endocrinol. Metab.* 93, 1980–1987. doi: 10.1210/jc.2007-2235

Lubkin, M., and Stricker-Krongrad, A. (1998). Independent feeding and metabolic actions of orexins in mice. *Biochem. Biophys. Res. Commun.* 253, 241–245. doi: 10.1006/bbrc.1998.9750

Luguet, S., Perez, F. A., Hnasko, T. S., and Palmiter, R. D. (2005). NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* 310, 683–685. doi: 10.1126/science.1115524

Maniam, J., and Morris, M. J. (2012). The link between stress and feeding behaviour. *Neuropharmacology* 63, 97–110. doi: 10.1016/j.neuropharm.2012.04.017

Marcheva, B., Ramsey, K. M., Buhr, E. D., Kobayashi, Y., Su, H., Ko, C. H., et al. (2010). Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature* 466, 627–631. doi: 10.1038/nature09253

Martinez-Merlos, M. T., Angeles-Castellanos, M., Diaz-Munoz, M., Aguilar-Roblero, R., Mendoza, J., and Escobar, C. (2004). Dissociation between adipose tissue signals, behavior and the food-entrained oscillator. *J. Endocrinol.* 181, 53–63. doi: 10.1677/joe.0.1810053

Mendoza, J., Angeles-Castellanos, M., and Escobar, C. (2005). Food entrainment by a palatable meal induces food-anticipatory activity and c-FOS expression in reward-related areas of the brain. *Neuroscience* 133, 293–303. doi: 10.1016/j.neuroscience.2005.01.064

Merkestein, M., Brans, M. A. D., Luijendijk, M. C., de Jong, J. W., Egecioglu, E., Dickson, S. L., et al. (2012). Ghrelin mediates anticipation to a palatable meal in rats. *Obesity* 20, 963–971. doi: 10.1038/oby.2011.389

Mieda, M., Williams, S. C., Richardson, J. A., Tanaka, K., and Yanagisawa, M. (2006). The dorsomedial hypothalamic nucleus as a putative food-entrainable circadian pacemaker. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12150–12155. doi: 10.1073/pnas.0604189103

Mieda, M., Williams, S. C., Sinton, C. M., Richardson, J. A., Sakurai, T., and Yanagisawa, N. (2004). Orexin neurons function in an efferent pathway of a food-entrainable circadian oscillator in eliciting food-anticipatory activity and wakefulness. *J. Neurosci.* 24, 10493–10501. doi: 10.1523/JNEUROSCI.3171-04.2004

Mistlberger, R. E. (1993). Effects of scheduled food and water access on circadian rhythms of hamsters in constant light, dark, and light:dark. *Physiol. Behav.* 53, 509–516. doi: 10.1016/0031-9384(93)90145-6

Mistlberger, R. E., Antle, M. C., Kilduff, T. S., and Jones, M. (2003). Food-and light-entrained circadian rhythms in rats with hypocretin-2-saporin ablations of the lateral hypothalamus. *Brain Res.* 980, 161–168. doi: 10.1016/S0006-8993(03)02755-0

Mistlberger, R. E., and Antle, M. C. (1999a). Neonatal monosodium glutamate alters circadian organization of feeding, food anticipatory activity and photic masking in the rat. *Brain Res.* 842, 73–83. doi: 10.1016/S0006-8993(99)01836-3

Mistlberger, R. E., and Marchant, E. G. (1999b). Enhanced food anticipatory rhythms in the genetically obese zucker rat. *Physiol. Behav.* 66, 329–335. doi: 10.1016/S0031-9384(98)00311-4

Mistlberger, R. E., Kent, B. A., Chan, S., Patton, D. F., Weinberg, A., and Parfyonov, M. (2012). Circadian clocks for all meal times: anticipation of 2 daily meals in rats. *PLoS ONE* 7:e31772. doi: 10.1371/journal.pone.0031772

Mistlberger, R. E., and Rusak, B. (1987). Palatable daily meals entrain anticipatory rhythms in free feeding rats: dependence on meal size and nutrient content. *Physiol. Behav.* 41, 219–226. doi: 10.1016/0031-9384(87)90356-8

Mistlberger, R. E., and Rusak, B. (1988). Food anticipatory circadian rhythms in paraventricular and lateral hypothalamic lesioned rats. *J. Biol. Rhythms* 3, 277–292. doi: 10.1177/074873048800300306

Mistlberger, R. E. (1994). Circadian food-anticipatory activity: formal models and physiological mechanisms. *Neurosci. Biobehav. Rev.* 18, 171–195. doi: 10.1016/0149-7634(94)90023-X

Mistlberger, R. E. (2011). Neurobiology of food anticipatory circadian rhythms. *Physiol. Behav.* 104, 535–545. doi: 10.1016/j.physbeh.2011.04.015

Mojsov, S., Kopczynski, M. G., and Habener, J. F. (1990). Both amidated and nonamidated forms of glucagon-like peptide I are synthesized in the rat intestine and pancreas. *J. Biol. Chem.* 265, 8001–8006.

Moorman, D. E., and Aston-Jones, G. (2010). Orexin/Hypocretin modulates response of ventral tegmental dopamine neurons to prefrontal activation: diurnal influences. *J. Neurosci.* 30, 15585–15599. doi: 10.1523/JNEUROSCI.2871-10.2010

Moriya, T., Aida, R., Kudo, T., Akiyama, M., Doi, M., Hayasaka, N., et al. (2009). The dorsomedial hypothalamic nucleus is not necessary for food-anticipatory circadian rhythms of behavior, temperature or clock gene expression in mice. *Eur. J. Neurosci.* 29, 1447–1460. doi: 10.1111/j.1460-9568.2009.06697.x

Mountjoy, K. G. (2010). Distribution and function of melanocortin receptors within the brain. *Adv. Exp. Med. Biol.* 681, 29–48. doi: 10.1007/978-1-4419-6354-3_3

Nakamura, T., Uramura, K., Nambu, T., Yada, T., Goto, K., Yanagisawa, M., et al. (2000). Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Res.* 873, 181–187. doi: 10.1016/S0006-8993(00)02555-5

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., et al. (2001). A role for ghrelin in the central regulation of feeding. *Nature* 409, 194–198. doi: 10.1038/35051587

Narita, S. G., Nagumo, Y., Hashimoto, S., Narita, M., Khotib, J., Miyatake, M., et al. (2006). Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors to morphine. *J. Neurosci.* 26, 398–405. doi: 10.1523/JNEUROSCI.2761-05.2006

Oishi, K., Kasamatsu, M., and Ishida, N. (2004). Gene- and tissue-specific alterations of circadian clock gene expression in streptozotocin-induced diabetic mice under restricted feeding. *Biochem. Biophys. Res. Commun.* 317, 330–334. doi: 10.1016/j.bbrc.2004.03.055

Oster, H., Damerow, S., Kiessling, S., Jakubcakova, V., Abraham, D., Tian, J., et al. (2006). The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab.* 4, 163–173. doi: 10.1016/j.cmet.2006.07.002

Otway, D. T., Frost, G., and Johnston, J. D. (2009). Circadian rhythmicity in murine pre-adipocyte and adipocyte cells. *Chronobiol. Int.* 26, 1340–1354. doi: 10.3109/07420520903412368

Patterson, Z. R., and Abizaid, A. (2013). Stress induced obesity: lessons from rodent models of stress. *Front. Neurosci.* 7:130. doi: 10.3389/fnins.2013.00130

Patton, D., Marchant, E., Parfyonov, M., Smit, A., Opiol, H., Patterson, Z., et al. (2012). Multiple daily meals: effects on behavior, peripheral oscillators and associated hormones. *Soc. Res. Biol. Rhythms Abstr.* 13, 130.

Peyron, C., Tighe, D. K., van den Pol, A. N., de Lecea, L., Heller, H. C., Sutcliffe, J. G., et al. (1998). Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.* 18, 9996–10015.

Pezuk, P., Mohawk, J. A., Wang, L. A., and Menaker, M. (2012). Glucocorticoids as entraining signals for peripheral circadian oscillators. *Endocrinology* 153, 4775–4783. doi: 10.1210/en.2012-1486

Reddy, A. B., Maywood, E. S., Karp, N. A., King, V. M., Inoue, Y., Gonzales, F. J., et al. (2007). Glucocorticoid signaling synchronizes the liver circadian transcriptome. *Hepatology* 45, 1478–1488. doi: 10.1002/hep.21571

Ribeiro, A. C., Ceccarini, G., Dupre, C., Friedman, J. M., Pfaff, D. W., and Mark, A. L. (2011). Contrasting effects of leptin on food anticipatory and total locomotor activity. *PLoS ONE* 6:e23364. doi: 10.1371/journal.pone.0023364

Roselli-Rehfuss, L., Mountjoy, K. G., Robbins, L. S., Mortrud, M. T., Low, M. J., Tatro, J. B., et al. (1993). Indentification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. *Proc. Natl. Acad. Sci. U.S.A.* 90, 8856–8860. doi: 10.1073/pnas.90.19.8856

Sadacca, L. A., Lamia, K. A., deLemos, A. S., Blum, B., and Weitz, C. J. (2011). An intrinsic circadian clock of the pancreas is required for normal insulin release and glucose homeostasis in mice. *Diabetologica* 54, 120–124. doi: 10.1007/s00125-010-1920-8

Sainsbury, A., Rohner-Jeanrenaud, F., Cusin, I., Zakrzewska, K. E., Halban, P. A., Gaillard, R. C., et al. (1997). Chronic central neuropeptide Y infusion in normal rats: status of the hypothalamo-pituitary-adrenal axis, and vagal mediation of hyperinsulinaemia. *Diabetologia* 40, 1269–1277. doi: 10.1007/s001250050820

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., et al. (1998). Orexins and orexin receptors: a family of hypothalamic neurpeptides and G-protein-coupled receptors that regulate feeding behavior. *Cell* 92, 573–585. doi: 10.1016/S0092-8674(00)80949-6

Segall, L. A., Verwy, M., and Amir, S. (2008). Timed restricted feeding restores the rhythms of expression of the clock protein, Period 2, in the oval nucleus of the bed nucleus of the stria terminalis and central nucleus of the amygdala in adrenalectomized rats. *Neuroscience* 157, 52–56. doi: 10.1016/j.neuroscience.2008.08.055

Shea, S. A., Hilton, M. F., Orlova, C., Ayers, R. T., and Mantzoros, C. S. (2005). Independent circadian and sleep/wake regulation of adipokines and glucose in humans. *J. Clin. Endocrinol. Metab.* 90, 2537–2544. doi: 10.1210/jc.2004-2232

Shughrue, P. J., Lane, M. V., and Merchenthaler, I. (1996). Glucagon-like peptide-1 receptor (GLP-1R) mRNA in the rat hypothalamus. *Endocrinology* 137. 5159–5162. doi: 10.1210/en.137.11.5159

Sinha, M. K., Ohannesian, J. P., Heiman, M. L., Kriauciunas, A., Stephans, T. W., Magosin, S., et al. (1996). Nocturnal rise in leptin in lean, obese, and non-indulin-dependent diabetes mellitus subjects. *J. Clin. Invest.* 97, 1344–1347. doi: 10.1172/JCI118551

Sinha, R., and Jastreboff, A. M. (2013). Stress as a common risk factor for obesity and addiction. *Biol. Psychiatry* 73, 827–835. doi: 10.1016/j.biopsych.2013.01.032

Small, C. J., Kim, M. S., Staley, S. A., Mitchell, J. R., Murphy, K., Morgan, D. G., et al. (2001). Effects of chronic central nervous system administration of agouti-related protein in pair fed animals. *Diabetes* 50, 248–254. doi: 10.2337/diabetes.50.2.248

Smart, D., Haynes, A. C., and Williams, G. (2002). Orexins and the treatment of obesity. *Eur. J. Pharmacol.* 440, 199–212. doi: 10.1016/S0014-2999(02)01429-2

Smialowska, M., Lewinska, G., and Tokarski, K. (1994). The role of α-1 adrenergic receptors in the stimulating effect of neuropeptide Y (NPY) on rat behavioural activity. *Neuropeptides* 26, 225–232. doi: 10.1016/0143-4179(94)90074-4

Stanley, B. G., and Leibowitz, S. F. (1984). Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life Sci.* 35, 2635–2642. doi: 10.1016/0024-3205(84)90032-8

Stephan, F. K., Schwann, J. M., and Sisk, C. L. (1979). Anticipation of 24 hr feeding schedules in rats with lesions of the suprachiasmatic nucleus. *Behav. Neural Biol.* 25, 346–363. doi: 10.1016/S0163-1047(79)90415-1

Stephan, F. K. (1989). Forced dissociation of activity entrained to T cycles of food access in rats with suprachiasmatic lesions. *J. Biol. Rhythms* 4, 467–479. doi: 10.1177/074873048900400406

Stephan, F. K. (2002). The "other" circadian system: food as a Zeitgeber. *J. Biol. Rhythms* 17, 284–292. doi: 10.1177/074873040201700402

Stokkan, K. A., Yamazaki, S., Tei, H., Sakaki, Y., and Menaker, M. (2001). Entrainment of the circadian clock in the liver by feeding. *Science* 291, 490–493. doi: 10.1126/science.291.5503.490

Sujino, M., Furukawa, J., Koinuma, S., Fujioka, A., Nagano, M., Iigo, M., et al. (2012). Differential entrainment of peripheral clocks in the rat by glucocorticoid and feeding. *Endocrinology* 153, 2277–2286. doi: 10.1210/en.2011-1794

Sutton, G. M., Perez-Tilve, D., Nogueiras, R., Fang, J., Kim, J. K., Cone, R. D., et al. (2008). The melanocortin-3 receptor is required for entrainment to meal intake. *J. Neurosci.* 28, 12946–12955. doi: 10.1523/JNEUROSCI.3615-08.2008

Sutton, G. M., Trevaskis, J. L., Hulver, M. W., McMillan, R. P., Markward, N. J., Babin, M. J., et al. (2006). Diet-genotype interactions in the development of the obese, insulin-resistant phenotype of c57BL/6J mice lacking melanocortin-3 or-4 receptors. *Endocrinology* 147, 2183–2196. doi: 10.1210/en.2005-1209

Szentirmai, É., Kapas, L., Sun, Y., Smith, R. G., and Krueger, J. M. (2010). Restricted feeding-induced sleep, activity, and body temperature changes in normal and preproghrelin-deficient mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 298, R467–R477. doi: 10.1152/ajpregu.00557.2009

Szentirmai, É. (2012). Central but not systemic administration of ghrelin induces wakefulness in mice. *PLoS ONE* 7:e41172. doi: 10.1371/journal.pone.0041172

Toshinai, K., Yamaguchi, H., Sun, Y., Smith, R. O., Yamanaka, A., Sakurai, T., et al. (2006). Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor. *Endocrinology* 147, 2306–2314. doi: 10.1210/en.2005-1357

Trapp, S., and Hisadome, K. (2011). Glugacon-like peptide 1 and the brain: central actions-central sources. *Auto. Neurosci.* 161, 14–19. doi: 10.1016/j.autneu.2010.09.008

Vahl, T. P., Drazen, D. L., Seeley, R. J., D'Alessio, D. A., and Woods, S. C. (2010). Meal-anticipatory glucagon-like peptide-1 secretion in rats. *Endocrinology* 151, 569–575. doi: 10.1210/en.2009-1002

Verhagen, L. A., Luijendijk, M. C., Adan, R. A. (2011a). Leptin reduces hyperactivity in an animal model for anorexia nervosa via the ventral tegmental area. *Eur. Neuropsychopharmacol.* 21, 274–281. doi: 10.1016/j.euroneuro.2010.11.006

Verhagen, L. A., Egecioglu, E., Luijendijk, M. C., Hillebrand, J. J., Adan, R. A., and Dickson, S. L. (2011b). Acute and chronic suppression of the central ghrelin signaling system reveals a role in food anticipatory activity. *Eur. Neuropsychopharmacol.* 21, 384–392. doi: 10.1016/j.euroneuro.2010.06.005

Verwey, M., and Amir, S. (2009). Food-entrainable circadian oscillators in the brain. *Eur. J. Neurosci.* 30, 1650–1657. doi: 10.1111/j.1460-9568.2009.06960.x

Verwey, M., Lam, G. Y., and Amir, S. (2009). Circadian rhythms of PERIOD1 expression in the dorsomedial hypothalamic nucleus in the absence of entrained food-anticipatory activity rhythms in rats. *Eur. J. Neurosci.* 29, 2217–2222. doi: 10.1111/j.1460-9568.2009.06766.x

Weaver, D. R. (1998). The suprachiasmatic nucleus: a 25-year retrospective. *J. Biol. Rhythms* 13, 100–112. doi: 10.1177/074873098128999952

Webb, I. C., Baltazar, R. M., Lehman, M. N., and Coolen, L. M. (2009a). Bidirectional interactions between the circadian and reward systems: is restricted food access a unique zeitgeber. *Eur. J. Neurosci.* 30, 1739–1748. doi: 10.1111/j.1460-9568.2009.06966.x

Webb, I. C., Baltazar, R. M., Wang, X., Pitchers, K. K., Coolen, L. C., and Lehman, M. N. (2009b). Diurnal variations in natural and drug reward, mesolimbic tyrosine hydroxylase, and clock gene expression in the male rat. *J. Biol. Rhythms* 24, 465–476. doi: 10.1177/0748730409346657

Webb, I. C., Patton, D. F., Hamson, D., and Mistlberger, R. E. (2008). Neural correlates of arousal-induced circadian clock resetting:Hypocretin/orexin and the intergeniculate leaflet. *Eur. J. Neurosci.* 27, 828–835. doi: 10.1111/j.1460-9568.2008.06074.x

Welsh, D. K., Takahashi, J. S., and Kay, S. A. (2010). Suprachiasmatic nucleus: cell automony and network properties. *Ann. Rev. Physiol.* 72, 551–577. doi: 10.1146/annurev-physiol-021909-135919

Wiley, J. H., and Leveille, G. A. (1970). Significance of insulin in the metabolic adaptation of rats to a meal ingestion. *J. Nutr.* 100, 1073–1080.

Woods, S. C., Hutton, R. A., and Makous, W. (1970). Conditioned insulin secretion in the albino rat. *Proc. Soc. Exp. Biol. Med.* 133, 964–968. doi: 10.3181/00379727-133-34605

Woods, S. C., Vasselli, J. R., Kaestner, E., Szakmary, G. A., Milburn, P., and Vitiello, M. V. (1977). Conditioned insulin secretion and meal feeding in rats. *J. Comp. Physiol. Psychol.* 91, 128–133. doi: 10.1037/h0077307

Xu, B., Kalra, P. S., Farmerie, W. G., and Kalra, S. P. (1999). Daily changes in hypothalamic gene expression of neuropeptide, Y, galanin, proopiomelanocortin, and adipocyte leptin gene expression and secretion: effects of food restriction. *Endocrinology* 140, 2868–2875. doi: 10.1210/en.140.6.2868

Yamanaka, A., Beuckmann, C. T., Willie, J. T., Hara, J., Tsujino, N., Mieda, M., et al. (2003). Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 38, 701–713. doi: 10.1016/S0896-6273(03)00331-3

Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R., Ueda, M., et al. (2000). Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 288, 682–685. doi: 10.1126/science.288.5466.682

Yoshihara, T., Honma, S., and Honma, K. (1996). Effects of restricted daily feeding on neuropeptide Y release in the rat paraventricular nucleus. *AJP Endocrinol. Metab.* 270, E589–E595.

Zhang, Y., Proeenca, R., Maffe, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* 372 425–432. doi: 10.1038/372425a0

Zvonic, S., Ptitsyn, A. A., Conrad, S. A., Scott, L. K., Floyd, Z. E., Kilroy, G., et al. (2006). Characterization of peripheral circadian clocks in adipose tissues. *Diabetes* 55, 962–970. doi: 10.2337/diabetes.55.04.06.db05-0873

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Circannual changes in stress and feeding hormones and their effect on food-seeking behaviors

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Seasonal fluctuations in food availability show a tight association with seasonal variations in body weight and food intake. Seasonal variations in food intake, energy storage, and expenditure appear to be a widespread phenomenon suggesting they may have evolved in anticipation for changing environmental demands. These cycles appear to be driven by changes in external daylength acting on neuroendocrine pathways. A number of neuroendocrine pathways, two of which are the endocrine mechanisms underlying feeding and stress, appear to show seasonal changes in both their circulating levels and reactivity. As such, variation in the level or reactivity to these hormones may be crucial factors in the control of seasonal variations in food-seeking behaviors. The present review examines the relationship between feeding behavior and seasonal changes in circulating hormones. We hypothesize that seasonal changes in circulating levels of glucocorticoids and the feeding-related hormones ghrelin and leptin contribute to seasonal fluctuations in feeding-related behaviors. This review will focus on the seasonal circulating levels of these hormones as well as sensitivity to these hormones in the modulation of food-seeking behaviors.

Keywords: leptin, glucocorticoids, ghrelin, seasonal variation, stress, feeding

GENERAL OVERVIEW

The purpose of this review is to examine seasonal variation in responses to and circulating levels of glucocorticoids and ghrelin and leptin in relation to their facilitation or attenuation of feeding- and reward-related behaviors during the summer and winter seasons. We became interested in determining whether seasonal fluctuations in either stress or feeding hormones would interfere with these behaviors in the winter or facilitate food- and reward-related behavioral output in the summer. As such, literature will be reviewed that has investigated circannual changes in responses to and circulating levels of glucocorticoids and the feeding hormones, ghrelin and leptin. While others have provided comprehensive reviews on circadian fluctuations in these hormones (Kalra et al., 2003; Engeland and Arnhold, 2005; Lightman, 2008; Dietrich and Horvath, 2009; Papadimitriou and Priftis, 2009; Mistlberger, 2011), this paper reviews literature on the circannual fluctuations in these hormone levels and how they might influence feeding and feeding-related behaviors (such as bar pressing). As well, while there may be interesting relationships between circannual and circadian rhythms in the control of feeding [for a review on this topic in fish, see Volkoff et al. (2010)], a thorough discussion of this topic is beyond the scope of this review paper, though will be touched on briefly. Therefore, this review will examine how otherwise enhanced food-related behaviors might be attenuated by seasonal fluctuations in glucocorticoids and ghrelin and leptin signaling. This review may provide insight into inconsistent findings in experimental literature that might be due, in part, to seasonal variation in these hormones.

SEASONAL VARIATION IN FOOD INTAKE

In the wild, food availability can show considerable seasonal fluctuation resulting in associated variation in body weight and food intake for animals (Loudon, 1994). Seasonal rhythms in energy storage and expenditure appear to have evolved in a way for the animal to anticipate annual changes in the external environment thereby allowing an animal to adjust its physiology and behavior in preparation for the changing seasons (Ebling and Barrett, 2008). These adjustments are clearly important adaptations to environmental change and in the wild, appear to be driven by changes in external day length signals (Rousseau et al., 2003). However, these seasonal fluctuations in food intake and body weight are also observed in the presence of *ad-libitum* food and are exhibited under standard laboratory conditions (Mercer, 1998). As it is the case that when food is kept constant and feeding-related behaviors show changes that are linked with a seasonal pattern (Ferguson and Maier, 2013), it suggests that other, hard-wired physiological responses may mediate seasonal changes in behavior. We hypothesize that changes in behavior may be brought about by fluctuations in the level or neural responses to circulating hormones.

The definition of season for the purposes of this review paper will be based on photoperiod length. As such, short day (SD) will be synonymous with autumn and winter months when daylight is typically less than 12 h. Long day (LD) will be used interchangeably with the summer months when daylight is typically more than 12 h. The general guiding behavioral principle is that during LD photoperiods, food intake is increased to accumulate fat and during SD photoperiods, there is a decrease in food

intake and a utilization of the excess fat (Schuhler and Ebling, 2006). As an example, Otsuka and colleagues found that during SD conditions (winter), rats have a lower body weight and epididymis fat mass compared to rats exposed to LD (summer) conditions (Otsuka et al., 2012). We hypothesize that seasonal changes in the sensitivity to and circulating levels of glucocorticoids and the feeding-related hormones ghrelin and leptin may be contributing factors to these seasonal variations in food intake. Indeed, non-hibernating species [e.g., sand rats (*Psammomys obesus*), Fischer 344 rats, golden hamsters (*Mesocricetus auratus*)] have been found to have higher circulating blood levels of glucocorticoids in the winter months than in the summer (Gutzler et al., 2009). Evidence also suggests that the sensitivity to and secretion of ghrelin and leptin are seasonally dependent, with relationships that are subject to photoperiodic regulation. In this respect, ghrelin concentrations decrease in the winter (discussed further below) in association with reduction in food intake showing seasonal variation in the level of circulating ghrelin (Bradley et al., 2010). As well, central or peripheral ghrelin administration increases food intake 2-fold after administration during summer months; an effect that is absent in the winter months showing changes in ghrelin sensitivity (Bradley et al., 2010). With respect to leptin, increased food intake and weight gain in LD are associated with high circulating leptin and decreased intake and weight loss in SD are associated with low circulating levels of leptin, showing changes in circulating levels (Rousseau et al., 2003). Seasonal leptin resistance has also been found showing higher sensitivity to leptin in the winter, thereby decreasing food intake, and lower sensitivity in the summer (Rousseau et al., 2003). Therefore, this review will examine the seasonal regulation of both stress- and feeding-related hormones as contributing factors in seasonal variation in feeding-related behaviors.

GLUCOCORTICOIDS

A stressor can be defined as a situation or event that an organism recognizes as threatening or challenging. Stressors can be either acute or chronic. An acute stress can be defined as a single exposure to a threatening situation (e.g., inescapable tailshock; Frank et al., 2013). Forms of chronic stress occur when an organism is exposed to a number of unpredictable stressors (e.g., restraint, water deprivation, food deprivation, circadian disruption) over an extended period of time [e.g., 1–2 weeks or longer (Frank et al., 2013)]. Both acute and chronic stressors elicit a series of physiological and behavioral reactions that mobilize stored energy and facilitate an organism's ability to cope (Anisman and Merali, 1999). The two most commonly studied physiological systems that respond to stress are the sympathetic nervous system and the neuroendocrine system (McEwen and Sapolsky, 1995; McEwen, 2000). The neuroendocrine system response to stress begins with an activation of the hypothalamic-pituitary-adrenal (HPA) axis. The hypothalamus secretes corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) to affect the anterior lobe of the pituitary gland. When CRH and AVP bind to the anterior pituitary, the secretion of adrenocorticotropic hormone (ACTH) ensues. This hormone travels through the bloodstream to the adrenal glands, which respond by releasing glucocorticoid hormones into general circulation. Glucocorticoids bind to receptors on cells throughout the body, triggering a number of metabolic reactions that help direct oxygen and nutrients to the stressed body site (Harbuz and Lightman, 1992; Anisman and Merali, 1999). Glucocorticoid binding in the brain modulates a variety of processes, including attention, feeding, mood, and memory (Fietta and Delsante, 2009). Because a stressful event usually elicits glucocorticoid secretion, glucocorticoid levels are often used as a relative index of HPA axis activity (Johnstone et al., 2012) and hence, an approximation of the stress response.

Stress is known to alter feeding-related behaviors with both increases and decreases being observed. Stress-induced changes in feeding responses are due to a number of contributing factors, including the level of circulating glucocorticoids (for review see Maniam and Morris, 2012). With this in mind, one possible reason for changes in food seeking behaviors in the winter may be due to either basal or stress-induced changes in circulating glucocorticoids.

SEASONAL VARIATION IN GLUCOCORTICOIDS

Many organisms have internal mechanisms that allow them to anticipate changing seasons (circannual rhythms) in order to prepare for migration, changes in food availability or periods of reproduction (Gwinner, 2003; Paul et al., 2008). Many species also show circadian rhythms that are typically synchronized to the environment by regular cues such as light and temperature (Dickmeis et al., 2013). The interaction between these two types of rhythms is not clear but it is interesting to speculate that circadian fluctuations in circulating levels of glucocorticoids may be an internal mechanism used to anticipate the changing of the seasons based on photoperiod. In addition to its stress reactivity, circulating levels of glucocorticoids show daily variation whereby levels peak prior to the active period (day for diurnal species and night for nocturnal species; Chung et al., 2011; Ota et al., 2012). As the photoperiod changes from LD to SD conditions, it is possible that the circadian fluctuation of circulating glucocorticoid levels will adjust in terms of overall plasma concentration and possibly, time of peak secretion (Matchock et al., 2007). A study by Vondrasova et al. (1997) looked at the LD effects of summer on human glucocorticoid rhythms and the diurnal fluctuations. They found that glucocorticoids rose earlier in the day during the summer than the winter months (Vondrasova et al., 1997). This may be a hard-wired, internal signaling mechanism for the organism to anticipate changes in food availability and start changing its feeding patterns. Once the SD period sets in, the question remains as to whether there are elevations in basal levels of glucocorticoids and whether these elevations in glucocorticoids (either basal or those brought on by a stressor) would serve to alter SD feeding patterns.

Animal studies

Changes in the levels of circulating glucocorticoids are often used to assess HPA activation in response to stressful stimuli. There is evidence indicating that mammals, reptiles, amphibians, and birds display seasonal changes in both basal and

stress-induced plasma glucocorticoid concentrations that may alter behavioral output (Romero, 2002; Romero et al., 2008). Studies on non-hibernating mammals [e.g., sand rat, adult male white-footed mouse (*Peromyscus leucopus*), subterranean rodent (*Ctenomys talarum*), bison bull (*Bison bison*), Belding's ground squirrel (*Spermophilus beldingi*)] in both the wild and in the laboratory have shown marked seasonal changes in both circulating basal levels of glucocorticoids and stress-induced changes in circulating levels of glucocorticoids (Amirat et al., 1980; Amirat and Brudieux, 1984, 1993; Mooring et al., 2006; Nunes et al., 2006; Pyter et al., 2007; Vera et al., 2011). Overall, these studies indicate that non-hibernating species have increased basal and stress-induced glucocorticoid levels in the fall and winter months compared to the spring and summer months (Amirat et al., 1980; Amirat and Brudieux, 1984, 1993).

Specifically, when blood samples were taken from the desert sand rat, basal levels of glucocorticoids were low during early June, increased during autumn and peaked to their highest levels during the winter months (Amirat et al., 1980; Amirat and Brudieux, 1993). In studies using rats and mice where light and dark cycles were manipulated to mimic winter (SD) and summer (LD) months, basal glucocorticoid levels tended to be higher during the SD periods (winter) than LD periods (summer) (Ottenweller et al., 1987; Pyter et al., 2007; Otsuka et al., 2012). When the photoperiod was manipulated in Syrian hamsters, they showed modification of plasma concentrations of glucocorticoids such that the peak level of circulating glucocorticoids during SD was lower compared to LD (Ottenweller et al., 1987).

In mice, SD periods are associated with an increased HPA-axis responsiveness to stressful stimuli (Pyter et al., 2007; Otsuka et al., 2012). SD photoperiods have also been shown to be associated with an increase in stress-induced circulating glucocorticoid levels in response to restraint stress (Pyter et al., 2007). Changes in glucocorticoid levels in response to exogenous administration of dexamethasone have also shown seasonal variations. After dexamethasone administration, plasma glucocorticoid levels showed a large, rapid increase that persisted for a longer timeframe during the winter (February) than the summer (June) (Amirat et al., 1980; Amirat and Brudieux, 1984, 1993). The work with animals all points to the tentative conclusion that circulating glucocorticoid levels—both basal and stress-induced—are higher during the winter months than in the summer months.

Human studies

Seasonal variation in basal glucocorticoid levels has also been noted in humans and has been examined in healthy participants using salivary swabs, urine collection, or blood plasma levels (Walker et al., 1997; King et al., 2000; Thorn et al., 2011). Overall, there is some consensus that basal levels of circulating glucocorticoids are lower during the spring and summer and peak during the fall and winter (Walker et al., 1997; King et al., 2000). Concentrations of urinary glucocorticoid levels were reported to be higher during December and January compared to the rest of the year (Hansen et al., 2001). A study by Vongrasova and colleagues manipulated the photoperiod in the summer (Vondrasova-Jelinkova et al., 1999). They found that by shortening the photoperiod in the summer to mimic day length as would occur in winter, they could match the elevated glucocorticoid levels as those observed during the winter. Due to the circadian nature of fluctuating glucocorticoid levels throughout the day, consistency in sample collection is an important factor in human studies. For example, the study by King and colleagues showed a significant effect of seasonality when they gathered saliva samples during both the morning and evening finding that basal levels of glucocorticoids were lowest in the spring and highest in the winter and fall (King et al., 2000). Therefore, there appear to be consistent findings of seasonal variation in human basal glucocorticoid levels with higher levels in SD conditions.

While there is a dearth of studies examining seasonal variation in stress-induced changes in glucocorticoid levels in humans, the available literature suggests more pronounced stress-reactivity in the winter than in the summer. In an examination of seasonal variation in stress-induced changes in circulating levels of glucocorticoids, hourly blood samples were obtained from medical students under the influence of a stressful event (an exam; Malarkey et al., 1995). These students reported that the examinations did result in a significant elevation in psychological stress. In addition, there was a significant effect of examination stress on the increase in mean daytime ACTH levels during autumn, but not during the spring. In a similar study, an academic stressor was found to increase circulating levels of glucocorticoids more so in the fall-winter months than in the summer months (Matalka and Sidki, 1998).

GLUCOCORTICOIDS AND FEEDING-RELATED BEHAVIOR

Circulating levels of glucocorticoids, either basal or stress-induced changes, have been shown to influence food choice, body weight and appetite in both humans and animals (Oliver and Wardle, 1999; Baran et al., 2009; Liu et al., 2011; Schwabe and Wolf, 2011) and this may be an important contributing factor to seasonal variation in food intake.

Animal studies

Animal studies in the laboratory have revealed that, overall, stress-induced changes in circulating glucocorticoids or exogenous administration of glucocorticoids (thereby elevating basal levels) leads to a decrease in food intake (Levine and Morley, 1981; Morley et al., 1983; Liu et al., 2011). In laboratory studies under free-feeding conditions, when rats were administered exogenous glucocorticoids over a period of days, mimicking a chronic elevation in basal levels of glucocorticoids as would be observed in SD, winter months, a decrease in food intake leading to a decrease in body weight was reported (De Vos et al., 1995; Liu et al., 2011). A study by Liu et al. (2011) administered a high dose (15 mg/kg/day) and a low dose (5 mg/kg/day) of hydrocortisone to rats to study food intake and body weight change (Liu et al., 2011). They found that after chronic administration of the high dose of hydrocortisone (similar to elevated basal levels of circulating glucocorticoids as observed in SD, winter, conditions), food intake was reduced [see also De Vos et al. (1995), Konno et al. (2008) for similar results of decreased food consumption

after dexamethasone treatment]. Because chronic hydrocortisone treatment mimics high levels of basal circulating glucocorticoids, as would be observed in SD conditions, results indicate that elevated circulating levels of basal glucocorticoids, rather than food scarcity (as occurs in SD conditions) may be a causal, contributing factor to the decreased food intake in laboratory rats.

Chronic and acute stressors appear to affect food intake differently. Chronic stressors tend to decrease food intake (Levine and Morley, 1981; Liu et al., 2011; Nyuyki et al., 2012), whereas acute stressors often lead to an increase in food intake (Levine and Morley, 1981; Morley et al., 1983). However, the severity of the stressor—either chronic or acute—may also be a critical factor in the modulation of food intake. For example, a sudden drop in food intake is likely the result of a severe, acute stressor in rats (Armario, 2006). A study by Levine and Morley (1981) used tail pinch as a mild, acute stressor to investigate food intake in rats in the laboratory. During the acute tail pinch procedure, rats ate steadily over a 5-minute period with increased eating being observed during a second trial, 15 min after the first. The chronic tail pinch group consumed less food than the acute group and exhibited leading to a decrease in body weight compared to controls (Levine and Morley, 1981). Another type of chronic stressor is chronic subordinate colony housing where four male rats are housed together with a larger male rat. This chronic stressor has been shown to decrease body weight gain and increase plasma glucocorticoids when compared to controls (Nyuyki et al., 2012). Although chronic stress appears to decrease food intake when only normal rat chow is present, palatable food (e.g., high fat, sucrose) consumption may increase in response to a chronic stressor (Dallman et al., 2003). Under chronic restraint stress conditions, when rats were given a choice between chow or dense lard and sucrose food (a palatable food), the rats undergoing chronic stress were more likely to choose and eat the palatable food but decrease intake of the regular chow (Pecoraro et al., 2004).

Human studies

In humans, stress is associated with both increases and decreases in food intake (Oliver and Wardle, 1999; Epel et al., 2001). The feeding response to stress can vary depending on individual differences, type of food available, and the type of stressor (chronic, acute, severe, moderate; Maniam and Morris, 2012). For example, acute stress affects food intake in humans subjects differently depending on the general state of chronic stress in their lives (Rutters et al., 2009; Tryon et al., 2013). Stress-induced changes in glucocorticoid levels are individualized as some individuals show a larger glucocorticoid response following a stressor than others (Epel et al., 2001; Rutters et al., 2009) and this in turn can affect feeding behavior. In humans, food intake is more likely to increase during a stressful event if a palatable food is readily available (Oliver and Wardle, 1999; Dallman et al., 2003, 2005; Zellner et al., 2007; Bennett et al., 2013). In a study by Oliver and Wardle (1999), an approximately equal number of participants reported eating more as eating less when stressed; however, the majority of participants reported an increase in snacking under stressful situations (Oliver and Wardle, 1999). It may be the case that stress alters food choice, with participants choosing more sweet snacks such as chocolate and cake rather than savory, meal-type foods (Oliver and Wardle, 1999; Dallman et al., 2003; Bennett et al., 2013). Gender may also play a role in food choice (Zellner et al., 2006, 2007). A study by Zellner et al. (2007) used unsolvable anagrams to stress participants. They placed four bowls of snack foods in the room; two healthy, two unhealthy. The men in the no-stress group ate more of the unhealthy foods (such as chocolate) than the men in the stress group (Zellner et al., 2007). Conversely, in a study investigating stress in women, it was found that under stress conditions, women were more likely to increase consumption of the unhealthy foods (Zellner et al., 2006).

GLUCOCORTICOID SUMMARY

Overall, while the animal data indicate that chronic or severe stress-induced elevations in circulating levels of glucocorticoids may reduce food intake, the picture is much more complicated in humans. However, the converging lines of evidence showing (1) reduced food intake in SD conditions, (2) elevated basal levels of circulating glucocorticoids in SD conditions, and (3) experimental findings showing stress-induced elevations in glucocorticoid levels and changes (reductions in animal/rodent models) in food intake point to the possibility that seasonal variation in food intake may be due, in part, to changes in circulating glucocorticoid levels. Elevations in basal levels of glucocorticoids, as happen during SD photoperiods, may be a factor in not simply reducing food intake, but, rather, changing food selection processes. If particular foods are not present, the organism may not be inclined to engage in feeding behavior and fat reserves may be depleted. On the other hand, if particular foods are present during the winter months, consumption of those foods may be elevated.

GHRELIN

While it seems that elevated levels of basal or stress-induced glucocorticoids during the winter months may be a factor in altering food intake, the contribution of hormones involved in feeding responses may show more robust seasonal fluctuations. In this case, levels of hormones that stimulate feeding would be predicted to be lower in the winter than in the summer. One such hormone is ghrelin.

Ghrelin, a 28- amino acid peptide, was the first identified circulating hormone that, when elevated either exogenously or endogenously, promotes feeding (Kojima et al., 1999). Ghrelin is synthesized primarily within the gastric oxyntic mucosa of the stomach and is an endogenous ligand for the growth hormone secretagogue G-protein coupled receptor (GHS-R). Within the central nervous system, GHS-R are expressed in the hypothalamus, the ventral tegmental area (VTA), the dorsal (DRN) and medial (MRN) raphe nuclei and the hippocampus (Zigman et al., 2006). The high expression of GHS-R in these regions may explain the role of ghrelin in meal initiation and weight regulation. These brain areas are involved in a number of homeostatic processes that underlie a variety of regulatory behaviors, such as feeding (Gil-Campos et al., 2006).

Ghrelin is released in response to acute and chronic changes in nutritional state and, while the factors involved in the regulation of ghrelin secretion are still under investigation, blood glucose

levels appear to contribute to the rise and fall of ghrelin levels (Shiiya et al., 2002). Endogenous ghrelin levels increase prior to feeding when an animal is hungry and decrease after feeding (Tschop et al., 2000; Theander-Carrillo et al., 2006). Decreased levels of ghrelin have been shown to decrease the motivation of rats to obtain reward (Skibicka and Dickson, 2011). Exogenous application of ghrelin has been shown to dose-dependently increase food intake 24 h after a single injection in the third ventricle, the DRN or the hippocampus (Carlini et al., 2002, 2004). Carlini et al. (2004) also looked at how soon after a ghrelin injection into the DRN or hippocampus food intake increased. They found that the increase in food intake after ghrelin injections could be seen as early as 1 h after injection. They also saw that at the highest dose (3.0 nmol/ul), food intake was double that of control animals (Carlini et al., 2002, 2004). Abizaid et al. (2006) used a repeated-fast protocol in wild type, GHS-R knock-out and ghrelin deficient mice. The repeated-fast procedure consisted of two phases. During the first phase, food was removed from the cage overnight then returned in the morning for 6 h. Food intake was measured once the food was replaced at various time points. For the second phase, food was removed after the sixth hour until the next morning when food was again re-placed for another 6 h. They found that both the ghrelin and GHS-R deficient groups showed lower food intake compared to the wild type group. These studies delineate an important role of ghrelin in stimulating appetite and increasing feeding behaviors as well as increasing the motivation to seek out food (for review see Hosoda et al., 2002; Kojima and Kangawa, 2002; Wells, 2009).

SEASONAL VARIATION IN GHRELIN

The mechanisms controlling the ability of animals to prepare for and anticipate seasonal changes seems to be hardwired—consisting of both hormonal and neuronal interactions that influence body mass, food intake and metabolism (Underwood, 1971; Haga, 1993; Korhonen and Saarela, 2005). Animals that live in climates with predictable seasonal changes in food availability must acquire metabolic strategies that compensate for these seasonal changes (Bradley et al., 2010). Animals typically increase food intake during the summer in an attempt to accumulate fat stores that can then be utilized in the winter when food is scarce (Fuglei et al., 2004; Bradley et al., 2010; Janzen et al., 2012). Ghrelin has been identified as a potential candidate for mediating seasonal changes in metabolism, behavior, and food intake. However, there is conflicting research as to the effects of seasonal variation in the responsiveness and expression of ghrelin, which appears to stem from species-specific effects of the hormone. Here we will focus on the effect of ghrelin in mammals.

In mammals, the seasonal effects of ghrelin are dependent on (1) the level of circulating ghrelin that is produced and (2) the neural and behavioral responsiveness to ghrelin. The Arctic fox displays seasonal fluctuations in circulating ghrelin levels in relation to seasonal adjustments in fat deposition, metabolic rate and food-related behaviors (Fuglei et al., 2004). In this species, plasma levels of ghrelin are lowest during winter when food intake is suppressed and highest in the summer when food is more abundant (Fuglei et al., 2004; Mustonen et al., 2005a). The low levels of circulating ghrelin that occur in the winter months may stimulate the utilization of fat stores as an adaption to food restriction as evidenced by a negative correlation between ghrelin levels and free fatty acids (FFA; Fuglei et al., 2004).

The finding of increased utilization of fat stores is supported by findings that there are large seasonal variations in body fat and weight in the Arctic fox. In summer months when food levels are highest, the deposition of fat takes place, as approximately 20% of their total body weight is fat. By the onset of winter, this accumulation of fat is rapidly mobilized and, as a result, fat levels are at their lowest by the winter's end (Fuglei et al., 2004). Interestingly, circulating ghrelin fluctuates from intermediate to high levels during autumn when voluntary food intake is shown to be highest and the animals are accumulating fat. These increased ghrelin levels may underlie the Arctic foxes ability to gather adequate energy stores for the winter season by increased food intake that increases fat stores in the autumn, a process known as wintering (Mustonen et al., 2005a).

In the Siberian hamster, ghrelin is a potential component of the neurohormonal mechanism that regulates seasonal changes in food intake. In this species, changes in day length have been shown to initiate changes in metabolism, behavior, body mass and food intake (Steinlechner and Heldmaier, 1982). During SD periods, these animals reduce the cost of thermoregulation by reducing food intake and body mass (Tups et al., 2004; Bradley et al., 2010). This appears to be achieved by a change in the responsiveness to ghrelin rather than a reduction in the level of circulating ghrelin as a change in day length did not alter fasting levels of ghrelin (Tups et al., 2004). Thus, during SD, body mass is reduced in part because of a decreased responsiveness to ghrelin which in turn attenuates feeding (Keen-Rhinehart and Bartness, 2005; Bradley et al., 2010). However, during LD periods (summer), there is an attempt to increase fat stores for winter survival. As such, the responsiveness to ghrelin increases leading to an increase in the motivation to seek food and feed (Bradley et al., 2010). Bradley et al. (2010) found that Siberian hamsters ate more during the LD than the SD following i.p. ghrelin injections (same dose). Although day length does not affect the level of circulating ghrelin in these animals, after fasting, hamsters in LD conditions ate more than hamsters in SD conditions (Bartness and Wade, 1985a; Tups et al., 2004) further suggesting a reduced responsiveness to ghrelin signaling. Possibly, a lower threshold for behavioral responsiveness to ghrelin exists which may lead to more frequent meals in LD conditions and increased motivation to feed in an attempt to increase fat stores for winter survival (Bradley et al., 2010).

GHRELIN AND FEEDING BEHAVIOR

Various studies have indicated that ghrelin has a powerful impact on a number of aspects of feeding-related behaviors. Operant conditioning has been used to assess the motivational properties of rewarding food by assessing the magnitude of acquired behaviors (i.e., nose pokes and bar presses) that are directed toward obtaining the food reward (Thorndike, 1911; Hodos, 1961; Hodos and Kalman, 1963). Numerous studies have shown that ghrelin increases operant lever pressing for various food rewards including high fat diet (HFD), sucrose, peanut butter and chocolate pellets in both mice and rats (Perello et al., 2010; Skibicka et al.,

2011). Further suggestive of an increased motivational state to obtain food reward, rats or mice that were given intra-VTA ghrelin infusions earned more food rewards during a progressive ratio task (Perello et al., 2010; Skibicka et al., 2011). This effect was matched by the finding that rats given ghrelin injections increased their chow intake when food was presented after conditioning while rats (hungry or satiated) injected with the GHS-R antagonist showed reduced operant responding (Skibicka et al., 2011).

Another behavioral task that has been used to highlight the contribution of ghrelin to feeding-related behaviors is the conditioned place preference (CPP) task. Using the CPP task, Disse et al. (2010) conducted a study using both wild type and GHS-R knock-out mice. They first looked at the effects of natural increases in ghrelin as would be brought about by food restriction (mice were restricted to 50% of their normal food intake during a 24 h period). They found that a short exposure to the food-paired side during training elicited a strong CPP in WT mice as measured by increased time spent in the food-paired side. This effect was absent in GHS-R knock-out mice, indicating that GHS-R KO reduced the facilitation of learning the CPP with short training exposures brought about by food restriction (Disse et al., 2010). The next part of the study investigated the effect of exogenously administered ghrelin on the CPP task by administering ghrelin i.p. 20 min before the conditioning sessions. It was found that pretreatment with ghrelin increased the amount of time spent in the food-paired side over and above the time displayed by the vehicle-treated group (Disse et al., 2010). Furthermore, by administering a GHS-R antagonist to rats prior to the conditioning sessions, (Egecioglu et al., 2010) found that the ability of a rewarding food to elicit a CPP was suppressed. These findings highlight the conclusion that ghrelin signaling may serve a critical role in the pathways involved in the motivation required for reward-related behaviors.

Ghrelin and dopamine

The mechanisms through which ghrelin promotes food intake and body weight regulation are multifaceted, stimulating not only food intake, but also enhancing the rewarding properties of food and the motivation to acquire food (for reviews see Skibicka and Dickson, 2011; Perello and Zigman, 2012). Feeding-associated reward is governed by the mesolimbic dopamine (DA) pathway that consists of cell bodies located in the VTA sending projections to multiple nodes in the Acb, amygdala, prefrontal cortex, and hippocampus (Nestler and Carlezon, 2006). These projections are associated with food reward and food seeking behavior (Richardson and Gratton, 1998; Bassareo and Di Chiara, 1999). In addition to DA projections in the mesolimbic pathway being activated by the ingestion of rewarding and palatable food, these projections are activated by ghrelin (Abizaid, 2009) as GHS-Rs are present on approximately 60% of VTA DA neurons (Abizaid et al., 2006; Jerlhag et al., 2006; Zigman et al., 2006).

Various experiments have investigated the influence of ghrelin on the DA-containing pathways in relation to reward-related behaviors. For example, Jerlhag et al. (2006, 2010, 2011b) measured locomotor activity after ghrelin injections into the third ventricle. They found that after ghrelin injections, locomotor activity increased; an indication of increased DA output. Following direct injections of ghrelin into the VTA or the third ventricle, an increase in the frequency of action potentials in VTA DA neurons was noted as well as increased DA turnover into the Acb (Abizaid et al., 2006; Jerlhag et al., 2006, 2010). In addition, increased feeding was observed when ghrelin was infused into the VTA, suggesting that ghrelin may modulate DA activity in the VTA reward pathway to enhance feeding responses (Abizaid et al., 2006).

GHRELIN SUMMARY

The regulation of ghrelin secretion appears to depend on nutritional state potentially arising from blood glucose levels; when an organism is hungry, and thereby low levels of blood glucose, circulating levels of ghrelin would rise and stimulate feeding behaviors via activation of receptors in the brain. In mammals, the seasonal effects of ghrelin are dependent on (1) the level of circulating ghrelin that is produced and (2) the neural and behavioral responsiveness to ghrelin. Circulating ghrelin levels are decreased in SD conditions and ghrelin reactivity is also decreased in the winter. Both of these physiological responses might ultimately impede DA function (neural activation and output) and impede food-seeking and food intake in SD conditions.

LEPTIN

Leptin is a peptide hormone that was first described in 1994 (Casanueva and Dieguez, 1999). Leptin is the product of the obese gene and is expressed and secreted exclusively by adipocytes in the periphery (Zhang et al., 1994). Leptin affects areas of the central nervous system involved in the regulation of energy balance with prompt feedback regarding the status of the bodies energy stores and energy flux to help modulate food intake and energy homeostasis (Zhang et al., 1994; Ahima and Flier, 2000; Perry et al., 2010). Leptin functions as an afferent signal in a negative feedback loop that controls adipose tissue mass and reduces food intake. The amount of leptin produced by adipocytes is proportional to body fat mass. Leptin is shown to decrease food intake, as exogenous leptin administration decreased food intake and body weight (Ahima and Flier, 2000; Friedman, 2004; Domingos et al., 2011). Leptin is thought to be another major factor in energy balance and is hypothesized as having the opposite effect as ghrelin on food intake and body weight (Tups et al., 2004; Schmid et al., 2005). A negative correlation between circulating ghrelin and leptin exists, as leptin appears to inhibit food intake and ghrelin has been shown to increase food intake. Thus, when leptin is increased, ghrelin is reduced and food intake is attenuated (Tups et al., 2004; Schmid et al., 2005). Barazzoni et al. (2003) showed that even under caloric restriction, which would naturally increase circulating ghrelin levels, subcutaneous leptin infusions in lean rats prevented this natural rise in serum ghrelin levels. This finding of reduced serum ghrelin when leptin is injected during food deprivation highlights the role of leptin in the negative feedback loop that controls body weight and food intake.

Leptin is released in response to acute and chronic changes in nutritional state. Endogenous leptin levels are low prior to

feeding when an animal is hungry and will increase as an animal becomes satiated (Tschop et al., 2000; Davis et al., 2011). Leptin appears to increase energy expenditure and decrease food intake which results in a decreased body weight and body fat mass (Campfield et al., 1995; Dileone, 2009; van Zessen et al., 2012). The role of leptin in maintaining energy homeostasis has also been shown to regulate reward- related behaviors (Davis et al., 2011). As leptin and its receptors have been found in the VTA, it is not surprising that leptin has been shown to regulate effort-based responding for food reward (Davis et al., 2011). Leptin is thought to accomplish this by decreasing DA release and by opposing the actions of ghrelin in the VTA (Davis et al., 2011). Viral knockdown of the leptin receptors in the VTA not only increased food intake of balanced or high fat diet, but also elevated the preference for sucrose or high fat diets (Hommel et al., 2006; van Zessen et al., 2012). It seems that in addition to being involved in the hypothalamic control of energy expenditures, body mass, and food intake, leptin is also involved in the mesolimbic dopaminergic system control over reward-based feeding and responding (Caro et al., 1996; Davis et al., 2011). The function of leptin to signal the central nervous system about the state of energy stores is extremely important as adequate reserves are important for survival (Mustonen et al., 2005a; Schmid et al., 2005).

SEASONAL VARIATION IN LEPTIN

Animals have developed both physiological and behavioral strategies to anticipate and overcome the different challenges that accompany the changing of seasons (Zhao and Wang, 2006). Photoperiod length differs across seasons, as winter has reduced periods of sunlight compared to summer. Photoperiod plays an important role in mediating the seasonal variation in body mass and energy homeostasis (Bartness and Wade, 1985b; Bartness and Goldman, 1989; Zhao and Wang, 2006). Similar to ghrelin, leptin fluctuates in accordance with the seasonal fluctuations in energy intake, body mass and fat mass. Seasonal changes in energy intake, body mass, body fat, and serum leptin levels occur independently from food availability, which suggests a hard-wired, evolutionarily conserved mechanism (Klingenspor et al., 1996, 2000; Zhao and Wang, 2006). It seems possible that during different seasons, leptin is utilized differently from those signals induced by negative energy balance, created by controlled food deprivation (Adam and Mercer, 2001). Here we will focus on the effect of leptin in seasonal non-hibernating mammals and how changes in photoperiod affect the responsiveness of leptin and the impact on energy intake and body fat mass.

In seasonal mammals, such as the Siberian hamster, weight loss resulting from the winter months is associated with low circulating levels of leptin compared to the summer months (Adam and Mercer, 2001). There also appear to be changes in the sensitivity to leptin feedback as expression of the leptin receptor gene in the hypothalamus is lower during the winter months whereas, under natural conditions of food deprivation, expression of the gene is increased (Adam and Mercer, 2001). The changes in leptin responsiveness or sensitivity and concentration during seasonal variation have been studied in a number of seasonal mammals such as Siberian hamsters, Arctic foxes, and Brandt's voles; all discussed here.

In the Siberian hamster, the effects and responsiveness of leptin during different seasons has been investigated via manipulating the photoperiod and infusing leptin at different time points. Under normal conditions, there is a robust positive correlation between total body fat mass and plasma leptin concentration (Korhonen et al., 2008). There is significant body weight loss, in terms of adipose tissue amount, during the transition from the obese LD phenotype to the leaner SD phenotype (Klingenspor et al., 1996). The mobilization of adipose tissue during the SD period is associated with decreased leptin plasma concentration. For example, leptin levels are 2–3 times higher in Siberian hamsters during the LD as opposed to the SD condition (Klingenspor et al., 1996; Tups et al., 2004). The appetite-depressing effects of leptin are greater in Siberian hamsters exposed to SD conditions than those in LD conditions suggesting that SD-exposed hamsters have increased sensitivity to leptin (Klingenspor et al., 2000). Likewise, Rousseau et al. (2002) demonstrated that, when all other physiological parameters were controlled, chronic leptin infusions only resulted in body weight and fat loss in Siberian hamsters exposed to a SD conditions [similar findings were reported by Atcha et al. (2000)]. Thus, it appears that photoperiod may alter the sensitivity to leptin-induced seasonal changes in body weight, food intake, and fat mass in the Siberian hamster.

Brandt voles also have an increased energy mobilization in SD periods compared to LD. Compared to LD voles, voles acclimated to SD conditions have decreased levels of serum leptin, decreased body fat mass and increased energy intake (Zhao and Wang, 2006). These variations in leptin serum levels, body composition, and energy intake between SD and LD were found to be independent from both temperature and food availability, suggesting that photoperiod length is a critical factor in this effect (Zhao and Wang, 2006). The decreased body weight and body mass in winter displayed by Brandt voles indicate that these animals have evolved a physiological adaption to maintain a low body mass in winter months (Flier, 1998; Li and Wang, 2005; Zhao et al., 2010). Additionally, the decrease in leptin plasma concentration during the SD photoperiod suggests that leptin may act as a starvation signal to increase energy intake to help cope with the conditions during SD photoperiods (Li and Wang, 2005).

The Arctic fox also expresses seasonal changes in leptin sensitivity (Mustonen et al., 2005a). The Arctic fox exhibits a pattern of seasonal fattening with fat accumulation starting in August and September and peaking in November and December (Fuglei et al., 2004). In the autumn months, voluntary food intake is increased, which leads to an accumulation of fat to prepare for the reduced food availability in winter (Fuglei et al., 2004; Mustonen et al., 2005a). The increased fat storage observed in autumn is accompanied by increased circulating plasma leptin concentrations (Mustonen et al., 2005a). This increase in plasma leptin concentrations does not seem to induce the normal loss of appetite, body weight and fat storage during autumnal fatting that is normally associated with leptin (Mustonen et al., 2005a). It seems that leptin does not act as an anorectic hormone during the autumnal fatting, as it is important for the Arctic fox to prepare for the harsh winter by maximizing feeding, with the goal of increasing body fat (Mustonen et al., 2005a). This decreased leptin sensitivity during autumn is not present in winter, as the

gradual reduction in body mass and fat stores are accompanied by decreased leptin levels. This suggests that in the Artic fox, leptin functions as an indicator of adiposity during the winter fat mobilization period (Mustonen et al., 2005a).

The seasonal variation in leptin seems to be an evolutionary mechanism to anticipate changing environments. Animals that live in climates with predictable seasonal changes in food availability must acquire metabolic strategies that change according to the season (Bradley et al., 2010). It seems that leptin may be an integral component of the physiological processes needed to regulate appetite in anticipation of changing seasons. However, as is seen in the Artic fox during autumn, there are other times when animals may be insensitive to changes in blood leptin levels (Mustonen et al., 2005a). Another possibility is that leptin does not function as an acute indicator of body adiposity in seasonal animals, but rather, as a signal of nutritional status. This is evidenced by the positive correlation between plasma leptin concentrations and body composition in the Arctic fox, Siberian hamster, and Brandt voles when all circannual data are analyzed together (Mustonen et al., 2005a).

LEPTIN AND FEEDING BEHAVIOR

The mechanisms for the ability of leptin to inhibit food intake and regulate body weight/adiposity composition are multifaceted. Leptin not only inhibits food intake but has also been shown to reduce both the rewarding properties of food and the motivation to acquire food (Davis et al., 2011). As with ghrelin, both operant conditioning and the CPP have been used to assess the role of leptin in the motivational properties of food. Injections of leptin have been shown to decrease progressive ratio responding for HFD and sucrose, as well as impair the acquisition and expression of a CPP for HFD (Figlewicz, 2003; Figlewicz et al., 2006; Hommel et al., 2006; Davis et al., 2011). These data suggest that motivation to acquire food is decreased by leptin. The ability of leptin to decrease the motivation to obtain food has been further studied by using knockdown of the leptin receptor (LepR) and LepR antagonists; both of which increase progressive ratio responding for a HFD (Davis et al., 2011). These findings suggest that leptin signaling and its receptors may play a critical role in the pathways involved in food-related behaviors.

Leptin and dopamine

Leptin can modify food-based behaviors by altering the function of the mesolimbic DA pathway (Figlewicz and Woods, 2000; Dileone, 2009; Perry et al., 2010). The rewarding aspects of feeding are governed by the mesolimbic DA pathways which are activated by the ingestion of rewarding and/or palatable food and are inhibited by leptin (Davis et al., 2011). LepR are expressed in the VTA and 75–90% of LepR-positive neurons are DA neurons (Hommel et al., 2006). Furthermore, direct application of leptin into the VTA has been shown to attenuate the activation of these DA neurons (Hommel et al., 2006).

Elevations in midbrain leptin are capable of modulating feeding behavior and DA responses (Krugel et al., 2003; Hommel et al., 2006). Injection of leptin into either the third ventricle or the VTA has been shown to decrease food consumption, whereas when the effect of leptin is decreased in the midbrain, feeding increases (Figlewicz et al., 2003; Krugel et al., 2003). In the VTA, both viral knock-down of LepR and administration of leptin receptor antagonists have been shown to increase both normal chow and HFD, as well as increase the preference for both sucrose and HFD (Hommel et al., 2006; Davis et al., 2011). These effects on feeding may be a result of leptin-induced changes in DA concentration and DA neuronal firing as leptin administration into the midbrain has been shown to attenuate the firing of DA neurons (Hommel et al., 2006; Davis et al., 2011). In addition, injections of leptin into the VTA reduce DA release into the Acb. This effect might result from a direct inhibitory effect of leptin on VTA DA neurons (Krugel et al., 2003; Hommel et al., 2006). Leptin's effects on DA firing and DA turnover into the Acb seem to directly oppose the actions of ghrelin (Dileone, 2009).

LEPTIN SUMMARY

Leptin functions as an afferent signal that is released in response to acute and chronic changes in nutritional state. Endogenous leptin levels are low prior to feeding when an animal is hungry and increase as an animal becomes satiated. With respect to seasonal changes in leptin function, there appears to be seasonal leptin resistance whereby there is a higher sensitivity to leptin in the winter, thereby resulting in decreased food intake, and lower sensitivity in the summer, thereby resulting in elevated food intake. Leptin signaling in the DA system may be one mechanism through which the motivation to seek out and consume food is reduced as would happen during SD photoperiods. With leptin receptors showing increased sensitivity during the SD winter months, activity in the DA mesolimbic system would be significantly reduced thereby reducing food seeking behaviors.

SYNTHESIS OF FINDINGS

We sought to investigate whether changes in circulating glucocorticoids or changes in the physiological effects of ghrelin and/or leptin during the winter would impede the propensity for an animal to engage in food-related behaviors such as seeking and consuming food. A review of the literature on seasonal variation in basal and stress-induced levels of circulating glucocorticoids and ghrelin and leptin levels and sensitivity reveals several plausible explanations for reduced food seeking and consuming behaviors during SD photoperiods.

GLUCOCORTICOID-MEDIATED ATTENUATION OF FOOD SEEKING BEHAVIORS IN WINTER

Studies reviewed indicate that non-hibernating species have increased basal and stress-induced glucocorticoid levels in the fall and winter months compared to the spring and summer months (Amirat et al., 1980; Amirat and Brudieux, 1984, 1993). While some animal studies have revealed that stress-induced increases in circulating glucocorticoids leads to a decrease in food intake (Levine and Morley, 1981; Morley et al., 1983; Liu et al., 2011) others have reported that repeated bouts of stress (i.e., chronic forms of stress) leads to increased food consumption (Pecoraro et al., 2004; Lutter et al., 2008; Pankevich et al., 2010). These

differences could be due to an acute/chronic stressor procedure whereby acute stress seems to decrease food consumption while chronic stress increases food consumption. Indeed, seasonal variation in glucocorticoid responses to chronic and acute stress also seems to vary (Vera et al., 2011).

Circulating levels of glucocorticoids are elevated in association with goal-directed, reward-related behaviors (Schwabe et al., 2011). As shown by others (Kerr et al., 1991; Arbel et al., 1994; Bodnoff et al., 1995), elevations in circulating glucocorticoid concentrations by systemic administration or glucocorticoid receptor agonists, impair learning and memory processes. The relationship between stress, DA, and food seeking behaviors may or may not support the idea that elevated stress can specifically impair DA-mediated increases in behavioral output. Stress-induced increases in levels of circulating glucocorticoids has been reported to increase DA concentrations in the Acb shell (Brake et al., 1999, 2000) particularly in conditions when the DA system is activated (Cador et al., 1993; Rouge-Pont et al., 1995; Marinelli and Piazza, 2002) as would be the case when animals are seeking food. An acute injection of dexamethasone (8 mg/kg but not 4 or 2 mg/kg) reduces locomotor activity in mice and acute or chronic dexamethasone treatment (mimicking increased levels of circulating glucocorticoids) reduces DA-dependent amphetamine-stimulated increases in locomotor activity (Wrobel et al., 2005). A report has also shown that repeated restraint stress enhances DA uptake into striatal synaptosomes within the Acb, characterized by increased transporter capacity, potentially diminishing DA function (Copeland et al., 2005). As well, stress has been shown to downregulate D1 receptor binding in the Acb shell and upregulate D1 receptor binding in the VTA (Czyrak et al., 2003). In this scenario, even with elevated DA being produced by elevated, stress-induced circulating glucocorticoids, DA function would be lessened by elevated re-uptake and downregulated postsynaptic actions. This would then attenuate food-seeking behavior as seen in SD photoperiods.

GHRELIN-MEDIATED ATTENUATION OF FOOD SEEKING BEHAVIORS IN WINTER

A second possibility is that seasonal fluctuations in ghrelin could decrease the propensity for the animals to engage in food-seeking behaviors via an interaction with DA. As reviewed, ghrelin levels are lower in the winter than in the summer and animals are more likely to eat in excess and in higher caloric amounts during the summer potentially in preparation for the winter (Fuglei et al., 2004; Keen-Rhinehart and Bartness, 2005; Mustonen et al., 2005a,b; Bradley et al., 2010). This suggests that food-seeking behaviors would be increased in the summer and decreased in the winter. Numerous studies have shown that ghrelin increases operant lever pressing for various food rewards including HFD, sucrose, peanut butter, and chocolate pellets in both mice and rats (Perello et al., 2010; Skibicka et al., 2011). In terms of the functional outcomes in response to decreased ghrelin activity, ghrelin receptor antagonist injections have been shown to reduce operant responding for a sucrose solution (Landgren et al., 2011). Therefore, in the winter when ghrelin levels are low, a reduction in food-seeking, and potentially, consuming, behaviors would be predicted. Conversely, in the summer, these behaviors might be increased due to the elevations in ghrelin activity.

It is hypothesized that the actions of ghrelin on food-seeking behavior are due to interactions with the DA system. The ability of ghrelin to potentiate the DA reward system was studied by Abizaid et al. (2006) who found that ghrelin increased the frequency of action potentials of DA neurons in the VTA and increased DA turnover into the Acb. The ability of ghrelin to stimulate action potential firing in VTA DA neurons requires glutamatergic excitatory inputs (Andrews, 2011). As such, blockade of glutamatergic signaling following intra-VTA administration of the NMDAr antagonist AP5, blocked ghrelin-induced DA output in the Acb along with ghrelin-induced locomotor elevation (Jerlhag et al., 2011a). In this scenario, with low levels of ghrelin in the winter, the VTA-Acb circuit would show overall less responsivity to reward-related signals and reduce the "higher-order" aspects of food acquisition, such as motivation and cue responses (Narayanan et al., 2010). This would set up a condition whereby the animals would be less likely to engage in food-seeking behaviors.

LEPTIN-MEDIATED ATTENUATION OF FOOD SEEKING BEHAVIORS IN WINTER

A third possibility is that seasonal variations in changes in the responsiveness to leptin during the winter months would contribute to reduced food-seeking behaviors and reduced food intake possibly, again, via interactions with the DA system. Increased food intake and weight gain in LD photoperiods are associated with a relative insensitivity to high circulating leptin serum levels while decreased intake and weight loss in SD photoperiods appear to be an outcome of low circulating leptin but high sensitivity (Adam and Mercer, 2004). This heightened sensitivity to leptin during the winter SDs would lead to a decreased propensity to engage in food-seeking behaviors either through reductions in DA output or increases in anxiety-like behaviors.

Leptin-receptor expressing neurons are located in a subset of DA neurons in the VTA as well as the substantia nigra (Figlewicz, 2003). Leptin signaling in the VTA may be one mechanism through which the motivation to seek out and consume food is reduced (Hommel et al., 2006) as would happen during SD photoperiods. Viral-mediated reduction in the leptin receptor on these midbrain DA neurons can prolong progressive ratio responding for sucrose thereby increasing the motivation to obtain food (Davis et al., 2011) and in wild-type mice, leptin administration can decrease striatal dopamine D2 receptor binding (Pfaffly et al., 2010). This suggests that leptin reduces DA function within the striatum to reduce the motivation to obtain food. Leptin administration may decrease the motivation to seek food by decreasing the intrinsic excitability of VTA DA neurons (Hommel et al., 2006) or by decreasing the probability of glutamate release onto VTA neurons leading to presynaptic inhibition ultimately inhibiting appetitive behavior for rewarding stimuli (Thompson and Borgland, 2013). With leptin receptors showing increased sensitivity during the SD winter months, activity in the DA mesolimbic system would

be significantly reduced thereby reducing food seeking behaviors.

Alternatively, because leptin receptors are expressed on VTA neurons that show a dense set of projections to the extended central amygdala (Leshan et al., 2010), it might also be feasible that increased sensitivity to leptin during the SD winter months more strongly activates activity in the central nucleus of the amygdala thereby resulting in anxiety-like behaviors (Hunter et al., 2007). This enhanced activity in the amygdala may then contribute to an enhanced HPA response leading to elevated circulating levels of glucocorticoids and ultimately decrease the propensity of the organism to seek reward or food in the environment.

CONCLUSION

We entertained the possibility that seasonal changes in stress-or feeding-related hormones might dampen increase in reward-seeking behaviors. While it seems plausible that increased levels of glucocorticoids, as found in the winter, could interfere with DA output to stimulate food-seeking behaviors, the mechanism is convoluted. Alternatively, decreased levels of ghrelin or increased levels of leptin, as found in the winter, seem to be likely candidates in interfering with food-seeking behaviors in a DA-dependent mechanism. On the other hand, it seems just as likely that increased ghrelin activity or decreased leptin activity, as found in the summer, would work to facilitate an increase in food-seeking behavior in a DA-dependent fashion. As well, a combination of increased glucocorticoid activity, decreased ghrelin activity and increased leptin activity in the winter may impede DA-mediated change in food-seeking behaviors. Finally, other seasonal fluctuations in physiological processes (e.g., melatonin/serotonin tone), not covered in this review, could also play important roles in modulating seasonal effects on feeding behavior. While not being able to make any decisive conclusions on underlying neural mechanisms that mediate seasonal variation in food-related behaviors, we hope that this review serves to generate hypotheses for future experiments.

REFERENCES

Abizaid, A. (2009). Ghrelin and dopamine: new insights on the peripheral regulation of appetite. *J. Neuroendocrinol.* 21, 787–793. doi: 10.1111/j.1365-2826.2009.01896.x

Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., et al. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J. Clin. Invest.* 116, 3229–3239. doi: 10.1172/JCI29867

Adam, C. L., and Mercer, J. G. (2001). Hypothalamic neuropeptide systems and anticipatory weight change in Siberian hamsters. *Physiol. Behav.* 74, 709–715. doi: 10.1016/S0031-9384(01)00614-X

Adam, C. L., and Mercer, J. G. (2004). Appetite regulation and seasonality: implications for obesity. *Proc. Nutr. Soc.* 63, 413–419. doi: 10.1079/PNS2004367

Ahima, R. S., and Flier, J. S. (2000). Leptin. *Annu. Rev. Physiol.* 62, 413–437. doi: 10.1146/annurev.physiol.62.1.413

Amirat, Z., and Brudieux, R. (1984). Seasonal changes in the metabolic clearance rate of cortisol in the adult male sand rat (*Psammomys obesus*). *Gen. Comp. Endocrinol.* 53, 232–234. doi: 10.1016/0016-6480(84)90247-8

Amirat, Z., and Brudieux, R. (1993). Seasonal changes in *in vivo* cortisol response to ACTH and in plasma and pituitary concentrations of ACTH in a desert rodent, the sand rat (*Psammomys obesus*). *Comp. Biochem. Physiol. Comp. Physiol.* 104, 29–34.

Amirat, Z., Khammar, F., and Brudieux, R. (1980). Seasonal changes in plasma and adrenal concentrations of cortisol, corticosterone, aldosterone, and electrolytes in the adult male sand rat (*Psammomys obesus*). *Gen. Comp. Endocrinol.* 40, 36–43. doi: 10.1016/0016-6480(80)90093-3

Andrews, Z. B. (2011). The extra-hypothalamic actions of ghrelin on neuronal function. *Trends Neurosci.* 34, 31–40. doi: 10.1016/j.tins.2010.10.001

Anisman, H., and Merali, Z. (1999). Understanding stress: characteristics and caveats. *Alcohol Res. Health* 23, 241–249.

Arbel, I., Kadar, T., Silbermann, M., and Levy, A. (1994). The effects of long-term corticosterone administration on hippocampal morphology and cognitive performance of middle-aged rats. *Brain Res.* 657, 227–235. doi: 10.1016/0006-8993(94)90972-5

Armario, A. (2006). The hypothalamic-pituitary-adrenal axis: what can it tell us about stressors? *CNS Neurol. Disord. Drug Targets* 5, 485–501. doi: 10.2174/187152706778559336

Atcha, Z., Cagampang, F. R., Stirland, J. A., Morris, I. D., Brooks, A. N., Ebling, F. J., et al. (2000). Leptin acts on metabolism in a photoperiod-dependent manner, but has no effect on reproductive function in the seasonally breeding Siberian hamster (*Phodopus sungorus*). *Endocrinology* 141, 4128–4135. doi: 10.1210/en.141.11.4128

Baran, S. E., Armstrong, C. E., Niren, D. C., Hanna, J. J., and Conrad, C. D. (2009). Chronic stress and sex differences on the recall of fear conditioning and extinction. *Neurobiol. Learn. Mem.* 91, 323–332. doi: 10.1016/j.nlm.2008.11.005

Barazzoni, R., Zanetti, M., Stebel, M., Biolo, G., Cattin, L., and Guarnieri, G. (2003). Hyperleptinemia prevents increased plasma ghrelin concentration during short-term moderate caloric restriction in rats. *Gastroenterology* 124, 1188–1192. doi: 10.1016/S0016-5085(03)00281-6

Bartness, T. J., and Goldman, B. D. (1989). Mammalian pineal melatonin: a clock for all seasons. *Experientia* 45, 939–945. doi: 10.1007/BF01953051

Bartness, T. J., and Wade, G. N. (1985a). Body weight, food intake and energy regulation in exercising and melatonin-treated Siberian hamsters. *Physiol. Behav.* 35, 805–808. doi: 10.1016/0031-9384(85)90415-9

Bartness, T. J., and Wade, G. N. (1985b). Photoperiodic control of seasonal body weight cycles in hamsters. *Neurosci. Biobehav. Rev.* 9, 599–612. doi: 10.1016/0149-7634(85)90006-5

Bassareo, V., and Di Chiara, G. (1999). Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. *Eur. J. Neurosci.* 11, 4389–4397. doi: 10.1046/j.1460-9568.1999.00843.x

Bennett, J., Greene, G., and Schwartz-Barcott, D. (2013). Perceptions of emotional eating behavior. A qualitative study of college students. *Appetite* 60, 187–192. doi: 10.1016/j.appet.2012.09.023

Bodnoff, S. R., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M., and Meaney, M. J. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *J. Neurosci.* 15, 61–69.

Bradley, S. P., Pattullo, L. M., Patel, P. N., and Prendergast, B. J. (2010). Photoperiodic regulation of the orexigenic effects of ghrelin in Siberian hamsters. *Horm. Behav.* 58, 647–652. doi: 10.1016/j.yhbeh.2010.06.009

Brake, W. G., Flores, G., Francis, D., Meaney, M. J., Srivastava, L. K., and Gratton, A. (2000). Enhanced nucleus accumbens dopamine and plasma corticosterone stress responses in adult rats with neonatal excitotoxic lesions to the medial prefrontal cortex. *Neuroscience* 96, 687–695. doi: 10.1016/S0306-4522(00)00002-6

Brake, W. G., Sullivan, R. M., Flores, G., Srivastava, L. K., and Gratton, A. (1999). Neonatal ventral hippocampal lesions attenuate the nucleus accumbens dopamine response to stress: an electrochemical study in the adult rat. *Brain Res.* 831, 25–32. doi: 10.1016/S0006-8993(99)01477-8

Cador, M., Dulluc, J., and Mormede, P. (1993). Modulation of the locomotor response to amphetamine by corticosterone. *Neuroscience* 56, 981–988. doi: 10.1016/0306-4522(93)90144-5

Campfield, L. A., Smith, F. J., Guisez, Y., Devos, R., and Burn, P. (1995). Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269, 546–549. doi: 10.1126/science.7624778

Carlini, V. P., Monzon, M. E., Varas, M. M., Cragnolini, A. B., Schioth, H. B., Scimonelli, T. N., et al. (2002). Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochem. Biophys. Res. Commun.* 299, 739–743. doi: 10.1016/S0006-291X(02)02740-7

Carlini, V. P., Varas, M. M., Cragnolini, A. B., Schioth, H. B., Scimonelli, T. N., and de Barioglio, S. R. (2004). Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. *Biochem. Biophys. Res. Commun.* 313, 635–641. doi: 10.1016/j.bbrc.2003.11.150

Caro, J. F., Sinha, M. K., Kolaczynski, J. W., Zhang, P. L., and Considine, R. V. (1996). Leptin: the tale of an obesity gene. *Diabetes* 45, 1455–1462.

Casanueva, F. F., and Dieguez, C. (1999). Neuroendocrine regulation and actions of leptin. *Front. Neuroendocrinol.* 20, 317–363. doi: 10.1006/frne.1999.0187

Chung, S., Son, G. H., and Kim, K. (2011). Circadian rhythm of adrenal glucocorticoid: its regulation and clinical implications. *Biochim. Biophys. Acta* 1812, 581–591. doi: 10.1016/j.bbadis.2011.02.003

Copeland, B. J., Neff, N. H., and Hadjiconstantinou, M. (2005). Enhanced dopamine uptake in the striatum following repeated restraint stress. *Synapse* 57, 167–174. doi: 10.1002/syn.20169

Czyrak, A., Mackowiak, M., Chocyk, A., Fijal, K., and Wedzony, K. (2003). Role of glucocorticoids in the regulation of dopaminergic neurotransmission. *Pol. J. Pharmacol.* 55, 667–674.

Dallman, M. F., Pecoraro, N. C., and La Fleur, S. E. (2005). Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain Behav. Immun.* 19, 275–280. doi: 10.1016/j.bbi.2004.11.004

Dallman, M. F., Pecoraro, N., Akana, S. F., La Fleur, S. E., Gomez, F., Houshyar, H., et al. (2003). Chronic stress and obesity: a new view of "comfort food". *Proc. Natl. Acad. Sci. U.S.A.* 100, 11696–11701. doi: 10.1073/pnas.1934666100

Davis, J. F., Choi, D. L., Schurdak, J. D., Fitzgerald, M. F., Clegg, D. J., Lipton, J. W., et al. (2011). Leptin regulates energy balance and motivation through action at distinct neural circuits. *Biol. Psychiatry* 69, 668–674. doi: 10.1016/j.biopsych.2010.08.028

De Vos, P., Saladin, R., Auwerx, J., and Staels, B. (1995). Induction of ob gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. *J. Biol. Chem.* 270, 15958–15961. doi: 10.1074/jbc.270.27.15958

Dickmeis, T., Weger, B. D., and Weger, M. (2013). The circadian clock and glucocorticoids - Interactions across many time scales. *Mol. Cell. Endocrinol.* doi: 10.1016/j.mce.2013.05.012. [Epub ahead of print].

Dietrich, M. O., and Horvath, T. L. (2009). Feeding signals and brain circuitry. *Eur. J. Neurosci.* 30, 1688–1696. doi: 10.1111/j.1460-9568.2009.06963.x

Dileone, R. J. (2009). The influence of leptin on the dopamine system and implications for ingestive behavior. *Int. J. Obes. (Lond.)* 33(Suppl. 2), S25–S29. doi: 10.1038/ijo.2009.68

Disse, E., Bussier, A. L., Veyrat-Durebex, C., Deblon, N., Pfluger, P. T., Tschop, M. H., et al. (2010). Peripheral ghrelin enhances sweet taste food consumption and preference, regardless of its caloric content. *Physiol. Behav.* 101, 277–281. doi: 10.1016/j.physbeh.2010.05.017

Domingos, A. I., Vaynshteyn, J., Voss, H. U., Ren, X., Gradinaru, V., Zang, F., et al. (2011). Leptin regulates the reward value of nutrient. *Nat. Neurosci.* 14, 1562–1568. doi: 10.1038/nn.2977

Ebling, F. J., and Barrett, P. (2008). The regulation of seasonal changes in food intake and body weight. *J. Neuroendocrinol.* 20, 827–833. doi: 10.1111/j.1365-2826.2008.01721.x

Egecioglu, E., Jerlhag, E., Salome, N., Skibicka, K. P., Haage, D., Bohlooly, Y. M., et al. (2010). Ghrelin increases intake of rewarding food in rodents. *Addict. Biol.* 15, 304–311. doi: 10.1111/j.1369-1600.2010.00216.x

Engeland, W. C., and Arnhold, M. M. (2005). Neural circuitry in the regulation of adrenal corticosterone rhythmicity. *Endocrine* 28, 325–332. doi: 10.1385/ENDO:28:3:325

Epel, E., Lapidus, R., McEwen, B., and Brownell, K. (2001). Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. *Psychoneuroendocrinology* 26, 37–49. doi: 10.1016/S0306-4530(00)00035-4

Ferguson, S. A., and Maier, K. L. (2013). A review of seasonal/circannual effects of laboratory rodent behavior. *Physiol. Behav.* 119, 130–136. doi: 10.1016/j.physbeh.2013.06.007

Fietta, P., and Delsante, G. (2009). Central nervous system effects of natural and synthetic glucocorticoids. *Psychiatry Clin. Neurosci.* 63, 613–622. doi: 10.1111/j.1440-1819.2009.02005.x

Figlewicz, D. P. (2003). Adiposity signals and food reward: expanding the CNS roles of insulin and leptin. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284, R882–R892.

Figlewicz, D. P., Bennett, J. L., Naleid, A. M., Davis, C., and Grimm, J. W. (2006). Intraventricular insulin and leptin decrease sucrose self-administration in rats. *Physiol. Behav.* 89, 611–616. doi: 10.1016/j.physbeh.2006.07.023

Figlewicz, D. P., Evans, S. B., Murphy, J., Hoen, M., and Baskin, D. G. (2003). Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Res.* 964, 107–115. doi: 10.1016/S0006-8993(02)04087-8

Figlewicz, D. P., and Woods, S. C. (2000). Adiposity signals and brain reward mechanisms. *Trends Pharmacol. Sci.* 21, 235–236. doi: 10.1016/S0165-6147(00)01488-7

Flier, J. S. (1998). Clinical review 94: what's in a name? In search of leptin's physiologic role. *J. Clin. Endocrinol. Metab.* 83, 1407–1413. doi: 10.1210/jc.83.5.1407

Frank, M. G., Watkins, L. R., and Maier, S. F. (2013). Stress-induced glucocorticoids as a neuroendocrine alarm signal of danger. *Brain Behav. Immun.* doi: 10.1016/j.bbi.2013.02.004. [Epub ahead of print].

Friedman, J. M. (2004). Modern science versus the stigma of obesity. *Nat. Med.* 10, 563–569. doi: 10.1038/nm0604-563

Fuglei, E., Mustonen, A. M., and Nieminen, P. (2004). Effects of season, food deprivation and re-feeding on leptin, ghrelin and growth hormone in arctic foxes (*Alopex lagopus*) on Svalbard, Norway. *J. Comp. Physiol. B* 174, 157–162. doi: 10.1007/s00360-003-0400-6

Gil-Campos, M., Aguilera, C. M., Canete, R., and Gil, A. (2006). Ghrelin: a hormone regulating food intake and energy homeostasis. *Br. J. Nutr.* 96, 201–226. doi: 10.1079/BJN20061787

Gutzler, S. J., Karom, M., Erwin, W. D., and Albers, H. E. (2009). Photoperiodic regulation of adrenal hormone secretion and aggression in female Syrian hamsters. *Horm. Behav.* 56, 481–489. doi: 10.1016/j.yhbeh.2009.08.007

Gwinner, E. (2003). Circannual rhythms in birds. *Curr. Opin. Neurobiol.* 13, 770–778. doi: 10.1016/j.conb.2003.10.010

Haga, O. E. (1993). *A Seasonal Study of the Energetics of Artic Foxes from Svalbard*. Tromsø: University of Tromsø.

Hansen, A. M., Garde, A. H., Skovgaard, L. T., and Christensen, J. M. (2001). Seasonal and biological variation of urinary epinephrine, norepinephrine, and cortisol in healthy women. *Clin. Chim. Acta* 309, 25–35. doi: 10.1016/S0009-8981(01)00493-4

Harbuz, M. S., and Lightman, S. L. (1992). Stress and the hypothalamo-pituitary-adrenal axis: acute, chronic and immunological activation. *J. Endocrinol.* 134, 327–339. doi: 10.1677/joe.0.1340327

Hodos, W. (1961). Progressive ratio as a measure of reward strength. *Science* 134, 943–944. doi: 10.1126/science.134.3483.943

Hodos, W., and Kalman, G. (1963). Effects of increment size and reinforcer volume on progressive ratio performance. *J. Exp. Anal. Behav.* 6, 387–392. doi: 10.1901/jeab.1963.6-387

Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z. W., Gao, X. B., et al. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 51, 801–810. doi: 10.1016/j.neuron.2006.08.023

Hosoda, H., Kojima, M., and Kangawa, K. (2002). Ghrelin and the regulation of food intake and energy balance. *Mol. Interv.* 2, 494–503. doi: 10.1124/mi.2.8.494

Hunter, R. G., Bellani, R., Bloss, E., Costa, A., Romeo, R. D., and McEwen, B. S. (2007). Regulation of CART mRNA by stress and corticosteroids in the hippocampus and amygdala. *Brain Res.* 1152, 234–240. doi: 10.1016/j.brainres.2007.03.042

Janzen, W. J., Duncan, C. A., and Riley, L. G. (2012). Cortisol treatment reduces ghrelin signaling and food intake in tilapia, *Oreochromis mossambicus*. *Domest. Anim. Endocrinol.* 43, 251–259. doi: 10.1016/j.domaniend.2012.04.003

Jerlhag, E., Egecioglu, E., Dickson, S. L., Andersson, M., Svensson, L., and Engel, J. A. (2006). Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. *Addict. Biol.* 11, 45–54. doi: 10.1111/j.1369-1600.2006.00002.x

Jerlhag, E., Egecioglu, E., Dickson, S. L., and Engel, J. A. (2010). Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release,

and conditioned place preference. *Psychopharmacology (Berl.)* 211, 415–422. doi: 10.1007/s00213-010-1907-7

Jerlhag, E., Egecioglu, E., Dickson, S. L., and Engel, J. A. (2011a). Glutamatergic regulation of ghrelin-induced activation of the mesolimbic dopamine system. *Addict. Biol.* 16, 82–91. doi: 10.1111/j.1369-1600.2010.00231.x

Jerlhag, E., Landgren, S., Egecioglu, E., Dickson, S. L., and Engel, J. A. (2011b). The alcohol-induced locomotor stimulation and accumbal dopamine release is suppressed in ghrelin knockout mice. *Alcohol* 45, 341–347. doi: 10.1016/j.alcohol.2010.10.002

Johnstone, C. P., Reina, R. D., and Lill, A. (2012). Interpreting indices of physiological stress in free-living vertebrates. *J. Comp. Physiol. B* 182, 861–879. doi: 10.1007/s00360-012-0656-9

Kalra, S. P., Bagnasco, M., Otukonyong, E. E., Dube, M. G., and Kalra, P. S. (2003). Rhythmic, reciprocal ghrelin and leptin signaling: new insight in the development of obesity. *Regul. Pept.* 111, 1–11. doi: 10.1016/S0167-0115(02)00305-1

Keen-Rhinehart, E., and Bartness, T. J. (2005). Peripheral ghrelin injections stimulate food intake, foraging, and food hoarding in Siberian hamsters. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, R716–R722. doi: 10.1152/ajpregu.00705.2004

Kerr, D. S., Campbell, L. W., Applegate, M. D., Brodish, A., and Landfield, P. W. (1991). Chronic stress-induced acceleration of electrophysiologic and morphometric biomarkers of hippocampal aging. *J. Neurosci.* 11, 1316–1324.

King, J. A., Rosal, M. C., Ma, Y., Reed, G., Kelly, T. A., Stanek, E. J. 3rd., et al. (2000). Sequence and seasonal effects of salivary cortisol. *Behav. Med.* 26, 67–73. doi: 10.1080/08964280009595753

Klingenspor, M., Dickopp, A., Heldmaier, G., and Klaus, S. (1996). Short photoperiod reduces leptin gene expression in white and brown adipose tissue of Djungarian hamsters. *FEBS Lett.* 399, 290–294. doi: 10.1016/S0014-5793(96)01343-9

Klingenspor, M., Niggemann, H., and Heldmaier, G. (2000). Modulation of leptin sensitivity by short photoperiod acclimation in the Djungarian hamster, *Phodopus sungorus*. *J. Comp. Physiol. B* 170, 37–43. doi: 10.1007/s003600050005

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402, 656–660. doi: 10.1038/45230

Kojima, M., and Kangawa, K. (2002). Ghrelin, an orexigenic signaling molecule from the gastrointestinal tract. *Curr. Opin. Pharmacol.* 2, 665–668. doi: 10.1016/S1471-4892(02)00220-5

Konno, J., Yoshida, S., Ina, A., Ohmomo, H., Shutoh, F., Nogami, H., et al. (2008). Upregulated expression of neuropeptide Y in hypothalamic-pituitary system of rats by chronic dexamethasone administration. *Neurosci. Res.* 60, 259–265. doi: 10.1016/j.neures.2007.11.005

Korhonen, T., Mustonen, A. M., Nieminen, P., and Saarela, S. (2008). Effects of cold exposure, exogenous melatonin and short-day treatment on the weight-regulation and body temperature of the Siberian hamster (*Phodopus sungorus*). *Regul. Pept.* 149, 60–66. doi: 10.1016/j.regpep.2007.09.033

Korhonen, T., and Saarela, S. (2005). Role of adiposity hormones in the mouse during fasting and winter-acclimatization. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 140, 217–223. doi: 10.1016/j.cbpb.2005.01.006

Krugel, U., Schraft, T., Kittner, H., Kiess, W., and Illes, P. (2003). Basal and feeding-evoked dopamine release in the rat nucleus accumbens is depressed by leptin. *Eur. J. Pharmacol.* 482, 185–187. doi: 10.1016/j.ejphar.2003.09.047

Landgren, S., Simms, J. A., Thelle, D. S., Strandhagen, E., Bartlett, S. E., Engel, J. A., et al. (2011). The ghrelin signalling system is involved in the consumption of sweets. *PLoS ONE* 6:e18170. doi: 10.1371/journal.pone.0018170

Leshan, R. L., Opland, D. M., Louis, G. W., Leinninger, G. M., Patterson, C. M., Rhodes, C. J., et al. (2010). Ventral tegmental area leptin receptor neurons specifically project to and regulate cocaine- and amphetamine-regulated transcript neurons of the extended central amygdala. *J. Neurosci.* 30, 5713–5723. doi: 10.1523/JNEUROSCI.1001-10.2010

Levine, A. S., and Morley, J. E. (1981). Stress-induced eating in rats. *Am. J. Physiol.* 241, R72–R76.

Li, X. S., and Wang, D. H. (2005). Regulation of body weight and thermogenesis in seasonally acclimatized Brandt's voles (Microtus brandti). *Horm. Behav.* 48, 321–328. doi: 10.1016/j.yhbeh.2005.04.004

Lightman, S. L. (2008). The neuroendocrinology of stress: a never ending story. *J. Neuroendocrinol.* 20, 880–884. doi: 10.1111/j.1365-2826.2008.01711.x

Liu, X. Y., Shi, J. H., Du, W. H., Fan, Y. P., Hu, X. L., Zhang, C. C., et al. (2011). Glucocorticoids decrease body weight and food intake and inhibit appetite regulatory peptide expression in the hypothalamus of rats. *Exp. Ther. Med.* 2, 977–984.

Loudon, A. S. (1994). Photoperiod and the regulation of annual and circannual cycles of food intake. *Proc. Nutr. Soc.* 53, 495–507. doi: 10.1079/PNS19940060

Lutter, M., Krishnan, V., Russo, S. J., Jung, S., McClung, C. A., and Nestler, E. J. (2008). Orexin signaling mediates the antidepressant-like effect of calorie restriction. *J. Neurosci.* 28, 3071–3075. doi: 10.1523/JNEUROSCI.5584-07.2008

Malarkey, W. B., Pearl, D. K., Demers, L. M., Kiecolt-Glaser, J. K., and Glaser, R. (1995). Influence of academic stress and season on 24-hour mean concentrations of ACTH, cortisol, and beta-endorphin. *Psychoneuroendocrinology* 20, 499–508. doi: 10.1016/0306-4530(94)00077-N

Maniam, J., and Morris, M. J. (2012). The link between stress and feeding behaviour. *Neuropharmacology* 63, 97–110. doi: 10.1016/j.neuropharm.2012.04.017

Marinelli, M., and Piazza, P. V. (2002). Interaction between glucocorticoid hormones, stress and psychostimulant drugs. *Eur. J. Neurosci.* 16, 387–394. doi: 10.1046/j.1460-9568.2002.02089.x

Matalka, K. Z., and Sidki, A. (1998). Academic stress - Influence on leukocyte distribution, cortisol, and prolactin. *Lab. Med.* 29, 697–702.

Matchock, R. L., Dorn, L. D., and Susman, E. J. (2007). Diurnal and seasonal cortisol, testosterone, and DHEA rhythms in boys and girls during puberty. *Chronobiol. Int.* 24, 969–990. doi: 10.1080/07420520701649471

McEwen, B. S. (2000). The neurobiology of stress: from serendipity to clinical relevance. *Brain Res.* 886, 172–189. doi: 10.1016/S0006-8993(00)02950-4

McEwen, B. S., and Sapolsky, R. M. (1995). Stress and cognitive function. *Curr. Opin. Neurobiol.* 5, 205–216. doi: 10.1016/0959-4388(95)80028-X

Mercer, J. G. (1998). Regulation of appetite and body weight in seasonal mammals. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 119, 295–303. doi: 10.1016/S0742-8413(98)00018-8

Mistlberger, R. E. (2011). Neurobiology of food anticipatory circadian rhythms. *Physiol. Behav.* 104, 535–545. doi: 10.1016/j.physbeh.2011.04.015

Mooring, M. S., Patton, M. L., Lance, V. A., Hall, B. M., Schaad, E. W., Fetter, G. A., et al. (2006). Glucocorticoids of bison bulls in relation to social status. *Horm. Behav.* 49, 369–375. doi: 10.1016/j.yhbeh.2005.08.008

Morley, J. E., Levine, A. S., and Rowland, N. E. (1983). Minireview. Stress induced eating. *Life Sci.* 32, 2169–2182. doi: 10.1016/0024-3205(83)90415-0

Mustonen, A. M., Pyykonen, T., Asikainen, J., Hanninen, S., Mononen, J., and Nieminen, P. (2005a). Circannual leptin and ghrelin levels of the blue fox (*Alopex lagopus*) in reference to seasonal rhythms of body mass, adiposity, and food intake. *J. Exp. Zool. A Comp. Exp. Biol.* 303, 26–36.

Mustonen, A. M., Pyykonen, T., Paakkonen, T., Ryokkynen, A., Asikainen, J., Aho, J., et al. (2005b). Adaptations to fasting in the American mink (Mustela vison): carbohydrate and lipid metabolism. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 140, 195–202. doi: 10.1016/j.cbpb.2004.12.004

Narayanan, N. S., Guarnieri, D. J., and Dileone, R. J. (2010). Metabolic hormones, dopamine circuits, and feeding. *Front. Neuroendocrinol.* 31, 104–112. doi: 10.1016/j.yfrne.2009.10.004

Nestler, E. J., and Carlezon, W. A. Jr. (2006). The mesolimbic dopamine reward circuit in depression. *Biol. Psychiatry* 59, 1151–1159. doi: 10.1016/j.biopsych.2005.09.018

Nunes, S., Pelz, K. M., Muecke, E. M., Holekamp, K. E., and Zucker, I. (2006). Plasma glucocorticoid concentrations and body mass in ground squirrels: seasonal variation and circannual organization. *Gen. Comp. Endocrinol.* 146, 136–143. doi: 10.1016/j.ygcen.2005.10.013

Nyuyki, K. D., Beiderbeck, D. I., Lukas, M., Neumann, I. D., and Reber, S. O. (2012). Chronic subordinate colony housing (CSC) as a model of chronic psychosocial stress in male rats. *PLoS ONE* 7:e52371. doi: 10.1371/journal.pone.0052371

Oliver, G., and Wardle, J. (1999). Perceived effects of stress on food choice. *Physiol. Behav.* 66, 511–515. doi: 10.1016/S0031-9384(98)00322-9

Ota, T., Fustin, J. M., Yamada, H., Doi, M., and Okamura, H. (2012). Circadian clock signals in the adrenal cortex. *Mol. Cell. Endocrinol.* 349, 30–37. doi: 10.1016/j.mce.2011.08.010

Otsuka, T., Goto, M., Kawai, M., Togo, Y., Sato, K., Katoh, K., et al. (2012). Photoperiod regulates corticosterone rhythms by altered adrenal sensitivity via melatonin-independent mechanisms in Fischer 344 rats and C57BL/6J mice. *PLoS ONE* 7:e39090. doi: 10.1371/journal.pone.0039090

Ottenweller, J. E., Tapp, W. N., Pitman, D. L., and Natelson, B. H. (1987). Adrenal, thyroid, and testicular hormone rhythms in male golden hamsters on long and short days. *Am. J. Physiol.* 253, R321–R328.

Pankevich, D. E., Teegarden, S. L., Hedin, A. D., Jensen, C. L., and Bale, T. L. (2010). Caloric restriction experience reprograms stress and orexigenic pathways and promotes binge eating. *J. Neurosci.* 30, 16399–16407. doi: 10.1523/JNEUROSCI.1955-10.2010

Papadimitriou, A., and Priftis, K. N. (2009). Regulation of the hypothalamic-pituitary-adrenal axis. *Neuroimmunomodulation* 16, 265–271. doi: 10.1159/000216184

Paul, M. J., Zucker, I., and Schwartz, W. J. (2008). Tracking the seasons: the internal calendars of vertebrates. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 341–361. doi: 10.1098/rstb.2007.2143

Pecoraro, N., Reyes, F., Gomez, F., Bhargava, A., and Dallman, M. F. (2004). Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. *Endocrinology* 145, 3754–3762. doi: 10.1210/en.2004-0305

Perello, M., Sakata, I., Birnbaum, S., Chuang, J. C., Osborne-Lawrence, S., Rovinsky, S. A., et al. (2010). Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. *Biol. Psychiatry* 67, 880–886. doi: 10.1016/j.biopsych.2009.10.030

Perello, M., and Zigman, J. M. (2012). The role of ghrelin in reward-based eating. *Biol. Psychiatry* 72, 347–353. doi: 10.1016/j.biopsych.2012.02.016

Perry, M. L., Leinninger, G. M., Chen, R., Luderman, K. D., Yang, H., Gnegy, M. E., et al. (2010). Leptin promotes dopamine transporter and tyrosine hydroxylase activity in the nucleus accumbens of Sprague-Dawley rats. *J. Neurochem.* 114, 666–674. doi: 10.1111/j.1471-4159.2010.06757.x

Pfaffly, J., Michaelides, M., Wang, G. J., Pessin, J. E., Volkow, N. D., and Thanos, P. K. (2010). Leptin increases striatal dopamine D2 receptor binding in leptin-deficient obese (ob/ob) mice. *Synapse* 64, 503–510. doi: 10.1002/syn.20755

Pyter, L. M., Adelson, J. D., and Nelson, R. J. (2007). Short days increase hypothalamic-pituitary-adrenal axis responsiveness. *Endocrinology* 148, 3402–3409. doi: 10.1210/en.2006-1432

Richardson, N. R., and Gratton, A. (1998). Changes in medial prefrontal cortical dopamine levels associated with response-contingent food reward: an electrochemical study in rat. *J. Neurosci.* 18, 9130–9138.

Romero, L. M. (2002). Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol.* 128, 1–24. doi: 10.1016/S0016-6480(02)00064-3

Romero, L. M., Meister, C. J., Cyr, N. E., Kenagy, G. J., and Wingfield, J. C. (2008). Seasonal glucocorticoid responses to capture in wild free-living mammals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R614–R622. doi: 10.1152/ajpregu.00752.2007

Rouge-Pont, F., Marinelli, M., Le Moal, M., Simon, H., and Piazza, P. V. (1995). Stress-induced sensitization and glucocorticoids. II. Sensitization of the increase in extracellular dopamine induced by cocaine depends on stress-induced corticosterone secretion. *J. Neurosci.* 15, 7189–7195.

Rousseau, K., Atcha, Z., Cagampang, F. R., Le Rouzic, P., Stirland, J. A., Ivanov, T. R., et al. (2002). Photoperiodic regulation of leptin resistance in the seasonally breeding Siberian hamster (*Phodopus sungorus*). *Endocrinology* 143, 3083–3095. doi: 10.1210/en.143.8.3083

Rousseau, K., Atcha, Z., and Loudon, A. S. (2003). Leptin and seasonal mammals. *J. Neuroendocrinol.* 15, 409–414. doi: 10.1046/j.1365-2826.2003.01007.x

Rutters, F., Nieuwenhuizen, A. G., Lemmens, S. G., Born, J. M., and Westerterp-Plantenga, M. S. (2009). Acute stress-related changes in eating in the absence of hunger. *Obesity (Silver Spring)* 17, 72–77. doi: 10.1038/oby.2008.493

Schmid, D. A., Held, K., Ising, M., Uhr, M., Weikel, J. C., and Steiger, A. (2005). Ghrelin stimulates appetite, imagination of food, GH, ACTH, and cortisol, but does not affect leptin in normal controls. *Neuropsychopharmacology* 30, 1187–1192. doi: 10.1038/sj.npp.1300670

Schuhler, S., and Ebling, F. J. (2006). Role of melanocortin in the long-term regulation of energy balance: lessons from a seasonal model. *Peptides* 27, 301–309. doi: 10.1016/j.peptides.2005.03.060

Schwabe, L., Dickinson, A., and Wolf, O. T. (2011). Stress, habits, and drug addiction: a psychoneuroendocrinological perspective. *Exp. Clin. Psychopharmacol.* 19, 53–63. doi: 10.1037/a0022212

Schwabe, L., and Wolf, O. T. (2011). Stress-induced modulation of instrumental behavior: from goal-directed to habitual control of action. *Behav. Brain Res.* 219, 321–328. doi: 10.1016/j.bbr.2010.12.038

Shiiya, T., Nakazato, M., Mizuta, M., Date, Y., Mondal, M. S., Tanaka, M., et al. (2002). Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J. Clin. Endocrinol. Metab.* 87, 240–244. doi: 10.1210/jc.87.1.240

Skibicka, K. P., and Dickson, S. L. (2011). Ghrelin and food reward: the story of potential underlying substrates. *Peptides* 32, 2265–2273. doi: 10.1016/j.peptides.2011.05.016

Skibicka, K. P., Hansson, C., Alvarez-Crespo, M., Friberg, P. A., and Dickson, S. L. (2011). Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* 180, 129–137. doi: 10.1016/j.neuroscience.2011.02.016

Steinlechner, S., and Heldmaier, G. (1982). Role of photoperiod and melatonin in seasonal acclimatization of the Djungarian hamster, *Phodopus sungorus*. *Int. J. Biometeorol.* 26, 329–337. doi: 10.1007/BF02219503

Theander-Carrillo, C., Wiedmer, P., Cettour-Rose, P., Nogueiras, R., Perez-Tilve, D., Pfluger, P., et al. (2006). Ghrelin action in the brain controls adipocyte metabolism. *J. Clin. Invest.* 116, 1983–1993. doi: 10.1172/JCI25811

Thompson, J. L., and Borgland, S. L. (2013). Presynaptic leptin action suppresses excitatory synaptic transmission onto ventral tegmental area dopamine neurons. *Biol. Psychiatry* 73, 860–868. doi: 10.1016/j.biopsych.2012.10.026

Thorn, L., Evans, P., Cannon, A., Hucklebridge, F., and Clow, A. (2011). Seasonal differences in the diurnal pattern of cortisol secretion in healthy participants and those with self-assessed seasonal affective disorder. *Psychoneuroendocrinology* 36, 816–823. doi: 10.1016/j.psyneuen.2010.11.003

Thorndike, E. L. (1911). *Animal Intelligence Experimental Studies.* New York, NY: Macmillan. doi: 10.5962/bhl.title.55072

Tryon, M. S., Decant, R., and Laugero, K. D. (2013). Having your cake and eating it too: a habit of comfort food may link chronic social stress exposure and acute stress-induced cortisol hyporesponsiveness. *Physiol. Behav.* 114–115, 32–37. doi: 10.1016/j.physbeh.2013.02.018

Tschop, M., Smiley, D. L., and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* 407, 908–913. doi: 10.1038/35038090

Tups, A., Helwig, M., Khorooshi, R. M., Archer, Z. A., Klingenspor, M., and Mercer, J. G. (2004). Circulating ghrelin levels and central ghrelin receptor expression are elevated in response to food deprivation in a seasonal mammal (*Phodopus sungorus*). *J. Neuroendocrinol.* 16, 922–928. doi: 10.1111/j.1365-2826.2004.01251.x

Underwood, L. S. (1971). *The Bioenergetics of the Artic Fox.* University Park: Pennsylvania State University.

van Zessen, R., van der Plasse, G., and Adan, R. A. (2012). Contribution of the mesolimbic dopamine system in mediating the effects of leptin and ghrelin on feeding. *Proc. Nutr. Soc.* 71, 435–445. doi: 10.1017/S0029665112000614

Vera, F., Antenucci, C. D., and Zenuto, R. R. (2011). Cortisol and corticosterone exhibit different seasonal variation and responses to acute stress and captivity in tuco-tucos (*Ctenomys talarum*). *Gen. Comp. Endocrinol.* 170, 550–557. doi: 10.1016/j.ygcen.2010.11.012

Volkoff, H., Hoskins, L. J., and Tuziak, S. M. (2010). Influence of intrinsic signals and environmental cues on the endocrine control of feeding in fish: potential application in aquaculture. *Gen. Comp. Endocrinol.* 167, 352–359. doi: 10.1016/j.ygcen.2009.09.001

Vondrasova, D., Hajek, I., and Illnerova, H. (1997). Exposure to long summer days affects the human melatonin and cortisol rhythms. *Brain Res.* 759, 166–170. doi: 10.1016/S0006-8993(97)00358-2

Vondrasova-Jelinkova, D., Hajek, I., and Illnerova, H. (1999). Adjustment of the human melatonin and cortisol rhythms to shortening of the natural summer photoperiod. *Brain Res.* 816, 249–253. doi: 10.1016/S0006-8993(98)01189-5

Walker, B. R., Best, R., Noon, J. P., Watt, G. C., and Webb, D. J. (1997). Seasonal variation in glucocorticoid activity in healthy men. *J. Clin. Endocrinol. Metab.* 82, 4015–4019. doi: 10.1210/jc.82.12.4015

Wells, T. (2009). Ghrelin - Defender of fat. *Prog. Lipid Res.* 48, 257–274. doi: 10.1016/j.plipres.2009.04.002

Wrobel, A., Zebrowska-Lupina, I., and Wielosz, M. (2005). Dexamethasone reduces locomotor stimulation induced by dopamine agonists in mice. *Pharmacol. Rep.* 57, 451–457.

Zellner, D. A., Loaiza, S., Gonzalez, Z., Pita, J., Morales, J., Pecora, D., et al. (2006). Food selection changes under stress. *Physiol. Behav.* 87, 789–793. doi: 10.1016/j.physbeh.2006.01.014

Zellner, D. A., Saito, S., and Gonzalez, J. (2007). The effect of stress on men's food selection. *Appetite* 49, 696–699. doi: 10.1016/j.appet.2007.06.013

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* 372, 425–432. doi: 10.1038/372425a0

Zhao, Z. J., Chen, J. F., and Wang, D. H. (2010). Diet-induced obesity in the short-day-lean Brandt's vole. *Physiol. Behav.* 99, 47–53. doi: 10.1016/j.physbeh.2009.10.008

Zhao, Z. J., and Wang, D. H. (2006). Short photoperiod influences energy intake and serum leptin level in Brandt's voles (Microtus brandtii). *Horm. Behav.* 49, 463–469. doi: 10.1016/j.yhbeh.2005.10.003

Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B., and Elmquist, J. K. (2006). Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J. Comp. Neurol.* 494, 528–548. doi: 10.1002/cne.20823

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Perinatal programming of neuroendocrine mechanisms connecting feeding behavior and stress

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Feeding behavior is closely regulated by neuroendocrine mechanisms that can be influenced by stressful life events. However, the feeding response to stress varies among individuals with some increasing and others decreasing food intake after stress. In addition to the impact of acute lifestyle and genetic backgrounds, the early life environment can have a life-long influence on neuroendocrine mechanisms connecting stress to feeding behavior and may partially explain these opposing feeding responses to stress. In this review I will discuss the perinatal programming of adult hypothalamic stress and feeding circuitry. Specifically I will address how early life (prenatal and postnatal) nutrition, early life stress, and the early life hormonal profile can program the hypothalamic-pituitary-adrenal (HPA) axis, the endocrine arm of the body's response to stress long-term and how these changes can, in turn, influence the hypothalamic circuitry responsible for regulating feeding behavior. Thus, over- or under-feeding and/or stressful events during critical windows of early development can alter glucocorticoid (GC) regulation of the HPA axis, leading to changes in the GC influence on energy storage and changes in GC negative feedback on HPA axis-derived satiety signals such as corticotropin-releasing-hormone. Furthermore, peripheral hormones controlling satiety, such as leptin and insulin are altered by early life events, and can be influenced, in early life and adulthood, by stress. Importantly, these neuroendocrine signals act as trophic factors during development to stimulate connectivity throughout the hypothalamus. The interplay between these neuroendocrine signals, the perinatal environment, and activation of the stress circuitry in adulthood thus strongly influences feeding behavior and may explain why individuals have unique feeding responses to similar stressors.

Keywords: hypothalamic-pituitary-adrenal axis, glucocorticoids, development, leptin, insulin

INTRODUCTION

How an individual responds to stress and how this influences their feeding behavior is governed by many factors, including genetic influence and the proximal environment. For instance, body mass index, an outcome closely associated with diet (Duvigneaud et al., 2007; Wan et al., 2009), is thought to be 40–70% heritable (Loos, 2009), but a person's social group also has significant influence over their food choices (Dabbaghian et al., 2012; Robinson and Higgs, 2012) and there is even seasonal variation in food intake, with people consuming more fat in the winter and spring (Van Staveren et al., 1986; Watson and McDonald, 2007). In addition to these examples, immediate life stress can influence feeding behavior. For example, perceived stress over long periods, such as economic difficulties and job-related demands, is associated with excess weight gain (Block et al., 2009; Fowler-Brown et al., 2009). Social status in humans, and chronic social subordination and reorganization of the social group in macaques, is linked with obesity, increased central (visceral) fat, and indices of metabolic syndrome (Shively and Clarkson, 1988; Jayo et al., 1993; Brunner et al., 1997; Shively, 1998; Shively et al., 2009). These types of stressors encourage negative eating behaviors that are likely to precipitate or contribute to weight gain. Thus, those with high job stress are more likely to eat until they are full and more likely to eat to control mood (Nishitani et al., 2009).

Stress and elevated glucocorticoids (GC) also tend to encourage appetite specifically for high energy highly palatable foods (La Fleur et al., 2004; Warne et al., 2006, 2009; Dallman, 2010). Rats exposed to chronic stress (daily 3 h restraint stress for 5 days) prefer calorically dense foods, such as lard and sucrose, relative to non-stressed rats (Pecoraro et al., 2004). Hypothalamic-pituitary-adrenal (HPA) axis responses to restraint are even attenuated by these high calorie foods, indicating such "comfort eating" can actually help control HPA axis reactivity to stress (Pecoraro et al., 2004). This ability of high energy foods to ameliorate stress responses is reinforced by the influence of foods on reward pathways (Bassareo and Di Chiara, 1997, 1999). Similar brain circuitry is recruited by calorically dense food as by drugs of addiction and, in this regard, the drive to eat highly palatable food is significantly correlated with the drive to consume drugs of abuse (Gosnell, 2000; Nieuwenhuizen and Rutters, 2008; Coccurello et al., 2009). These reward outcomes are largely mediated by the nucleus accumbens. Nucleus accumbens dopaminergic, opioid, and glutamatergic transmission, for instance, are important for mediating reward-associated information, irrespective of whether it is related to drugs (Di Chiara and Imperato, 1988; Koob and

Le Moal, 2001; Ito et al., 2004), sex (Balfour et al., 2004), or palatable food (Berridge, 2009). Additionally, the only peptide known to stimulate food intake, ghrelin (Hosoda et al., 2006), promotes the rewarding feeling food gives (Egecioglu et al., 2010) and is also involved in the feeling of reward elicited by alcohol and psychostimulant drugs (Jerlhag et al., 2009, 2010).

Despite direct lifestyle factors influencing feeding, it is also clear that given similar diets, activity levels, and life stress, individuals do not necessarily respond with the same feeding style or maintain the same body weights (Eilat-Adar et al., 2005; Matsuo et al., 2009). It is now evident the perinatal environment is equally, if not more, important in programming how an individual responds to stress, its feeding patterns, and how stress and feeding influence one another. The early life environment may therefore account for some of these inter-personal differences. This review will discuss the impact of the perinatal environment on the interplay between stress and feeding behavior.

EARLY LIFE STRESS PROGRAMS THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS AND HYPOTHALAMIC FEEDING CIRCUITRY TO INFLUENCE FEEDING BEHAVIOR LONG TERM

PRENATAL STRESS

It is now clear that early life stress is an important programming factor in a number of aspects of physiology. Foetal, neonatal, and postnatal stress are certainly important in determining adult vulnerability to dysfunction in the neuroendocrine systems regulating both the HPA axis and food intake under stressed and non-stressed conditions.

During pregnancy the foetus is relatively protected from the effects of stress experienced by the mother. In pregnancy, progesterone and its metabolite, allopregnanolone, are increased in the maternal brain. Allopregnanolone usually inhibits the noradrenergic input to the paraventricular nucleus of the hypothalamus (PVN) from the nucleus of the solitary tract, which stimulates the HPA axis response to stress (systemic interleukin 1β). This inhibition is enhanced with the higher concentrations of allopregnanolone, and the response to stress further inhibited (Brunton et al., 2005, 2009; Brunton and Russell, 2011). Thus, pregnant animals have a smaller increase in corticosterone in response to stress than non-pregnant animals do (Brunton et al., 2005; Slattery and Neumann, 2008; Brunton et al., 2009). In addition, during pregnancy the placenta produces significant amounts of 11β-hydroxysteroid dehydrogenase (HSD)2, which converts GC from the active to the inactive form before they can reach the foetus, protecting it from GC in the maternal circulation (Lucassen et al., 2009).

Such elaborate mechanisms to protect the foetus from the effects of stress would suggest such effects are likely to be dangerous, and certainly in cases of severe or chronic maternal stress excess GC may still negatively affect the foetus. Severe stress (e.g., death of a close relative in humans or restraint in rodents) during pregnancy can affect foetal brain development (Henry et al., 1994; Rossi-George et al., 2009), can influence mood regulation and predispose to anxiety behaviors (Vallee et al., 1997), can disrupt learning and memory (Lordi et al., 1997; Entringer et al., 2009), and can enhance sensitivity to drug abuse (Morley-Fletcher et al., 2004; Thomas et al., 2009).

Prenatal stress can also influence feeding behavior long-term. Babies born to mothers who experienced the death of a family member immediately before or during pregnancy have a significantly increased risk they will gain excess weight in childhood (Li et al., 2010). These prenatal stress effects are also evident in animal models (Tamashiro et al., 2009). Rat pups born to dams that were exposed to chronic variable stress during pregnancy are hyperphagic immediately after birth (Purcell et al., 2011) and throughout life when fed normal rat chow (Pankevich et al., 2009).

These effects of prenatal stress are associated with short- and long-term changes in GC regulation and the HPA axis. In the short term, chronic variable stress reduces 11β-HSD2 levels in the placenta of the female foetus, allowing GC access to influence the foetus (Pankevich et al., 2009). Prenatal restraint stress given to rat dams also leads to dysregulated corticosterone responses to mild stress in the offspring when they are tested in adulthood (Henry et al., 1994; Maccari et al., 1995, 2003; Koehl et al., 1999; Rossi-George et al., 2009). This effect on HPA axis function is likely to be due to reduced mineralocorticoid and glucocorticoid receptor (GR) expression at the level of the hippocampus (Henry et al., 1994; Maccari et al., 1995) and is linked with susceptibility to obesity and indices of diabetes if the animals are later fed a high fat diet (Tamashiro et al., 2009). Similarly, prenatal dexamethasone (a synthetic GC) leads to hyperactivity of the HPA axis and to obesity long-term (Dahlgren et al., 2001).

POSTNATAL STRESS

Postnatal stress can also lead to a predisposition to HPA axis dysfunction, appetite dysregulation, and obesity in adulthood. Early psychological trauma in humans such as sexual abuse, the death of a mother or significant family member, or parental stress, is a significant risk factor for the development of obesity in later life (Koch et al., 2008; D'Argenio et al., 2009). Children from families reporting high levels of stress are significantly more likely to be obese than those from families without such stress (Koch et al., 2008; Moens et al., 2009). Similarly, exposure to war-related events during childhood has been associated with a higher body mass index later in life (Llabre and Hadi, 2009).

In rodents, the postnatal period is also one of particular vulnerability to the programming effects of stress and GC. In the rat, the first to second weeks of life are distinctive as a stress hypo-responsive period (Sapolsky and Meaney, 1986). At this time, the rat has low basal corticosterone and adrenocorticotropic hormone (ACTH) concentrations. It also does not respond to stress to the same degree as adult rats do (Levine, 2002), an effect that is likely due to an extremely efficient GC negative feedback response (Dent et al., 2000; Vazquez et al., 2006). In humans, this period is approximately analogous to the first 12 months of life (Gunnar and Donzella, 2002). The stress hypo-responsive period is contingent upon the continued presence of and feeding from the dam (Tilbrook et al., 2006) and parenting style during this time, categorized by high- or low-intensity nursing and grooming, can predict later-life responses of the HPA axis to stress (Liu et al., 1997;

Champagne and Meaney, 2001). The stress hypo-responsive period and HPA axis development can also be affected by early life stress.

In rodents, separation from the mother is a significant psychological stress to the neonate and has long-lasting physiological effects. It leads to anxiety in adulthood, affects basal plasma corticosterone and ACTH, alters GR expression in the brain, and exacerbates the corticosterone response to stress (Lehmann et al., 2002a,b; Barna et al., 2003; Xu et al., 2011). Maternal separation of this type in early life can lead to a life-long reduction in food intake and a significant aversion to foods high in carbohydrates, compared with no maternal separation (Penke et al., 2001). Maternal separation also increases vulnerability to the effects of stress on feeding regulation. So, in a study where maternal separation or social isolation alone were insufficient to affect feeding behavior, food intake was significantly increased, as was weight gain, in socially isolated rats that had previously undergone maternal separation (Ryu et al., 2009).

Perinatal stress thus clearly influences HPA axis function long-term and this can significantly impact upon feeding behavior. However, other early life perturbations can also influence this circuitry. Perinatal nutrition is an important example.

EARLY LIFE NUTRITION PROGRAMS HPA AXIS AND HYPOTHALAMIC FEEDING CIRCUITRY TO INFLUENCE FEEDING BEHAVIOR LONG TERM

PRENATAL NUTRITION

Before an infant is even exposed to the semi-direct influence of diet or stress from the mother, it inherits a dietary legacy from the father that can contribute to the development of stress- and feeding-related pathways. Obesity in the father, for instance, can damage the sperm; reducing concentration, motility, and morphology, and contributing to DNA damage (Kasturi et al., 2008), damage that can be ameliorated by diet and exercise interventions (Palmer et al., 2012). Paternal obesity increases the risk a baby will be born small (Power et al., 2003) and, in girls, increases the risk of higher adiposity levels pre-puberty (Figueroa-Colon et al., 2000). High fat diet in the sire in rodents can also induce β-cell dysfunction long-term in the offspring contributing to the development of diabetes symptoms (Ng et al., 2010).

Unsurprisingly, maternal diet and adiposity also contribute to the offspring's development (Guillaume et al., 1995; Parsons et al., 2001). There is a clearly established relationship between maternal obesity and offspring obesity (Dabelea et al., 2000; Ruager-Martin et al., 2010) and the children of obese mothers are more likely to develop metabolic complications such as diabetes in later life (Dabelea et al., 2000; Boney et al., 2005). There is also evidence to suggest obesity *per se* is not necessary to influence offspring feeding patterns and metabolism. A maternal diet that is high in fat or a maternal "junk food" diet leads to malformation of central reward pathways in the offspring. The rewarding nature of food is heightened and these offspring come to preferentially select high fat, high sucrose foods (Ong and Muhlhausler, 2011; Gugusheff et al., 2013). This diet in the mother leads to hyperinsulinaemia, insulin resistance, and increased fat deposition in the offspring (Albuquerque et al., 2006; Srinivasan et al., 2006; Ashino et al., 2012).

Severe prenatal malnutrition is a unique type of nutritional stress that can also program changes to the HPA axis and feeding circuitry. The Dutch Famine, or Dutch Hunger Winter, of 1944–1945 was a devastating period of serious malnutrition and starvation as a result of the final battles and aftermath of World War II that affected much of the population of the Netherlands. Studies of victims of this disaster have revealed that inadequate nutrition in the first trimester of gestation (but not the last trimester) leads to significant obesity and metabolic sequelae in young adult males (Ravelli et al., 1976) and middle-aged females (Ravelli et al., 1999; Roseboom et al., 2000a,b). This prenatal under-nutrition also predisposes people to prefer a diet that is high in fat (Lussana et al., 2008). These findings are mirrored by similar results in animal models of intrauterine growth restriction. Thus, intrauterine growth-restricted rodents eat more than controls and have a preference for highly palatable fatty foods (Vickers et al., 2000; Bellinger et al., 2004; Bellinger and Langley-Evans, 2005). For example, rats born to dams fed a low protein diet during pregnancy chose to eat more of a high fat and less of a high carbohydrate diet than control rats did (Bellinger et al., 2004). *In utero* malnutrition can also lead to a reduction in physical activity after birth (Vickers et al., 2003; Sebert et al., 2009), both these factors leading to obesity and comorbidities (Jimenez-Chillaron and Patti, 2007).

It is likely that, as with maternal stress, maternal nutrition regulates placental 11β-HSD2 levels to influence foetal exposure to GC (Stocker et al., 2004, 2005). Food restriction in general, or protein restriction specifically, during pregnancy, can reduce 11β-HSD2 in the placenta (Langley-Evans et al., 1996; Lesage et al., 2006). The foetus is therefore vulnerable to over-exposure to GC, which disrupts development of the HPA axis and leads to elevated hippocampal mineralocorticoid receptor expression and an exacerbated corticosterone response to stress in later life (Lesage et al., 2002, 2006). Some of these long-term effects of foetal exposure to GC can be prevented by exogenous inhibitors of GC synthesis (Langley-Evans et al., 1996).

POSTNATAL NUTRITION

It is currently hypothesized that hyperphagia and obesity after *in utero* growth restriction are due to a mismatch between the developmental and subsequent environments leading to excessive "catch up growth" and associated changes in feeding behavior (Gluckman and Hanson, 2004; Wadhwa et al., 2009). Indeed, rapid weight gain in humans in the first week after birth is a highly significant risk factor for obesity in later life (Ong et al., 2000; Stettler et al., 2005). Remarkably, for every 100 g of weight gained in the first week of life, the risk of becoming obese as an adult increases by 28% (Stettler et al., 2005).

In rodents, as well as humans, the timing as well as the rate of catch up growth is important in determining its influence on development. If the *in utero* food restriction is continued after birth, by continuing to food-restrict the dam during lactation, the growth-restricted phenotype is exacerbated. That is, the animals remain small during the suckling period but by 9 months of age

they are normal-weight, have normal levels of fat and leptin, and have improved glucose tolerance and insulin sensitivity (Desai et al., 2005; Berleze et al., 2009). Conversely, if the *in utero* food-restricted pups are suckled by an *ad-libitum*-fed dam, rapid catch up growth occurs and the pups become larger and fatter in adulthood than controls, and have elevated leptin levels (Desai et al., 2005). These rodent studies thus suggest preventing or delaying catch up growth removes the increased propensity to develop obesity (Desai et al., 2005; Ross and Desai, 2005). Supporting this are data from the 1941–1944 Siege of Leningrad famine. Because of the long duration of the famine and general food shortage and poor living standards before and after it (Leon et al., 1997; Roseboom et al., 2000a), it is likely those people subjected to *in utero* malnutrition continued to be fed a similar diet after birth and did not have significant catch up growth. In this cohort there was, consequently, no association between perinatal malnutrition and adult obesity or risk of disease (Stanner et al., 1997).

In rodents, a useful model for altered postnatal nutrition is to manipulate the litter sizes in which the pups are raised (McCance, 1962; Plagemann et al., 1999b; Schmidt et al., 2001; Morris et al., 2005; Plagemann, 2006; Chen et al., 2008; Rodel et al., 2008; Morris and Chen, 2009; Spencer and Tilbrook, 2009). Thus, rats and mice can be allocated to litters that are smaller than usual; usually three or four pups per litter compared with approximately 12 as a control. In this environment small-litter rodents drink more milk, and milk that is higher in fat and has a greater energy content than those from control litters (Fiorotto et al., 1991). Rodents that are suckled in small litters for the duration of the suckling period weigh significantly more in adulthood than those from control litters (McCance, 1962; Plagemann et al., 1999b; Schmidt et al., 2001; Morris et al., 2005; Plagemann, 2006; Chen et al., 2008; Rodel et al., 2008; Morris and Chen, 2009; Spencer and Tilbrook, 2009). They have more body fat in adulthood and have indices of metabolic disturbances (Plagemann et al., 1992). These small-litter rats also have pronounced changes in HPA axis function (Spencer and Tilbrook, 2009). Their HPA axes mature faster than in rats from control litters (Boullu-Ciocca et al., 2005) and they have exacerbated HPA axis responses to psychological (females) (Spencer and Tilbrook, 2009) and physical (females and males) stress (Clarke et al., 2012).

In conjunction with causing life-long weight gain and enhanced fat mass, many groups have seen small-litter rearing causes hyperphagia and increased appetite throughout life (Plagemann et al., 1999b; Biddinger and Fox, 2010). Mice raised in small litters eat more at each meal, have impaired satiety, and they eat their first meal faster following a mild food deprivation (Biddinger and Fox, 2010). This effect of postnatal overfeeding is likely to be due to changes in the hypothalamic circuitry that regulates feeding. It is interesting, however, that not all groups have seen hyperphagia in this model (Stefanidis and Spencer, 2012), again suggesting multiple influences are necessary to regulate feeding behavior.

The converse model to suckling rodents in small litters is to allocate them to large litters where their access to milk is reduced. Rats suckled in this manner have slower growth and remain smaller throughout life (Velkoska et al., 2008; Bulfin et al., 2011). They also have long-term changes in HPA axis function (Hernandez et al., 2010; Bulfin et al., 2011). Their HPA axis responses to stress (restraint) are attenuated, with corticosterone concentrations returning to baseline more quickly indicating the response may be more efficient in these animals (Bulfin et al., 2011).

Dietary composition is an important factor that can also potentially influence growth and development generally, and specifically development of the central circuitry that regulates feeding. The World Health Organization now recommends exclusive breast-feeding of infants for at least 6 months (WHO, 2002) and some investigations have illustrated this has a protective effect against obesity. For instance, exclusive breast-feeding has been linked with slower weight gain and a reduced long-term obesity risk compared with formula-feeding (e.g., Kramer et al., 2004; Holmes et al., 2011). There is some controversy to these findings and it may be that socio-economic status, related lifestyle factors, or the nutritional content of the formula are also important (Durmus et al., 2011). For instance, these differences in weight gain have not been shown in babies fed low-protein formula (Koletzko et al., 2009). In this regard, the content of the breast-milk also seems to be important (Rist et al., 2007). For instance conjugated linoleic acid isomers (found in organic dairy and meat products) can prevent fat deposition in some human trials (Racine et al., 2010) and these are increased in the breast-milk if the mother has a high dietary intake (Rist et al., 2007). Even the frequency of feeding may also affect the baby's risk of developing obesity (Erlanson-Albertsson and Zetterstrom, 2005; Toschke et al., 2005), as can the timing of the introduction of formula or solid food (Seach et al., 2010).

The perinatal period is thus extremely vulnerable to programming by nutritional influence and stress. Excess stress and/or inadequate nutrition during development can interact to influence feeding and stress responses long-term. Although the mechanism(s) by which these early life challenges influence this circuitry have not been definitively determined, we do have some indications as to how this occurs.

MECHANISMS BY WHICH THE PERINATAL ENVIRONMENT INFLUENCES FEEDING AND STRESS RESPONSES LONG TERM

The HPA axis is the principal neuroendocrine mechanism by which the body responds to a stressful event. When an animal perceives an actual (physical) or potential (psychological) threat (Dayas et al., 2001), medial parvocellular cells in the PVN at the apex of the HPA axis are activated. Corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are then released into the median eminence, followed by ACTH release from the anterior pituitary into the blood stream. ACTH acts at melanocortin 2 receptors to stimulate GC release from the adrenal cortex. GC are the principal mediators of the stress response, encouraging, for example, memory formation, blood flow to skeletal muscle, and suppression of further HPA axis activity [reviewed in Sapolsky et al. (2000); Papadimitriou and Priftis (2009)].

The HPA axis generally, and GC in particular, are also crucial for appetite regulation. In the first minutes after a stressful event,

appetite is typically suppressed; a mechanism that discourages food seeking and feeding when more pressing actions such as escape or defense are more prudent. This early stress-induced anorexia is mediated by CRH (Heinrichs and Richard, 1999; Richard et al., 2002), and other molecules of the same family, such as urocortins (Weninger et al., 1999; Richard et al., 2002). CRH influences food intake by acting at a number of appetite-regulatory brain regions including PVN, perifornical and ventromedial regions of the hypothalamus, lateral septum, and parabrachial nucleus (Richard et al., 2002), as well as indirectly influencing the dorsal anterior bed nucleus of the stria terminalis (Ciccocioppo et al., 2003). CRH and urocortins probably inhibit the activity of neuropeptide Y (NPY) neurons at the hypothalamus, which would normally stimulate food intake (Heinrichs et al., 1993; Currie, 2003). In this regard, any change in expression of CRH or its receptor is therefore likely to influence appetite (**Figure 1**). For instance, rats suckled in small litters, where they develop long-term hyperphagia and obesity have suppressed hypothalamic CRH expression, potentially contributing to less appetite suppression than in controls (Boullu-Ciocca et al., 2005).

Although CRH stimulated by stress acutely suppresses appetite, GC have the opposite effect in that they stimulate feeding (Santana et al., 1995; Dallman et al., 2004; **Figure 1**). GC are released downstream from CRH and can stimulate appetite for hours to days after a stressful event. In humans, a peripheral injection of CRH stimulates food intake 1 h later and this food consumption is directly related to the size of the peak cortisol response (George et al., 2010). After an acute stressor this encourages the animal to replace energy stores that were lost coping with the stress. If the HPA axis is hyperactive, however, with more GC secreted in response to stress, this can lead to a chronically stimulated appetite and increased feeding (De Vriendt et al., 2009).

Thus, one explanation for how perinatal stress and nutritional challenge can influence the neuroendocrine mechanisms regulating feeding behavior long-term is that they can permanently alter the sensitivity of the HPA axis and thus the levels of GC the animal is exposed to. As discussed, despite a number of mechanisms to protect the foetus, maternal GC can cross the placenta and influence foetal GC levels, receptor expression (Edwards et al., 1993), and 11β-HSD2 (Clifton et al., 2006). Foetal 11β-HSD2 levels are important in programming subsequent stress responses and 11β-HSD2$^{-/-}$ mice have greater anxiety levels and lower birth weights than wildtype littermates (Holmes et al., 2006). Similarly, stress (e.g., maternal separation) in the postnatal period can lead to a disruption of the stress hypo-responsive period and elevated GC (Wigger and Neumann, 1999; Lajud et al., 2012).

Excess GC in the foetus or neonate have a number of effects. They interfere with synaptic pruning during brain development in regions that are important for the control of the HPA axis; the prefrontal cortex and hippocampus (Jacobson and Sapolsky, 1991; Spencer et al., 2005). Thus, when dams are given restraint stress during pregnancy, the offspring have reduced levels of growth-associated protein of 43 kDa (GAP-43), which is normally responsible for establishing appropriate synaptic connectivity during development (Pfenninger et al., 1991; Larsson, 2006; Jutapakdeegul et al., 2009). Excess foetal or postnatal GC can also lead to reduced expression of GRs in the hypothalamus and hippocampus resulting in a less efficient GC negative feedback (Liu et al., 1997). This effect is likely to be, at least in part, epigenetically mediated. For example, rats given little parental attention (low intensity nursing) have a smaller increase in nerve growth factor inducible factor A (NGFI-A) expression when they are groomed (Hellstrom et al., 2012). NGFI-A increases histone acetylation of the GR, which facilitates demethylation of the GR promoter and thus receptor activity. With low levels of maternal attention, this activity is reduced, and this is reflected in hypersensitive HPA axis responses to stress in these animals (Champagne and Meaney, 2001). The opposite occurs with high levels of

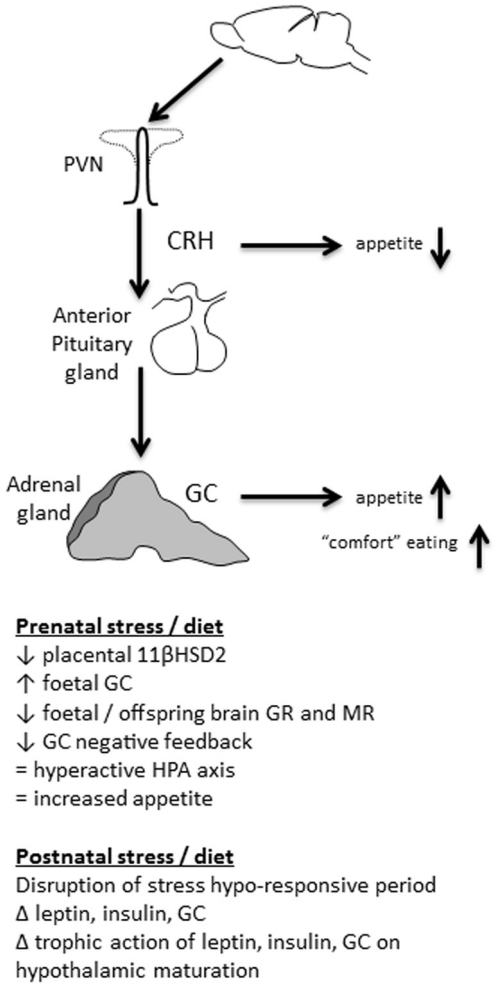

FIGURE 1 | The HPA axis can be programmed by early life stress and diet. When an animal is stressed, medial parvocellular cells in the paraventricular nucleus of the hypothalamus (PVN) are activated. Corticotropin-releasing hormone (CRH) is released into the median eminence, followed by adrenocorticotropic hormone (ACTH) release from the anterior pituitary into the blood stream. ACTH stimulates glucocorticoid (GC) release from the adrenal cortex. Early in the stress response CRH mediates anorexia. As the stress response progresses, GC stimulate appetite, particularly for highly palatable food. Early life events that disrupt HPA axis function can therefore influence feeding behavior long-term.

maternal attention (Fish et al., 2004; Meaney and Szyf, 2005). Interestingly, these changes in HPA axis function due to differences in maternal care seem to be insufficient to alter feeding behavior under basal conditions, as no differences in adiposity or feeding have been reported in these studies (Connor et al., 2012). Epigenetic processes are likely to mediate many changes in HPA axis function as a result of early life events. For instance, in addition to the studies of Meaney and colleagues, early life maternal separation stress in mice leads to changes in DNA methylation leading to increased AVP expression in the PVN, elevated basal GC, and changes in stress-coping style (Murgatroyd et al., 2009; Murgatroyd and Spengler, 2011). There is also some evidence to suggest epigenetic modifications can influence the interaction between stress and feeding behavior. Thus, maternal undernutrition results in increased histone acetylation and hypomethylation of the GR in the offspring's hypothalamus, with a substantial increase in GR expression in this region. These changes are closely linked with enhanced weight gain in these offspring (Stevens et al., 2010; Begum et al., 2012).

During development and in adulthood, GC also modulate the activity of other hormones involved in regulating feeding, including leptin (Spencer, 2012). In adults, leptin, secreted from adipocytes acts centrally, particularly at the arcuate nucleus of the hypothalamus (ARC) to suppress appetite (Schwartz et al., 2000). Although GC stimulate leptin release from adipose tissue, which would normally lead to appetite suppression, they also reduce the sensitivity of the brain to leptin, contributing to leptin resistance (Zakrzewska et al., 1997, 1999; Jequier, 2002). Additionally, in the developing animal, leptin is an important trophic factor, stimulating the development of the brain pathways that regulate feeding (Vickers et al., 2005, 2008; Bouret and Simerly, 2007), and excess GC can interfere with this development (**Figure 2**).

From birth in rodents and from approximately gestational day 21 in humans, the hypothalamic pathways subserving feeding start to fully mature (Koutcherov et al., 2002; Bouret and Simerly, 2006). In rodents, connectivity between the ARC and the dorsomedial hypothalamus (DMH) are established by postnatal day (P) 6, the ARC and PVN by P8-P10, and the ARC and lateral hypothalamus (LH) by P12 (Bouret et al., 2004a,b). This connectivity is dependent upon a surge in circulating leptin that occurs between P4 and P16 (Ahima et al., 1998), the leptin acting on the brain to trigger these communication pathways to mature. If this leptin surge does not occur at the crucial time, this connectivity is improperly formed, permanently affecting feeding-regulation (Bouret and Simerly, 2007). Leptin-deficient mice (Lep^{ob}/Lep^{ob}) that do not have this surge have impaired connectivity between the ARC, DMH, PVN, and LH, with a significant decrease in axon density between these regions. They are also hyperphagic and develop obesity in later life. This connectivity can be rescued by daily supplementation with leptin between, but not after, P4 and P12 (Bouret et al., 2004a,b; Vickers et al., 2005, 2008).

The leptin surge and the connectivity of this hypothalamic circuitry can be disrupted by maternal and postnatal diet and by early life stress. For instance, *in utero* growth restriction can lead to a premature leptin surge and a disruption of these

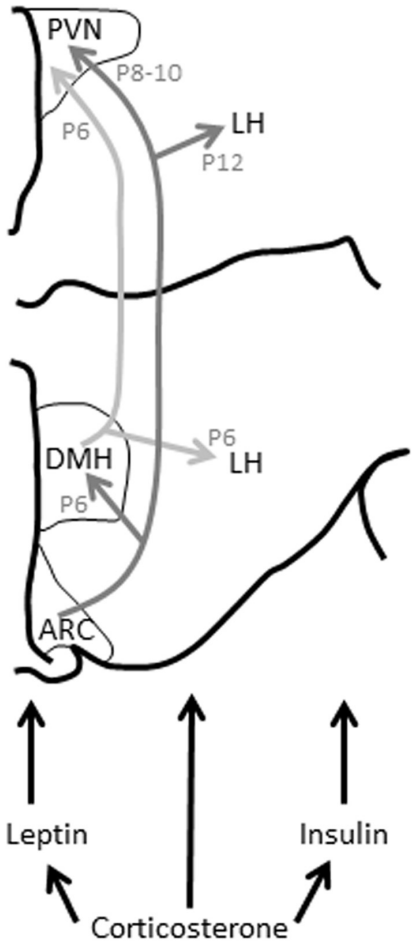

FIGURE 2 | Leptin, insulin, and glucocorticoids modulate hypothalamic connectivity during development. Changes to these hormonal profiles in early life can influence the development of this connectivity and thus feeding behavior and, probably, HPA axis function long-term. ARC, arcuate nucleus of the hypothalamus; DMH, dorsomedial nucleus of the hypothalamus; LH, lateral hypothalamus; P, postnatal day (day by which this connectivity has developed in the rat); PVN, paraventricular nucleus of the hypothalamus. Figure adapted from Bouret and Simerly (2006).

hypothalamic pathways, an effect that is mimicked by exogenous leptin administration from P5 to P10 (Yura et al., 2005). Similarly, rats born to obese dams have elevated plasma leptin in the first 3 weeks of life, and this is associated with resistance to the anorexigenic effects of leptin, hyperphagia, and obesity long-term (Kirk et al., 2009). Postnatal rodents receive much of their leptin from their mother's milk (Fiorotto et al., 1991). Therefore, pups raised by hyperleptinemic mothers, or in small litters where they drink more milk, may be exposed to more leptin than is optimal. It is likely this excess leptin triggers a premature leptin surge, thus reprogramming these feeding-regulatory pathways. Additionally, postnatal overfeeding, or even a genetic susceptibility to diet-induced obesity, can lead to an insensitivity of the ARC to leptin (Davidowa and Plagemann, 2000; Bouret et al., 2008). For example, ARC explants from diet induced obesity-susceptible rats are less responsive to leptin, developing less neurite outgrowth after

leptin application than controls (Bouret et al., 2008). In humans, the equivalent period of development is likely the third trimester of gestation. In humans, intrauterine growth restricted babies or those born small for gestational age have reduced circulating leptin compared with normal babies (Ren and Shen, 2010). Conversely, babies born to obese mothers may be exposed to high levels of leptin too early (Catalano et al., 2009). In either case, if the timing or magnitude of the leptin surge is disrupted, the development of the necessary hypothalamic circuitry may not take place appropriately and feeding behavior would be permanently altered.

As well as an effect of early diet on leptin, it is clear excess GC during the perinatal period can influence the leptin-dependent development of this circuitry. Administration of the synthetic GC, dexamethasone, from postnatal day (P)3 to P6 can elevate plasma leptin in rats, which may interfere with the leptin surge (Bruder et al., 2005). In humans, betamethasone given to pregnant women significantly enhances their plasma leptin concentrations and also elevates leptin levels in the foetus and neonate (Marinoni et al., 2008). Conversely, elevated leptin during the neonatal period can alter HPA axis function long-term, increasing GR levels in the PVN and hippocampus, and enhancing the efficiency of the GC negative feedback response to stress (Proulx et al., 2001).

In addition to modulating the effects of leptin, GC also contribute to insulin secretion from the pancreas and can modify insulin's action on the brain (Strack et al., 1995). Insulin usually acts to suppress appetite via its actions at the hypothalamus and suppresses dopamine-mediated reward at the ventral tegmental area (Figlewicz et al., 2008). However, insulin also enhances preference for high fat, high sucrose "comfort foods" (La Fleur et al., 2004; Warne et al., 2006, 2009). So, chronically elevated GC or a hyperactive GC response to stress programmed by early life events can contribute to comfort eating. Elevated GC also reduce insulin's ability to inhibit feeding-stimulatory pathways in the brain, again leading to inappropriate feeding behavior (Asensio et al., 2004).

Insulin signaling in the adult is sensitive to the early life developmental environment and maternal over-nutrition leads to long-term changes in insulin secretion and sensitivity (Plagemann, 2008). As with leptin, insulin also acts as a trophic factor during development to stimulate connectivity of appetite-regulating brain pathways (**Figure 2**). For instance, excess insulin prenatally, due to hyperinsulinemia in the mother, or postnatally due to overfeeding, can lead to higher insulin concentrations at the level of the hypothalamus, and this may result in changes in brain cell morphology (Plagemann et al., 1998). In particular, the neurons of the ventromedial hypothalamus are vulnerable to insulin, and excess central insulin can lead to a permanent increase in orexigenic NPY and galanin in the ARC and PVN at weaning and in later life (Plagemann et al., 1992, 1999a,b).

It is not clear how, or if, perinatal stress can influence the programming effects of insulin on the circuitry regulating feeding. As with leptin, perinatal stress can stimulate an increase in circulating insulin (Moyer-Mileur et al., 2011). Perinatal stress also clearly affects adult HPA axis function and this can significantly alter how the brain responds to insulin. Future experiments are necessary to determine if acute changes in insulin as the result of stress in early life are sufficient to alter insulin-dependent brain development, or the effects of early life stress are less direct.

In addition to leptin and insulin, early life stress can influence feeding behavior long-term by influencing sensitivity to serotonin. For instance, *in utero* protein restriction combined with *ad-libitum* feeding postnatally can lead to a reduction in the anorexic effects of serotonin (Lopes De Souza et al., 2008). Serotonin sensitivity is also closely regulated by stress (Asan et al., 2013). GC may also further influence appetite regulation via their effects on ghrelin. Ghrelin's principal function is to stimulate feeding, but levels of this hormone are increased during stress and can modulate responses to several stressors (Hosoda et al., 2006; Kristenssson et al., 2006; Spencer et al., 2012). Conceivably, changes in HPA axis function and GC production as a result of the early life environment would alter ghrelin's ability to stimulate feeding. Details of the roles of serotonin and ghrelin in integrating stress and feeding behavior remain to be investigated.

Encouragingly, there is significant potential for effects of perinatal programming on endocrine mechanisms connecting feeding behavior and stress to be reversed or ameliorated by concomitant or later dietary or other interventions. For instance, a postnatal diet high in omega-3 fatty acids ameliorates hyperleptinemia and hypertension associated with *in utero* exposure to dexamethasone (Wyrwoll et al., 2006). Interventions to normalize leptin have also been particularly successful in animal models. Thus, the reduction in 11β-HSD2 associated with a maternal low protein diet during pregnancy can be normalized by giving the dams leptin throughout pregnancy and lactation (Stocker et al., 2004). Neonatal treatment with leptin, from P3 to P13, can also reduce the hyperleptinemia, hyperinsulinemia, hyperphagia, and obesity associated with *in utero* growth restriction and subsequent high fat diet, albeit with some sex differences (Vickers et al., 2005, 2008). Such treatments can even be effective into adulthood, with insulin-like growth factor-1 treatment in adult rats also reducing the long-term effects of *in utero* growth restriction on feeding and metabolic sequelae (Vickers et al., 2001).

In summary, the early life environment has a critical role in programming the circuitry that later integrates stress and feeding behavior. A hyperactive HPA axis, programmed as the result of a stressful early life environment, can lead to excess GC and an exacerbation of GC's typical appetite-stimulatory effects. GC's interactions with feeding-related hormones such as leptin and insulin are also affected. In addition, the early life environment also has specific influences on brain development, such as ensuring appropriate connectivity between the various parts of the hypothalamus necessary for regulating feeding. Stress and the early life nutritional environment can acutely affect this brain development leading to abnormal feeding behavior long-term.

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REFERENCES

Ahima, R. S., Prabakaran, D., and Flier, J. S. (1998). Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. Implications for energy homeostasis and neuroendocrine function. *J. Clin. Invest.* 101, 1020–1027. doi: 10.1172/JCI1176

Albuquerque, K. T., Sardinha, F. L., Telles, M. M., Watanabe, R. L., Nascimento, C. M., Tavares Do Carmo, M. G., et al. (2006). Intake of trans fatty acid-rich hydrogenated fat during pregnancy and lactation inhibits the hypophagic effect of central insulin in the adult offspring. *Nutrition* 22, 820–829. doi: 10.1016/j.nut.2006.04.009

Asan, E., Steinke, M., and Lesch, K. P. (2013). Serotonergic innervation of the amygdala: targets, receptors, and implications for stress and anxiety. *Histochem. Cell Biol.* 139, 785–812. doi: 10.1007/s00418-013-1081-1

Asensio, C., Muzzin, P., and Rohner-Jeanrenaud, F. (2004). Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance. *Int. J. Obes. Relat. Metab. Disord.* 28(Suppl. 4), S45–S52. doi: 10.1038/sj.ijo.0802856

Ashino, N. G., Saito, K. N., Souza, F. D., Nakutz, F. S., Roman, E. A., Velloso, L. A., et al. (2012). Maternal high-fat feeding through pregnancy and lactation predisposes mouse offspring to molecular insulin resistance and fatty liver. *J. Nutr. Biochem.* 23, 341–348. doi: 10.1016/j.jnutbio.2010.12.011

Balfour, M. E., Yu, L., and Coolen, L. M. (2004). Sexual behavior and sex-associated environmental cues activate the mesolimbic system in male rats. *Neuropsychopharmacology* 29, 718–730. doi: 10.1038/sj.npp.1300350

Barna, I., Balint, E., Baranyi, J., Bakos, N., Makara, G. B., and Haller, J. (2003). Gender-specific effect of maternal deprivation on anxiety and corticotropin-releasing hormone mRNA expression in rats. *Brain Res. Bull.* 62, 85–91. doi: 10.1016/S0361-923000216-8

Bassareo, V., and Di Chiara, G. (1997). Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed *ad libitum*. *J. Neurosci.* 17, 851–861.

Bassareo, V., and Di Chiara, G. (1999). Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. *Eur. J. Neurosci.* 11, 4389–4397. doi: 10.1046/j.1460-9568.1999.00843.x

Begum, G., Stevens, A., Smith, E. B., Connor, K., Challis, J. R., Bloomfield, F., et al. (2012). Epigenetic changes in fetal hypothalamic energy regulating pathways are associated with maternal undernutrition and twinning. *FASEB J.* 26, 1694–1703. doi: 10.1096/fj.11-198762

Bellinger, L., and Langley-Evans, S. C. (2005). Fetal programming of appetite by exposure to a maternal low-protein diet in the rat. *Clin. Sci.* 109, 413–420. doi: 10.1042/CS20050127

Bellinger, L., Lilley, C., and Langley-Evans, S. C. (2004). Prenatal exposure to a maternal low-protein diet programmes a preference for high-fat foods in the young adult rat. *Br. J. Nutr.* 92, 513–520. doi: 10.1079/BJN20041224

Berleze, K. J., Muller, A. P., Schweigert, I. D., Longoni, A., Sordi, F., De Assis, A. M., et al. (2009). Gestational and postnatal low protein diet alters insulin sensitivity in female rats. *Exp. Biol. Med. (Maywood)* 234, 1437–1444. doi: 10.3181/0903-RM-111

Berridge, K. C. (2009). 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. *Physiol. Behav.* 97, 537–550. doi: 10.1016/j.physbeh.2009.02.044

Biddinger, J. E., and Fox, E. A. (2010). Meal parameters and vagal gastrointestinal afferents in mice that experienced early postnatal overnutrition. *Physiol. Behav.* 101, 184–191. doi: 10.1016/j.physbeh.2010.04.011

Block, J. P., He, Y., Zaslavsky, A. M., Ding, L., and Ayanian, J. Z. (2009). Psychosocial stress and change in weight among US adults. *Am. J. Epidemiol.* 170, 181–192. doi: 10.1093/aje/kwp104

Boney, C. M., Verma, A., Tucker, R., and Vohr, B. R. (2005). Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 115, e290–e296. doi: 10.1542/peds.2004-1808

Boullu-Ciocca, S., Dutour, A., Guillaume, V., Achard, V., Oliver, C., and Grino, M. (2005). Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood: its relationship with the metabolic syndrome. *Diabetes* 54, 197–203. doi: 10.2337/diabetes.54.1.197

Bouret, S. G., Draper, S. J., and Simerly, R. B. (2004a). Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. *J. Neurosci.* 24, 2797–2805. doi: 10.1523/JNEUROSCI.5369-03.2004

Bouret, S. G., Draper, S. J., and Simerly, R. B. (2004b). Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science* 304, 108–110. doi: 10.1126/science.1095004

Bouret, S. G., Gorski, J. N., Patterson, C. M., Chen, S., Levin, B. E., and Simerly, R. B. (2008). Hypothalamic neural projections are permanently disrupted in diet-induced obese rats. *Cell Metab.* 7, 179–185. doi: 10.1016/j.cmet.2007.12.001

Bouret, S. G., and Simerly, R. B. (2006). Developmental programming of hypothalamic feeding circuits. *Clin. Genet.* 70, 295–301. doi: 10.1111/j.1399-0004.2006.00684.x

Bouret, S. G., and Simerly, R. B. (2007). Development of leptin-sensitive circuits. *J. Neuroendocrinol.* 19, 575–582. doi: 10.1111/j.1365-2826.2007.01563.x

Bruder, E. D., Jacobson, L., and Raff, H. (2005). Plasma leptin and ghrelin in the neonatal rat: interaction of dexamethasone and hypoxia. *J. Endocrinol.* 185, 477–484. doi: 10.1677/joe.1.06159

Brunner, E. J., Marmot, M. G., Nanchahal, K., Shipley, M. J., Stansfeld, S. A., Juneja, M., et al. (1997). Social inequality in coronary risk: central obesity and the metabolic syndrome. Evidence from the Whitehall II study. *Diabetologia* 40, 1341–1349. doi: 10.1007/s001250050830

Brunton, P. J., and Russell, J. A. (2011). Allopregnanolone and suppressed hypothalamo-pituitary-adrenal axis stress responses in late pregnancy in the rat. *Stress* 14, 6–12. doi: 10.3109/10253890.2010.482628

Brunton, P. J., McKay, A. J., Ochedalski, T., Piastowska, A., Rebas, E., Lachowicz, A., et al. (2009). Central opioid inhibition of neuroendocrine stress responses in pregnancy in the rat is induced by the neurosteroid allopregnanolone. *J. Neurosci.* 29, 6449–6460. doi: 10.1523/JNEUROSCI.0708-09.2009

Brunton, P. J., Meddle, S. L., Ma, S., Ochedalski, T., Douglas, A. J., and Russell, J. A. (2005). Endogenous opioids and attenuated hypothalamic-pituitary-adrenal axis responses to immune challenge in pregnant rats. *J. Neurosci.* 25, 5117–5126. doi: 10.1523/JNEUROSCI.0866-05.2005

Bulfin, L. J., Clarke, M. A., Buller, K. M., and Spencer, S. J. (2011). Anxiety and hypothalamic-pituitary-adrenal axis responses to psychological stress are attenuated in male rats made lean by large litter rearing. *Psychoneuroendo* 36, 1080–1091. doi: 10.1016/j.psyneuen.2011.01.006

Catalano, P. M., Presley, L., Minium, J., and Hauguel-De Mouzon, S. (2009). Fetuses of obese mothers develop insulin resistance *in utero*. *Diabetes Care* 32, 1076–1080. doi: 10.2337/dc08-2077

Champagne, F., and Meaney, M. J. (2001). Like mother, like daughter: evidence for non-genomic transmission of parental behavior and stress responsivity. *Prog. Brain Res.* 133, 287–302. doi: 10.1016/S0079-612333022-4

Chen, H., Simar, D., Lambert, K., Mercier, J., and Morris, M. J. (2008). Maternal and postnatal overnutrition differentially impact appetite regulators and fuel metabolism. *Endocrinology* 149, 5348–5356. doi: 10.1210/en.2008-0582

Ciccocioppo, R., Fedeli, A., Economidou, D., Policani, F., Weiss, F., and Massi, M. (2003). The bed nucleus is a neuroanatomical substrate for the anorectic effect of corticotropin-releasing factor and for its reversal by nociceptin/orphanin FQ. *J. Neurosci.* 23, 9445–9451.

Clarke, M. A., Stefanidis, A., and Spencer, S. J. (2012). Postnatal overfeeding leads to obesity and exacerbated febrile responses to lipopolysaccharide throughout life. *J. Neuroendocrinol.* 24, 511–524. doi: 10.1111/j.1365-2826.2011.02269.x

Clifton, V. L., Rennie, N., and Murphy, V. E. (2006). Effect of inhaled glucocorticoid treatment on placental 11beta-hydroxysteroid dehydrogenase type 2 activity and neonatal birthweight in pregnancies complicated by asthma. *Aust. N. Z. J. Obstet. Gynaecol.* 46, 136–140. doi: 10.1111/j.1479-828X.2006.00543.x

Coccurello, R., D'amato, F. R., and Moles, A. (2009). Chronic social stress, hedonism and vulnerability to obesity: lessons from rodents. *Neurosci. Biobehav. Rev.* 33, 537–550. doi: 10.1016/j.neubiorev.2008.05.018

Connor, K. L., Vickers, M. H., Beltrand, J., Meaney, M. J., and Sloboda, D. M. (2012). Nature, nurture or nutrition? Impact of maternal nutrition on maternal care,

offspring development and reproductive function. *J. Physiol.* 590, 2167–2180. doi: 10.1113/jphysiol.2011.223305

Currie, P. J. (2003). Integration of hypothalamic feeding and metabolic signals: focus on neuropeptide Y. *Appetite* 41, 335–337. doi: 10.1016/j.appet.2003.08.011

D'Argenio, A., Mazzi, C., Pecchioli, L., Di Lorenzo, G., Siracusano, A., and Troisi, A. (2009). Early trauma and adult obesity: is psychological dysfunction the mediating mechanism? *Physiol. Behav.* 98, 543–546. doi: 10.1016/j.physbeh.2009.08.010

Dabbaghian, V., Mago, V. K., Wu, T., Fritz, C., and Alimadad, A. (2012). Social interactions of eating behaviour among high school students: a cellular automata approach. *BMC Med. Res. Methodol.* 12:155. doi: 10.1186/1471-2288-12-155

Dabelea, D., Hanson, R. L., Lindsay, R. S., Pettitt, D. J., Imperatore, G., Gabir, M. M., et al. (2000). Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes* 49, 2208–2211. doi: 10.2337/diabetes.49.12.2208

Dahlgren, J., Nilsson, C., Jennische, E., Ho, H. P., Eriksson, E., Niklasson, A., et al. (2001). Prenatal cytokine exposure results in obesity and gender-specific programming. *Am. J. Physiol. Endocrinol. Metab.* 281, E326–E334.

Dallman, M. F. (2010). Stress-induced obesity and the emotional nervous system. *Trends Endocrinol. Metab.* 21, 159–165. doi: 10.1016/j.tem.2009.10.004

Dallman, M. F., La Fleur, S. E., Pecoraro, N. C., Gomez, F., Houshyar, H., and Akana, S. F. (2004). Minireview: glucocorticoids–food intake, abdominal obesity, and wealthy nations in 2004. *Endocrinology* 145, 2633–2638. doi: 10.1210/en.2004-0037

Davidowa, H., and Plagemann, A. (2000). Decreased inhibition by leptin of hypothalamic arcuate neurons in neonatally overfed young rats. *Neuroreport* 11, 2795–2798. doi: 10.1097/00001756-200008210-00037

Dayas, C. V., Buller, K. M., Crane, J. W., Xu, Y., and Day, T. A. (2001). Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. *Eur. J. Neurosci.* 14, 1143–1152. doi: 10.1046/j.0953-816x.2001.01733.x

Dent, G. W., Smith, M. A., and Levine, S. (2000). Rapid induction of corticotropin-releasing hormone gene transcription in the paraventricular nucleus of the developing rat. *Endocrinology* 141, 1593–1598. doi: 10.1210/en.141.5.1593

Desai, M., Gayle, D., Babu, J., and Ross, M. G. (2005). Programmed obesity in intrauterine growth-restricted newborns: modulation by newborn nutrition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, R91–R96. doi: 10.1152/ajpregu.00340.2004

De Vriendt, T., Moreno, L. A., and De Henauw, S. (2009). Chronic stress and obesity in adolescents: scientific evidence and methodological issues for epidemiological research. *Nutr. Metab. Cardiovasc. Dis.* 19, 511–519. doi: 10.1016/j.numecd.2009.02.009

Di Chiara, G., and Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. U.S.A.* 85, 5274–5278. doi: 10.1073/pnas.85.14.5274

Durmus, B., Van Rossem, L., Duijts, L., Arends, L. R., Raat, H., Moll, H. A., et al. (2011). Breast-feeding and growth in children until the age of 3 years: the Generation R Study. *Br. J. Nutr.* 105, 1704–1711. doi: 10.1017/S0007114510005374

Duvigneaud, N., Wijndaele, K., Matton, L., Deriemaeker, P., Philippaerts, R., Lefevre, J., et al. (2007). Socio-economic and lifestyle factors associated with overweight in Flemish adult men and women. *BMC Public Health* 7:23. doi: 10.1186/1471-2458-7-23

Edwards, C. R., Benediktsson, R., Lindsay, R. S., and Seckl, J. R. (1993). Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? *Lancet* 341, 355–357. doi: 10.1016/0140-673690148-A

Egecioglu, E., Jerlhag, E., Salome, N., Skibicka, K. P., Haage, D., Bohlooly, Y. M., et al. (2010). Ghrelin increases intake of rewarding food in rodents. *Addict. Biol.* 15, 304–311. doi: 10.1111/j.1369-1600.2010.00216.x

Eilat-Adar, S., Goldbourt, U., Resnick, H. E., and Howard, B. V. (2005). Intentional weight loss, blood lipids and coronary morbidity and mortality. *Curr. Opin. Lipidol.* 16, 5–9. doi: 10.1097/00041433-200502000-00003

Entringer, S., Buss, C., Kumsta, R., Hellhammer, D. H., Wadhwa, P. D., and Wust, S. (2009). Prenatal psychosocial stress exposure is associated with subsequent working memory performance in young women. *Behav. Neurosci.* 123, 886–893. doi: 10.1037/a0016265

Erlanson-Albertsson, C., and Zetterstrom, R. (2005). The global obesity epidemic: snacking and obesity may start with free meals during infant feeding. *Acta Paediatr.* 94, 1523–1531. doi: 10.1080/08035250500323780

Figlewicz, D. P., Bennett, J. L., Aliakbari, S., Zavosh, A., and Sipols, A. J. (2008). Insulin acts at different CNS sites to decrease acute sucrose intake and sucrose self-administration in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295, R388–R394. doi: 10.1152/ajpregu.90334.2008

Figueroa-Colon, R., Arani, R. B., Goran, M. I., and Weinsier, R. L. (2000). Paternal body fat is a longitudinal predictor of changes in body fat in premenarcheal girls. *Am. J. Clin. Nutr.* 71, 829–834.

Fiorotto, M. L., Burrin, D. G., Perez, M., and Reeds, P. J. (1991). Intake and use of milk nutrients by rat pups suckled in small, medium, or large litters. *Am. J. Physiol.* 260, R1104–R1113.

Fish, E. W., Shahrokh, D., Bagot, R., Caldji, C., Bredy, T., Szyf, M., et al. (2004). Epigenetic programming of stress responses through variations in maternal care. *Ann. N.Y. Acad. Sci.* 1036, 167–180. doi: 10.1196/annals.1330.011

Fowler-Brown, A. G., Bennett, G. G., Goodman, M. S., Wee, C. C., Corbie-Smith, G. M., and James, S. A. (2009). Psychosocial stress and 13-year BMI change smong blacks: the pitt county study. *Obesity (Silver Spring).* 17, 2106–2109. doi: 10.1038/oby.2009.130

George, S. A., Khan, S., Briggs, H., and Abelson, J. L. (2010). CRH-stimulated cortisol release and food intake in healthy, non-obese adults. *Psychoneuroendo* 35, 607–612. doi: 10.1016/j.psyneuen.2009.09.017

Gluckman, P. D., and Hanson, M. A. (2004). Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. *Pediatr. Res.* 56, 311–317. doi: 10.1203/01.PDR.0000135998.08025.FB

Gosnell, B. A. (2000). Sucrose intake predicts rate of acquisition of cocaine self-administration. *Psychopharmacology (Berl.)* 149, 286–292. doi: 10.1007/s002130000375

Gugusheff, J. R., Ong, Z. Y., and Muhlhausler, B. S. (2013). A maternal "junk-food" diet reduces sensitivity to the opioid antagonist naloxone in offspring postweaning. *FASEB J.* 27, 1275–1284. doi: 10.1096/fj.12-217653

Guillaume, M., Lapidus, L., Beckers, F., Lambert, A., and Bjorntorp, P. (1995). Familial trends of obesity through three generations: the Belgian-Luxembourg child study. *Int. J. Obes. Relat. Metab. Disord.* 19(Suppl. 3), S5–S9.

Gunnar, M. R., and Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology* 27, 199–220. doi: 10.1016/S0306-453000045-2

Heinrichs, S. C., and Richard, D. (1999). The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. *Neuropeptides* 33, 350–359. doi: 10.1054/npep.1999.0047

Heinrichs, S. C., Menzaghi, F., Pich, E. M., Hauger, R. L., and Koob, G. F. (1993). Corticotropin-releasing factor in the paraventricular nucleus modulates feeding induced by neuropeptide Y. *Brain Res.* 611, 18–24. doi: 10.1016/0006-899391771-J

Hellstrom, I. C., Dhir, S. K., Diorio, J. C., and Meaney, M. J. (2012). Maternal licking regulates hippocampal glucocorticoid receptor transcription through a thyroid hormone-serotonin-NGFI-A signalling cascade. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 2495–2510. doi: 10.1098/rstb.2012.0223

Henry, C., Kabbaj, M., Simon, H., Le Moal, M., and Maccari, S. (1994). Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *J. Neuroendocrinol.* 6, 341–345. doi: 10.1111/j.1365-2826.1994.tb00591.x

Hernandez, C. E., Matthews, L. R., Oliver, M. H., Bloomfield, F. H., and Harding, J. E. (2010). Effects of sex, litter size and periconceptional ewe nutrition on offspring behavioural and physiological response to isolation. *Physiol. Behav.* 101, 588–594. doi: 10.1016/j.physbeh.2010.08.020

Holmes, A. V., Auinger, P., and Howard, C. R. (2011). Combination feeding of breast milk and formula: evidence for shorter breast-feeding duration from the National Health and Nutrition Examination Survey. *J. Pediatr.* 159, 186–191. doi: 10.1016/j.jpeds.2011.02.006

Holmes, M. C., Abrahamsen, C. T., French, K. L., Paterson, J. M., Mullins, J. J., and Seckl, J. R. (2006). The mother or the fetus? 11beta-hydroxysteroid dehydrogenase type 2 null mice provide evidence for direct fetal programming of behavior by endogenous glucocorticoids.

J. Neurosci. 26, 3840–3844. doi: 10.1523/JNEUROSCI.4464-05.2006

Hosoda, H., Kojima, M., and Kangawa, K. (2006). Biological, physiological, and pharmacological aspects of ghrelin. *J. Pharmacol. Sci.* 100, 398–410. doi: 10.1254/jphs.CRJ06002X

Ito, R., Robbins, T. W., and Everitt, B. J. (2004). Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. *Nat. Neurosci.* 7, 389–397. doi: 10.1038/nn1217

Jacobson, L., and Sapolsky, R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr. Rev.* 12, 118–134. doi: 10.1210/edrv-12-2-118

Jayo, J. M., Shively, C. A., Kaplan, J. R., and Manuck, S. B. (1993). Effects of exercise and stress on body fat distribution in male cynomolgus monkeys. *Int. J. Obes. Relat. Metab. Disord.* 17, 597–604.

Jequier, E. (2002). Leptin signaling, adiposity, and energy balance. *Ann. N.Y. Acad. Sci.* 967, 379–388. doi: 10.1111/j.1749-6632.2002.tb04293.x

Jerlhag, E., Egecioglu, E., Dickson, S. L., and Engel, J. A. (2010). Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference. *Psychopharmacology (Berl.)* 211, 415–422. doi: 10.1007/s00213-010-1907-7

Jerlhag, E., Egecioglu, E., Landgren, S., Salome, N., Heilig, M., Moechars, D., et al. (2009). Requirement of central ghrelin signaling for alcohol reward. *Proc. Natl. Acad. Sci. U.S.A.* 106, 11318–11323. doi: 10.1073/pnas.0812809106

Jimenez-Chillaron, J. C., and Patti, M. E. (2007). To catch up or not to catch up: is this the question? Lessons from animal models. *Curr. Opin. Endocrinol. Diabetes Obes.* 14, 23–29. doi: 10.1097/MED.0b013e328013da8e

Jutapakdeegul, N., Polboon, N., Afadlal, S., Phansuwan-Pujito, P., and Govitrapong, P. (2009). Repeated restraint stress and corticosterone injections during late pregnancy alter GAP-43 expression in the hippocampus and prefrontal cortex of rat pups. *Int. J. Dev. Neurosci.* 28, 83–90. doi: 10.1016/j.ijdevneu.2009.09.003

Kasturi, S. S., Tannir, J., and Brannigan, R. E. (2008). The metabolic syndrome and male infertility. *J. Androl.* 29, 251–259. doi: 10.2164/jandrol.107.003731

Kirk, S. L., Samuelsson, A. M., Argenton, M., Dhonye, H., Kalamatianos, T., Poston, L., et al. (2009). Maternal obesity induced by diet in rats permanently influences central processes regulating food intake in offspring. *PLoS ONE* 4:e5870. doi: 10.1371/journal.pone.0005870

Koch, F. S., Sepa, A., and Ludvigsson, J. (2008). Psychological stress and obesity. *J. Pediatr.* 153, 839–844. doi: 10.1016/j.jpeds.2008.06.016

Koehl, M., Darnaudery, M., Dulluc, J., Van Reeth, O., Le Moal, M., and Maccari, S. (1999). Prenatal stress alters circadian activity of hypothalamo-pituitary-adrenal axis and hippocampal corticosteroid receptors in adult rats of both gender. *J. Neurobiol.* 40, 302–315. doi: 10.1002/(SICI)1097-469540, 3<302::AID-NEU3>3.0.CO;2-7

Koletzko, B., Von Kries, R., Monasterolo, R. C., Subias, J. E., Scaglioni, S., Giovannini, M., et al. (2009). Infant feeding and later obesity risk. *Adv. Exp. Med. Biol.* 646, 15–29. doi: 10.1007/978-1-4020-9173-5_2

Koob, G. F., and Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* 24, 97–129. doi: 10.1016/S0893-133X(00)00195-0

Koutcherov, Y., Mai, J. K., Ashwell, K. W., and Paxinos, G. (2002). Organization of human hypothalamus in fetal development. *J. Comp. Neurol.* 446, 301–324. doi: 10.1002/cne.10175

Kramer, M. S., Guo, T., Platt, R. W., Vanilovich, I., Sevkovskaya, Z., Dzikovich, I., et al. (2004). Feeding effects on growth during infancy. *J. Pediatr.* 145, 600–605. doi: 10.1016/j.jpeds.2004.06.069

Kristenssson, E., Sundqvist, M., Astin, M., Kjerling, M., Mattsson, H., Dornonville De La Cour, C., et al. (2006). Acute psychological stress raises plasma ghrelin in the rat. *Regul. Pept.* 134, 114–117. doi: 10.1016/j.regpep.2006.02.003

La Fleur, S. E., Akana, S. F., Manalo, S. L., and Dallman, M. F. (2004). Interaction between corticosterone and insulin in obesity: regulation of lard intake and fat stores. *Endocrinology* 145, 2174–2185. doi: 10.1210/en.2003-1359

Lajud, N., Roque, A., Cajero, M., Gutierrez-Ospina, G., and Torner, L. (2012). Periodic maternal separation decreases hippocampal neurogenesis without affecting basal corticosterone during the stress hyporesponsive period, but alters HPA axis and coping behavior in adulthood. *Psychoneuroendo* 37, 410–420. doi: 10.1016/j.psyneuen.2011.07.011

Langley-Evans, S. C., Gardner, D. S., and Jackson, A. A. (1996). Maternal protein restriction influences the programming of the rat hypothalamic-pituitary-adrenal axis. *J. Nutr.* 126, 1578–1585.

Larsson, C. (2006). Protein kinase C and the regulation of the actin cytoskeleton. *Cell. Signal.* 18, 276–284. doi: 10.1016/j.cellsig.2005.07.010

Lehmann, J., Pryce, C. R., Jongen-Relo, A. L., Stohr, T., Pothuizen, H. H., and Feldon, J. (2002a). Comparison of maternal separation and early handling in terms of their neurobehavioral effects in aged rats. *Neurobiol. Aging* 23, 457–466. doi: 10.1016/S0197-458000320-7

Lehmann, J., Russig, H., Feldon, J., and Pryce, C. R. (2002b). Effect of a single maternal separation at different pup ages on the corticosterone stress response in adult and aged rats. *Pharmacol. Biochem. Behav.* 73, 141–145. doi: 10.1016/S0091-305700788-8

Leon, D. A., Chenet, L., Shkolnikov, V. M., Zakharov, S., Shapiro, J., Rakhmanova, G., et al. (1997). Huge variation in Russian mortality rates 1984-94: artefact, alcohol, or what? *Lancet* 350, 383–388. doi: 10.1016/S0140-673603360-6

Lesage, J., Dufourny, L., Laborie, C., Bernet, F., Blondeau, B., Avril, I., et al. (2002). Perinatal malnutrition programs sympathoadrenal and hypothalamic-pituitary-adrenal axis responsiveness to restraint stress in adult male rats. *J. Neuroendocrinol.* 14, 135–143. doi: 10.1046/j.0007-1331.2001.00753.x

Lesage, J., Sebaai, N., Leonhardt, M., Dutriez-Casteloot, I., Breton, C., Deloof, S., et al. (2006). Perinatal maternal undernutrition programs the offspring hypothalamo-pituitary-adrenal (HPA) axis. *Stress* 9, 183–198. doi: 10.1080/10253890601056192

Levine, S. (2002). Regulation of the hypothalamic-pituitary-adrenal axis in the neonatal rat: the role of maternal behavior. *Neurotox. Res.* 4, 557–564. doi: 10.1080/10298420290030569

Li, J., Olsen, J., Vestergaard, M., Obel, C., Baker, J. L., and Sorensen, T. I. (2010). Prenatal stress exposure related to maternal bereavement and risk of childhood overweight. *PLoS ONE* 5:e11896. doi: 10.1371/journal.pone.0011896

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., et al. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 277, 1659–1662. doi: 10.1126/science.277.5332.1659

Llabre, M. M., and Hadi, F. (2009). War-related exposure and psychological distress as predictors of health and sleep: a longitudinal study of Kuwaiti children. *Psychosom. Med.* 71, 776–783. doi: 10.1097/PSY.0b013e3181ae6aee

Loos, R. J. (2009). Recent progress in the genetics of common obesity. *Br. J. Clin. Pharmacol.* 68, 811–829. doi: 10.1111/j.1365-2125.2009.03523.x

Lopes De Souza, S., Orozco-Solis, R., Grit, I., Manhaes De Castro, R., and Bolanos-Jimenez, F. (2008). Perinatal protein restriction reduces the inhibitory action of serotonin on food intake. *Eur. J. Neurosci.* 27, 1400–1408. doi: 10.1111/j.1460-9568.2008.06105.x

Lordi, B., Protais, P., Mellier, D., and Caston, J. (1997). Acute stress in pregnant rats: effects on growth rate, learning, and memory capabilities of the offspring. *Physiol. Behav.* 62, 1087–1092. doi: 10.1016/S0031-938400261-8

Lucassen, P. J., Bosch, O. J., Jousma, E., Kromer, S. A., Andrew, R., Seckl, J. R., et al. (2009). Prenatal stress reduces postnatal neurogenesis in rats selectively bred for high, but not low, anxiety: possible key role of placental 11beta-hydroxysteroid dehydrogenase type 2. *Eur. J. Neurosci.* 29, 97–103. doi: 10.1111/j.1460-9568.2008.06543.x

Lussana, F., Painter, R. C., Ocke, M. C., Buller, H. R., Bossuyt, P. M., and Roseboom, T. J. (2008). Prenatal exposure to the Dutch famine is associated with a preference for fatty foods and a more atherogenic lipid profile. *Am. J. Clin. Nutr.* 88, 1648–1652. doi: 10.3945/ajcn.2008.26140

Maccari, S., Darnaudery, M., Morley-Fletcher, S., Zuena, A. R., Cinque, C., and Van Reeth, O. (2003). Prenatal stress and long-term consequences: implications of glucocorticoid hormones. *Neurosci. Biobehav. Rev.* 27, 119–127. doi: 10.1016/S0149-763400014-9

Maccari, S., Piazza, P. V., Kabbaj, M., Barbazanges, A., Simon, H., and Le Moal, M. (1995). Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. *J. Neurosci.* 15, 110–116.

Marinoni, E., Letizia, C., Ciardo, F., Corona, G., Moscarini, M., and Di Iorio, R. (2008). Effects of prenatal betamethasone administration on leptin and adiponectin concentrations in maternal and fetal circulation. *Am. J. Obstet. Gynecol.* 199, 141 e141–e146. doi: 10.1016/j.ajog.2008.02.047

Matsuo, T., Nakata, Y., Katayama, Y., Iemitsu, M., Maeda, S., Okura, T., et al. (2009). PPARG genotype accounts for part of individual variation in body weight reduction in response to calorie restriction. *Obesity (Silver Spring)* 17, 1924–1931. doi: 10.1038/oby.2009.199

McCance, R. A. (1962). Food, growth, and time. *Lancet* 2, 671–676. doi: 10.1016/S0140-673690499-3

Meaney, M. J., and Szyf, M. (2005). Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues Clin. Neurosci.* 7, 103–123.

Moens, E., Braet, C., Bosmans, G., and Rosseel, Y. (2009). Unfavourable family characteristics and their associations with childhood obesity: a cross-sectional study. *Eur. Eat. Disord. Rev.* 17, 315–323. doi: 10.1002/erv.940

Morley-Fletcher, S., Puopolo, M., Gentili, S., Gerra, G., Macchia, T., and Laviola, G. (2004). Prenatal stress affects 3, 4-methylenedioxymethamphetamine pharmacokinetics and drug-induced motor alterations in adolescent female rats. *Eur. J. Pharmacol.* 489, 89–92. doi: 10.1016/j.ejphar.2004.02.028

Morris, M. J., and Chen, H. (2009). Established maternal obesity in the rat reprograms hypothalamic appetite regulators and leptin signaling at birth. *Int. J. Obes. (Lond.)* 33, 115–122. doi: 10.1038/ijo.2008.213

Morris, M. J., Velkoska, E., and Cole, T. J. (2005). Central and peripheral contributions to obesity-associated hypertension: impact of early overnourishment. *Exp. Physiol.* 90, 697–702. doi: 10.1113/expphysiol.2005.030783

Moyer-Mileur, L. J., Haley, S., Gulliver, K., Thomson, A., Slater, H., Barrett, B., et al. (2011). Mechanical-tactile stimulation (MTS) during neonatal stress prevents hyperinsulinemia despite stress-induced adiposity in weanling rat pups. *Early Hum. Dev.* 87, 159–163. doi: 10.1016/j.earlhumdev.2010.12.001

Murgatroyd, C., and Spengler, D. (2011). Epigenetic programming of the HPA axis: early life decides. *Stress* 14, 581–589. doi: 10.3109/10253890.2011.602146

Murgatroyd, C., Patchev, A. V., Wu, Y., Micale, V., Bockmuhl, Y., Fischer, D., et al. (2009). Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nat. Neurosci.* 12, 1559–1566. doi: 10.1038/nn.2436

Ng, S. F., Lin, R. C., Laybutt, D. R., Barres, R., Owens, J. A., and Morris, M. J. (2010). Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. *Nature* 467, 963–966. doi: 10.1038/nature09491

Nieuwenhuizen, A. G., and Rutters, F. (2008). The hypothalamic-pituitary-adrenal-axis in the regulation of energy balance. *Physiol. Behav.* 94, 169–177. doi: 10.1016/j.physbeh.2007.12.011

Nishitani, N., Sakakibara, H., and Akiyama, I. (2009). Eating behavior related to obesity and job stress in male Japanese workers. *Nutrition* 25, 45–50. doi: 10.1016/j.nut.2008.07.008

Ong, K. K., Ahmed, M. L., Emmett, P. M., Preece, M. A., and Dunger, D. B. (2000). Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ* 320, 967–971. doi: 10.1136/bmj.320.7240.967

Ong, Z. Y., and Muhlhausler, B. S. (2011). Maternal "junk-food" feeding of rat dams alters food choices and development of the mesolimbic reward pathway in the offspring. *FASEB J.* 25, 2167–2179. doi: 10.1096/fj.10-178392

Palmer, N. O., Bakos, H. W., Owens, J. A., Setchell, B. P., and Lane, M. (2012). Diet and exercise in an obese mouse fed a high-fat diet improve metabolic health and reverse perturbed sperm function. *Am. J. Physiol. Endocrinol. Metab.* 302, E768–E780. doi: 10.1152/ajpendo.00401.2011

Pankevich, D. E., Mueller, B. R., Brockel, B., and Bale, T. L. (2009). Prenatal stress programming of offspring feeding behavior and energy balance begins early in pregnancy. *Physiol. Behav.* 98, 94–102. doi: 10.1016/j.physbeh.2009.04.015

Papadimitriou, A., and Priftis, K. N. (2009). Regulation of the hypothalamic-pituitary-adrenal axis. *Neuroimmunomodulation* 16, 265–271. doi: 10.1159/000216184

Parsons, T. J., Power, C., and Manor, O. (2001). Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study. *BMJ* 323, 1331–1335. doi: 10.1136/bmj.323.7325.1331

Pecoraro, N., Reyes, F., Gomez, F., Bhargava, A., and Dallman, M. F. (2004). Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. *Endocrinology* 145, 3754–3762. doi: 10.1210/en.2004-0305

Penke, Z., Felszeghy, K., Fernette, B., Sage, D., Nyakas, C., and Burlet, A. (2001). Postnatal maternal deprivation produces long-lasting modifications of the stress response, feeding and stress-related behaviour in the rat. *Eur. J. Neurosci.* 14, 747–755. doi: 10.1046/j.0953-816x.2001.01691.x

Pfenninger, K. H., De La Houssaye, B. A., Helmke, S. M., and Quiroga, S. (1991). Growth-regulated proteins and neuronal plasticity. A commentary. *Mol. Neurobiol.* 5, 143–151. doi: 10.1007/BF02935543

Plagemann, A. (2006). Perinatal nutrition and hormone-dependent programming of food intake. *Horm. Res.* 65(Suppl. 3), 83–89. doi: 10.1159/000091511

Plagemann, A. (2008). A matter of insulin: developmental programming of body weight regulation. *J. Matern. Fetal. Neonatal. Med.* 21, 143–148. doi: 10.1080/14767050801929869

Plagemann, A., Harder, T., Rake, A., Janert, U., Melchior, K., Rohde, W., et al. (1999a). Morphological alterations of hypothalamic nuclei due to intrahypothalamic hyperinsulinism in newborn rats. *Int. J. Dev. Neurosci.* 17, 37–44. doi: 10.1016/S0736-574800064-1

Plagemann, A., Harder, T., Rake, A., Voits, M., Fink, H., Rohde, W., et al. (1999b). Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergic neurons, and syndrome x-like alterations in adulthood of neonatally overfed rats. *Brain Res.* 836, 146–155. doi: 10.1016/S0006-899301662-5

Plagemann, A., Heidrich, I., Gotz, F., Rohde, W., and Dorner, G. (1992). Obesity and enhanced diabetes and cardiovascular risk in adult rats due to early postnatal overfeeding. *Exp. Clin. Endocrinol.* 99, 154–158. doi: 10.1055/s-0029-1211159

Plagemann, A., Rake, A., Harder, T., Melchior, K., Rohde, W., and Dorner, G. (1998). Reduction of cholecystokinin-8S-neurons in the paraventricular hypothalamic nucleus of neonatally overfed weanling rats. *Neurosci. Lett.* 258, 13–16. doi: 10.1016/S0304-394000823-4

Power, C., Li, L., Manor, O., and Davey Smith, G. (2003). Combination of low birth weight and high adult body mass index: at what age is it established and what are its determinants? *J. Epidemiol. Community Health* 57, 969–973. doi: 10.1136/jech.57.12.969

Proulx, K., Clavel, S., Nault, G., Richard, D., and Walker, C. D. (2001). High neonatal leptin exposure enhances brain GR expression and feedback efficacy on the adrenocortical axis of developing rats. *Endocrinology* 142, 4607–4616. doi: 10.1210/en.142.11.4607

Purcell, R. H., Sun, B., Pass, L. L., Power, M. L., Moran, T. H., and Tamashiro, K. L. (2011). Maternal stress and high-fat diet effect on maternal behavior, milk composition, and pup ingestive behavior. *Physiol. Behav.* 104, 474–479. doi: 10.1016/j.physbeh.2011.05.012

Racine, N. M., Watras, A. C., Carrel, A. L., Allen, D. B., McVean, J. J., Clark, R. R., et al. (2010). Effect of conjugated linoleic acid on body fat accretion in overweight or obese children. *Am. J. Clin. Nutr.* 91, 1157–1164. doi: 10.3945/ajcn.2009.28404

Ravelli, A. C., Der Meulen, J. H., Osmond, C., Barker, D. J., and Bleker, O. P. (1999). Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am. J. Clin. Nutr.* 70, 811–816.

Ravelli, G. P., Stein, Z. A., and Susser, M. W. (1976). Obesity in young men after famine exposure *in utero* and early infancy. *N. Engl. J. Med.* 295, 349–353. doi: 10.1056/NEJM197608122950701

Ren, R. X., and Shen, Y. (2010). A meta-analysis of relationship between birth weight and cord blood leptin levels in newborns. *World J. Pediatr.* 6, 311–316. doi: 10.1007/s12519-010-0216-x

Richard, D., Lin, Q., and Timofeeva, E. (2002). The corticotropin-releasing factor family of peptides and CRF receptors: their roles in the regulation of energy balance. *Eur. J. Pharmacol.* 440, 189–197. doi: 10.1016/S0014-299901428-0

Rist, L., Mueller, A., Barthel, C., Snijders, B., Jansen, M., Simoes-Wust, A. P., et al. (2007). Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands. *Br. J. Nutr.* 97, 735–743. doi: 10.1017/S0007114507433074

Robinson, E., and Higgs, S. (2012). Liking food less: the impact of social influence on food liking evaluations in female students. *PLoS ONE* 7:e48858. doi: 10.1371/journal.pone.0048858

Rodel, H. G., Prager, G., Stefanski, V., Von Holst, D., and Hudson, R. (2008). Separating maternal and litter-size effects on early postnatal growth in two species of altricial small mammals. *Physiol. Behav.* 93, 826–834. doi: 10.1016/j.physbeh.2007.11.047

Roseboom, T. J., Van Der Meulen, J. H., Osmond, C., Barker, D. J., Ravelli, A. C., and Bleker, O. P. (2000a). Plasma lipid profiles in adults after prenatal exposure to the Dutch famine. *Am. J. Clin. Nutr.* 72, 1101–1106.

Roseboom, T. J., Van Der Meulen, J. H., Osmond, C., Barker, D. J., Ravelli, A. C., Schroeder-Tanka, J. M., et al. (2000b). Coronary heart disease after prenatal exposure to the Dutch famine, 1944–1945. *Heart* 84, 595–598. doi: 10.1136/heart.84.6.595

Ross, M. G., and Desai, M. (2005). Gestational programming: population survival effects of drought and famine during pregnancy. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, R25–R33. doi: 10.1152/ajpregu.00418.2004

Rossi-George, A., Virgolini, M. B., Weston, D., and Cory-Slechta, D. A. (2009). Alterations in glucocorticoid negative feedback following maternal Pb, prenatal stress and the combination: a potential biological unifying mechanism for their corresponding disease profiles. *Toxicol. Appl. Pharmacol.* 234, 117–127. doi: 10.1016/j.taap.2008.10.003

Ruager-Martin, R., Hyde, M. J., and Modi, N. (2010). Maternal obesity and infant outcomes. *Early Hum. Dev.* 86, 715–722. doi: 10.1016/j.earlhumdev.2010.08.007

Ryu, V., Yoo, S. B., Kang, D. W., Lee, J. H., and Jahng, J. W. (2009). Post-weaning isolation promotes food intake and body weight gain in rats that experienced neonatal maternal separation. *Brain Res.* 1295, 127–134. doi: 10.1016/j.brainres.2009.08.006

Santana, P., Akana, S. F., Hanson, E. S., Strack, A. M., Sebastian, R. J., and Dallman, M. F. (1995). Aldosterone and dexamethasone both stimulate energy acquisition whereas only the glucocorticoid alters energy storage. *Endocrinology* 136, 2214–2222. doi: 10.1210/en.136.5.2214

Sapolsky, R. M., Romero, L. M., and Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21, 55–89. doi: 10.1210/er.21.1.55

Sapolsky, R. M., and Meaney, M. J. (1986). Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Res.* 396, 64–76. doi: 10.1016/0165-017390010-X

Schmidt, I., Fritz, A., Scholch, C., Schneider, D., Simon, E., and Plagemann, A. (2001). The effect of leptin treatment on the development of obesity in overfed suckling Wistar rats. *Int. J. Obes. Relat. Metab. Disord.* 25, 1168–1174. doi: 10.1038/sj.ijo.0801669

Schwartz, M. W., Woods, S. C., Porte, D. Jr., Seeley, R. J., and Baskin, D. G. (2000). Central nervous system control of food intake. *Nature* 404, 661–671. doi: 10.1038/35007534

Seach, K. A., Dharmage, S. C., Lowe, A. J., and Dixon, J. B. (2010). Delayed introduction of solid feeding reduces child overweight and obesity at 10 years. *Int. J. Obes. (Lond.)* 34, 1475–1479. doi: 10.1038/ijo.2010.101

Sebert, S. P., Hyatt, M. A., Chan, L. L., Patel, N., Bell, R. C., Keisler, D., et al. (2009). Maternal nutrient restriction between early and midgestation and its impact upon appetite regulation after juvenile obesity. *Endocrinology* 150, 634–641. doi: 10.1210/en.2008-0542

Shively, C. A. (1998). Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys. *Biol. Psychiatry* 44, 882–891. doi: 10.1016/S0006-322300437-X

Shively, C. A., and Clarkson, T. B. (1988). Regional obesity and coronary artery atherosclerosis in females: a non-human primate model. *Acta Med. Scand. Suppl.* 723, 71–78.

Shively, C. A., Register, T. C., and Clarkson, T. B. (2009). Social stress, visceral obesity, and coronary artery atherosclerosis in female primates. *Obesity (Silver Spring)* 17, 1513–1520. doi: 10.1038/oby.2009.74

Slattery, D. A., and Neumann, I. D. (2008). No stress please! Mechanisms of stress hyporesponsiveness of the maternal brain. *J. Physiol.* 586, 377–385. doi: 10.1113/jphysiol.2007.145896

Spencer, S. J. (2012). Early life programming of obesity: the impact of the perinatal environment on the development of obesity and metabolic dysfunction in the offspring. *Curr. Diabetes Rev.* 8, 55–68. doi: 10.2174/157339912798829214

Spencer, S. J., Buller, K. M., and Day, T. A. (2005). Medial prefrontal cortex control of the paraventricular hypothalamic nucleus response to psychological stress: possible role of the bed nucleus of the stria terminalis. *J. Comp. Neurol.* 481, 363–376. doi: 10.1002/cne.20376

Spencer, S. J., and Tilbrook, A. (2009). Neonatal overfeeding alters adult anxiety and stress responsiveness. *Psychoneuroendocrinology* 34, 1133–1143. doi: 10.1016/j.psyneuen.2009.02.013

Spencer, S. J., Xu, L., Clarke, M. A., Lemus, M., Reichenbach, A., Geenen, B., et al. (2012). Ghrelin regulates the hypothalamic-pituitary-adrenal axis and restricts anxiety after acute stress. *Biol. Psychiatry* 72, 457–465. doi: 10.1016/j.biopsych.2012.03.010

Srinivasan, M., Katewa, S. D., Palaniyappan, A., Pandya, J. D., and Patel, M. S. (2006). Maternal high-fat diet consumption results in fetal malprogramming predisposing to the onset of metabolic syndrome-like phenotype in adulthood. *Am. J. Physiol. Endocrinol. Metab.* 291, E792–E799. doi: 10.1152/ajpendo.00078.2006

Stanner, S. A., Bulmer, K., Andres, C., Lantseva, O. E., Borodina, V., Poteen, V. V., et al. (1997). Does malnutrition *in utero* determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study. *BMJ* 315, 1342–1348. doi: 10.1136/bmj.315.7119.1342

Stefanidis, A., and Spencer, S. J. (2012). Effects of neonatal overfeeding on juvenile and adult feeding and energy expenditure in the rat. *PLoS ONE* 7:e52130. doi: 10.1371/journal.pone.0052130

Stettler, N., Stallings, V. A., Troxel, A. B., Zhao, J., Schinnar, R., Nelson, S. E., et al. (2005). Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula. *Circulation* 111, 1897–1903. doi: 10.1161/01.CIR.0000161797.67671.A7

Stevens, A., Begum, G., Cook, A., Connor, K., Rumball, C., Oliver, M., et al. (2010). Epigenetic changes in the hypothalamic proopiomelanocortin and glucocorticoid receptor genes in the ovine fetus after periconceptional undernutrition. *Endocrinology* 151, 3652–3664. doi: 10.1210/en.2010-0094

Stocker, C. J., Arch, J. R., and Cawthorne, M. A. (2005). Fetal origins of insulin resistance and obesity. *Proc. Nutr. Soc.* 64, 143–151. doi: 10.1079/PNS2005417

Stocker, C., O'dowd, J., Morton, N. M., Wargent, E., Sennitt, M. V., Hislop, D., et al. (2004). Modulation of susceptibility to weight gain and insulin resistance in low birthweight rats by treatment of their mothers with leptin during pregnancy and lactation. *Int. J. Obes. Relat. Metab. Disord.* 28, 129–136. doi: 10.1038/sj.ijo.0802476

Strack, A. M., Sebastian, R. J., Schwartz, M. W., and Dallman, M. F. (1995). Glucocorticoids and insulin: reciprocal signals for energy balance. *Am. J. Physiol.* 268, R142–R149.

Tamashiro, K. L., Terrillion, C. E., Hyun, J., Koenig, J. I., and Moran, T. H. (2009). Prenatal stress or high-fat diet increases susceptibility to diet-induced obesity in rat offspring. *Diabetes* 58, 1116–1125. doi: 10.2337/db08-1129

Thomas, M. B., Hu, M., Lee, T. M., Bhatnagar, S., and Becker, J. B. (2009). Sex-specific susceptibility to cocaine in rats with a history of prenatal stress. *Physiol. Behav.* 97, 270–277. doi: 10.1016/j.physbeh.2009.02.025

Tilbrook, A. J., Turner, A. I., Ibbott, M. D., and Clarke, I. J. (2006). Activation of the hypothalamo-pituitary-adrenal axis by isolation and restraint stress during lactation in ewes: effect of the presence of the lamb and suckling. *Endocrinology* 147, 3501–3509. doi: 10.1210/en.2005-1632

Toschke, A. M., Kuchenhoff, H., Koletzko, B., and Von Kries, R. (2005). Meal frequency and childhood obesity. *Obes. Res.* 13, 1932–1938. doi: 10.1038/oby.2005.238

Vallee, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., and Maccari, S. (1997). Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. *J. Neurosci.* 17, 2626–2636.

Van Staveren, W. A., Deurenberg, P., Burema, J., De Groot, L. C., and Hautvast, J. G. (1986). Seasonal variation in food intake, pattern of physical activity and change in body weight in a group of young adult Dutch women consuming self-selected diets. *Int. J. Obes.* 10, 133–145.

Vazquez, D. M., Bailey, C., Dent, G. W., Okimoto, D. K., Steffek, A.,

Lopez, J. F., et al. (2006). Brain corticotropin-releasing hormone (CRH) circuits in the developing rat: effect of maternal deprivation. *Brain Res.* 1121, 83–94. doi: 10.1016/j.brainres.2006.08.104

Velkoska, E., Cole, T. J., Dean, R. G., Burrell, L. M., and Morris, M. J. (2008). Early undernutrition leads to long-lasting reductions in body weight and adiposity whereas increased intake increases cardiac fibrosis in male rats. *J. Nutr.* 138, 1622–1627.

Vickers, M. H., Breier, B. H., Cutfield, W. S., Hofman, P. L., and Gluckman, P. D. (2000). Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *Am. J. Physiol. Endocrinol. Metab.* 279, E83–E87.

Vickers, M. H., Breier, B. H., McCarthy, D., and Gluckman, P. D. (2003). Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 285, R271–R273. doi: 10.1152/ajpregu.00051.2003

Vickers, M. H., Gluckman, P. D., Coveny, A. H., Hofman, P. L., Cutfield, W. S., Gertler, A., et al. (2005). Neonatal leptin treatment reverses developmental programming. *Endocrinology* 146, 4211–4216. doi: 10.1210/en.2005-0581

Vickers, M. H., Gluckman, P. D., Coveny, A. H., Hofman, P. L., Cutfield, W. S., Gertler, A., et al. (2008). The effect of neonatal leptin treatment on postnatal weight gain in male rats is dependent on maternal nutritional status during pregnancy. *Endocrinology* 149, 1906–1913. doi: 10.1210/en.2007-0981

Vickers, M. H., Ikenasio, B. A., and Breier, B. H. (2001). IGF-I treatment reduces hyperphagia, obesity, and hypertension in metabolic disorders induced by fetal programming. *Endocrinology* 142, 3964–3973. doi: 10.1210/en.142.9.3964

Wadhwa, P. D., Buss, C., Entringer, S., and Swanson, J. M. (2009). Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. *Semin. Reprod. Med.* 27, 358–368. doi: 10.1055/s-0029-1237424

Wan, Y. P., Xu, R. Y., Wu, Y. J., Chen, Z. Q., and Cai, W. (2009). Diet intervention on obese children with hypertension in China. *World J. Pediatr.* 5, 269–274. doi: 10.1007/s12519-009-0051-0

Warne, J. P., Akana, S. F., Ginsberg, A. B., Horneman, H. F., Pecoraro, N. C., and Dallman, M. F. (2009). Disengaging insulin from corticosterone: roles of each on energy intake and disposition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1366–R1375. doi: 10.1152/ajpregu.91016.2008

Warne, J. P., Horneman, H. F., Wick, E. C., Bhargava, A., Pecoraro, N. C., Ginsberg, A. B., et al. (2006). Comparison of superior mesenteric versus jugular venous infusions of insulin in streptozotocin-diabetic rats on the choice of caloric intake, body weight, and fat stores. *Endocrinology* 147, 5443–5451. doi: 10.1210/en.2006-0702

Watson, P. E., and McDonald, B. W. (2007). Seasonal variation of nutrient intake in pregnancy: effects on infant measures and possible influence on diseases related to season of birth. *Eur. J. Clin. Nutr.* 61, 1271–1280. doi: 10.1038/sj.ejcn.1602644

Weninger, S. C., Muglia, L. J., Jacobson, L., and Majzoub, J. A. (1999). CRH-deficient mice have a normal anorectic response to chronic stress. *Regul. Pept.* 84, 69–74. doi: 10.1016/S0167-011500070-1

Wigger, A., and Neumann, I. D. (1999). Periodic maternal deprivation induces gender-dependent alterations in behavioral and neuroendocrine responses to emotional stress in adult rats. *Physiol. Behav.* 66, 293–302. doi: 10.1016/S0031-938400300-X

World Health Organisation. (2002). http://www.who.int/nutrition/topics/infantfeeding_recommendation/en/

Wyrwoll, C. S., Mark, P. J., Mori, T. A., Puddey, I. B., and Waddell, B. J. (2006). Prevention of programmed hyperleptinemia and hypertension by postnatal dietary omega-3 fatty acids. *Endocrinology* 147, 599–606. doi: 10.1210/en.2005-0748

Xu, H., Hu, W., Zhang, X., Gao, W., Liang, M., Chen, T., et al. (2011). The effect of different maternal deprivation paradigms on the expression of hippocampal glucocorticoid receptors, calretinin and calbindin-D28k in male and female adolescent rats. *Neurochem. Int.* 59, 847–852. doi: 10.1016/j.neuint.2011.07.010

Yura, S., Itoh, H., Sagawa, N., Yamamoto, H., Masuzaki, H., Nakao, K., et al. (2005). Role of premature leptin surge in obesity resulting from intrauterine undernutrition. *Cell Metab.* 1, 371–378. doi: 10.1016/j.cmet.2005.05.005

Zakrzewska, K. E., Cusin, I., Sainsbury, A., Rohner-Jeanrenaud, F., and Jeanrenaud, B. (1997). Glucocorticoids as counterregulatory hormones of leptin: toward an understanding of leptin resistance. *Diabetes* 46, 717–719. doi: 10.2337/diabetes.46.4.717

Zakrzewska, K. E., Cusin, I., Stricker-Krongrad, A., Boss, O., Ricquier, D., Jeanrenaud, B., et al. (1999). Induction of obesity and hyperleptinemia by central glucocorticoid infusion in the rat. *Diabetes* 48, 365–370. doi: 10.2337/diabetes.48.2.365

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Stress induced obesity: lessons from rodent models of stress

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Stress was once defined as the non-specific result of the body to any demand or challenge to homeostasis. A more current view of stress is the behavioral and physiological responses generated in the face of, or in anticipation of, a perceived threat. The stress response involves activation of the sympathetic nervous system and recruitment of the hypothalamic-pituitary-adrenal (HPA) axis. When an organism encounters a stressor (social, physical, etc.), these endogenous stress systems are stimulated in order to generate a fight-or-flight response, and manage the stressful situation. As such, an organism is forced to liberate energy resources in attempt to meet the energetic demands posed by the stressor. A change in the energy homeostatic balance is thus required to exploit an appropriate resource and deliver useable energy to the target muscles and tissues involved in the stress response. Acutely, this change in energy homeostasis and the liberation of energy is considered advantageous, as it is required for the survival of the organism. However, when an organism is subjected to a prolonged stressor, as is the case during chronic stress, a continuous irregularity in energy homeostasis is considered detrimental and may lead to the development of metabolic disturbances such as cardiovascular disease, type II diabetes mellitus and obesity. This concept has been studied extensively using animal models, and the neurobiological underpinnings of stress induced metabolic disorders are beginning to surface. However, different animal models of stress continue to produce divergent metabolic phenotypes wherein some animals become anorexic and lose body mass while others increase food intake and body mass and become vulnerable to the development of metabolic disturbances. It remains unclear exactly what factors associated with stress models can be used to predict the metabolic outcome of the organism. This review will explore a variety of rodent stress models and discuss the elements that influence the metabolic outcome in order to further extend our understanding of stress-induced obesity.

Keywords: stress, obesity, animal models, hormones, feeding behavior, hypothalamus

INTRODUCTION

It is now generally accepted that obesity has reached epidemic proportions. To some medical professionals, obesity is considered the leading preventable cause of death worldwide. More worrisome is the fact that the prevalence of obesity has drastically increased in the past 30 years, where ∼10% of the population was considered overweight in the 1970's, a number that has now risen to over 35% in the United States (Flegal et al., 2010, 2012). This estimate is accompanied by a wealth of statistics demonstrating that individuals who are overweight or obese have a much higher susceptibility to diseases such as Type II diabetes, cardiovascular disease and metabolic syndrome. In fact, some studies show that life expectancy decreases significantly as a function of weight gain, and that individuals suffering from obesity die up to a decade sooner than those of a normal weight (Peeters et al., 2003). For this reason, obesity is one of the most pressing health issues seen in the twenty-first century.

It is clear that there is a strong genetic predisposition to obesity and metabolic disorders. Nevertheless, environmental and social factors influence amounts of food consumed or energy spent and ultimately impact phenotype. Arguably, stress may be the most important environmental factor affecting metabolism and feeding behavior to promote obesity. Indeed, there is an association between adiposity, particularly abdominal adiposity, and psychological distress (Raikkonen et al., 1996; Chrousos and Gold, 1998; Bjorntorp, 2001). Furthermore, and in parallel with the increasing rates of obesity, subjects from industrialized communities report higher levels of workplace stress and sleep deprivation in recent years relative to the past (Bjorntorp, 2001; Caruso, 2006; Grosch et al., 2006).

The physiological and behavioral responses to stress are necessary for survival. During an acute stress response, activation of the hypothalamic-pituitary-adrenal (HPA) axis entails a cascade of events that begins with the release of corticotropin-releasing factor (CRF) from specialized neurons in the paraventricular nucleus of the hypothalamus (PVN) into the portal blood neighboring the anterior pituitary gland. CRF secretion in turn leads to the synthesis and release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the general circulation, which ultimately stimulates the release of glucocorticoids from the adrenal

cortex. ACTH exerts negative feedback control over CRF at the level of the hypothalamus and, along with CRF, orchestrates the body's response to a homeostatic challenge (Aguilera, 1994). Glucocorticoid release from the adrenal glands is the final output stage of the HPA axis, which represents, amongst other things, an organism's attempt to mobilize energy stores to fuel the brain, skeletal and cardiac muscles.

The primary glucocorticoids secreted in response to stress are cortisol and corticosterone in humans and rodents, respectively. In many tissues, glucocorticoids serve an opposing function to insulin's attempt at storing energy, in part by suppressing insulin secretion (Dinneen et al., 1993). By inducing a transient state of insulin resistance, glucocorticoids restrain hepatic glucose production and regulate glucose delivery to peripheral tissue thereby producing an acute state of hyperglycemia (Rizza et al., 1982; Mangos et al., 2000). The peripheral tissues involved in the stress response use the acute hyperglycemic environment to meet the energetic demands of the stressor.

The aforementioned secretion of CRF and subsequent release of glucocorticoids is under the control of a finely tuned negative feedback loop, wherein elevated circulating glucocorticoids travel back to the hypothalamus (Jones et al., 1977; Di et al., 2003, 2005), hippocampus (Sapolsky et al., 1984, 2000), medial prefrontal cortex (Diorio et al., 1993; Radley et al., 2008) and pituitary gland (Mahmoud et al., 1984; Cole et al., 2000) to inhibit further stimulation of the HPA axis (McEwen, 2007; Ulrich-Lai and Herman, 2009). At the level of the hypothalamus, glucocorticoids have been shown to cause rapid suppression of excitatory synaptic inputs to CRF neurons within the PVN (Di et al., 2003), an effect that is thought to be mediated by the retrograde action of endocannabinoids on upstream glutamate neurons (Di et al., 2005). In contrast, glucocorticoid feedback at the level of the hippocampus increases excitatory post-synaptic potentials via modulation of membrane-bound mineralocorticoid receptors (Olijslagers et al., 2008). Rapid excitation of efferent hippocampal neurons, in turn, leads to the activation of inhibitory GABAergic input onto the neuroendocrine PVN neurons (Boudaba et al., 1996; Herman et al., 2002) and is therefore implicated in the negative feedback regulation of the HPA axis (Furay et al., 2008). Furthermore, the medial prefrontal cortex (mPFC) is also responsive to stress-induced secretion of glucocorticoids and is capable of directly modulating the activity of secretory PVN neurons (Radley et al., 2008). Injections of corticosterone (CORT) into the mPFC results in significant reductions of CRF and ACTH release following restraint stress in rats (Diorio et al., 1993), demonstrating a role for the mPFC in the regulation of HPA axis activity. After answering the energetic demands posed by a stressor, the negative feedback loop of the HPA axis ensures an efficient return to homeostatic balance following cessation of the stressor and it is therefore considered a mediator of allostasis.

The acute response to stress is one that is both necessary and beneficial because it mediates the behavioral and physiological adaptations necessary for homeostatic maintenance under stressful conditions. Protracted stressors, on the other hand, lead to an excessive energetic burden resulting in long lasting physiological changes, a process referred to as allostatic overload (McEwen, 2004). Failure to adjust to the energetic demands posed by this overload, or failure to reduce the stressor itself, may lead to negative health consequences, both metabolic and psychiatric. Increasing rates of chronic daily stress, regardless of their origin, in combination with increased availability of foods high in calories creates an environment conducive to the development of obesity and other metabolic disturbances. In turn, the rising incidence of obesity is detrimental to health care as it exerts a financial burden to taxpayers while saturating medical services that would otherwise be available. The interaction between neural circuits involved in regulating energy homeostasis and body composition with the physiological responses to stressors has therefore become pertinent and clinically relevant. In attempt to shed light on this matter, scientists must turn to one of the most widely used tools for studying the molecular underpinnings of disease, which is the use of animal models. The present review aims to investigate the use of animal models in the study of stress and to highlight some of the factors that contribute to the metabolic outcome of these various stress paradigms.

STRESS, EATING AND ENERGY HOMEOSTASIS

Stress influences feeding behaviors and energy homeostasis in both humans and rodents. However, the neural mechanisms that are recruited by the stress response are complex and involve a number of circuits that include hypothalamic networks regulating energy balance, brain stem networks regulating visceral function, sympathetic and parasympathetic mechanisms, midbrain nuclei involved in reward and arousal, and corticolimbic structures that mediate emotional and cognitive aspects related to both stress and food intake. Furthermore, stress produces dramatic endocrine and neuroendocrine responses that in themselves affect energy balance and food intake. Among these, the best-characterized responses are the activation of the sympathetic nervous system and that of the HPA axis. The former is elicited to generate rapid physiological responses including increased heart rate and respiration, glucose uptake in muscle and decreased visceral function, all important for generating a fight or flight response. Subsequent activation of the HPA axis, as previously discussed, represents a hormonal cascade that culminates in the secretion of glucocorticoids. These steroid hormones have a multitude of effects, both in the periphery and the brain that among other things alter metabolism, promote appetite and increase the locomotor activities associated with food seeking behaviors in rodents (McEwen, 2004).

Numerous studies have shown that stress modulates metabolism, but these effects are not always consistent and can range from marked decreases in both body weight and food intake, to increases in body weight and calorie consumption (Dallman et al., 2005; Depke et al., 2008; Nieuwenhuizen and Rutters, 2008). For example, acute psychogenic or systemic stressors results in a rapid decline of food intake and the successive breakdown of nutrient stores (Depke et al., 2008). Similarly, exposure to chronic stressors can also lead to decreased body weight and food intake (Torres and Nowson, 2007; Depke et al., 2008). While these animals appear to lose weight, they do by using nutrients derived from tissues other than fat in spite of decreasing their body weight (Akana et al., 1999; Depke

et al., 2008). Metabolically, these animals show altered glucose regulation, an early indicator of insulin resistance (Black, 2006).

Interestingly repeated exposure to psychogenic stressors, in particular social stressors, generates increases in body weight, adiposity, and in the intake of high calorie "comfort" meals in a number of species including non-human and human primates (Pecoraro et al., 2004; Dallman et al., 2005; Tamashiro et al., 2007a; Coccurello et al., 2008; O'Connor et al., 2008; Wilson et al., 2008). These data are of particular relevance considering that in human populations stress is highly associated with social interactions (Tamashiro et al., 2007a,b; Michaud et al., 2008; O'Connor et al., 2008; Wilson et al., 2008). The mechanisms underlying metabolic changes in response to social stressors have commonly been associated with the negative consequences of repeated stimulation of the HPA axis (Anisman and Matheson, 2005). In the case of obesity, elevated levels of glucocorticoids appear to be associated with abdominal adiposity (Black, 2006). Indeed, clinical conditions characterized by high cortisol levels, as seen in patients with Cushing's or Prader–Willi syndrome, are also associated with abdominal obesity and type II diabetes (Nieuwenhuizen and Rutters, 2008; Weaver, 2008). In contrast, removal of the adrenal glands in experimental animals results in decreased body weight and food intake that can be restored by corticosterone replacement (Saito and Bray, 1984; Pralong et al., 1993; Makimura et al., 2000).

Finally stress also affects psychological processes that could lead to the preference of foods that have a comfort value. For instance, some stressors have been associated with a loss of control over food intake and an increased drive to eat highly palatable non-nutritious foods (Groesz et al., 2012). This active seeking of highly palatable foods is thought to be a form of self-medication for both humans and rodents, wherein HPA axis activation is down regulated in response to the rewarding properties of such foods ultimately creating a preference, and potentially cravings, for these foods (Dallman et al., 2003, 2005; Pecoraro et al., 2004; Tomiyama et al., 2011). Interestingly, while all stressors are known to simulate the HPA axis, there is a documented, bi-directional effect on food intake. Physical stress, for example, may cause a reduction in food intake (Popper et al., 1989) while psychological stress can increase or decrease food intake (Huhman, 2006).

REGULATION OF FOOD INTAKE AND ENERGY BALANCE: A LOOK INSIDE THE BRAIN

To understand how stress influences metabolism and feeding we first need to consider how feeding and metabolism are regulated and then how stress can influence these mechanisms. Within the brain there exists a very complex organization of nuclei that collectively control food intake and energy homeostasis. For a more comprehensive review on the many brain circuits regulating energy homeostasis and the integration of afferent metabolic signals see Horvath et al. (2004), Abizaid and Horvath (2008).

Traditionally, the hypothalamus is considered the focal point for processing and integrating both afferent and efferent metabolic information (Elmquist, 2000; Schwartz et al., 2000; Dhillo and Bloom, 2001; Cummings and Shannon, 2003). Within the hypothalamus, these nuclei can be roughly divided into two distinct groups based on their tendencies to promote (lateral hypothalamus, LH) or inhibit food intake (ventromedial nucleus and paraventricular nucleus; VMH and PVN, respectively). Furthermore, at the base of the hypothalamus the arcuate nucleus (ARC) lies within an area that is less protected by the blood brain barrier and is thus capable of detecting peripherally circulating metabolic signals (Ciofi, 2011; Morita and Miyata, 2012, 2013). With this real estate, and the expression of both orexigenic and anorectic peptides, the ARC is thought to play an intimate role in the regulation of food intake and energy homeostasis (Schwartz et al., 2000; Cone et al., 2001; Cowley et al., 2001, 2003). The influence of the ARC on feeding behaviors is mediated by a sub-set of first-order neurons that either co-express the orexigenic transcripts neuropeptide Y (NPY) and agouti-related peptide (AgRP), or the anorectic transcripts proopiomelanocortin (POMC) and cocaine-amphetamine related transcript (CART). NPY and AgRP are two potent orexigenic peptides found predominantly in the ARC and whose expression and secretions are mediated through impairments in energy balance such as depletions of body fat stores, reductions in circulating glucose and/or altered metabolic hormone signaling in the brain (White and Kershaw, 1990; Kalra et al., 1991; Wilding et al., 1993; Schwartz et al., 2000). POMC and CART are also major regulators of energy balance, however, in contrast to NPY and AgRP, they produce a potent and long-lasting anorexigenic effect (Kristensen et al., 1998; Hagan et al., 1999; Larsen et al., 2000; Cowley et al., 2001). The expression and secretions of POMC and CART are mediated by signals of an energy surplus such as overfeeding and increases in body weight (Hagan et al., 1999). NPY/AgRP and POMC/CART neurons are profoundly interconnected and differentially regulated by other hypothalamic nuclei involved in energy homeostasis (Broberger et al., 1997; Parker and Herzog, 1999). Recently, it has been shown that selective activation of NPY/AgRP neurons in the ARC causes a potent suppression of POMC/CART neuronal activity, and a subsequent increase in long-term food intake (Atasoy et al., 2012). Thus, in addition to being influenced by peripheral signals indicating energy availability, NPY/AgRP neurons are capable of directly regulating activity of other neuronal subpopulations involved in food intake (Wahlestedt et al., 1986; Broberger et al., 1997; Parker and Herzog, 1999; Cowley et al., 2001; Atasoy et al., 2012). Furthermore, satiety centers such as the VMH have been shown to send excitatory inputs onto POMC/CART neurons, providing a more complex inhibition of food intake (Sternson et al., 2005). Interestingly, the excitatory glutamanergic neurons in the VMH express CRF receptors (Chalmers et al., 1995) and are thus stimulated by stress-induced CRF secretion, as well as the structurally related urocortin peptides (Chen et al., 2010; Kuperman et al., 2010). Once stimulated, these neurons project from the VMH to arcuate POMC/CART neurons and therefore initiate an anorectic signaling cascade downstream of the VMH (Chen et al., 2010). In contrast, NPY/AgRP neurons are regulated by weak inhibitory inputs from within the ARC but do not respond to the VMH (Sternson et al., 2005) and are not controlled by anorectic signals.

Peripheral signals ascending via the third ventricle and/or the vagus nerve are capable of directly and/or indirectly updating the first order arcuate neurons with information pertaining to the organism's nutritional status. Once stimulated, these first

order NPY/AgRP and POMC/CART neurons project to second order hypothalamic centers located predominantly in the PVN, LH, and brain stem nuclei (Clark et al., 1984; Ramos et al., 2005; Sternson et al., 2005; Palmiter, 2007; Atasoy et al., 2012; Wu et al., 2012) to regulate meal frequency (Marin Bivens et al., 1998) and meal size, respectively (Kalra et al., 1988). NPY/AgRP neurons, for example, project directly to the PVN where they are capable of producing prolonged inhibitory post-synaptic currents and a robust feeding bout (Atasoy et al., 2012). In addition, NPY/AgRP neurons project to the LH where they serve to promote the secretion of other orexigenic transcripts such as hypocretin/orexin in response to hunger signals (Cone et al., 2001). POMC/CART neurons, on the other hand, are capable of down regulating activity in these same areas. POMC/CART project to the LH and PVN to reduce food intake and increase energy expenditure, respectively (Cone et al., 2001; Cowley et al., 2001).

STRESS AND AUTONOMIC FUNCTION

Integration of circulating metabolic and stress signals also occurs in several brain stem nuclei that are part of the autonomic and enteric nervous system (Berthoud et al., 2006). As such, these nuclei are critical for the responses that follow the activation of the stress axis by psychogenic, metabolic, or immunological challenges (Smith and Vale, 2006). Within the brain stem, the nucleus of the solitary tract (NTS) is the main target for information ascending from the gut via the vagus nerve and the enteric nervous system. The NTS contains noradrenergic neurons that modulate the activity of hypothalamic and limbic structures implicated in feeding, including the PVN and central nucleus of the amygdala (CeA) (Treece et al., 2000; Grill and Kaplan, 2001, 2002). Another brain stem region important for the regulation of food intake and stress is the area postrema (AP). The AP lies outside of the blood brain barrier and is sensitive to blood born nutritional signals including hormones like leptin, insulin, cholecystokinin (CCK) and ghrelin, as well as being sensitive to changes in circulating levels of glucose. Signals are relayed from the AP to the NTS where they are integrated with ascending visceral signals, as well as to the PVN where they may elicit feeding and autonomic responses (Ellenberger and Feldman, 1990; Woulfe et al., 1990; Ferguson, 1991; Castaneyra-Perdomo et al., 1992; Diaz-Regueira and Anadon, 1992; Cai et al., 1996). In addition to the regulation of feeding responses, the brain stem plays a key role in the generation of the autonomic responses, including increases in blood pressure, respiration, hepatic glucose production, vasoconstriction, lipolysis, and thermogenesis (Bamshad et al., 1998; Krukoff, 1998; Dunn et al., 2004).

Given these roles, it is not surprising that noradrenergic cells in the brain stem are affected by chronic stress. Indeed, many of the effects of chronic stress on this system are controlled by medications that block noradrenergic receptors (i.e., β-blockers) (Grassi, 2007). Moreover, NPY released from sympathetic cells into peripheral circulation may be critical for the adipogenic effects of stress, primarily by acting directly on NPY-Y2 receptors located on adipocytes (Kuo et al., 2007).

STRESS AND COMFORT FOODS: THE ROLE OF REWARD

The midbrain contains a number of cell groups implicated in brain functions underlying reward seeking, learning and memory, affective states and the generation of locomotor responses. The ventral tegmentum area (VTA) has re-emerged as an important structure in the modulation of food intake (Simerly, 2006). Dopamine (DA) cells within this region project to the striatum, prefrontal cortex, hippocampus and amygdala (Margolis et al., 2006; Fields et al., 2007; Robinson et al., 2007) where they produce behaviors to get rewards such as food and sex, or behaviors to escape aversive stimuli (Wang et al., 2004; Wise, 2004, 2006; Volkow and Wise, 2005; Baler and Volkow, 2006). These cells also play a key role in the development of addiction (Wise, 2004, 2006; Volkow and Wise, 2005). Interestingly, fasting increases DA tone and re-feeding produces an increase of DA release into the nucleus accumbens (Wang et al., 2002). Anticipation of food and other rewards also produce DA release from the VTA into the nucleus accumbens (Blackburn et al., 1992; Richardson and Gratton, 1996). In addition to DA-related processes, serotonergic components of the raphe nucleus have been implicated in the regulation of food intake (Heisler et al., 2003; Halford et al., 2007). Indeed, there are serotonin (5-HT) projections to hypothalamic and limbic centers involved in the regulation of food intake (Heym and Gladfelter, 1982; Vertes et al., 1999; Brown and Molliver, 2000; Silva et al., 2002; Jankowski and Sesack, 2004). Several lines of evidence demonstrate that 5-HT has an inhibitory effect on food intake, including clinical and experimental data on the anorectic effects of 5-HT reuptake inhibitors (i.e., SSRIs) (Halford et al., 2007). The effects of 5-HT on food intake and metabolic function are mediated, at least in part, through the stimulation of POMC cells in the hypothalamic ARC, as well as through direct effects of 5-HT onto cells of the PVN (Heisler et al., 2003).

Stress influences these midbrain regions, and chronic stress produces long lasting changes in the monoaminergic neurotransmitters produced and released by cells in these structures (Anisman et al., 2008). The way in which these systems are changed by chronic stress are numerous and may include inflammatory responses, direct and indirect glucocorticoid action and a number of associative processes that enhance the salience of external stimuli related to the stressor (Anisman and Matheson, 2005; Anisman et al., 2008). Nevertheless, stimulation of DA and 5-HT seem to be counter regulatory responses to the stress and appear to promote behaviors associated with coping including the consumption of palatable comfort foods to reduce stress (Dallman et al., 2006).

STRESS AND METABOLIC HORMONES INTERACT TO REGULATE ENERGY BALANCE

In response to stress, there are several peptides and hormones released both centrally and peripherally that are capable of acting on many hypothalamic centers, which will inevitably dictate the feeding behaviors generated in response to a stressor. There are countless transmitters, peptides and hormones released directly, or indirectly, in response to stressful stimuli (See Anisman and Matheson, 2005 or Black, 2006 for reviews). Here, we will review

a subset of these messengers and discuss briefly how they relate to feeding responses following stress.

Glucocorticoids

Both human and rodent obesity, with an emphasis on visceral obesity, have been associated with increased HPA axis activity and subsequent rises in glucocorticoid concentrations that can affect both the brain and peripheral tissues (Weaver et al., 1993). Excess production of glucocorticoids, as seen in patients with Cushing's disease for example, leads to increases in central adiposity and additional metabolic complications (Bjorntorp and Rosmond, 1999, 2000). Like other steroid hormones, the lipophilic glucocorticoids gain access to the brain via the blood brain barrier (Castonguay, 1991) and influence the expression of hypothalamic peptides involved in the regulation of food intake and energy homeostasis (Cavagnini et al., 2000; Savontaus et al., 2002). Expression of glucocorticoid receptors (GR's) has been shown in many brain regions heavily implicated in energy homeostasis such as the ARC, LH and PVN (Morimoto et al., 1996). Given that glucocorticoids are capable of regulating behaviors that control energy input and energy expenditure, they are regarded as a key factor in the link between stress and obesity. To strengthen this association, it has been shown that bilateral adrenalectomy in rats decreases glucocorticoid concentrations as well as food intake (Germano et al., 2007), and glucocorticoid replacement in adrenalectomized animals restores food intake (Green et al., 1992; Jacobson, 1999). However, the association between glucocorticoid concentration and obesity may not be as straightforward as it was once thought to be. Glucocorticoids have been shown to stimulate expression, inhibit expression and interact with, other metabolically active hormones (e.g., insulin, leptin, CRF, and others), in turn providing a much more complex control over feeding behaviors (Brindley and Rolland, 1989; Cavagnini et al., 2000; La Fleur, 2006; Jahng et al., 2008). As previously mentioned, all stressors possess the ability to elicit glucocorticoid secretion. No two stressors, however, elicit the same response from the HPA axis or the same feeding response. The degree to which the HPA axis becomes activated, and thus the amount of glucocorticoids produced in response to the stressor, depends in large part on the length and severity of the stressor at hand (Marti et al., 1994; Harris et al., 1998; Valles et al., 2000).

Ghrelin

Despite glucocorticoids being considered the primary hormonal culprit behind the development of stress-induced metabolic disturbances, other metabolically activate hormones may also play a role in this process. One of these potential hormones is the gut derived hormone ghrelin. Ghrelin is a 28 amino acid hormone peptide produced in the X/A-like cells of the gastric oxyntic mucosa lining the stomach (Dornonville De La Cour et al., 2001). Acutely, ghrelin has the unique ability to promote food intake (Nakazato et al., 2001; Currie et al., 2005; Date et al., 2006) an effect that is mediated by the stimulation of distinct hypothalamic, brain stem, and midbrain nuclei (Cowley et al., 2003; Seoane et al., 2003; Abizaid et al., 2006). While there are countless neuropeptides that carry out anorexic functions, to date, ghrelin is the only known gut-brain peptide that encourages food intake. In addition, ghrelin contributes to the control of long-term energy homeostasis by regulating body weight and adiposity presumably by reducing lipid oxidation (Tschop et al., 2000; Choi et al., 2003; Theander-Carrillo et al., 2006; Patterson et al., 2013).

The only known ghrelin receptor, growth hormone secretagogue receptor-1a (GHSR-1a) is widely expressed in both rodents and humans, with the highest expression found in the hypothalamus and the pituitary gland, both are areas heavily invested in energy homeostasis and the physiological response to stress. Interestingly, plasma ghrelin levels have been shown to rise in parallel with glucocorticoids in response to both acute and chronic stress, and ghrelin levels remain elevated for an extended period of time after the cessation of the stressor (Asakawa et al., 2001; Kristenssson et al., 2006; Lutter et al., 2008b; Ochi et al., 2008; Patterson et al., 2010, 2013; Chuang et al., 2011). For example, acute water avoidance stress in rats has been shown to elicit an 85% increase in circulating ghrelin 1 h after cessation of the stressor (Kristenssson et al., 2006). Similarly, 24 h after the cessation of a 2-week chronic unpredictable stress paradigm stressed animals show a significant increase in both CORT and plasma acylated ghrelin compared to non-stressed animals (Patterson et al., 2010). Finally, chronic social defeat stress has been shown to increase circulating acylated ghrelin levels (Lutter et al., 2008b; Chuang et al., 2011; Patterson et al., 2013), a change that persists up to 30 days after the stress paradigm has been terminated (Lutter et al., 2008b). Administration of ghrelin, both centrally and peripherally, has been shown to indirectly stimulate hypothalamic CRF neurons, leading to HPA axis activation (Cabral et al., 2012). Ghrelin deficient mice show a reduction in the number of CRF expressing cells in the PVN and demonstrate a hyporesponsive HPA axis in response to acute stress (Spencer et al., 2012). Interruption of ghrelin signaling, both genetically and pharmacologically, during times of chronic stress can improve the metabolic outcome of these animals suggesting that ghrelin does in fact contribute to the development of stress-induced metabolic disturbances (Lutter et al., 2008b; Chuang et al., 2011; Patterson et al., 2013). Although it is generally accepted that ghrelin stimulates food intake in response to a stressor, it remains unclear what type of food is encouraged following a stress-induced secretion of ghrelin. The caloric content of foods ingested in response to a stressor will ultimately shape the metabolic phenotype of that organism. Data from our laboratory suggests that ghrelin promotes the intake of carbohydrates while simultaneously decreasing the intake of a high-fat diet, an effect thought to reflect an acute need for rapid energy resources (Patterson et al., 2013). This idea originates from findings that stressed mice display a shift toward preferentially breaking down carbohydrates as a fuel source, while protecting adipose tissue, in a ghrelin-dependent manner (Patterson et al., 2013). In contrast, others have demonstrated ghrelin-mediated increases in high-fat diet consumption, along with a stronger conditioned place preference toward a high-fat diet, following a chronic stressor (Chuang et al., 2011). Differences between these data may reflect differences in diet availability during the stress paradigm as well as differences in the time in which the food was presented relative to the stressor itself. Thus, mice may engage in reward-based eating of comfort foods and develop a conditioned place preference to such foods after

being stressed (Chuang et al., 2011), however, this may not be the case if they are tested during the stress paradigms, as is the case in our laboratory where they show a marked preference toward a carbohydrate rich diet (Patterson et al., 2013). Furthermore, an association between the presentation of the high-fat diet and the onset of the social stressor may contribute the lack of high-fat consumption seen in wild-type animals (Patterson et al., 2013).

Leptin

Leptin is another peripherally derived hormone, expressed in accordance to the size and amounts of adipocytes that is known to exert some control of feeding behaviors and energy homeostasis. Once secreted from peripheral adipocytes, leptin acts on hypothalamic centers (e.g., ARC) to reduce food intake and increase energy expenditure, thus encouraging a negative energy balance (Friedman, 2002). It has been suggested that leptin is also involved in the metabolic consequences of stress. Circulating glucocorticoids, for example, increase leptin mRNA expression and its secretion from adipocytes (Hardie et al., 1996; Russell et al., 1998; Williams et al., 2000). This pattern of direct glucocorticoid-mediated stimulation of leptin secretion has been shown in both humans and rodents (Miell et al., 1996; Mostyn et al., 2001). Once secreted, leptin directly stimulates anorectic POMC/CART neurons in the ARC and mediates the ability of other metabolically active hormones like ghrelin, for example, to depolarize these neurons (Elias et al., 1998; Cowley et al., 2001). Furthermore, it has been shown that leptin shares an overlapping physiological and intracellular signaling cascade with hormones such as insulin (Niswender and Schwartz, 2003) thus exerting control of feeding circuits from multiple standpoints. There is, however, contradicting data regarding interactions with leptin and glucocorticoids thus making it difficult to interpret the effects of leptin in the context of stress-induced feeding and/or changes in body weight. One group has shown that IP administration of glucocorticoids (mimicking levels seen following an acute stressor) increases leptin concentrations and therefore resulted in a decrease in food intake and body weight (Zakrzewska et al., 1999a). However, the same group demonstrated an opposing role for leptin when glucocorticoids were administered centrally, wherein NPY expression was increased and so too were food intake and body weight (Zakrzewska et al., 1999b). It remains unclear how leptin contributes to stress induced metabolic disorders and more studies are required in order to dissociate the interactions between central vs. peripheral leptin and glucocorticoids in response to stressful stimuli.

Others

In addition to corticosterone, ghrelin and leptin, there are a host of hormones that are stimulated by stress and that contribute directly to the regulation of food intake and energy balance. For instance, cytokines, both inflammatory and anti-inflammatory, are secreted following acute stressors (Black, 2006; Anisman et al., 2008). Chronic stressors, however, result in high levels of cytokines like IL-6 and TNF-α, both of which may contribute to obesity by causing peripheral and central leptin resistance (Velloso et al., 2008). Furthermore, while insulin may not be directly related to the stress response, its interactions with glucocorticoids allow it to participate in shaping an organism's phenotype in response to stress. For example, in the presence of insulin glucocorticoids shift their focus from mobilization of lipids to lipid accumulation (Ottosson et al., 2000). In additions, the interaction between insulin and elevated levels of glucocorticoids has been linked to increased obesity following overconsumption of comfort foods (Dallman et al., 2005). For a more detailed review on the neurochemical control of food intake see (Maniam and Morris, 2012).

STRESS MODELS

There are many disorders/diseases that are studied through the use of animal models, and obesity is no different. Over the past century, many different stress models have been designed, some in attempt to study the effects of stress on depression or anxiety, and others to directly study the effects of stress on food intake and obesity. While it is well-documented that stress is associated with changes in body weight in rodents (Barnett, 1958; Barnett et al., 1960; Marti et al., 1994) it is not always the case that animal models mimic human reality and the basis of dissimilar findings remains poorly understood. Here, we will examine several different stress models used in the context of obesity and discuss the benefits and pitfalls of each.

VISIBLE BURROW SYSTEM

The Visible Burrow System (VBS) was originally developed as a naturalistic social stressor in attempt to investigate agonistic behaviors in rats (Blanchard et al., 1977, 1995). This model reproduces an underground burrow system, which is thought to mimic the natural habitat of a rat. Traditionally, four adult male rats, and two adult female rats, are group housed in the VBS and a social hierarchy is rapidly formed within days yielding one dominant and three submissive male animals (Tamashiro et al., 2006). This provides a very unique scenario for scientists interested in studying aggressive behaviors in rodents in a "naturalistic" setting wherein there is little requirement for intervention by the experimenter. Since its inception, others who are more interested in studying the neuroendocrine systems involved with stress, feeding and energy homeostasis have adopted the VBS. Inside the VBS, submissive animals show increased basal CORT (Blanchard et al., 1993, 1995; McKittrick et al., 1995, 2000), increases in testosterone (Blanchard et al., 1995; Hardy et al., 2002; Tamashiro et al., 2004) and reliably lose ∼10–15% of their body weight (Blanchard et al., 1995; Hardy et al., 2002; Tamashiro et al., 2004). This reduction in body weight has been carefully attributed to decreases in food intake and changes in metabolic rate, while other factors such as basal locomotor activity have been eliminated as contributing factors (Tamashiro et al., 2004, 2006). The molecular underpinnings of such a robust and reliable reduction of food intake are not entirely clear. The VBS has been shown to elicit no change in the expression of orexigenic peptides or hormones, but rather elicits a reduction of leptin and insulin in submissive animals. More experiments are required for a detailed description of changes in hypothalamic feedings circuits that underlie the metabolic outcome of this stress paradigm. As a result of decreased food intake and changes in metabolic rate, submissive animals in the VBS show reductions of adiposity and a decline in lean body mass (Tamashiro et al., 2004). Interestingly,

if the animals are removed from the VBS and allowed to recover, in attempt to correct their metabolic phenotype, they show a restoration of most physiological and endocrine measures, but will not regain body weight to levels equivalent to dominant or non-stressed control animals (Tamashiro et al., 2006). Animals immediately become hyperphagic and it has been shown that the gradual accumulation of body weight during a 3 week recovery from the VBS is the result of increased visceral adipose tissue (Tamashiro et al., 2006). Interestingly, the animals display persistent anhedonia and will consume less sucrose during the recovery period, compared to controls (Tamashiro et al., 2007a).

In this regard, it would appear that the VBS is not an ideal stress paradigm for the study of obesity. While there are certainly some well-documented increases in adipose tissue observed in submissive animals, this is a relative measurement to where the animals were following the participation in the VBS. In absolute terms, these animals show an overall reduction of food intake, body weight and visceral adipose tissue and therefore do not mimic human cases of stress-induced obesity. Perhaps these findings are of no surprise given the endocrine profile of these animals. Increased testosterone, for example, as seen in submissive animals has been shown to predict reduced adipose tissue (Krotkiewski et al., 1980). Interestingly, in the absence of females inside the VBS, submissive animals do not show any changes in body weight (Tamashiro et al., 2007a). It is possible that competition for resources (food, water, enrichment, females, etc.) results in submissive animals lacking energetic resources required to develop an obese-like phenotype.

RESIDENT INTRUDER

The resident intruder paradigm, also known as social defeat stress, has long been used as a method for studying the effects of social dominance and subordination in animals (Ginsburg and Allee, 1942; Miczek, 1979; Miczek et al., 1982). Social hierarchies result in the expression of dominant and submissive behavioral and neuroendocrine patterns that can be used to study the effects of chronic stressors. When compared to other types of stress paradigms such as foot shock or restraint stress, the resident intruder paradigm appears to elicit the most robust physiological response, as measured by changes in hormonal and cardiovascular function (Koolhaas et al., 1997; Sgoifo et al., 1999; Keeney et al., 2006). The territorial nature of rodents allows experimenters to manipulate social hierarchies in attempt to study the physiological consequences of chronic social stressors. When an animal is single housed for ∼1 week, there is a rapid development of territorial behavior within the home cage and this animal is said to be the resident (Bartolomucci et al., 2001). Traditionally, in the resident intruder paradigm, the experimenter presents a novel, non-littermate rodent to the resident inside the home cage. The novel animal is thus considered an intruder and will have to fight for his (typically performed in males only) position in the social hierarchy within that cage. It has been shown that housing animals with littermates, while permitting the formation of a social hierarchy, has no influence on CORT production or anxiety-like behaviors in submissive animals and is thus considered non-stressful (Bartolomucci et al., 2001). In contrast, when housed with a novel, non-sibling rodent, submissive animals show signs of stress such as decreased immune responses (Bartolomucci et al., 2001), increased food intake, increased body weight and fat mass (Haller et al., 1999; Bhatnagar and Vining, 2003; Foster et al., 2006; Patterson et al., 2013). It is therefore necessary to use non-familiar animals when attempting to elicit metabolic changes as a consequence of social defeat stress.

Normally, this paradigm consists of two male rodents, one resident and one intruder. There are several variants of the resident intruder paradigm, all of which reflect differences in predictability, habituation, intensity and ultimately the consequent phenotype. Typically, the intruder is presented in the home cage of the resident and they are allowed to interact until one animal subdues the other. Following interaction, animals are separated by a central divider hemisecting the home cage of the resident (Bartolomucci et al., 2001; Patterson et al., 2013). This divider permits the exchange of sensory information but forbids any physical contact between the animals. By lifting this divider and allowing the two animals to interact, the experiment presents an opportunity for the animals to establish a social hierarchy, creating four possible groups: resident-dominants, resident-subordinates, intruder-dominant and intruder-subordinate (Bartolomucci et al., 2001). The physiological outcome in animals exposed to the resident intruder paradigm seems highly dependent on the nature of the social hierarchy, in particular the change in position within the social hierarchy (Bartolomucci et al., 2001, 2004, 2005; Solomon et al., 2007). Reversal of social status in mice, for example, seen when a dominant resident becomes submissive to the intruder has been associated with immunosuppression (Devoino et al., 2003) and dramatic increases in body weight (Bartolomucci et al., 2004), while residents who did not lose their dominant status showed no changes in immune function (Devoino et al., 2003) and in some cases show reductions in body weight (Bartolomucci et al., 2004). The emergence of aggressive and dominant behavior in non-defeated intruders is considered an immunostimulant (Devoino et al., 2003) and these animals show resistance to the development of obesity, as well as predictable allocation of adipose depots (Solomon et al., 2007). Therefore, status in the social hierarchy within the resident-intruder paradigm, as seen in the VBS, can be used to predict the metabolic outcome of this stress paradigm and can even predict the location wherein animals will store adipose tissue (Solomon et al., 2007). In the context of chronic social defeat stress, some laboratories choose novel residents each day of defeat (Bhatnagar and Vining, 2003; Devoino et al., 2003; Foster et al., 2006; Keeney et al., 2006; Krishnan et al., 2007; Lutter et al., 2008a,b; Chuang et al., 2011). By employing novel residents, the experimental subjects are faced with a new social hierarchy within each unique episode of defeat. In contrast, other laboratories reuse the resident, thus maintaining an exclusive social hierarchy for the duration of the experiment (Bartolomucci et al., 2001, 2004, 2010; Moles et al., 2006; Solomon et al., 2007; Patterson et al., 2013). The former prevents habituation through predictability and the latter leads to eventual habituation and is thus considered a milder form of chronic social defeat.

Interestingly, the metabolic phenotypes generated in concert with positions in social hierarchies can be substantiated by the hormonal profile of the animals. Submissive animals in

the resident intruder paradigm, for example show elevations in CORT and blood glucose, but also increases in circulating orexigenic/adipogenic plasma ghrelin (Lutter et al., 2008b; Patterson et al., 2013) and increased expression of hypothalamic NPY and AgRP (Patterson et al., 2013). Increases in ghrelin signaling leading to subsequent increases in hypothalamic NPY/AgRP results in stimulation of food intake and adiposity, with an emphasis on visceral adiposity leading to obesity. Interruption in ghrelin signaling, via pharmacological blockage or genetic manipulation, can blunt the stress-induced increases of food intake, body weight gain and adiposity seen in submissive animals (Patterson et al., 2013). Furthermore, hormonal changes that influence metabolic phenotype may vary across time throughout a chronic social defeat stress paradigms. Some physiological changes, for example, are seen immediately following an episode of defeat (Bhatnagar and Vining, 2003; Bartolomucci et al., 2004, 2005; Foster et al., 2006; Keeney et al., 2006; Lutter et al., 2008b) whereas others do not emerge until the subject has been removed from the stress paradigm and allowed to recover (Keeney et al., 2006; Moles et al., 2006; Solomon et al., 2007; Lutter et al., 2008b; Chuang et al., 2011; Patterson et al., 2013).

There are several advantages to using the resident intruder paradigm in attempt to study the physiological consequences of chronic stress. One distinct advantage is there has been a clear dissociation between food intake and body weight changes, where stressed animas will show distinct changes in body weight relative to non-stressed controls with no changes in food intake, thus suggesting that stress-induced changes in body weight and body composition are not solely attributed to increases or decreases in food intake (Haller et al., 1999; Bhatnagar and Vining, 2003; Bartolomucci et al., 2004; Moles et al., 2006; Solomon et al., 2007). Some groups have taken this one step further and dissociated the social stress aspect from the physical stress aspect of an episode of defeat and shown that social stress alone can produce changes in caloric intake (Moles et al., 2006). Interestingly, these changes in metabolism appear to extend beyond the last social interaction between resident and intruder. Subordinate animals in the resident intruder paradigm, for example, continue to show increases in body mass even weeks after last social defeat (Solomon et al., 2007; Patterson et al., 2013), when levels of food intake were equivalent to non-stressed controls (Solomon et al., 2007) suggesting that this stress paradigm left an imprint on the metabolism of these animals. This increase in body mass has been attributed to the ghrelin-dependent shift in carbohydrate utilization resulting in an increase in adipose visceral adipose tissue (Bartolomucci et al., 2001, 2004; Patterson et al., 2013) and increase overall lipid content (Moles et al., 2006; Solomon et al., 2007).

Another advantage to using the resident intruder paradigm is its apparent external validity. It has been well-documented in human cases that a higher position on a social hierarchy (as measured by socioeconomic status, for example) is associated with decreased reported stress levels (Cohen et al., 2006; Sujoldzic and De Lucia, 2007; Burdette and Hill, 2008), healthier diets (McEwen, 1998; Cartwright et al., 2003; Burdette and Hill, 2008), decreased body mass index (BMI) (George et al., 2005; Brunner et al., 2007; Burdette and Hill, 2008), etc. On the contrary, being lower on the socioeconomic hierarchy has been associated with increased stress and increased BMI (Hellerstedt and Jeffery, 1997; Laitinen et al., 2002; George et al., 2005; Cohen et al., 2006), thus shaping the trends in obesity such that humans are likely to be healthier and more fit then those beneath them on the socioeconomic ladder, regardless of their starting position. While the VBS also involves a similar type of stressor, one major difference between these two paradigms is food availability. Because a divider in the resident intruder paradigm separates the animals, they are granted unimpeded access to *ad libitum* calories, as is seen in human cases of chronic social stress.

Finally, while some stressors have anorectic effects, this particular stress paradigm causes submissive animals to overeat (Patterson et al., 2013). Overall, animals placed in the resident intruder paradigm eat more foods that are high in fat relative to non-stressed controls, but subordinate animals show increases in high fat diet intake relative to their dominant counterparts (Moles et al., 2006). However, it should be noted that the consumption of a high fat diet as a consequence of chronic stress varies from one study to another and may depend on other factors such as diet availability (time and location), gender and animal strain.

While acute social defeat is considered to be a potent stressor by means of HPA axis activity (ACTH, CORT, immune function, etc.) within hours of a defeat episode (Koolhaas et al., 1997; Sgoifo et al., 1999; Keeney et al., 2006) chronic social defeat stress results in significant habituation (Keeney et al., 2006; Patterson et al., 2013) wherein defeat stress elicits a bi-phasic response from the HPA axis. Within the first 24 h, a dramatic increase in CORT is evident following an episode of defeat. If the paradigm is continued, the rise in CORT in response to an episode of defeat is blunted for the next 2 weeks, but will show another spike if the paradigm is continued beyond 2 weeks (Keeney et al., 2006). Furthermore, there is a discrepancy in food intake, hypothalamic CRF expression and glucocorticoid secretion between acute vs. chronically defeated animals (Keeney et al., 2006; Moles et al., 2006; Patterson et al., 2013). These data are indicative of an adaptive stress response, whose ability to downplay the potency of the stressor is limited and may become detrimental in response to chronic social defeat (Keeney et al., 2006).

CHRONIC MILD STRESSORS

Chronic mild stress (CMS) has been well-documented for its ability to produce depression and the wide range of accompanying physical, behavioral and neurochemical changes that accompany depression (e.g., Anhedonia) (Willner et al., 1992; Willner, 1997; Stohr et al., 2000). Generally speaking, chronic mild stress is composed of daily stressors generated randomly from a select list of possible stressors, however, CMS protocols can vary substantially from one laboratory to the next. Daily stressors could be housing the animal in a small cage or a cage with wet bedding, housing in an overcrowded cage, cage tilt, foot shock, tail pinch, changes to light/dark cycle (24 h of light, for example), housing in a soiled cage, predator odor, loud noises and more (Willner, 1991, 1997; Muscat and Willner, 1992; Willner et al., 1992; Stohr et al., 2000; Duncko et al., 2001; Westenbroek et al., 2003; Patterson et al., 2010).

The most notable physiological consequence of CMS is the induction of anhedonia, however, this model is also capable of producing impairments in conditioned place preference, reductions in brain stimulation reward, decreased sexual behavior, compromised immune function and irregular sleep architecture (Willner, 1997). These consequences of CMS are reminiscent of the physiological and neurochemical/neuroendocrine changes observed in human cases of depression, and many consequences of CMS can be corrected with *chronic* anti-depressant administration (Willner et al., 1992; Moreau et al., 1994). Because chronic, but not acute, treatment with generic antidepressants can consistently reduce the consequences of CMS, it is considered one of the best animal models of depression.

However, very few studies that use CMS elicit an obese like state in their experimental animals. Instead, it has been shown that CMS elicits decreases in the consumption of highly palatable foods (Muscat and Willner, 1992; Patterson et al., 2010) and subsequently body weight (Muscat and Willner, 1992; Westenbroek et al., 2003; Patterson et al., 2010). It is difficult to map out the hormonal and neurochemical responses as a consequence of CMS due to the inherent variability of stress paradigms. Within any given CMS protocol exists multiple forms of stressors, each capable of eliciting a unique feeding response. Furthermore, it is difficult to draw similarities between animal models of CMS and any type of human chronic stress. As previously mentioned, any given stressor poses a distinct physiological challenge to an organism and therefore elicits a distinct response. One must consider what type of CMS can be applied to a human scenario before attempting to model human obesity.

PHYSICAL STRESSORS

Despite the growing body of evidence suggesting that psychological stress is one of the largest sources of human stress and may in fact contribute to the obesity epidemic, most animals models aimed at investigating the role of stress on metabolic processes focus on physical, rather then psychosocial, stressors (Tamashiro et al., 2006). Physical stressors are often used in tandem with other forms of stressors in chronic stress models (e.g., UCMS) or, more frequently, as acute stressors on their own.

Tail pinch/foot shock

Tail pinch and foot shock, for example, are two physical stressors used commonly and have both been shown to have an impact on feeding behaviors in rodents. It was shown very early on that chronic tail pinch in rats is capable of eliciting the consumption of an energy rich diet (Rowland and Antelman, 1976). In this particular experiment the rats were encouraged to consume calories through the presence of a highly palatable, energy rich diet. It is unclear if tail pinch is sufficient enough to induce physiological changes capable of influencing dietary habits. Rather, it has been suggested that these feeding responses are a product of general arousal as opposed to an induction of motivated feeding behaviors (Greeno and Wing, 1994). Furthermore, it has been shown that an increase in feeding behavior is not observed in all subjects exposed to tail pinch, and is therefore thought to produce unreliable feeding responses in rodents (Levine and Morley, 1981).

Foot shock, on the other hand, has been shown to increase circulating plasma glucose, insulin and leptin in rats (Farias-Silva et al., 2002; Solomon et al., 2007) suggesting an anorectic hormonal profile. The hyperglycemia and hyperleptinemia observed in animals exposed to foot shock has been attributed to insulin subsensitivity in adipose tissue (Farias-Silva et al., 2002). Despite the anorectic hormonal profile in response to repeated foot shock, animals show no change in food intake but still maintain a positive energy balance and show greater cumulative weight gain relative to non-stressed controls (Solomon et al., 2007). The changes in body mass and body composition following repeated foot shocks are said to be reminiscent of the metabolic consequences of subordinate animals in the intruder resident paradigm, albeit occurred at a much faster rate (Solomon et al., 2007).

Restraint stress/immobilization

Physical restraint and immobilization are other commonly used methods of acute physical stressors. Restraint stress is regarded as a form of mild stress, as indicated by physiologic and neuroendocrine responses, however, it has been shown to increase expression of orexigenic peptides in the hypothalamus nonetheless (Chagra et al., 2011). Interestingly, the expression of orexigenic peptides seems to increase when restraint stress is repeated over time (Chagra et al., 2011) and it has been shown that feeding responses to restraint stress are maintained in the absence of the stressor (Tamashiro et al., 2007a). Mild restraint stress has the ability to increase the number of AgRP-expressing neurons, while simultaneously reducing α-MSH receptors, in the ARC (Chagra et al., 2011). The pattern of hypothalamic activation seems to change with repeated exposure to restraint stress. Specifically, cFos activation in the PVN, ARC and LH can be observed following acute restraint, but activation is subsided with repeated exposures suggesting habituation to this stress paradigm (Chagra et al., 2011).

Exposure to an immobilization stress, on the other hand, elicits a dramatic increase in CORT secretion and is considered to be one of the most potent stressors with respect to HPA axis hyperactivity (Haque et al., 2012). Following immobilization stress, rats have been shown to decrease their food-intake and body weight (Haque et al., 2012; Wang et al., 2012), an indication of the stressors potency. Similarly, immobilization stress has been shown to increase anorectic leptin and leptin receptor (ob-R) expression in the rat hypothalamus, particularly the ARC (Wang et al., 2012). Furthermore, no changes in NPY expression are observed following immobilization stress (Wang et al., 2012), providing hormonal data to support the feeding responses to this type of stress paradigm. It is unclear as to whether chronic exposure to immobilization stress exaggerates or blunts the feeding responses seen following acute exposure. There exists conflicting data pertaining to the potential for immobilization stress habituation (Marquez et al., 2002; Dal-Zotto et al., 2004; Wang et al., 2012).

Cold stress

Exposure to cold (cold stress) has been used in both rodent and human models of stress. In humans, a cold stress produces a significant rise in cortisol levels at about 15 min following the onset of the stressor (Geliebter et al., 2012). Normally, in stress protocols that are capable of eliciting an obese like phenotype, cortisol

secretion can be correlated to subsequent caloric intake. However, the cortisol secretion observed in human patients following a cold stress do not correlate with subsequent caloric intake and these subjects show an overall decrease in the amount of calories consumed following the stressor relative to non-stressed controls (Geliebter et al., 2012). In contrast, cold stress in rodents has been shown to increase CORT secretion and CORT secretion was directly correlated to the upregulation of hypothalamic orexigenic peptides (e.g., NPY/AgRP) (Kuo et al., 2007). When cold stress was combined with the presence of a high-fat diet, there appears to be a significant increase in NPY and its receptor (Y2R) in white adipose tissue (Kuo et al., 2007). Furthermore, these changes were thought to elicit proliferation and differentiation of adipocytes, ultimately producing obesity and a metabolic syndrome-like condition (Kuo et al., 2007). Similar increases in Y2R in white adipose tissue have been shown following subordination stress (Patterson et al., 2013), however, it remains unclear if changes in NPY circuitry in response to cold stress are an attempt to insulate/thermoregulate or if they are due to marked changes in metabolism leading to an obese like state.

Given that cold stress can be consistently applied to both human and animal experimental subjects, but produce opposing metabolic outcomes, it is not considered to be a good model for studying stress-induced obesity. Decreasing body temperature, as can be expected during a cold stress paradigm, is metabolically expensive to an organism, as autonomic processes will immediately attempt to thermoregulate. Thermoregulation is metabolically expensive and might therefore elicit a feeding response to replenish the organisms energy stores. This in itself can be considered a major confound when attempting to study stress-induced obesity. Further studies are required to better define the role of orexigenic transcripts in response to cold stress.

FACTORS INFLUENCING METABOLIC OUTCOME

One hurdle faced by those using animal models to study stress-induced obesity is choosing the right model, but also the right parameters. One of the biggest difficulties when comparing results between laboratories is that their stress paradigms are often associated with different parameters (predictability, presence/absence of a calorically dense diet, presence/absence of a recovery period, time of day, etc.) Undoubtedly, there are many variables that can be manipulated by an experimenter when using any of the aforementioned stress paradigms, all of which appear to influence the metabolic outcome of the stress paradigm.

PREDICTABILITY

Predictability of a stressor will occur when experimental routines are repeated multiple times, at the same time of day in accordance with the light/dark schedule. It is well-known that the stress response adapts quickly to the presence of a stressor (McEwen, 1998, 2004; McEwen and Wingfield, 2010), and it has therefore been suggested that unpredictability of stressors will exaggerate the normal physiological response to that stressor. Intermittent bouts of social defeat, for example, have been shown to elicit very large CORT responses (Koolhaas et al., 1997; Sgoifo et al., 1999; Moles et al., 2006) and greater increases in adipose tissue deposition in rodents when compared to repeated bouts in chronic social defeat (Foster et al., 2006). Repeated bouts, on the other hand, become habituated to and elicit a dampened HPA axis response and metabolic adaptations (Keeney et al., 2006; Patterson et al., 2013). By varying the time lapse between aggressive episodes in the resident-intruder paradigm, habituation of the physiological response to the stressor cannot occur. If sustained for an extended period of time, this response may overwhelm the homeostatic mechanisms and increase the potency of that stress paradigm.

Similarly, intermittent, or acute, foot shock has been shown to cause a significant reduction in food intake and body weight, as do most physical stressors. Interestingly, when chronic foot shocks are delivered, animals are able to predict their presence, and they show no changes in caloric consumption or body weight (Griffiths et al., 1992), again lending the experimenter control over the metabolic outcome through predictability.

One must consider the evolutionary purpose of the physiological response to stress when discussing how predictability influences the outcome of a particular stressor. The physiological responses to stress are generated in attempt to meet the energetic demands of the particular stressor, liberate the appropriate energy resources while temporarily down regulating any non-pertinent bodily functions. As previously discussed, the acute stress response is an adaptive response and is one that is necessary for the survival and well-being of an organism. However, this may not be the case when dealing with protracted stressors and even more so to unpredictable protracted stressors. Habituation of the CORT response and other secreted hormones may be indicative that the demand for energy in response to the stressor may also have familiarized, thus avoiding any unnecessary changes to metabolism. However, if the animal cannot predict the presence of the stressor, then metabolic habituation is not possible which exerts the largest expense on the organism and ultimately produces the greatest metabolic consequence.

INTENSITY

The intensity of a stressor can be gauged through several objective measures such as glucocorticoid secretion (i.e., CORT), circulating CRF/ACTH levels, cFos activation in stress related brain regions, etc. There are limited number of studies investigating the relationship between stress severity and metabolic outcome, however, as a rule of thumb, the magnitude of food intake reduction as a consequence of stress can be used as a general marker of stress severity. For example, in an experiment wherein 3 types of physical stressors were used to represent low, medium and high intensity (handling, restraint, and immobilization) there was a graduated anorexic response as the intensity of the stressors increased (Marti et al., 1994). Interestingly, in this particular experiment they also employed a restraint stress (medium intensity) for different durations of time and showed no differences in reactivity, suggesting that it is in fact dependent on the stressor itself and not the length of time it is administered (Marti et al., 1994). The same pattern has been shown when comparing feeding responses between a mild food shock (1.5 mA) and a potent immobilization stressor, where immobilization leads to a significantly greater reduction in food intake, body weight and increased CORT secretion (Rabasa et al., 2011). Similar findings

have been reported from human cases, wherein eating patterns of US Marine's are negatively correlated with severity of stress exposure (Popper et al., 1989). Stress severity not only influences feeding response during time of stress, but it may also predict the persistent feeding behaviors during the recovery period (Harris et al., 1998; Valles et al., 2000). This suggests that stress severity, even in acute stressors, may in fact shape metabolic outcome of stress paradigm for the long-term.

Physiologically speaking, what makes one stressor more potent then the next? Of course one would have to consider varying levels of glucocorticoid secretion, or other metabolically active hormones, given the extent to which each stressor activates CRF neurons in the PVN. However, this may not be the case. It has been shown that many different stress paradigms, both physical and psychological stressors, with seemingly different potencies elicit indiscriminate activation of the HPA axis, with measurable differences in amygdala activation (Dayas et al., 2001). Furthermore, it is well-documented that the melanocortin system is an integral component to the anorexic responses to stress. Interestingly MC4 receptors are densely expressed in the amygdala, and when activated, these particular receptors potently inhibit food intake (Liu et al., 2013). Therefore, perhaps analysis of regional activation, outside of the HPA axis, in areas like the amygdala can better predict the intensity of a stressor and therefore the metabolic outcome. More experiments need to be conducted to better understand this phenomena.

GENDER DIFFERENCES

Like many other disorders (e.g., schizophrenia, major depression, anxiety, etc.), gender seems to play a key role in determining the overarching consequences of stress on body weight management. While there exists evidence suggesting that, in humans, there is no sex difference in stress exposure (Young and Korszun, 2010), there also exists evidence suggesting that women are more likely to acknowledge and/or recognize an event as being stressful (Goldman et al., 2005). In humans, women often report more stress-related eating (Greeno and Wing, 1994) and more consumption of foods high in fats and sugars in response to stressful situations (Wansink et al., 2003) compared to men. In support of these data, it has been suggested that perhaps the feeding responses to stressors is different between men and women as a result of their coping strategies (Grunberg and Straub, 1992). Men, for example, tend to display the typical fight or flight response to a stressor while women are more likely to seek out social support following stress, a strategy termed "tend-befriend" (Taylor et al., 2000).

Once again, we look to the use of animal models to further clarify the role of gender in stress induced metabolic dysfunctions. Under basal conditions, female rats show higher levels of CORT then do males (Stohr et al., 2000) which sets the stage for divergent phenotypes in response to stress. There is an abundance of evidence demonstrating gender-specific effects of social defeat stress (Haller et al., 1999), social instability stress (Haller et al., 1999), chronic mild stress (Stohr et al., 2000; Dalla et al., 2005, 2008; Shors et al., 2007), foot shock (Iwasaki-Sekino et al., 2009) acute forced swim test and the development of learned helplessness (Shors et al., 2007; Dalla et al., 2008, 2010). There is very limited data pertaining to the effect of social stressors on females. In the context of social defeat, males seem to be more vulnerable to changes in body weight and food intake then do females (Haller et al., 1999). It is possible that the aggressive nature of males renders them more susceptible to detrimental effects of social stressors with an aggressive component. In comparison, social stressors that lack aggression (e.g., overcrowded housing or social isolation, unpredictable social environments) elicit a greater response in female rats compared to males (Haller et al., 1999; Schmidt et al., 2010).

Given that, in humans, women are twice as likely to develop major depression then men (Kendler et al., 2002; Marcus et al., 2005), the majority of animal work done investigating gender differences in response to stress have employed the use of CMS and acute physical stressors due to their superiority in modeling human depression. It has been shown that both CMS and acute foot shock elicits an exaggerated CORT response in females compared to males (Dalla et al., 2005; Iwasaki-Sekino et al., 2009), suggesting that gender affects stress-related regions of the brain. Additionally, female rats show disruption of estrous cycles, increased floating time in a forced swim test, no reduction of body weight and dramatic changes in hippocampal serotonergic and dopaminergic neurotransmission compared to male rats, in response to CMS (Dalla et al., 2005). Interestingly, these data reflect, to some extent, human conditions of depression wherein hypo-serotonergic function in the hippocampus may lead to depressive like symptoms and correction with antidepressants, whom increase serotonergic neurotransmission, can alleviate atrophy seen in hippocampal neurons. Here we see that female rats showed reduced hippocampal serotonin turnover in response to CMS, whereas males showed no changes (Dalla et al., 2005). As would be expected, these same female rats show reduced locomotion in an open field test and increased immobility in a forced swim test (Dalla et al., 2005), behaviors reminiscent of human depression.

GENETICS AND GENETIC MODELS

It is not surprising that genetics also contribute to the metabolic outcome of some stress paradigms. When discussing rodent models of stress, different rodent strains will reliably acquire distinct metabolic phenotypes in response to equivalent stressors. It is well-documented that different strains of rodents show different effects of social stress (Berton et al., 1998) and unpredictable chronic mild stressors including both physical and psychological components (Stohr et al., 2000; Mineur et al., 2003; Pothion et al., 2004; Yalcin et al., 2008). Under basal conditions, for example, Fischer rats show a hyperactive HPA axis, as evidenced by elevated CORT levels, compared to the hypoactive HPA axis of the Lewis rat (Stohr et al., 2000). This provides a unique opportunity for experimenters to investigate the relationship between HPA axis activity and metabolic outcome of stress paradigms. For example, it has been shown that Fischer rats are more susceptible to the anxiogenic effects of CMS compared to Lewis rats, as would be expected based on their genetic differences (Stohr et al., 2000). Fischer rats also demonstrate differences in their peak CORT response and negative feedback loops associated with the HPA axis as measured by a two fold increase in latency to return to basal

Table 1 | Summary of stress paradigms, hormonal responses and physiological outcome.

Stress paradigm	Duration	Species	Intensity	Predict-ability	Physiological response	Food intake/body composition	Reference(s)
Visual burrow system	Chronic (3 weeks)	Long evans rats	+	+++	Increased CORT[1]/ decreased CORT[2], reduced leptin and insulin[2]	Decrease in food intake resulting in 10–15% body weight reduction	[1]Blanchard et al., 1995 [2]Tamashiro et al., 2004
Resident intruder paradigm	Intermittent	Syrian hamsters	+++	+	Increased leptin	Increased food intake, body weight and adiposity	Foster et al., 2006
Resident intruder paradigm	[1]Chronic (10 days) [2,3]Chronic (21 days)	[1]C57 BL6/J Mice [2]CD-1 Mice [3]C57 BL6/J Mice	+++ ++ ++	+ +++ +++	Increases in CORT, ghrelin[1,3], NPY[3], AgRP[3]	Increased food intake; no change in body weight[1]; Increased carbohydrate intake, body weight and adiposity[2,3]	[1]Lutter et al., 2008a,b [2]Bartolomucci et al., 2004, 2009 [3]Patterson et al., 2013
Chronic mild stress	Chronic	[1]Lister hooded rats [2]C57 BL6/J-DBA Mice	++	+	Increase CORT[1,2] and ghrelin[2],	Anhedonia and depression[1]; Decreased consumption of palatable diet and body weight[1,2]	[1]Muscat and Willner, 1992 [2]Patterson et al., 2010
Foot shock	Acute (1 day)	Syrian hamsters	++	+	Increased leptin, no change in insulin	Increased body mass, feed efficiency and adipose tissue	Solomon et al., 2007
Foot shock	Chronic (3–14 days)	[1]Wistar rats [2]C57 BL6/J Mice [2]BALB/c Mice	++	++	[1]Increased glucose, increased insulin	[1]Increased lipolysis [2]Decrease in caloric consumption; no change in body weight	[1]Farias-Silva et al., 2002; [2]Griffiths et al., 1992
Tail pinch	Chronic ($6 \cdot days^{-1} \times 5$ days)	Sprague–Dawley rats	+	+++	n/a	Increased caloric intake and body weight	Rowland and Antelman, 1976
Restraint	[1]Acute (1 day) [2]Acute (15 min)	[1]Sprague–Dawley rats [2]C57 BL6/J Mice	+	+	Increase cFOS in PVN[1,2], LH[1] and ARC[1]; Increased CORT[2]	Orexigenic effects[1] Anxiogenic effects[2]	[1]Chagra et al., 2011 [2]Spencer et al., 2012
Restraint	Chronic (14 days)	Sprague–Dawley rats	+	+++	Increased LH AgRP; Decreased MC4-R in ARC and LH	Increased feeding-related behaviors and redistribution of energy stores	Chagra et al., 2011

(Continued)

Table 1 | Continued

Stress paradigm	Duration	Species	Intensity	Predictability	Physiological response	Food intake/body composition	Reference(s)
Immobilization	Acute (2 h)	Albino wistar rats	+++	+	Dramatic increase in CORT	Decrease food intake and body weight	Haque et al., 2012
Immobilization	[1]Chronic (1 h × 7 days) [2]Chronic (3 h × 21 days)	Sprague–Dawley rats	+++	+	Dramatic increase in CORT; increase in leptin[2]; no changes in NPY[2]	Decrease food intake and body weight	[1]Rabasa et al., 2011 [2]Wang et al., 2012
Cold stress	Chronic (14 days–3 months)	C57 BL6/J mice	+	+++	Increase CORT and NPY/AgRP, Y2R	Increased adiposity	Kuo et al., 2007

+, *Mild;* ++, *Moderate;* +++, *High.*

CORT levels following a stressor (Stohr et al., 2000). Interestingly, in this study Lewis rats showed greater startle responses and more risk assessment behaviors as compared to Fischer rats (Stohr et al., 2000), an effect thought to be mediated by CRF.

The same phenomena has been demonstrated in different mouse strains, wherein BALB/c and C3H/He strains appear more sensitive to an unpredictable CMS compared to C57BL/6J mice, for example (Mineur et al., 2003). Similarly, differences in body weight changes, sucrose consumption, water maze performance, grooming behaviors, coat characteristics and responses to forced swim following unpredictable CMS vary significantly over different strains of mice (Mineur et al., 2003; Pothion et al., 2004; Yalcin et al., 2008). In a study by Pothion et al. (2004), it was demonstrated that sucrose preference over water, and baseline body weights, were consistent across 11 different strains of mice. When exposed to an unpredictable CMS, however, some obvious strain differences were observed wherein stressed mice consumed significantly less sucrose (CBA/H and C57 BL/6J) and gained significantly less weight (CBA/H only) relative to control mice, a phenomena that did not occur in any other strain (Pothion et al., 2004). Similarly, others have shown a reduction in sucrose consumption in CD-1, DBA/2J, C57 BL/6J and BALB/C mice in response to acute footshock while this effect was entirely absent in A/J mice (Griffiths et al., 1992). Furthermore, the extent of sucrose consumption decrease varied across different strains of mice where CD-1, DBA/2J and BALB/C mice showed a greater reduction compared to that of C57BL/6J and A/J mice (Griffiths et al., 1992).

In addition to comparing how different rodent strains respond to a variety of stress paradigms, many have used knock-out models, wherein the gene encoding a specific peptide or receptor has been removed, to explore the roles and contributions of these peptide signaling systems in response to stress. The use of ghrelin and ghrelin receptor knockout mice has been instrumental in determining the role of central ghrelin in mediating the orexigenic, adipogenic and anxiolytic effects of ghrelin following chronic social defeat stress (Lutter et al., 2008b; Patterson et al., 2013). Others have used the well-defined leptin deficient ob/ob mouse model to determine leptin's role in stress-induced feeding and anxiety. The obese-like phenotype in ob/ob mice is characterized by hypercorticosteronemia and leptin injections given to ob/ob mice reverse this hormonal profile (Ahima et al., 1996) suggesting that leptin plays a key role in the regulation of the HPA axis. It should be of no surprise that knock-out models that interrupt normal ghrelin and/or leptin signaling will influence the metabolic response to a stressor given the dense expression of ghrelin and leptin receptors on neurons containing NPY/AgRP, POMC/CART, CRF, ACTH and more. Similarly, urocortin knock-out animals have been used to help characterize the role or urocortins in mediating the physiological response to stress. Urocortin-2 deficient mice, for example, show elevations in basal stress hormones (i.e., ACTH) and do not show a rebound feeding in response to a 2 h caloric restriction test (Chen et al., 2006). Furthermore, urocortin-2 deficient mice display an antidepressant-like phenotype when exposed to both a forced swim test and a tail-suspension test (Chen et al., 2006). CRF2 receptor deficient mice also show premature inactivation of the HPA axis (Coste et al., 2000), both illustrating urocortin's influence on HPA axis activation as well as stress induced food intake and anxiety (Coste et al., 2000; Chen et al., 2006). The same patterns of diverse physiological responses can be expected from other rodent models wherein the normal expression of hypothalamic peptides is interrupted genetically.

Interestingly, the responses to antidepressants can also vary across different rodent strains and genetically modified rodent models (Yalcin et al., 2008) suggesting not only a genetic component to stress susceptibility but to the treatment of stress-related disorders as well. For example, it has been shown that hypothalamic orexigenic peptides such as ghrelin and orexin mediate the depressive-like symptoms and antidepressant-like effects of calorie restriction following chronic stress (Lutter et al., 2008a,b). These findings are relevant given the association between stress, depression and feeding. Calorically restricted orexin-null mice, for example, show anxiety like behaviors following chronic social

defeat where wild-type littermates do not (Lutter et al., 2008a). Similarly ghrelin is capable of dampening the depressive like symptoms that normally arise following chronic social defeat (Lutter et al., 2008b). These data highlight the ability of hypothalamic feeding circuitry to influence the physiological response to stress and alter the behavioral and metabolic responses generated.

CONCLUSION

It has been well-established that stressful environments are conducive to the development of obesity and related metabolic disorders. Indeed, the stress response represents a metabolic challenge that is followed by physiological and behavioral responses in attempt to meet this challenge. Evolutionary pressures have shaped these homeostatic and allostatic responses, yet continued exposure to stressors and the constant struggle to meet these energetic demands may ultimately serve as detrimental. Chronic stress may therefore alter the response to stressors and tilt the energy homeostatic scale toward an increase in energy consumption and adiposity. A detailed understanding of the interaction between the stress response, the nature of the stressors and their impact on food intake and energy balance regulation may allow for more personalized treatments of obesity, be it pharmacologically or psychologically based. The use of animal models will certainly increase our understanding of these types of interactions and aid in defining the neurobiological underpinnings of consequent metabolic disorders. However, a comprehensive understanding of the wide array of stress paradigms poses its own challenges. Using variants, or derivatives, of established stress paradigms makes any type of direct comparison of data between laboratories difficult. As a result, many different stressors are broadly categorized (e.g., **Table 1**) and may not reflect the physiological differences between them. Perhaps an attempt can be made to better distinguish the details surrounding these stress paradigms or implement some type of standardization. One thing is for certain, however, in that many different stressors pose distinctive energetic challenges on an organism, and while metabolic processes are adjusted to compensate for these challenges, many result in the conservation of adipose tissue.

REFERENCES

Abizaid, A., and Horvath, T. L. (2008). Brain circuits regulating energy homeostasis. *Regul. Pept.* 149, 3–10. doi: 10.1016/j.regpep.2007.10.006

Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., et al. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J. Clin. Invest.* 116, 3229–3239. doi: 10.1172/JCI29867

Aguilera, G. (1994). Regulation of pituitary ACTH secretion during chronic stress. *Front. Neuroendocrinol.* 15, 321–350. doi: 10.1006/frne.1994.1013

Ahima, R. S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E., et al. (1996). Role of leptin in the neuroendocrine response to fasting. *Nature* 382, 250–252. doi: 10.1038/382250a0

Akana, S. F., Strack, A. M., Hanson, E. S., Horsley, C. J., Milligan, E. D., Bhatnagar, S., et al. (1999). Interactions among chronic cold, corticosterone and puberty on energy intake and deposition. *Stress* 3, 131–146. doi: 10.3109/10253899909001118

Anisman, H., and Matheson, K. (2005). Stress, depression, and anhedonia: caveats concerning animal models. *Neurosci. Biobehav. Rev.* 29, 525–546. doi: 10.1016/j.neubiorev.2005.03.007

Anisman, H., Merali, Z., and Hayley, S. (2008). Neurotransmitter, peptide and cytokine processes in relation to depressive disorder: comorbidity between depression and neurodegenerative disorders. *Prog. Neurobiol.* 85, 1–74. doi: 10.1016/j.pneurobio.2008.01.004

Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Fujimiya, M., et al. (2001). A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. *Neuroendocrinology* 74, 143–147. doi: 10.1159/000054680

Atasoy, D., Betley, J. N., Su, H. H., and Sternson, S. M. (2012). Deconstruction of a neural circuit for hunger. *Nature* 488, 172–177. doi: 10.1038/nature11270

Baler, R. D., and Volkow, N. D. (2006). Drug addiction: the neurobiology of disrupted self-control. *Trends Mol. Med.* 12, 559–566. doi: 10.1016/j.molmed.2006.10.005

Bamshad, M., Aoki, V. T., Adkison, M. G., Warren, W. S., and Bartness, T. J. (1998). Central nervous system origins of the sympathetic nervous system outflow to white adipose tissue. *Am. J. Physiol.* 275, R291–R299.

Barnett, S. A. (1958). Physiological effects of social stress in wild rats. I. The adrenal cortex. *J. Psychosom. Res.* 3, 1–11. doi: 10.1016/0022-399990012-6

Barnett, S. A., Eaton, J. C., and McCallum, C. H. (1960). Physiological effects of "social stress" in wild rats–II. Liver glycogen and blood glucose. *J. Psychosom. Res.* 4, 251–260. doi: 10.1016/0022-399990001-5

Bartolomucci, A., Cabassi, A., Govoni, P., Ceresini, G., Cero, C., Berra, D., et al. (2009). Metabolic consequences and vulnerability to diet-induced obesity in male mice under chronic social stress. *PLoS ONE* 4:e4331. doi: 10.1371/journal.pone.0004331

Bartolomucci, A., Carola, V., Pascucci, T., Puglisi-Allegra, S., Cabib, S., Lesch, K. P., et al. (2010). Increased vulnerability to psychosocial stress in heterozygous serotonin transporter knockout mice. *Dis. Model. Mech.* 3, 459–470. doi: 10.1242/dmm.004614

Bartolomucci, A., Palanza, P., Gaspani, L., Limiroli, E., Panerai, A. E., Ceresini, G., et al. (2001). Social status in mice: behavioral, endocrine and immune changes are context dependent. *Physiol. Behav.* 73, 401–410. doi: 10.1016/S0031-938400453-X

Bartolomucci, A., Palanza, P., Sacerdote, P., Panerai, A. E., Sgoifo, A., Dantzer, R., et al. (2005). Social factors and individual vulnerability to chronic stress exposure. *Neurosci. Biobehav. Rev.* 29, 67–81. doi: 10.1016/j.neubiorev.2004.06.009

Bartolomucci, A., Pederzani, T., Sacerdote, P., Panerai, A. E., Parmigiani, S., and Palanza, P. (2004). Behavioral and physiological characterization of male mice under chronic psychosocial stress. *Psychoneuroendocrinology* 29, 899–910. doi: 10.1016/j.psyneuen.2003.08.003

Berthoud, H. R., Sutton, G. M., Townsend, R. L., Patterson, L. M., and Zheng, H. (2006). Brainstem mechanisms integrating gut-derived satiety signals and descending forebrain information in the control of meal size. *Physiol. Behav.* 89, 517–524. doi: 10.1016/j.physbeh.2006.08.018

Berton, O., Aguerre, S., Sarrieau, A., Mormede, P., and Chaouloff, F. (1998). Differential effects of social stress on central serotonergic activity and emotional reactivity in Lewis and spontaneously hypertensive rats. *Neuroscience* 82, 147–159. doi: 10.1016/S0306-452200282-0

Bhatnagar, S., and Vining, C. (2003). Facilitation of hypothalamic-pituitary-adrenal responses to novel stress following repeated social stress using the resident/intruder paradigm. *Horm. Behav.* 43, 158–165. doi: 10.1016/S0018-506X(02)00011-9

Bjorntorp, P. (2001). Do stress reactions cause abdominal obesity and comorbidities. *Obes. Rev.* 2, 73–86. doi: 10.1046/j.1467-789x.2001.00027.x

Bjorntorp, P., and Rosmond, R. (1999). Hypothalamic origin of the metabolic syndrome X. *Ann. N.Y. Acad. Sci.* 892, 297–307. doi: 10.1111/j.1749-6632.1999.tb07803.x

Bjorntorp, P., and Rosmond, R. (2000). The metabolic syndrome–a neuroendocrine disorder. *Br. J. Nutr.* 83(Suppl. 1), S49–S57. doi: 10.1017/S0007114500000957

Black, P. H. (2006). The inflammatory consequences of psychologic stress: relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. *Med. Hypotheses* 67, 879–891. doi: 10.1016/j.mehy.2006.04.008

Blackburn, J. R., Pfaus, J. G., and Phillips, A. G. (1992). Dopamine functions in appetitive and defensive behaviours. *Prog. Neurobiol.*

39, 247–279. doi: 10.1016/0301-008290018-A

Blanchard, D. C., Sakai, R. R., McEwen, B., Weiss, S. M., and Blanchard, R. J. (1993). Subordination stress: behavioral, brain, and neuroendocrine correlates. *Behav. Brain Res.* 58, 113–121. doi: 10.1016/0166-432890096-9

Blanchard, D. C., Spencer, R. L., Weiss, S. M., Blanchard, R. J., McEwen, B., and Sakai, R. R. (1995). Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. *Psychoneuroendocrinology* 20, 117–134. doi: 10.1016/0306-4530E0045-B

Blanchard, R. J., Blanchard, D. C., Takahashi, T., and Kelley, M. J. (1977). Attack and defensive behaviour in the albino rat. *Anim. Behav.* 25, 622–634. doi: 10.1016/0003-347290113-0

Boudaba, C., Szabo, K., and Tasker, J. G. (1996). Physiological mapping of local inhibitory inputs to the hypothalamic paraventricular nucleus. *J. Neurosci.* 16, 7151–7160.

Brindley, D. N., and Rolland, Y. (1989). Possible connections between stress, diabetes, obesity, hypertension and altered lipoprotein metabolism that may result in atherosclerosis. *Clin. Sci. (Lond.)* 77, 453–461.

Broberger, C., Landry, M., Wong, H., Walsh, J. N., and Hokfelt, T. (1997). Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. *Neuroendocrinology* 66, 393–408. doi: 10.1159/000127265

Brown, P., and Molliver, M. E. (2000). Dual serotonin (5-HT) projections to the nucleus accumbens core and shell: relation of the 5-HT transporter to amphetamine-induced neurotoxicity. *J. Neurosci.* 20, 1952–1963.

Brunner, E. J., Chandola, T., and Marmot, M. G. (2007). Prospective effect of job strain on general and central obesity in the Whitehall II Study. *Am. J. Epidemiol.* 165, 828–837. doi: 10.1093/aje/kwk058

Burdette, A. M., and Hill, T. D. (2008). An examination of processes linking perceived neighborhood disorder and obesity. *Soc. Sci. Med.* 67, 38–46. doi: 10.1016/j.socscimed.2008.03.029

Cabral, A., Suescun, O., Zigman, J. M., and Perello, M. (2012). Ghrelin indirectly activates hypophysiotropic CRF neurons in rodents. *PLoS ONE* 7:e31462. doi: 10.1371/journal.pone.0031462

Cai, Y., Hay, M., and Bishop, V. S. (1996). Synaptic connections and interactions between area postrema and nucleus tractus solitarius. *Brain Res.* 724, 121–124. doi: 10.1016/0006-899300282-X

Cartwright, M., Wardle, J., Steggles, N., Simon, A. E., Croker, H., and Jarvis, M. J. (2003). Stress and dietary practices in adolescents. *Health Psychol.* 22, 362–369. doi: 10.1037/0278-6133.22.4.362

Caruso, C. C. (2006). Possible broad impacts of long work hours. *Ind. Health* 44, 531–536. doi: 10.2486/indhealth.44.531

Castaneyra-Perdomo, A., Perez-Delgado, M. M., Montagnese, C., and Coen, C. W. (1992). Brainstem projections to the medial preoptic region containing the luteinizing hormone-releasing hormone perikarya in the rat. An immunohistochemical and retrograde transport study. *Neurosci. Lett.* 139, 135–139. doi: 10.1016/0304-394090875-8

Castonguay, T. W. (1991). Glucocorticoids as modulators in the control of feeding. *Brain Res. Bull.* 27, 423–428. doi: 10.1016/0361-923090136-8

Cavagnini, F., Croci, M., Putignano, P., Petroni, M. L., and Invitti, C. (2000). Glucocorticoids and neuroendocrine function. *Int. J. Obes. Relat. Metab. Disord.* 24(Suppl. 2), S77–S79. doi: 10.1038/sj.ijo.0801284

Chagra, S. L., Zavala, J. K., Hall, M. V., and Gosselink, K. L. (2011). Acute and repeated restraint differentially activate orexigenic pathways in the rat hypothalamus. *Regul. Pept.* 167, 70–78. doi: 10.1016/j.regpep.2010.11.006

Chalmers, D. T., Lovenberg, T. W., and De Souza, E. B. (1995). Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. *J. Neurosci.* 15, 6340–6350.

Chen, A., Zorrilla, E., Smith, S., Rousso, D., Levy, C., Vaughan, J., et al. (2006). Urocortin 2-deficient mice exhibit gender-specific alterations in circadian hypothalamus-pituitary-adrenal axis and depressive-like behavior. *J. Neurosci.* 26, 5500–5510. doi: 10.1523/JNEUROSCI.3955-05.2006

Chen, P., Vaughan, J., Donaldson, C., Vale, W., and Li, C. (2010). Injection of Urocortin 3 into the ventromedial hypothalamus modulates feeding, blood glucose levels, and hypothalamic POMC gene expression but not the HPA axis. *Am. J. Physiol. Endocrinol. Metab.* 298, E337–E345. doi: 10.1152/ajpendo.00402.2009

Choi, K., Roh, S. G., Hong, Y. H., Shrestha, Y. B., Hishikawa, D., Chen, C., et al. (2003). The role of ghrelin and growth hormone secretagogues receptor on rat adipogenesis. *Endocrinology* 144, 754–759. doi: 10.1210/en.2002-220783

Chrousos, G. P., and Gold, P. W. (1998). A healthy body in a healthy mind–and vice versa–the damaging power of "uncontrollable" stress. *J. Clin. Endocrinol. Metab.* 83, 1842–1845. doi: 10.1210/jc.83.6.1842

Chuang, J. C., Perello, M., Sakata, I., Osborne-Lawrence, S., Savitt, J. M., Lutter, M., et al. (2011). Ghrelin mediates stress-induced food-reward behavior in mice. *J. Clin. Invest.* 121, 2684–2692. doi: 10.1172/JCI57660

Ciofi, P. (2011). The arcuate nucleus as a circumventricular organ in the mouse. *Neurosci. Lett.* 487, 187–190. doi: 10.1016/j.neulet.2010.10.019

Clark, J. T., Kalra, P. S., Crowley, W. R., and Kalra, S. P. (1984). Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. *Endocrinology* 115, 427–429. doi: 10.1210/endo-115-1-427

Coccurello, R., D'amato, F. R., and Moles, A. (2008). Chronic social stress, hedonism and vulnerability to obesity: lessons from Rodents. *Neurosci. Biobehav. Rev.* 31, 623–633.

Cohen, S., Doyle, W. J., and Baum, A. (2006). Socioeconomic status is associated with stress hormones. *Psychosom. Med.* 68, 414–420. doi: 10.1097/01.psy.0000221236.37158.b9

Cole, M. A., Kim, P. J., Kalman, B. A., and Spencer, R. L. (2000). Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. *Psychoneuroendocrinology* 25, 151–167. doi: 10.1016/S0306-453000045-1

Cone, R. D., Cowley, M. A., Butler, A. A., Fan, W., Marks, D. L., and Low, M. J. (2001). The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *Int. J. Obes. Relat. Metab. Disord.* 25(Suppl. 5), S63–S67. doi: 10.1038/sj.ijo.0801913

Coste, S. C., Kesterson, R. A., Heldwein, K. A., Stevens, S. L., Heard, A. D., Hollis, J. H., et al. (2000). Abnormal adaptations to stress and impaired cardiovascular function in mice lacking corticotropin-releasing hormone receptor-2. *Nat. Genet.* 24, 403–409. doi: 10.1038/74255

Cowley, M. A., Smart, J. L., Rubinstein, M., Cerdan, M. G., Diano, S., Horvath, T. L., et al. (2001). Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* 411, 480–484. doi: 10.1038/35078085

Cowley, M. A., Smith, R. G., Diano, S., Tschop, M., Pronchuk, N., Grove, K. L., et al. (2003). The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37, 649–661. doi: 10.1016/S0896-627300063-1

Cummings, D. E., and Shannon, M. H. (2003). Roles for ghrelin in the regulation of appetite and body weight. *Arch. Surg.* 138, 389–396. doi: 10.1001/archsurg.138.4.389

Currie, P. J., Mirza, A., Fuld, R., Park, D., and Vasselli, J. R. (2005). Ghrelin is an orexigenic and metabolic signaling peptide in the arcuate and paraventricular nuclei. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R353–R358. doi: 10.1152/ajpregu.00756.2004

Dalla, C., Antoniou, K., Drossopoulou, G., Xagoraris, M., Kokras, N., Sfikakis, A., et al. (2005). Chronic mild stress impact: are females more vulnerable. *Neuroscience* 135, 703–714. doi: 10.1016/j.neuroscience.2005.06.068

Dalla, C., Antoniou, K., Kokras, N., Drossopoulou, G., Papathanasiou, G., Bekris, S., et al. (2008). Sex differences in the effects of two stress paradigms on dopaminergic neurotransmission. *Physiol. Behav.* 93, 595–605. doi: 10.1016/j.physbeh.2007.10.020

Dalla, C., Pitychoutis, P. M., Kokras, N., and Papadopoulou-Daifoti, Z. (2010). Sex differences in animal models of depression and antidepressant response. *Basic Clin. Pharmacol. Toxicol.* 106, 226–233. doi: 10.1111/j.1742-7843.2009.00516.x

Dallman, M. F., Pecoraro, N., Akana, S. F., La Fleur, S. E., Gomez, F., Houshyar, H., et al. (2003). Chronic stress and obesity: a new view of "comfort food". *Proc. Natl. Acad. Sci. U.S.A.* 100, 11696–11701. doi: 10.1073/pnas.1934666100

Dallman, M. F., Pecoraro, N. C., and La Fleur, S. E. (2005). Chronic stress and comfort foods: self-medication and abdominal obesity.

Brain Behav. Immun. 19, 275–280. doi: 10.1016/j.bbi.2004.11.004

Dallman, M. F., Pecoraro, N. C., La Fleur, S. E., Warne, J. P., Ginsberg, A. B., Akana, S. F., et al. (2006). Glucocorticoids, chronic stress, and obesity. *Prog. Brain Res.* 153, 75–105. doi: 10.1016/S0079-612353004-3

Dal-Zotto, S., Marti, O., Delgado, R., and Armario, A. (2004). Potentiation of glucocorticoid release does not modify the long-term effects of a single exposure to immobilization stress. *Psychopharmacology (Berl.)* 177, 230–237. doi: 10.1007/s00213-004-1939-y

Date, Y., Shimbara, T., Koda, S., Toshinai, K., Ida, T., Murakami, N., et al. (2006). Peripheral ghrelin transmits orexigenic signals through the noradrenergic pathway from the hindbrain to the hypothalamus. *Cell Metab.* 4, 323–331. doi: 10.1016/j.cmet.2006.09.004

Dayas, C. V., Buller, K. M., Crane, J. W., Xu, Y., and Day, T. A. (2001). Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. *Eur. J. Neurosci.* 14, 1143–1152. doi: 10.1046/j.0953-816x.2001.01733.x

Depke, M., Fusch, G., Domanska, G., Geffers, R., Volker, U., Schuett, C., et al. (2008). Hypermetabolic syndrome as a consequence of repeated psychological stress in mice. *Endocrinology* 149, 2714–2723. doi: 10.1210/en.2008-0038

Devoino, L., Alperina, E., and Pavina, T. (2003). Immunological consequences of the reversal of social status in C57BL/6J mice. *Brain Behav. Immun.* 17, 28–34. doi: 10.1016/S0889-159100037-5

Dhillo, W. S., and Bloom, S. R. (2001). Hypothalamic peptides as drug targets for obesity. *Curr. Opin. Pharmacol.* 1, 651–655. doi: 10.1016/S1471-489200110-2

Di, S., Malcher-Lopes, R., Halmos, K. C., and Tasker, J. G. (2003). Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. *J. Neurosci.* 23, 4850–4857.

Di, S., Malcher-Lopes, R., Marcheselli, V. L., Bazan, N. G., and Tasker, J. G. (2005). Rapid glucocorticoid-mediated endocannabinoid release and opposing regulation of glutamate and gamma-aminobutyric acid inputs to hypothalamic magnocellular neurons. *Endocrinology* 146, 4292–4301. doi: 10.1210/en.2005-0610

Diaz-Regueira, S., and Anadon, R. (1992). Central projections of the vagus nerve in Chelon labrosus Risso (Teleostei, O. Perciformes). *Brain. Behav. Evol.* 40, 297–310. doi: 10.1159/000113920

Dinneen, S., Alzaid, A., Miles, J., and Rizza, R. (1993). Metabolic effects of the nocturnal rise in cortisol on carbohydrate metabolism in normal humans. *J. Clin. Invest.* 92, 2283–2290. doi: 10.1172/JCI116832

Diorio, D., Viau, V., and Meaney, M. J. (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J. Neurosci.* 13, 3839–3847.

Dornonville De La Cour, C., Bjorkqvist, M., Sandvik, A. K., Bakke, I., Zhao, C. M., Chen, D., et al. (2001). A-like cells in the rat stomach contain ghrelin and do not operate under gastrin control. *Regul. Pept.* 99, 141–150. doi: 10.1016/S0167-011500243-9

Duncko, R., Kiss, A., Skultetyova, I., Rusnak, M., and Jezova, D. (2001). Corticotropin-releasing hormone mRNA levels in response to chronic mild stress rise in male but not in female rats while tyrosine hydroxylase mRNA levels decrease in both sexes. *Psychoneuroendocrinology* 26, 77–89. doi: 10.1016/S0306-453000040-8

Dunn, A. J., Swiergiel, A. H., and Palamarchouk, V. (2004). Brain circuits involved in corticotropin-releasing factor-norepinephrine interactions during stress. *Ann. N.Y. Acad. Sci.* 1018, 25–34. doi: 10.1196/annals.1296.003

Elias, C. F., Lee, C., Kelly, J., Aschkenasi, C., Ahima, R. S., Couceyro, P. R., et al. (1998). Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron* 21, 1375–1385. doi: 10.1016/S0896-627380656-X

Ellenberger, H. H., and Feldman, J. L. (1990). Brainstem connections of the rostral ventral respiratory group of the rat. *Brain Res.* 513, 35–42. doi: 10.1016/0006-899391086-V

Elmquist, J. K. (2000). Anatomic basis of leptin action in the hypothalamus. *Front. Horm. Res.* 26, 21–41. doi: 10.1159/000061020

Farias-Silva, E., Sampaio-Barros, M. M., Amaral, M. E., Carneiro, E. M., Boschero, A. C., Grassi-Kassisse, D. M., et al. (2002). Subsensitivity to insulin in adipocytes from rats submitted to foot-shock stress. *Can. J. Physiol. Pharmacol.* 80, 783–789. doi: 10.1139/y02-104

Ferguson, A. V. (1991). The area postrema: a cardiovascular control centre at the blood-brain interface. *Can. J. Physiol. Pharmacol.* 69, 1026–1034. doi: 10.1139/y91-153

Fields, H. L., Hjelmstad, G. O., Margolis, E. B., and Nicola, S. M. (2007). Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. *Annu. Rev. Neurosci.* 30, 289–316. doi: 10.1146/annurev.neuro.30.051606.094341

Flegal, K. M., Carroll, M. D., Kit, B. K., and Ogden, C. L. (2012). Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA* 307, 491–497. doi: 10.1001/jama.012.39

Flegal, K. M., Carroll, M. D., Ogden, C. L., and Curtin, L. R. (2010). Prevalence and trends in obesity among US adults, 1999-2008. *JAMA* 303, 235–241. doi: 10.1001/jama.2009.2014

Foster, M. T., Solomon, M. B., Huhman, K. L., and Bartness, T. J. (2006). Social defeat increases food intake, body mass, and adiposity in Syrian hamsters. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290, R1284–R1293. doi: 10.1152/ajpregu.00437.2005

Friedman, J. M. (2002). The function of leptin in nutrition, weight, and physiology. *Nutr. Rev.* 60, S1–S14. discussion S68–S84, 85–17.

Furay, A. R., Bruestle, A. E., and Herman, J. P. (2008). The role of the forebrain glucocorticoid receptor in acute and chronic stress. *Endocrinology* 149, 5482–5490. doi: 10.1210/en.2008-0642

Geliebter, A., Gibson, C. D., Hernandez, D. B., Atalayer, D., Kwon, A., Lee, M. I., et al. (2012). Plasma cortisol levels in response to a cold pressor test did not predict appetite or *ad libitum* test meal intake in obese women. *Appetite* 59, 956–959. doi: 10.1016/j.appet.2012.08.025

George, G. C., Milani, T. J., Hanss-Nuss, H., and Freeland-Graves, J. H. (2005). Compliance with dietary guidelines and relationship to psychosocial factors in low-income women in late postpartum. *J. Am. Diet. Assoc.* 105, 916–926. doi: 10.1016/j.jada.2005.03.009

Germano, C. M., De Castro, M., Rorato, R., Laguna, M. T., Antunes-Rodrigues, J., Elias, C. F., et al. (2007). Time course effects of adrenalectomy and food intake on cocaine- and amphetamine-regulated transcript expression in the hypothalamus. *Brain Res.* 1166, 55–64. doi: 10.1016/j.brainres.2007.05.077

Ginsburg, B., and Allee, W. C. (1942). Some effects of conditioning on social dominance and subordination in inbred strains of mice. *Physiol. Zool.* 15, 485–506.

Goldman, N., Glei, D. A., Seplaki, C., Liu, I. W., and Weinstein, M. (2005). Perceived stress and physiological dysregulation in older adults. *Stress* 8, 95–105. doi: 10.1080/10253890500141905

Grassi, G. (2007). Adrenergic overdrive as the link among hypertension, obesity, and impaired thermogenesis: lights and shadows. *Hypertension* 49, 5–6. doi: 10.1161/01.HYP.0000251688.60327.37

Green, P. K., Wilkinson, C. W., and Woods, S. C. (1992). Intraventricular corticosterone increases the rate of body weight gain in underweight adrenalectomized rats. *Endocrinology* 130, 269–275. doi: 10.1210/en.130.1.269

Greeno, C. G., and Wing, R. R. (1994). Stress-induced eating. *Psychol. Bull.* 115, 444–464. doi: 10.1037/0033-2909.115.3.444

Griffiths, J., Shanks, N., and Anisman, H. (1992). Strain-specific alterations in consumption of a palatable diet following repeated stressor exposure. *Pharmacol. Biochem. Behav.* 42, 219–227. doi: 10.1016/0091-305790519-L

Grill, H. J., and Kaplan, J. M. (2001). Interoceptive and integrative contributions of forebrain and brainstem to energy balance control. *Int. J. Obes. Relat. Metab. Disord.* 25(Suppl 5), S73–S77. doi: 10.1038/sj.ijo.0801917

Grill, H. J., and Kaplan, J. M. (2002). The neuroanatomical axis for control of energy balance. *Front. Neuroendocrinol.* 23, 2–40. doi: 10.1006/frne.2001.0224

Groesz, L. M., McCoy, S., Carl, J., Saslow, L., Stewart, J., Adler, N., et al. (2012). What is eating you. Stress and the drive to eat. *Appetite* 58, 717–721. doi: 10.1016/j.appet.2011.11.028

Grosch, J. W., Caruso, C. C., Rosa, R. R., and Sauter, S. L. (2006). Long hours of work in the U.S.: associations with demographic and organizational characteristics, psychosocial working conditions, and health. *Am. J. Ind. Med.* 49, 943–952. doi: 10.1002/ajim.20388

Grunberg, N. E., and Straub, R. O. (1992). The role of gender and taste class in the effects of stress on eating. *Health Psychol.* 11, 97–100. doi: 10.1037/0278-6133.11.2.97

Hagan, M. M., Rushing, P. A., Schwartz, M. W., Yagaloff, K. A., Burn, P., Woods, S. C., et al. (1999). Role of the CNS melanocortin system in the response to overfeeding. *J. Neurosci.* 19, 2362–2367.

Halford, J. C., Harrold, J. A., Boyland, E. J., Lawton, C. L., and Blundell, J. E. (2007). Serotonergic drugs: effects on appetite expression and use for the treatment of obesity. *Drugs* 67, 27–55. doi: 10.2165/00003495-200767010-00004

Haller, J., Fuchs, E., Halasz, J., and Makara, G. B. (1999). Defeat is a major stressor in males while social instability is stressful mainly in females: towards the development of a social stress model in female rats. *Brain Res. Bull.* 50, 33–39. doi: 10.1016/S0361-923000087-8

Haque, Z., Akbar, N., Yasmin, F., Haleem, M. A., and Haleem, D. J. (2012). Inhibition of immobilization stress-induced anorexia, behavioral deficits, and plasma corticosterone secretion by injected leptin in rats. *Stress* 6, 353–362. doi: 10.3109/10253890.2012.736047

Hardie, L. J., Guilhot, N., and Trayhurn, P. (1996). Regulation of leptin production in cultured mature white adipocytes. *Horm. Metab. Res.* 28, 685–689. doi: 10.1055/s-2007-979878

Hardy, M. P., Sottas, C. M., Ge, R., McKittrick, C. R., Tamashiro, K. L., McEwen, B. S., et al. (2002). Trends of reproductive hormones in male rats during psychosocial stress: role of glucocorticoid metabolism in behavioral dominance. *Biol. Reprod.* 67, 1750–1755. doi: 10.1095/biolreprod.102.006312

Harris, R. B., Zhou, J., Youngblood, B. D., Rybkin, Ii, Smagin, G. N., et al. (1998). Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets. *Am. J. Physiol.* 275, R1928–R1938.

Heisler, L. K., Cowley, M. A., Kishi, T., Tecott, L. H., Fan, W., Low, M. J., et al. (2003). Central serotonin and melanocortin pathways regulating energy homeostasis. *Ann. N.Y. Acad. Sci.* 994, 169–174. doi: 10.1111/j.1749-6632.2003.tb03177.x

Hellerstedt, W. L., and Jeffery, R. W. (1997). The association of job strain and health behaviours in men and women. *Int. J. Epidemiol.* 26, 575–583. doi: 10.1093/ije/26.3.575

Herman, J. P., Tasker, J. G., Ziegler, D. R., and Cullinan, W. E. (2002). Local circuit regulation of paraventricular nucleus stress integration: glutamate-GABA connections. *Pharmacol. Biochem. Behav.* 71, 457–468. doi: 10.1016/S0091-305700681-5

Heym, J., and Gladfelter, W. E. (1982). Locomotor activity and ingestive behavior after damage to ascending serotonergic systems. *Physiol. Behav.* 29, 459–467. doi: 10.1016/0031-938490267-0

Horvath, T. L., Diano, S., and Tschop, M. (2004). Brain circuits regulating energy homeostasis. *Neuroscientist* 10, 235–246. doi: 10.1177/1073858403262151

Huhman, K. L. (2006). Social conflict models: can they inform us about human psychopathology. *Horm. Behav.* 50, 640–646. doi: 10.1016/j.yhbeh.2006.06.022

Iwasaki-Sekino, A., Mano-Otagiri, A., Ohata, H., Yamauchi, N., and Shibasaki, T. (2009). Gender differences in corticotropin and corticosterone secretion and corticotropin-releasing factor mRNA expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala in response to footshock stress or psychological stress in rats. *Psychoneuroendocrinology* 34, 226–237. doi: 10.1016/j.psyneuen.2008.09.003

Jacobson, L. (1999). Glucocorticoid replacement, but not corticotropin-releasing hormone deficiency, prevents adrenalectomy-induced anorexia in mice. *Endocrinology* 140, 310–317. doi: 10.1210/en.140.1.310

Jahng, J. W., Kim, N. Y., Ryu, V., Yoo, S. B., Kim, B. T., Kang, D. W., et al. (2008). Dexamethasone reduces food intake, weight gain and the hypothalamic 5-HT concentration and increases plasma leptin in rats. *Eur. J. Pharmacol.* 581, 64–70. doi: 10.1016/j.ejphar.2007.11.029

Jankowski, M. P., and Sesack, S. R. (2004). Prefrontal cortical projections to the rat dorsal raphe nucleus: ultrastructural features and associations with serotonin and gamma-aminobutyric acid neurons. *J. Comp. Neurol.* 468, 518–529. doi: 10.1002/cne.10976

Jones, M. T., Hillhouse, E. W., and Burden, J. L. (1977). Structure-activity relationships of corticosteroid feedback at the hypothalamic level. *J. Endocrinol.* 74, 415–424. doi: 10.1677/joe.0.0740415

Kalra, S. P., Dube, M. G., and Kalra, P. S. (1988). Continuous intraventricular infusion of neuropeptide Y evokes episodic food intake in satiated female rats: effects of adrenalectomy and cholecystokinin. *Peptides* 9, 723–728. doi: 10.1016/0196-978190112-X

Kalra, S. P., Dube, M. G., Sahu, A., Phelps, C. P., and Kalra, P. S. (1991). Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *Proc. Natl. Acad. Sci. U.S.A.* 88, 10931–10935. doi: 10.1073/pnas.88.23.10931

Keeney, A., Jessop, D. S., Harbuz, M. S., Marsden, C. A., Hogg, S., and Blackburn-Munro, R. E. (2006). Differential effects of acute and chronic social defeat stress on hypothalamic-pituitary-adrenal axis function and hippocampal serotonin release in mice. *J. Neuroendocrinol.* 18, 330–338. doi: 10.1111/j.1365-2826.2006.01422.x

Kendler, K. S., Gardner, C. O., and Prescott, C. A. (2002). Toward a comprehensive developmental model for major depression in women. *Am. J. Psychiatry* 159, 1133–1145. doi: 10.1176/appi.ajp.159.7.1133

Koolhaas, J. M., Meerlo, P., De Boer, S. F., Strubbe, J. H., and Bohus, B. (1997). The temporal dynamics of the stress response. *Neurosci. Biobehav. Rev.* 21, 775–782. doi: 10.1016/S0149-763400057-7

Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., et al. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* 131, 391–404. doi: 10.1016/j.cell.2007.09.018

Kristensen, P., Judge, M. E., Thim, L., Ribel, U., Christjansen, K. N., Wulff, B. S., et al. (1998). Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* 393, 72–76. doi: 10.1038/29993

Kristenssson, E., Sundqvist, M., Astin, M., Kjerling, M., Mattsson, H., Dornonville De La Cour, C., et al. (2006). Acute psychological stress raises plasma ghrelin in the rat. *Regul. Pept.* 134, 114–117. doi: 10.1016/j.regpep.2006.02.003

Krotkiewski, M., Kral, J. G., and Karlsson, J. (1980). Effects of castration and testosterone substitution on body composition and muscle metabolism in rats. *Acta Physiol. Scand.* 109, 233–237. doi: 10.1111/j.1748-1716.1980.tb06592.x

Krukoff, T. L. (1998). Central regulation of autonomic function: no brakes. *Clin. Exp. Pharmacol. Physiol.* 25, 474–478. doi: 10.1111/j.1440-1681.1998.tb02238.x

Kuo, L. E., Kitlinska, J. B., Tilan, J. U., Li, L., Baker, S. B., Johnson, M. D., et al. (2007). Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nat. Med.* 13, 803–811. doi: 10.1038/nm1611

Kuperman, Y., Issler, O., Regev, L., Musseri, I., Navon, I., Neufeld-Cohen, A., et al. (2010). Perifornical Urocortin-3 mediates the link between stress-induced anxiety and energy homeostasis. *Proc. Natl. Acad. Sci. U.S.A.* 107, 8393–8398. doi: 10.1073/pnas.1003969107

La Fleur, S. E. (2006). The effects of glucocorticoids on feeding behavior in rats. *Physiol. Behav.* 89, 110–114. doi: 10.1016/j.physbeh.2006.01.028

Laitinen, J., Ek, E., and Sovio, U. (2002). Stress-related eating and drinking behavior and body mass index and predictors of this behavior. *Prev. Med.* 34, 29–39. doi: 10.1006/pmed.2001.0948

Larsen, P. J., Vrang, N., Petersen, P. C., and Kristensen, P. (2000). Chronic intracerebroventricular administration of recombinant CART(42-89) peptide inhibits and causes weight loss in lean and obese Zucker (fa/fa) rats. *Obes. Res.* 8, 590–596. doi: 10.1038/oby.2000.76

Levine, A. S., and Morley, J. E. (1981). Stress-induced eating in rats. *Am. J. Physiol.* 241, R72–R76.

Liu, J., Garza, J. C., Li, W., and Lu, X. Y. (2013). Melanocortin-4 receptor in the medial amygdala regulates emotional stress-induced anxiety-like behaviour, anorexia and corticosterone secretion. *Int. J. Neuropsychopharmacol.* 16, 105–120. doi: 10.1017/S146114571100174X

Lutter, M., Krishnan, V., Russo, S. J., Jung, S., McClung, C. A., and Nestler, E. J. (2008a). Orexin signaling mediates the antidepressant-like effect of calorie restriction. *J. Neurosci.* 28, 3071–3075. doi: 10.1523/JNEUROSCI.5584-07.2008

Lutter, M., Sakata, I., Osborne-Lawrence, S., Rovinsky, S. A., Anderson, J. G., Jung, S., et al. (2008b). The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat. Neurosci.* 11, 752–753. doi: 10.1038/nn.2139

Mahmoud, S. N., Scaccianoce, S., Scraggs, P. R., Nicholson, S. A., Gillham, B., and Jones, M. T. (1984). Characteristics of corticosteroid inhibition of adrenocorticotrophin release from the anterior pituitary gland of the rat. *J. Endocrinol.* 102, 33–42. doi: 10.1677/joe.0.1020033

Makimura, H., Mizuno, T. M., Roberts, J., Silverstein, J., Beasley, J., and Mobbs, C. V. (2000). Adrenalectomy

reverses obese phenotype and restores hypothalamic melanocortin tone in leptin-deficient ob/ob mice. *Diabetes* 49, 1917–1923. doi: 10.2337/diabetes.49.11.1917

Mangos, G. J., Walker, B. R., Kelly, J. J., Lawson, J. A., Webb, D. J., and Whitworth, J. A. (2000). Cortisol inhibits cholinergic vasodilation in the human forearm. *Am. J. Hypertens.* 13, 1155–1160. doi: 10.1016/S0895-706101201-2

Maniam, J., and Morris, M. J. (2012). The link between stress and feeding behaviour. *Neuropharmacology* 63, 97–110. doi: 10.1016/j.neuropharm.2012.04.017

Marcus, S. M., Young, E. A., Kerber, K. B., Kornstein, S., Farabaugh, A. H., Mitchell, J., et al. (2005). Gender differences in depression: findings from the STAR*D study. *J. Affect. Disord.* 87, 141–150. doi: 10.1016/j.jad.2004.09.008

Margolis, E. B., Lock, H., Hjelmstad, G. O., and Fields, H. L. (2006). The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons. *J. Physiol.* 577, 907–924. doi: 10.1113/jphysiol.2006.117069

Marin Bivens, C. L., Thomas, W. J., and Stanley, B. G. (1998). Similar feeding patterns are induced by perifornical neuropeptide Y injection and by food deprivation. *Brain Res.* 782, 271–280. doi: 10.1016/S0006-899301289-4

Marquez, C., Belda, X., and Armario, A. (2002). Post-stress recovery of pituitary-adrenal hormones and glucose, but not the response during exposure to the stressor, is a marker of stress intensity in highly stressful situations. *Brain Res.* 926, 181–185. doi: 10.1016/S0006-899303112-2

Marti, O., Marti, J., and Armario, A. (1994). Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. *Physiol. Behav.* 55, 747–753. doi: 10.1016/0031-938490055-8

McEwen, B. S. (1998). Stress, adaptation, and disease. Allostasis and allostatic load. *Ann. N.Y. Acad. Sci.* 840, 33–44. doi: 10.1111/j.1749-6632.1998.tb09546.x

McEwen, B. S. (2004). Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann. N.Y. Acad. Sci.* 1032, 1–7. doi: 10.1196/annals.1314.001

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* 87, 873–904. doi: 10.1152/physrev.00041.2006

McEwen, B. S., and Wingfield, J. C. (2010). What is in a name. Integrating homeostasis, allostasis and stress. *Horm. Behav.* 57, 105–111. doi: 10.1016/j.yhbeh.2009.09.011

McKittrick, C. R., Blanchard, D. C., Blanchard, R. J., McEwen, B. S., and Sakai, R. R. (1995). Serotonin receptor binding in a colony model of chronic social stress. *Biol. Psychiatry* 37, 383–393. doi: 10.1016/0006-322300152-S

McKittrick, C. R., Magarinos, A. M., Blanchard, D. C., Blanchard, R. J., McEwen, B. S., and Sakai, R. R. (2000). Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin transporter sites. *Synapse* 36, 85–94.

Michaud, K., Matheson, K., Kelly, O., and Anisman, H. (2008). Impact of stressors in a natural context on release of cortisol in healthy adult humans: a meta-analysis. *Stress* 11, 177–197. doi: 10.1080/10253890701727874

Miczek, K. A. (1979). A new test for aggression in rats without aversive stimulation: differential effects of d-amphetamine and cocaine. *Psychopharmacology (Berl.)* 60, 253–259. doi: 10.1007/BF00426664

Miczek, K. A., Thompson, M. L., and Shuster, L. (1982). Opioid-like analgesia in defeated mice. *Science* 215, 1520–1522. doi: 10.1126/science.7199758

Miell, J. P., Englaro, P., and Blum, W. F. (1996). Dexamethasone induces an acute and sustained rise in circulating leptin levels in normal human subjects. *Horm. Metab. Res.* 28, 704–707. doi: 10.1055/s-2007-979882

Mineur, Y. S., Prasol, D. J., Belzung, C., and Crusio, W. E. (2003). Agonistic behavior and unpredictable chronic mild stress in mice. *Behav. Genet.* 33, 513–519. doi: 10.1023/A:1025770616068

Moles, A., Bartolomucci, A., Garbugino, L., Conti, R., Caprioli, A., Coccurello, R., et al. (2006). Psychosocial stress affects energy balance in mice: modulation by social status. *Psychoneuroendocrinology* 31, 623–633. doi: 10.1016/j.psyneuen.2006.01.004

Moreau, J. L., Bourson, A., Jenck, F., Martin, J. R., and Mortas, P. (1994). Curative effects of the atypical antidepressant mianserin in the chronic mild stress-induced anhedonia model of depression. *J. Psychiatry Neurosci.* 19, 51–56.

Morimoto, M., Morita, N., Ozawa, H., Yokoyama, K., and Kawata, M. (1996). Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: an immunohistochemical and in situ hybridization study. *Neurosci. Res.* 26, 235–269. doi: 10.1016/S0168-010201105-4

Morita, S., and Miyata, S. (2012). Different vascular permeability between the sensory and secretory circumventricular organs of adult mouse brain. *Cell Tissue Res.* 349, 589–603. doi: 10.1007/s00441-012-1421-9

Morita, S., and Miyata, S. (2013). Accessibility of low-molecular-mass molecules to the median eminence and arcuate hypothalamic nucleus of adult mouse. *Cell Biochem. Funct.* doi: 10.1002/cbf.2953. [Epub ahead of print].

Mostyn, A., Keisler, D. H., Webb, R., Stephenson, T., and Symonds, M. E. (2001). The role of leptin in the transition from fetus to neonate. *Proc. Nutr. Soc.* 60, 187–194. doi: 10.1079/PNS200086

Muscat, R., and Willner, P. (1992). Suppression of sucrose drinking by chronic mild unpredictable stress: a methodological analysis. *Neurosci. Biobehav. Rev.* 16, 507–517. doi: 10.1016/S0149-763480192-7

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., et al. (2001). A role for ghrelin in the central regulation of feeding. *Nature* 409, 194–198. doi: 10.1038/35051587

Nieuwenhuizen, A. G., and Rutters, F. (2008). The hypothalamic-pituitary-adrenal-axis in the regulation of energy balance. *Physiol. Behav.* 94, 169–177. doi: 10.1016/j.physbeh.2007.12.011

Niswender, K. D., and Schwartz, M. W. (2003). Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front. Neuroendocrinol.* 24, 1–10. doi: 10.1016/S0091-302200105-X

O'Connor, D. B., Jones, F., Conner, M., McMillan, B., and Ferguson, E. (2008). Effects of daily hassles and eating style on eating behavior. *Health Psychol.* 27, S20–S31. doi: 10.1037/0278-6133.27.1.S20

Ochi, M., Tominaga, K., Tanaka, F., Tanigawa, T., Shiba, M., Watanabe, T., et al. (2008). Effect of chronic stress on gastric emptying and plasma ghrelin levels in rats. *Life Sci.* 82, 862–868. doi: 10.1016/j.lfs.2008.01.020

Olijslagers, J. E., De Kloet, E. R., Elgersma, Y., Van Woerden, G. M., Joels, M., and Karst, H. (2008). Rapid changes in hippocampal CA1 pyramidal cell function via pre- as well as postsynaptic membrane mineralocorticoid receptors. *Eur. J. Neurosci.* 27, 2542–2550. doi: 10.1111/j.1460-9568.2008.06220.x

Ottosson, M., Lonnroth, P., Bjorntorp, P., and Eden, S. (2000). Effects of cortisol and growth hormone on lipolysis in human adipose tissue. *J. Clin. Endocrinol. Metab.* 85, 799–803. doi: 10.1210/jc.85.2.799

Palmiter, R. D. (2007). Is dopamine a physiologically relevant mediator of feeding behavior. *Trends Neurosci.* 30, 375–381. doi: 10.1016/j.tins.2007.06.004

Parker, R. M., and Herzog, H. (1999). Regional distribution of Y-receptor subtype mRNAs in rat brain. *Eur. J. Neurosci.* 11, 1431–1448. doi: 10.1046/j.1460-9568.1999.00553.x

Patterson, Z. R., Ducharme, R., Anisman, H., and Abizaid, A. (2010). Altered metabolic and neurochemical responses to chronic unpredictable stressors in ghrelin receptor-deficient mice. *Eur. J. Neurosci.* 32, 632–639. doi: 10.1111/j.1460-9568.2010.07310.x

Patterson, Z. R., Khazall, R., Mackay, H., Anisman, H., and Abizaid, A. (2013). Central ghrelin signaling mediates the metabolic response of C57BL/6 male mice to chronic social defeat stress. *Endocrinology* 154, 1080–1091. doi: 10.1210/en.2012-1834

Pecoraro, N., Reyes, F., Gomez, F., Bhargava, A., and Dallman, M. F. (2004). Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. *Endocrinology* 145, 3754–3762. doi: 10.1210/en.2004-0305

Peeters, A., Barendregt, J. J., Willekens, F., Mackenbach, J. P., Al Mamun, A., Bonneux, L., et al. (2003). Obesity in adulthood and its consequences for life expectancy: a life-table analysis. *Ann. Intern. Med.* 138, 24–32. doi: 10.7326/0003-4819-138-1-200301070-00008

Popper, R., Smits, G., Meiselman, H. L., and Hirsch, E. (1989). Eating in combat: a survey of U.S. Marines. *Mil. Med.* 154, 619–623.

Pothion, S., Bizot, J. C., Trovero, F., and Belzung, C. (2004). Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. *Behav.*

Brain Res. 155, 135–146. doi: 10.1016/j.bbr.2004.04.008

Pralong, F. P., Corder, R., and Gaillard, R. C. (1993). The effects of chronic glucocorticoid excess, adrenalectomy and stress on neuropeptide Y in individual rat hypothalamic nuclei. *Neuropeptides* 25, 223–231. doi: 10.1016/0143-417990107-L

Rabasa, C., Munoz-Abellan, C., Daviu, N., Nadal, R., and Armario, A. (2011). Repeated exposure to immobilization or two different footshock intensities reveals differential adaptation of the hypothalamic-pituitary-adrenal axis. *Physiol. Behav.* 103, 125–133. doi: 10.1016/j.physbeh.2011.02.022

Radley, J. J., Williams, B., and Sawchenko, P. E. (2008). Noradrenergic innervation of the dorsal medial prefrontal cortex modulates hypothalamo-pituitary-adrenal responses to acute emotional stress. *J. Neurosci.* 28, 5806–5816. doi: 10.1523/JNEUROSCI.0552-08.2008

Raikkonen, K., Keltikangas-Jarvinen, L., Adlercreutz, H., and Hautanen, A. (1996). Psychosocial stress and the insulin resistance syndrome. *Metab. Clin. Exp.* 45, 1533–1538. doi: 10.1016/S0026-049590184-5

Ramos, E. J., Meguid, M. M., Campos, A. C., and Coelho, J. C. (2005). Neuropeptide, Y, alpha-melanocyte-stimulating hormone, and monoamines in food intake regulation. *Nutrition* 21, 269–279. doi: 10.1016/j.nut.2004.06.021

Richardson, N. R., and Gratton, A. (1996). Behavior-relevant changes in nucleus accumbens dopamine transmission elicited by food reinforcement: an electrochemical study in rat. *J. Neurosci.* 16, 8160–8169.

Rizza, R. A., Mandarino, L. J., and Gerich, J. E. (1982). Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor detect of insulin action. *J. Clin. Endocrinol. Metab.* 54, 131–138. doi: 10.1210/jcem-54-1-131

Robinson, S., Rainwater, A. J., Hnasko, T. S., and Palmiter, R. D. (2007). Viral restoration of dopamine signaling to the dorsal striatum restores instrumental conditioning to dopamine-deficient mice. *Psychopharmacology (Berl.)* 191, 567–578. doi: 10.1007/s00213-006-0579-9

Rowland, N. E., and Antelman, S. M. (1976). Stress-induced hyperphagia and obesity in rats: a possible model for understanding human obesity. *Science* 191, 310–312. doi: 10.1126/science.1246617

Russell, C. D., Petersen, R. N., Rao, S. P., Ricci, M. R., Prasad, A., Zhang, Y., et al. (1998). Leptin expression in adipose tissue from obese humans: depot-specific regulation by insulin and dexamethasone. *Am. J. Physiol.* 275, E507–E515.

Saito, M., and Bray, G. A. (1984). Adrenalectomy and food restriction in the genetically obese (ob/ob) mouse. *Am. J. Physiol.* 246, R20–R25.

Sapolsky, R. M., Krey, L. C., and McEwen, B. S. (1984). Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. *Proc. Natl. Acad. Sci. U.S.A.* 81, 6174–6177. doi: 10.1073/pnas.81.19.6174

Sapolsky, R. M., Romero, L. M., and Munck, A. U. (2000). How do glucocorticoids influence stress responses. Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21, 55–89. doi: 10.1210/er.21.1.55

Savontaus, E., Conwell, I. M., and Wardlaw, S. L. (2002). Effects of adrenalectomy on AGRP, POMC, NPY and CART gene expression in the basal hypothalamus of fed and fasted rats. *Brain Res.* 958, 130–138. doi: 10.1016/S0006-899303674-0

Schmidt, M. V., Scharf, S. H., Liebl, C., Harbich, D., Mayer, B., Holsboer, F., et al. (2010). A novel chronic social stress paradigm in female mice. *Horm. Behav.* 57, 415–420. doi: 10.1016/j.yhbeh.2010.01.010

Schwartz, M. W., Woods, S. C., Porte, D. Jr., Seeley, R. J., and Baskin, D. G. (2000). Central nervous system control of food intake. *Nature* 404, 661–671.

Seoane, L. M., Lopez, M., Tovar, S., Casanueva, F. F., Senaris, R., and Dieguez, C. (2003). Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. *Endocrinology* 144, 544–551. doi: 10.1210/en.2002-220795

Sgoifo, A., Koolhaas, J., De Boer, S., Musso, E., Stilli, D., Buwalda, B., et al. (1999). Social stress, autonomic neural activation, and cardiac activity in rats. *Neurosci. Biobehav. Rev.* 23, 915–923. doi: 10.1016/S0149-763400025-1

Shors, T. J., Mathew, J., Sisti, H. M., Edgecomb, C., Beckoff, S., and Dalla, C. (2007). Neurogenesis and helplessness are mediated by controllability in males but not in females. *Biol. Psychiatry* 62, 487–495. doi: 10.1016/j.biopsych.2006.10.033

Silva, R. C., Cruz, A. P., Avanzi, V., Landeira-Fernandez, J., and Brandao, M. L. (2002). Distinct contributions of median raphe nucleus to contextual fear conditioning and fear-potentiated startle. *Neural Plast.* 9, 233–247. doi: 10.1155/NP.2002.233

Simerly, R. (2006). Feeding signals and drugs meet in the midbrain. *Nat. Med.* 12, 1244–1246. doi: 10.1038/nm1106-1244

Smith, S. M., and Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin. Neurosci.* 8, 383–395.

Solomon, M. B., Foster, M. T., Bartness, T. J., and Huhman, K. L. (2007). Social defeat and footshock increase body mass and adiposity in male Syrian hamsters. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R283–R290. doi: 10.1152/ajpregu.00330.2006

Spencer, S. J., Xu, L., Clarke, M. A., Lemus, M., Reichenbach, A., Geenen, B., et al. (2012). Ghrelin regulates the hypothalamic-pituitary-adrenal axis and restricts anxiety after acute stress. *Biol. Psychiatry* 72, 457–465. doi: 10.1016/j.biopsych.2012.03.010

Sternson, S. M., Shepherd, G. M., and Friedman, J. M. (2005). Topographic mapping of VMH –> arcuate nucleus microcircuits and their reorganization by fasting. *Nat. Neurosci.* 8, 1356–1363. doi: 10.1038/nn1550

Stohr, T., Szuran, T., Welzl, H., Pliska, V., Feldon, J., and Pryce, C. R. (2000). Lewis/Fischer rat strain differences in endocrine and behavioural responses to environmental challenge. *Pharmacol. Biochem. Behav.* 67, 809–819. doi: 10.1016/S0091-305700426-3

Sujoldzic, A., and De Lucia, A. (2007). A cross-cultural study of adolescents–BMI, body image and psychological well-being. *Coll. Antropol.* 31, 123–130.

Tamashiro, K. L., Hegeman, M. A., and Sakai, R. R. (2006). Chronic social stress in a changing dietary environment. *Physiol. Behav.* 89, 536–542. doi: 10.1016/j.physbeh.2006.05.026

Tamashiro, K. L., Hegeman, M. A., Nguyen, M. M., Melhorn, S. J., Ma, L. Y., Woods, S. C., et al. (2007a). Dynamic body weight and body composition changes in response to subordination stress. *Physiol. Behav.* 91, 440–448. doi: 10.1016/j.physbeh.2007.04.004

Tamashiro, K. L., Nguyen, M. M., Ostrander, M. M., Gardner, S. R., Ma, L. Y., Woods, S. C., et al. (2007b). Social stress and recovery: implications for body weight and body composition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293, R1864–R1874. doi: 10.1152/ajpregu.00371.2007

Tamashiro, K. L., Nguyen, M. M., Fujikawa, T., Xu, T., Yun Ma, L., Woods, S. C., and Sakai, R. R. (2004). Metabolic and endocrine consequences of social stress in a visible burrow system. *Physiol. Behav.* 80, 683–693. doi: 10.1016/j.physbeh.2003.12.002

Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A., and Updegraff, J. A. (2000). Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol. Rev.* 107, 411–429. doi: 10.1037/0033-295X.107.3.411

Theander-Carrillo, C., Wiedmer, P., Cettour-Rose, P., Nogueiras, R., Perez-Tilve, D., Pfluger, P., et al. (2006). Ghrelin action in the brain controls adipocyte metabolism. *J. Clin. Invest.* 116, 1983–1993. doi: 10.1172/JCI25811

Tomiyama, A. J., Dallman, M. F., and Epel, E. S. (2011). Comfort food is comforting to those most stressed: evidence of the chronic stress response network in high stress women. *Psychoneuroendocrinology* 36, 1513–1519. doi: 10.1016/j.psyneuen.2011.04.005

Torres, S. J., and Nowson, C. A. (2007). Relationship between stress, eating behavior, and obesity. *Nutrition* 23, 887–894. doi: 10.1016/j.nut.2007.08.008

Treece, B. R., Ritter, R. C., and Burns, G. A. (2000). Lesions of the dorsal vagal complex abolish increases in meal size induced by NMDA receptor blockade. *Brain Res.* 872, 37–43. doi: 10.1016/S0006-899302432-X

Tschop, M., Smiley, D. L., and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* 407, 908–913. doi: 10.1038/35038090

Ulrich-Lai, Y. M., and Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nat. Rev. Neurosci.* 10, 397–409. doi: 10.1038/nrn2647

Valles, A., Marti, O., Garcia, A., and Armario, A. (2000). Single exposure to stressors causes long-lasting, stress-dependent reduction of food intake in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 279, R1138–R1144.

Velloso, L. A., Araujo, E. P., and De Souza, C. T. (2008). Diet-induced inflammation of the hypothalamus in obesity. *Neuroimmunomodulation* 15, 189–193. doi: 10.1159/000153423

Vertes, R. P., Fortin, W. J., and Crane, A. M. (1999). Projections of the median raphe nucleus in the rat. *J. Comp. Neurol.* 407, 555–582.

Volkow, N. D., and Wise, R. A. (2005). How can drug addiction help us understand obesity. *Nat. Neurosci.* 8, 555–560. doi: 10.1038/nn1452

Wahlestedt, C., Yanaihara, N., and Hakanson, R. (1986). Evidence for different pre-and post-junctional receptors for neuropeptide Y and related peptides. *Regul. Pept.* 13, 307–318. doi: 10.1016/0167-011590048-0

Wang, G. J., Volkow, N. D., and Fowler, J. S. (2002). The role of dopamine in motivation for food in humans: implications for obesity. *Expert Opin. Ther. Targets* 6, 601–609. doi: 10.1517/14728222.6.5.601

Wang, G. J., Volkow, N. D., Thanos, P. K., and Fowler, J. S. (2004). Similarity between obesity and drug addiction as assessed by neurofunctional imaging: a concept review. *J. Addict. Dis.* 23, 39–53. doi: 10.1300/J069v23n03_04

Wang, S. X., Chen, J. X., Yue, G. X., Bai, M. H., Kou, M. J., and Jin, Z. Y. (2012). Xiaoyaosan decoction regulates changes in neuropeptide y and leptin receptor in the rat arcuate nucleus after chronic immobilization stress. *Evid. Based. Complement. Alternat. Med.* 2012, 381278.

Wansink, B., Cheney, M. M., and Chan, N. (2003). Exploring comfort food preferences across age and gender. *Physiol. Behav.* 79, 739–747. doi: 10.1016/S0031-938400203-8

Weaver, J. U. (2008). Classical endocrine diseases causing obesity. *Front. Horm. Res.* 36, 212–228. doi: 10.1159/000115367

Weaver, J. U., Kopelman, P. G., McLoughlin, L., Forsling, M. L., and Grossman, A. (1993). Hyperactivity of the hypothalamo-pituitary-adrenal axis in obesity: a study of ACTH, AVP, beta-lipotrophin and cortisol responses to insulin-induced hypoglycaemia. *Clin. Endocrinol. (Oxf.)* 39, 345–350. doi: 10.1111/j.1365-2265.1993.tb02375.x

Westenbroek, C., Ter Horst, G. J., Roos, M. H., Kuipers, S. D., Trentani, A., and Den Boer, J. A. (2003). Gender-specific effects of social housing in rats after chronic mild stress exposure. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 27, 21–30. doi: 10.1016/S0278-584600310-X

White, J. D., and Kershaw, M. (1990). Increased hypothalamic neuropeptide Y expression following food deprivation. *Mol. Cell. Neurosci.* 1, 41–48. doi: 10.1016/1044-743190040-B

Wilding, J. P., Gilbey, S. G., Bailey, C. J., Batt, R. A., Williams, G., Ghatei, M. A., et al. (1993). Increased neuropeptide-Y messenger ribonucleic acid (mRNA) and decreased neurotensin mRNA in the hypothalamus of the obese (ob/ob) mouse. *Endocrinology* 132, 1939–1944. doi: 10.1210/en.132.5.1939

Williams, L. B., Fawcett, R. L., Waechter, A. S., Zhang, P., Kogon, B. E., Jones, R., et al. (2000). Leptin production in adipocytes from morbidly obese subjects: stimulation by dexamethasone, inhibition with troglitazone, and influence of gender. *J. Clin. Endocrinol. Metab.* 85, 2678–2684. doi: 10.1210/jc.85.8.2678

Willner, P. (1991). Animal models as simulations of depression. *Trends Pharmacol. Sci.* 12, 131–136. doi: 10.1016/0165-614790529-2

Willner, P. (1997). Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl.)* 134, 319–329. doi: 10.1007/s002130050456

Willner, P., Muscat, R., and Papp, M. (1992). Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci. Biobehav. Rev.* 16, 525–534. doi: 10.1016/S0149-763480194-0

Wilson, M. E., Fisher, J., Fischer, A., Lee, V., Harris, R. B., and Bartness, T. J. (2008). Quantifying food intake in socially housed monkeys: social status effects on caloric consumption. *Physiol. Behav.* 94, 586–594. doi: 10.1016/j.physbeh.2008.03.019

Wise, R. A. (2004). Dopamine, learning and motivation. *Nat. Rev. Neurosci.* 5, 483–494. doi: 10.1038/nrn1406

Wise, R. A. (2006). The parsing of food reward. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 291, R1234–R1235. doi: 10.1152/ajpregu.00443.2006

Woulfe, J. M., Flumerfelt, B. A., and Hrycyshyn, A. W. (1990). Efferent connections of the A1 noradrenergic cell group: a DBH immunohistochemical and PHA-L anterograde tracing study. *Exp. Neurol.* 109, 308–322. doi: 10.1016/S0014-488680022-6

Wu, Q., Clark, M. S., and Palmiter, R. D. (2012). Deciphering a neuronal circuit that mediates appetite. *Nature* 483, 594–597. doi: 10.1038/nature10899

Yalcin, I., Belzung, C., and Surget, A. (2008). Mouse strain differences in the unpredictable chronic mild stress: a four-antidepressant survey. *Behav. Brain Res.* 193, 140–143. doi: 10.1016/j.bbr.2008.04.021

Young, E., and Korszun, A. (2010). Sex, trauma, stress hormones and depression. *Mol. Psychiatry* 15, 23–28. doi: 10.1038/mp.2009.94

Zakrzewska, K. E., Cusin, I., Stricker-Krongrad, A., Boss, O., Ricquier, D., Jeanrenaud, B., et al. (1999a). Induction of obesity and hyperleptinemia by central glucocorticoid infusion in the rat. *Diabetes* 48, 365–370. doi: 10.2337/diabetes.48.2.365

Zakrzewska, K. E., Sainsbury, A., Cusin, I., Rouru, J., Jeanrenaud, B., and Rohner-Jeanrenaud, F. (1999b). Selective dependence of intracerebroventricular neuropeptide Y-elicited effects on central glucocorticoids. *Endocrinology* 140, 3183–3187. doi: 10.1210/en.140.7.3183

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Interruption of ghrelin signaling in the PVN increases high-fat diet intake and body weight in stressed and non-stressed C57BL6J male mice

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Chronic social stress has been associated with increased caloric intake and adiposity. These effects have been linked to stress induced changes in the secretion of ghrelin, a hormone that targets a number of brain regions to increase food intake and energy expenditure and promote increased body fat content. One of the brain sites targeted by ghrelin is the hypothalamic paraventricular nucleus (PVN), a region critical for both the regulation of the stress response and the regulation of energy balance. Given these data, we examined the contribution of ghrelin receptors in the PVN to the metabolic and behavioral changes that are seen during chronic social stress in mice. To do this, mice were implanted with cannulae attached to osmotic minipumps and delivering either vehicle or the ghrelin receptor (growth hormone secretagogue receptor) antagonist [D-Lys-3]-GHRP-6 (20 nmol/day/mouse). Following a week of recovery, half of the animals in each group were exposed to chronic social defeat stress for a period of 3 weeks whereas the other half were left undisturbed. During this time, all animals were given *ad libitum* access to standard laboratory chow and presented a high-fat diet for 4 h during the day. Results showed that the ghrelin receptor antagonism did not decrease stressed induced caloric intake, but paradoxically increased the intake of the high fat diet. This would suggest that ghrelin acts on the PVN to promote the intake of carbohydrate rich diets while decreasing fat intake and blockade of ghrelin receptors in the PVN leads to more consumption of foods that are high in fat.

Keywords: ghrelin, PVN, food intake, body weight, hypothalamus, stress, social defeat

INTRODUCTION

In mammals, environmental stressors result in the activation of a number of homeostatic and allostatic physiological mechanisms that enable the organism to deal with the impending threat (McEwen, 2007). One of these mechanisms involves the activation of the hypothalamic pituitary adrenal (HPA) axis, a major neuroendocrine system that controls the response to stress. Thus, in the face of a stressor, parvocellular cells in the paraventricular nucleus (PVN) of the hypothalamus secrete corticotropin releasing hormone (CRH) onto the portal system in the median eminence and onto the anterior pituitary. Here CRH acts on corticotropes to elicit the secretion of adrenal corticotropin releasing hormone (ACTH) into the blood stream (Smith and Vale, 2006). Increased circulating levels of ACTH stimulate cells in the adrenal cortex to release glucocorticoids (Stratakis and Chrousos, 1995; Smith and Vale, 2006). Increased levels of glucocorticoids are important for a number of reasons that include the release of glucose and glycogen stores from the liver and muscle, and the release of insulin from the pancreas facilitating the generation of energy required to meet the energetic demands posed by the stressor (Black, 2006). Glucocorticoids then provide a negative feedback signal to the hypothalamus and hippocampus to terminate any further activation of the HPA axis (McEwen, 2007).

Recently, the orexigenic hormone ghrelin has emerged as another hormone that is secreted in response to stressors and one that may produce some of the metabolic changes required to deal with the energetic demands posed by the stressors (Patterson et al., 2013). In addition to increasing food intake and growth hormone secretion, ghrelin promotes the utilization of carbohydrates as a fuel source, while preventing the use of lipids, resulting in increases in body weight due to increases in body fat (Kojima et al., 1999; Tschop et al., 2000). Moreover, these effects are produced by ghrelin acting centrally (Tschop et al., 2000; Nakazato et al., 2001; Patterson et al., 2013). Under normal conditions, ghrelin is secreted in circadian patterns, or in anticipation of scheduled meals (Cummings et al., 2001; Drazen et al., 2006). Nevertheless, ghrelin is also secreted in response to acute stressors, and circulating ghrelin levels are higher in chronically stressed animals (Lutter et al., 2008; Patterson et al., 2009, 2013). Thus, animals that are exposed to repeated social stressors often show increased caloric intake, body weight, and adiposity in tandem with increased ghrelin concentrations (Lutter et al., 2008; Bartolomucci et al., 2009; Kumar et al., 2013; Patterson et al., 2013). More importantly, ghrelin mediates these effects through its action on brain sites that contain the GHSR (Patterson et al., 2013). These data support the notion that stress generates a metabolic

challenge, and that ghrelin is released as part of the physiological mechanisms that are generated to meet this challenge. Interestingly, mice with mutations to the only known ghrelin receptor, the growth hormone secretagogue receptor (GHSR), are more susceptible to develop depressive like behaviors after chronic social stress compared to their wild type (WT) littermates (Lutter et al., 2008).

While it is clear that stress-induced ghrelin secretion affects food intake and energy balance through central actions (Patterson et al., 2013), not much is known about the relative contribution of different ghrelin-sensitive brain regions, many of which are implicated in the behavioral and metabolic responses to stress. Among these regions, the PVN stands out given its role in the regulation of the HPA axis as well as in the modulation of sympathetic responses, and its connections with peripheral tissues such as the liver, pancreas and adipose tissues (Hill, 2013). Ghrelin receptors are found within the PVN although it appears that they do not co-localize with CRH producing neurons (Guan et al., 1997; Zigman et al., 2006; Cabral et al., 2012). Nevertheless, ghrelin acting on other cells in the PVN could be responsible for some of the behavioral and metabolic alterations that are produced by exposure to chronic social stress (Cabral et al., 2012). To examine this possibility, we exposed mice to chronic social defeat stress for a period of 21 days while being infused with a ghrelin receptor antagonist ([D-Lys-3]-GHRP-6) or vehicle (0.9% saline) and compared their metabolic phenotypes with non-stressed controls receiving the same drug or vehicle infusions.

MATERIALS AND METHODS

ANIMALS

Male C57BL6J mice weighing 20–22 g were obtained from Charles River farms, St. Constant, Quebec as experimental subjects. Mice ($N = 48$) were housed under standard laboratory conditions with *ad libitum* access to mouse chow (3.3 kcal/g, with 70% of calories derived from carbohydrates) and tap water in addition to daily 4-h high fat diet containing 60% caloric content from fat (TD 06414, Harlan). The calculated metabolized energy of the high fat diet was 5.1 kcal/g with 60.0% calories from fat. Male CD-1 retired breeder mice weighing 40–50 g, also obtained from Charles River Farms, were used as aggressors in the chronic social defeat stress paradigm. Food intake (both standard laboratory chow and high-fat diet) and body weight were weighed and recorded daily by the experimenters at 9:00 AM. The high-fat diet was available from 10:00 AM to 2:00 PM daily. Following the baseline period, mice were separated into 4 experimental groups as follows: vehicle stressed ($n = 12$), vehicle non-stressed ($n = 12$), [D-Lys-3]-GHRP-6 non-stressed ($n = 12$) and [D-Lys-3]-GHRP-6 stressed ($n = 12$). Due to attrition of animals throughout the 28-day cannula-minipump infusion process, as well as misplaced cannulae, the final group numbers used for data analysis were as follows: vehicle stressed ($n = 6$), vehicle non-stressed ($n = 10$), [D-Lys-3]-GHRP-6 non-stressed ($n = 4$) and [D-Lys-3]-GHRP-6 stressed ($n = 6$). All procedures documented were approved by the Carleton University Animal Care Committee and the guidelines of the Canadian Council on Animal Care were followed.

STEREOTAXIC SURGERY—CHRONIC DELIVERY OF GHRELIN RECEPTOR ANTAGONIST INTO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS

Prior to surgery, we conducted a 2-week baseline where measures of food intake (both standard laboratory chow and high fat diet) and body weight were recorded daily. After baseline, mice were anesthetized using isofluorane mixed with oxygen (4%) and implanted with an intracranial cannula connected to an osmotic minipump delivering either saline or the ghrelin receptor antagonist [D-Lys-3]-GHRP-6. To do this, the mouse's head was shaved and secured onto a mouse stereotaxic apparatus while anaesthetized (Kopf Instruments, Tujunga, CA). The scalp was cleaned with surgiprep and privodine to provide an aseptic canvas. Tear gel was applied to prevent dehydration of the eyes. A midline incision was made and the skin was retracted for a clear visualization of bregma. A 28 gauge stainless steel unilateral cannula (Alzet Brain Infusion Kit; Order #004760) coupled to an osmotic mini-pump (Alzet Mini-Osmotic Pump—Model 1004; flow rate: 0.11 μL/h for 28 days) using a polyethylene catheter and was implanted into the PVN of the hypothalamus. Stereotactic coordinates of the cannula, relative to bregma, were AP −0.94 mm, ML −1.75 mm and DV 4.83 mm (Paxinos and Franklin, 1997). Mini-pumps were filled with 100 μL of sterile saline (0.9% NaCl) to the control group while the experimental group had mini-pumps filled with 100 μL of the ghrelin receptor antagonist ([D-Lys-3]-GHRP-6) solution (Peptides International; 20 nmol/day/mouse). The implant was secured with contact and dental cement. When the cement was dry, the dorsal portion of the skin was separated from the muscle using blunt dissection to implant the mini-pump subcutaneously. The incision was closed using silk surgical sutures. Polysporin and Lidocaine were applied topically to the surgical site to prevent bacterial infection and pain, respectively. Mice were also injected with a low dose of meloxicam (Metacam, 0.1 mL of 5 mg/mL) to provide postoperative analgesia. Following surgery, mice were moved to a recovery area in a clean cage with a heating pad. Upon wakening, mice were monitored closely for optimal recovery for a total of 7 days following surgery.

CHRONIC SOCIAL DEFEAT STRESS PARADIGM

Following the 1 week recovery period after surgery, half of the saline and half of the ghrelin receptor antagonist infused mice were taken from their room and transported to another room where each of them was housed in a cage inhabited by a much larger sexually experience male CD-1 mouse for the next 21 days (Patterson et al., 2013). The experimental mice were protected from the aggressive CD-1 mice by an acrylic divider and wire mesh, yet olfactory, visual, and auditory contact was maintained with the resident for the entire 21-day stress period. Each day, the divider was removed and the animals were permitted to interact until the experimental mouse was subdued or until 15 min had passed. Measurements of regular chow intake, high fat diet intake, and body weight were recorded each day during the stress period.

BLOOD PLASMA COLLECTION

At the end of the 21 days of chronic social defeat stress, mice in the stress group were sacrificed by rapid decapitation, the

morning following the last social interaction with the dominant mouse. Non-stressed mice were sacrificed on the same day. Glucose concentrations from trunk blood samples were recorded using Accuchek® (Aviva) glucose meter and strips. Blood samples were also collected for analyses of plasma corticosterone (CORT) content. These samples were analyzed using a radioimmunoassay kit according to the manufacturer's protocol (ICN Biomedicals, CA, USA). Inter assay variability was less than 10%.

STATISTICS

All data were analyzed using 2×2 ANOVAs with drug ([D-Lys-3]-GHRP-6 vs. 0.9% saline) and treatment (stress vs. non-stress) as the between group factors, unless otherwise stated. Data from animals with misplaced cannula were not included in the analyses. All analyses were preceded with a Levene's Test of Quality of Error Variance to ensure the homogeneity of variance assumption was satisfied. The limit for statistical significance was set at $\alpha = 0.05$. All mice with misplaced cannula were eliminated from all statistical analysis.

RESULTS

HISTOLOGY

Postmortem analyses of cannulae placements showed that six mice had misplaced cannulae (Vehicle non-stressed miss, $n = 1$; Vehicle stressed miss, $n = 1$; [D-Lys-3]-GHRP-6 non-stressed miss, $n = 2$; [D-Lys-3]-GHRP-6 stressed miss, $n = 2$). The location of the cannulae for animals in each group is shown in **Figure 1**. As seen in this figure, most placements were located in the dorsolateral portion of the PVN. Because the cannulae were inserted on an angle (18°), most missed cannulae were placed too far lateral to the PVN, with only a few placed too far dorsal. The data from these animals were not included in the analyses given that there were only one or two animals from each group that had misplaced cannulae. Nevertheless, none of the animals with misplaced cannulae and receiving [D-Lys-3]-GHRP-6 showed the effects observed below suggesting that these effects were caused by localized action of the drug onto the PVN and not at sites outside of this region.

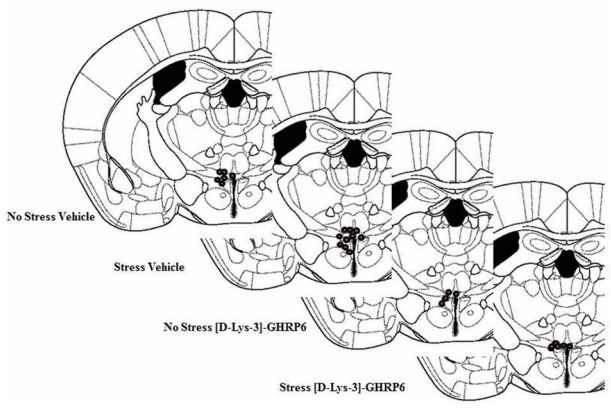

FIGURE 1 | Location of cannulae placement in animals from each group. Animals with missed cannulae placements were not included in this figure or in the analyses.

STRESS INCREASES CALORIC INTAKE IN MICE AND THIS EFFECT IS NOT ATTENUATED BY GHSR ANTAGONISM IN THE PVN

Total caloric intake was calculated daily throughout the study by adding the caloric content of standard laboratory chow and high-fat diet. At the end of the baseline period, mice were assigned to each of the experimental groups after being matched for total caloric intake. As such, there were no group differences in the amount of calories consumed during the baseline (data not shown). During the week that followed the surgical implantation of the cannulae, mice continued to eat similar amounts of calories and there were no differences between any of the groups. During the stress period, there was no significant interaction between drug and treatment on caloric consumption [interaction, $F_{(1,\ 22)} = 0.019$, $p > 0.05$], nor was there an effect of [D-Lys-3] GHRP-6 treatment [main effect of drug, $F_{(1,\ 22)} = 3.099$, $p > 0.05$]. There was, however, a significant treatment effect where stressed mice increased their total daily caloric intake, and this effect was not attenuated by GHSR antagonism [main effect of stress, $F_{(1,\ 22)} = 11.77$, $p < 0.05$; See **Figure 2A**].

The increase in caloric intake seen in stressed animals was due primarily to an increase in the daily intake of regular chow. Both vehicle and [D-Lys-3] GHRP-6 treated mice consumed equivalent amounts of chow before the stress period began ($p > 0.05$). Following the stress period, there was no significant interaction between drug and treatment on the consumption of regular chow [interaction, $F_{(1,\ 22)} = 0.063$, $p > 0.05$]. However, we did observe a significant treatment effect, where stressed mice rapidly increased their intake of regular laboratory chow regardless of the infusion they received in the PVN [main effect of stress, $F_{(1,\ 22)} = 10.48$, $p < 0.05$; See **Figure 2B**].

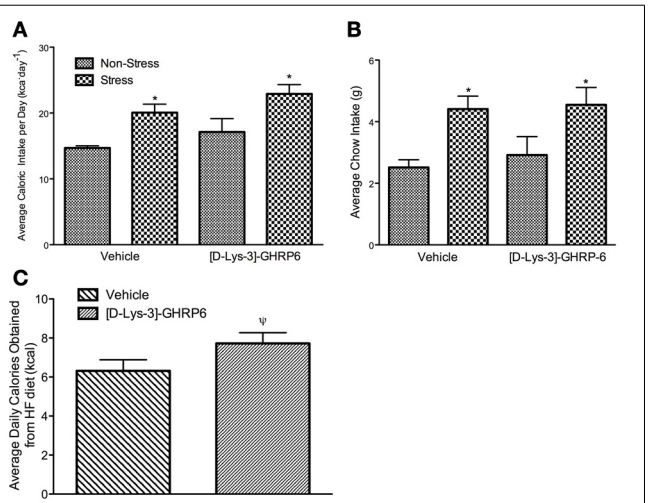

FIGURE 2 | (A) Average daily caloric intake (calculated as the sum of calories consumed from standard laboratory chow and high-fat diet), **(B)** average daily standard laboratory chow intake, **(C)** average number of calories consumed from the high fat diet per day for animals receiving either vehicle (0.9% saline) or 20 nmol/day of [D-Lys3-]-GHRP-6; stressed and non-stressed animals are collapse to illustrate main effect of drug. All values are expressed as mean ± s.e.m. $^{*}p < 0.05$ relative to non-stressed controls. $^{\psi}p = 0.05$ relative to vehicle treated animals.

BLOCKING GHSR IN THE PVN INCREASES THE INTAKE OF THE HIGH FAT DIET

Given that stress has been associated with increased consumption of diets that are calorically dense and highly palatable, we next examined if chronic social defeat stress would alter the intake of a high-fat diet. Mice in all groups ate little of the high fat diet early in the baseline period but increased their intake steadily over time, so that by the end of the baseline period, they were consuming about 37% ± 4.7 of their daily caloric intake from this diet. There was no significant interaction between treatment and drug on the proportion of calories obtained from the high-fat diet during the stress period [interaction, $F_{(1,\ 22)} = 0.411$, $p > 0.05$]. However, there was a significant treatment effect wherein the proportion of calories obtained from the high fat diet decreased in all animals that were stressed [main effect of stress, $F_{(1,\ 22)} = 4.32$, $p < 0.05$; data not shown]. Interestingly, there was also an effect of drug wherein mice receiving [D-Lys-3] GHRP-6 infusions into the PVN increased the total amount of high fat diet consumed, relative to animals receiving vehicle infusions regardless of their treatment conditions [main effect for drug, $F_{(1,\ 22)} = 4.25$, $p = 0.051$; See **Figure 2C**].

The increase in the total amount of fat consumed by animals infused with [D-Lys-3] GHRP-6 into the PVN was reflected in their body weight at the end of the stress period. There was no significant interaction between drug and treatment on the change in body weight during the stress period [interaction, $F_{(1,\ 22)} = 0.00$, $p > 0.05$] or on the average body weight at the end of the stress period [interaction, $F_{(1,\ 22)} = 0.425$, $p > 0.05$]. There was, however, a significant effect of drug infusions on the change in body weight, wherein mice treated chronically with [D-Lys-3] GHRP-6 weighed significantly more at the end of the stress period compared to animals treated with vehicle, regardless of their treatment group [main effect of drug, $F_{(1,\ 22)} = 12.49$, $p < 0.05$; See **Figure 3A**].

The effects of [D-Lys-3]-GHRP-6 on body weight were more pronounced when the data were expressed as a change from baseline, and a significant drug effect demonstrates that animals treated with [D-Lys-3]-GHRP-6 gained more weight than those treated with saline during the stress period [main effect for drug, $F_{(1,\ 22)} = 7.32$, $p < 0.05$; See **Figure 3B**]. Furthermore, there was a significant main effect of treatment on body weight gain. As expected, stressed mice gained significantly more weight than their non-stressed controls, however, this occurred independently of their drug treatment [main effect of stress, $F_{(1,\ 22)} = 4.65$, $p < 0.05$; See **Figure 3B**].

[D-Lys-3]-GHRP6 INFUSIONS INTO THE PVN DOES NOT INFLUENCE STRESS-INDUCED HYPERGLYCEMIA BUT DOES MEDIATE STRESS-INDUCED CORT SECRETION

Following sacrifice by rapid decapitation, trunk blood was analyzed for blood glucose and corticosterone. There was no interaction between drug and treatment on circulating blood glucose at the time of sacrifice [interaction, $F_{(1,\ 22)} = 1.238$, $p > 0.05$]. As expected, mice exposed to chronic social defeat stress had higher basal glucose levels at the time of sacrifice [**Figure 4A**; main effect of stress, $F_{(1,\ 22)} = 5.6$, $p < 0.05$], and this effect was not altered by [D-Lys-3] GHRP-6 infusions into the PVN ($p > 0.05$). Similarly, stressed mice had significantly higher levels of circulating CORT at the time of sacrifice compared to non-stressed controls, independent of drug treatment [main effect of stress, $F_{(1,\ 22)} = 12.263$, $p < 0.01$]. Furthermore, animals receiving [D-Lys-3]-GHRP-6 infusions into the PVN tended to have higher circulating CORT at the time of sacrifice regardless of their treatment group [main effect of drug, $F_{(1,\ 22)} = 4.118$, $p = 0.058$], and this main effect appeared to come from increased plasma CORT in samples collected from stressed [D-Lys-3]-GHRP-6 infused animals (**Figure 4B**). There was, however, no interaction effect between drug and treatment on circulating plasma CORT at the time of sacrifice [interaction, $F_{(1,\ 22)} = 2.788$, $p = 0.110$].

DISCUSSION

Ghrelin is a peptide that acts centrally to increase food intake and to alter metabolism by promoting the oxidation of carbohydrates while sparing the oxidation of fatty acids as substrate, ultimately increasing adipose tissue accumulation (Tschop et al., 2000). The effects of ghrelin on food intake and carbohydrate utilization have been mimicked by direct infusions of ghrelin onto the PVN (Currie et al., 2005). Similar to chronic delivery of ghrelin, acute and chronic social defeat stress increase the concentrations of the active form of ghrelin in plasma, but also result in higher caloric intake, and in some cases increased body weight, effects that are attenuated in socially defeated ghrelin receptor KO mice (Lutter et al., 2008; Davies et al., 2009; Raspopow et al., 2010; Kumar et al., 2013; Patterson et al., 2013). Interestingly, this type

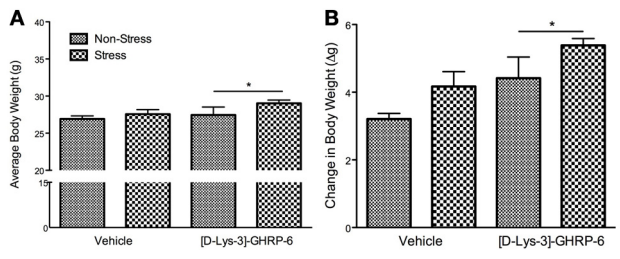

FIGURE 3 | (A) Body weight at the end of the 3 week chronic social defeat paradigm, and **(B)** the change of body weight during the stress period. All values are expressed as mean ± s.e.m. $^*p < 0.05$ relative to animals receiving vehicle infusions.

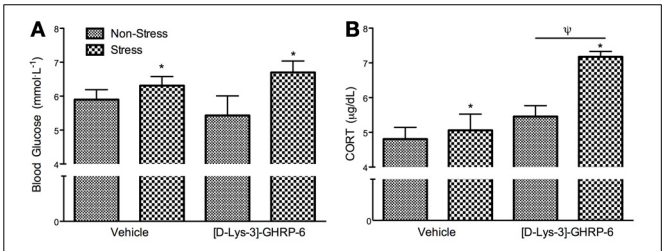

FIGURE 4 | (A) Plasma blood glucose levels and **(B)** plasma corticosterone levels in trunk blood collected at the time of sacrifice. All values are expressed as mean ± s.e.m. $^*p < 0.05$ relative to non-stressed animals; $^\Psi p < 0.05$ relative to vehicle treated animals.

of social stress causes mice to alter their metabolism to promote the utilization of carbohydrates, ultimately leading to increased adipose stores (Davies et al., 2009; Patterson et al., 2013). In contrast, ghrelin receptor KO mice continue to use fat as a substrate for nutrients and tend to lose body fat when chronically stressed, ultimately showing depletion of adipose tissue (Patterson et al., 2013). Furthermore, ghrelin seems to produce these metabolic effects centrally as chronic delivery of [D-Lys-3]-GHRP-6 into the cerebral ventricles decreases stress induced food intake and body weight gain (Patterson et al., 2013).

In order to produce these effects, ghrelin may be targeting a number of central sites that contain the ghrelin receptor and that are involved in food intake and metabolic rate, as well as in the regulation of the stress response including the hypothalamic PVN (Guan et al., 1997; Zigman et al., 2006). The present study was designed to examine the contribution of ghrelin receptors in the hypothalamic PVN in modulating some of the metabolic effects caused by chronic social defeat stress in mice. The results from the current experiment indicate that chronic ghrelin receptor blockade in the PVN via infusions of [D-Lys-3]-GHRP-6, does not attenuate stress induced increases in caloric intake or body weight, nor does it affect plasma levels of glucose. Interestingly, while stress decreased the intake of a high fat diet that was provided for 4 h during the day, [D-Lys-3]-GHRP-6 treated mice tended to consume more of this diet compared to animals receiving vehicle infusions and this effect was independent from stress. Not surprisingly, [D-Lys-3]-GHRP-6 treated mice weighed more than vehicle treated mice. Furthermore, these animals also tended to have elevated levels of circulating CORT regardless of the treatment. These effects were not observed in the few animals that received [D-Lys-3]-GHRP-6 infusions into areas outside the PVN (i.e., missed cannula placements) supporting the evidence that GHSR signaling in the PVN selectively mediates these effects.

Given that acute direct infusion of ghrelin into the PVN increase food intake in rats (Currie et al., 2005) we expected that stress induced increases in caloric intake would be attenuated by chronic blockade of ghrelin receptors using [D-Lys-3]-GHRP-6. Nevertheless, [D-Lys-3]-GHRP-6 was ineffective in decreasing caloric intake. In contrast, work from our lab has demonstrated that [D-Lys-3]-GHRP-6 delivered into the ventricles while animals are being stressed does decrease stress induced caloric intake (Patterson et al., 2013). It is therefore likely that, when infused into the ventricles, [D-Lys-3]-GHRP-6 blocks the ghrelin receptor in regions of the brain other than the PVN to reduce feeding including the ventromedial hypothalamus (VMH), arcuate nucleus (ARC), ventral tegmental area (VTA) and brain stem nuclei like the parabrachial nucleus and nucleus of the solitary tract (NTS), all implicated in the feeding responses to ghrelin (Tschop et al., 2000; Nakazato et al., 2001; Faulconbridge et al., 2003; Naleid et al., 2005; Abizaid et al., 2006; Skibicka et al., 2011). In this sense, central blockade of ghrelin receptors may attenuate the release of agouti related peptide (AgRP) and neuropeptide Y (NPY) from the ARC, attenuate dopaminergic tone from the VTA and noradrenergic tone from the brain stem to decrease food intake all resulting in overall attenuated feeding responses to chronic social stress. In our study, however, chronic blockade of the ghrelin receptor in the PVN was not sufficient to attenuate overall caloric intake.

In contrast, we did observe an increase in the intake of the palatable diet in those animals that received [D-Lys-3]-GHRP-6 into the PVN regardless of whether they were stressed or not. This would suggest that ghrelin receptors in the PVN, while not necessary for the overall feeding response seen during chronic social defeat, may be necessary for the switch in dietary preference that has been observed in stressed animals in previous studies (Patterson et al., 2013). In these studies, mice with restricted access to a high fat diet to 4 h during the day gradually increase the proportion of calories consumed from this high fat diet while decreasing the proportion of calories consumed from a regular diet, indicating a bias toward the calorically dense and highly palatable diet. When stressed, however, these mice increased the intake of regular chow while decreasing the intake of the high fat diet (Patterson et al., 2013). It is therefore, possible that stress induced ghrelin secretion acts on the PVN to inhibit the intake of high fat without altering total caloric intake. This would be in accordance with data showing that the PVN is important for dietary preference for carbohydrates and lesions to the PVN result in increased preference for high fat diets (Aravich and Sclafani, 1983; Leibowitz, 1988; Hoebel et al., 1989). Interestingly, ghrelin decreases inhibitory post synaptic currents in parvocellular neurons within the PVN, primarily cells that secrete CRH (although also in some TRH and other cells of unknown phenotype), suggesting that ghrelin facilitates indirect activation of these neuroendocrine cell groups (Cowley et al., 2003). Furthermore, central ghrelin infusions increase extracellular norepinephrine content in the PVN (Kawakami et al., 2008). Of course these effects are acute and it is not known if they are the same in animals subjected to chronic stress. Thus, while plasma ghrelin and CORT levels correlate during acute stress, ghrelin secretion remains elevated during chronic stress but the CORT response become blunted (Zheng et al., 2009). Interestingly, our data shows that chronic GHSR antagonism in the PVN results in higher plasma CORT levels, an effect that seems more pronounced in stressed animals (although not statistically significant). This would suggest that ghrelin may be important in the adaptive mechanisms that bring CORT levels down in the face of chronic stress. As such, one would expect that chronic blockade of GHSR in the PVN would make mice more vulnerable to stress induced pathological conditions, a state previously shown in GHSR KO mice (Lutter et al., 2008).

Finally, it is important to note that in our studies cannulae placements were closer to the magnocellular portion of the PVN, where cells that produce oxytocin, vasopressin, and galanin are located (Akabayashi et al., 1994). All of these cell groups have been associated with feeding, and ghrelin may influence the expression of these peptides in the PVN directly or indirectly (Akabayashi et al., 1994; Sclafani et al., 2007; Beck and Max, 2008). Thus, one could argue that stress induced ghrelin secretion targets the PVN to alter the preference of high fat diets via modulation of oxytocin, vasopressin and galanin, a hypothesis that needs further study.

Possible limitations of this study are the use of [D-Lys-3]-GHRP-6 to antagonize the ghrelin receptor. For instance, [D-Lys-3]-GHRP-6 appears to interact with the 5-HT_{2b} receptors in the

gut (Depoortere et al., 2006) although this interaction needs to be further demonstrated, as brain expression of this receptor is low compared to the gut (Hayes and Greenshaw, 2011). Given this finding, and the fact that other ghrelin receptor antagonists appear to act centrally to cause weight gain in spite of blocking the somatotropic effects of ghrelin (Halem et al., 2005), support the notion that the effects of [D-Lys-3]-GHRP-6, are related to their interaction with the ghrelin receptor and not to other receptors.

In summary, our results further demonstrate that chronic social defeat stress results in increased caloric intake and preference for carbohydrate rich diets in mice. This preference for carbohydrate (but not the overall increase in caloric intake) is mediated by direct action of stress induced ghrelin secretion on the PVN. Blockade of GHSR in the PVN biases animals toward increasing their intake of high fat, whether they are stressed or not. As such, the stress induced increases in caloric intake seen in vehicle treated mice, and the increases in the intake of the high fat diet are independent from each other and only the preference for fat is related to blockade of ghrelin receptors in the PVN.

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REFERENCES

Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., et al. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J. Clin. Invest.* 116, 3229–3239. doi: 10.1172/JCI29867

Akabayashi, A., Koenig, J. I., Watanabe, Y., Alexander, J. T., and Leibowitz, S. F. (1994). Galanin-containing neurons in the paraventricular nucleus: a neurochemical marker for fat ingestion and body weight gain. *Proc. Natl. Acad. Sci. U.S.A.* 91, 10375–10379. doi: 10.1073/pnas.91.22.10375

Aravich, P. F., and Sclafani, A. (1983). Paraventricular hypothalamic lesions and medial hypothalamic knife cuts produce similar hyperphagia syndromes. *Behav. Neurosci.* 97, 970–983. doi: 10.1037/0735-7044.97.6.970

Bartolomucci, A., Cabassi, A., Govoni, P., Ceresini, G., Cero, C., Berra, D., et al. (2009). Metabolic consequences and vulnerability to diet-induced obesity in male mice under chronic social stress. *PLoS ONE* 4:e4331. doi: 10.1371/journal.pone.0004331

Beck, B., and Max, J. P. (2008). Neuropeptide Y in the arcuato-paraventricular pathway and diet selection in the vasopressin-deficient Brattleboro rat. *Brain Res. Bull.* 76, 454–457. doi: 10.1016/j.brainresbull.2008.03.006

Black, P. H. (2006). The inflammatory consequences of psychologic stress: relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. *Med. Hypotheses.* 67, 879–891. doi: 10.1016/j.mehy.2006.04.008

Cabral, A., Suescun, O., Zigman, J. M., and Perello, M. (2012). Ghrelin indirectly activates hypophysiotropic CRF neurons in rodents. *PLoS ONE* 7:e31462. doi: 10.1371/journal.pone.0031462

Cowley, M. A., Smith, R. G., Diano, S., Tschop, M., Pronchuk, N., Grove, K. L., et al. (2003). The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37, 649–661. doi: 10.1016/S0896-6273(03)00063-1

Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., and Weigle, D. S. A. (2001). Preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50, 1714–1719. doi: 10.2337/diabetes.50.8.1714

Currie, P. J., Mirza, A., Fuld, R., Park, D., and Vasselli, J. R. (2005). Ghrelin is an orexigenic and metabolic signaling peptide in the arcuate and paraventricular nuclei. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R353–R358. doi: 10.1152/ajpregu.00756.2004

Davies, J. S., Kotokorpi, P., Eccles, S. R., Barnes, S. K., Tokarczuk, P. F., Allen, S. K., et al. (2009). Ghrelin induces abdominal obesity via GHS-R-dependent lipid retention. *Mol. Endocrinol.* 23, 914–924. doi: 10.1210/me.2008-0432

Depoortere, I., Thijs, T., and Peeters, T. (2006). The contractile effect of the ghrelin receptor antagonist, D-Lys3-GHRP-6, in rat fundic strips is mediated through 5-HT receptors. *Eur. J. Pharmacol.* 537, 160–165. doi: 10.1016/j.ejphar.2006.03.043

Drazen, D. L., Vahl, T. P., D'Alessio, D. A., Seeley, R. J., and Woods, S. C. (2006). Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status. *Endocrinology* 147, 23–30. doi: 10.1210/en.2005-0973

Faulconbridge, L. F., Cummings, D. E., Kaplan, J. M., and Grill, H. J. (2003). Hyperphagic effects of brainstem ghrelin administration. *Diabetes* 52, 2260–2265. doi: 10.2337/diabetes.52.9.2260

Guan, X. M., Yu, H., Palyha, O. C., McKee, K. K., Feighner, S. D., Sirinathsinghji, D. J., et al. (1997). Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res. Mol. Brain Res.* 48, 23–29. doi: 10.1016/S0169-328X(97)00071-5

Halem, H. A., Taylor, J. E., Dong, J. Z., Shen, Y., Datta, R., Abizaid, A., et al. (2005). A novel growth hormone secretagogue-1a receptor antagonist that blocks ghrelin-induced growth hormone secretion but induces increased body weight gain. *Neuroendocrinology* 81, 339–349. doi: 10.1159/000088796

Hayes, D. J., and Greenshaw, A. J. (2011). 5-HT receptors and reward-related behaviour: a review. *Neurosci. Biobehav. Rev.* 35, 1419–1449. doi: 10.1016/j.neubiorev.2011.03.005

Hill, J. W. (2013). PVN pathways controlling energy homeostasis. *Indian J. Endocrinol. Metab.* 16, 562–568. doi: 10.4103/2230-8210.105581

Hoebel, B. G., Hernandez, L., Schwartz, D. H., Mark, G. P., and Hunter, G. A. (1989). Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior. Theoretical and clinical implications. *Ann. N.Y. Acad. Sci.* 575, 171–91. discussion: 92–93. doi: 10.1111/j.1749-6632.1989.tb53242.x

Kawakami, A., Okada, N., Rokkaku, K., Honda, K., Ishibashi, S., and Onaka, T. (2008). Leptin inhibits and ghrelin augments hypothalamic noradrenaline release after stress. *Stress* 11, 363–369. doi: 10.1080/10253890701820257

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402, 656–660. doi: 10.1038/45230

Kumar, J., Chuang, J. C., Na, E. S., Kuperman, A., Gillman, A. G., Mukherjee, S., et al. (2013). Differential effects of chronic social stress and fluoxetine on meal patterns in mice. *Appetite* 64, 81–88. doi: 10.1016/j.appet.2012.12.023

Leibowitz, S. F. (1988). Hypothalamic paraventricular nucleus: interaction between alpha 2-noradrenergic system and circulating hormones and nutrients in relation to energy balance. *Neurosci. Biobehav. Rev.* 12, 101–109. doi: 10.1016/S0149-7634(88)80002-2

Lutter, M., Sakata, I., Osborne-Lawrence, S., Rovinsky, S. A., Anderson, J. G., Jung, S., et al. (2008). The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat. Neurosci.* 11, 752–753. doi: 10.1038/nn.2139

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* 87, 873–904. doi: 10.1152/physrev.00041.2006

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., et al. (2001). A role for ghrelin in the central regulation of feeding. *Nature* 409, 194–198. doi: 10.1038/35051587

Naleid, A. M., Grace, M. K., Cummings, D. E., and Levine, A. S. (2005). Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. *Peptides* 26, 2274–2279. doi: 10.1016/j.peptides.2005.04.025

Patterson, Z., Khazall, R., Anisman, H., and Abizaid, A. (2009). "Ghrelin mediates the metabolic alterations seen following exposure to chronic stress," in *13th Meeting of the Society*

for Behavioral Neuroendocrinology (East Lansing, MI).

Patterson, Z. R., Khazall, R., Mackay, H., Anisman, H., and Abizaid, A. (2013). Central ghrelin signaling mediates the metabolic response of C57BL/6 male mice to chronic social defeat stress. *Endocrinology* 154, 1080–1091. doi: 10.1210/en.2012-1834

Paxinos, G., and Franklin, K. B. G. (1997). *The Mouse Brain in Streotaxic Coordinates*. San Diego, CA: Academic Press.

Raspopow, K., Abizaid, A., Matheson, K., and Anisman, H. (2010). Psychosocial stressor effects on cortisol and ghrelin in emotional and non-emotional eaters: influence of anger and shame. *Horm. Behav.* 58, 677–684. doi: 10.1016/j.yhbeh.2010.06.003

Sclafani, A., Rinaman, L., Vollmer, R. R., and Amico, J. A. (2007). Oxytocin knockout mice demonstrate enhanced intake of sweet and nonsweet carbohydrate solutions. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 292, R1828–R1833. doi: 10.1152/ajpregu.00826.2006

Skibicka, K. P., Hansson, C., Alvarez-Crespo, M., Friberg, P. A., and Dickson, S. L. (2011). Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* 180, 129–137. doi: 10.1016/j.neuroscience.2011.02.016

Smith, S. M., and Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin. Neurosci.* 8, 383–395.

Stratakis, C. A., and Chrousos, G. P. (1995). Neuroendocrinology and pathophysiology of the stress system. *Ann. N.Y. Acad. Sci.* 771, 1–18. doi: 10.1111/j.1749-6632.1995.tb44666.x

Tschop, M., Smiley, D. L., and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* 407, 908–913. doi: 10.1038/35038090

Zheng, J., Dobner, A., Babygirija, R., Ludwig, K., and Takahashi, T. (2009). Effects of repeated restraint stress on gastric motility in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1358–R1365. doi: 10.1152/ajpregu.90928.2008

Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B., and Elmquist, J. K. (2006). Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J. Comp. Neurol.* 494, 528–548. doi: 10.1002/cne.20823

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Ghrelin and eating behavior: evidence and insights from genetically-modified mouse models

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Ghrelin is an octanoylated peptide hormone, produced by endocrine cells of the stomach, which acts in the brain to increase food intake and body weight. Our understanding of the mechanisms underlying ghrelin's effects on eating behaviors has been greatly improved by the generation and study of several genetically manipulated mouse models. These models include mice overexpressing ghrelin and also mice with genetic deletion of ghrelin, the ghrelin receptor [the growth hormone secretagogue receptor (GHSR)] or the enzyme that post-translationally modifies ghrelin [ghrelin O-acyltransferase (GOAT)]. In addition, a GHSR-null mouse model in which GHSR transcription is globally blocked but can be cell-specifically reactivated in a Cre recombinase-mediated fashion has been generated. Here, we summarize findings obtained with these genetically manipulated mice, with the aim to highlight the significance of the ghrelin system in the regulation of both homeostatic and hedonic eating, including that occurring in the setting of chronic psychosocial stress.

Keywords: hedonic eating, homeostatic eating, GOAT, GHSR

INTRODUCTION

Ghrelin is a 28-amino acid octanoylated peptide hormone secreted predominantly from a distinct population of endocrine "ghrelin" cells located within the gastric oxyntic mucosa, previously referred to as P/D1-type cells in humans and A like-type cells in rodents (Kojima and Kangawa, 2010). Ghrelin is the only known naturally occurring peptide to be modified by Ser^3 O-octanoylation, a reaction catalyzed by the enzyme ghrelin O-acyl transferase (GOAT) (Yang et al., 2008; Kojima and Kangawa, 2010). This post-translational modification is essential for the binding of ghrelin to and subsequently activation of its specific receptor, the growth hormone secretagogue receptor type 1a (GHSR). GHSR is a 7 transmembrane protein coupled to the Gq subfamily of heterotrimeric GTP-binding proteins that activates phospholipase C and in turn, induces release of calcium from intracellular stores (Howard et al., 1996; Damian et al., 2012). Stimulation of other signal transduction cascades also have been demonstrated upon activation of GHSR, although much of that data was characterized using transfected cell systems (Cruz and Smith, 2008). GHSR is expressed in several regions of the central nervous system and some peripheral organs (Cruz and Smith, 2008).

Ghrelin's actions are diverse. Ghrelin is a potent growth hormone (GH) secretagogue and orexigenic peptide (Kojima and Kangawa, 2010). Ghrelin also prevents falls in blood glucose upon extreme caloric restriction and minimizes depressive-like behavior upon chronic psychosocial stress (Lutter et al., 2008; Goldstein et al., 2011). Moreover, ghrelin stimulates motor activity in the gastrointestinal tract and accelerates gastric emptying (Peeters, 2003). Although desacyl-ghrelin, the non-octanoylated form, does not bind to GHSR, some studies report that it can modify certain physiological responses, including food intake and glucose homeostasis (Delhanty et al., 2012).

Genetically-modified mouse models have been instrumental in dissecting out the physiological roles of ghrelin, as well as the specific sites of ghrelin action. Genetic modifications in mice can be grouped in two classes: (1) Standard transgenic models, in which a foreign DNA construct is randomly integrated into the

mouse genome (commonly used for overexpression models) and (2) Gene-targeted transgenic models, in which a gene disruption or manipulation is introduced in a single gene via homologous recombination between the foreign DNA and the endogenous gene (commonly used for "knockout" and "knock-in" models) (Davey and Maclean, 2006). In this review, we discuss ghrelin's action on eating behaviors as determined using mice with genetic manipulations in the ghrelin signaling system (**Tables 1** and **2**). We first focus on the role of ghrelin in homeostatic eating and subsequently discuss the role of ghrelin in hedonic eating.

GHRELIN AND HOMEOSTATIC EATING

Homeostatic eating is defined as food intake driven by necessity, due to energy deficiency as perceived by the brain and body. Ghrelin is the only known circulating peptide hormone

Table 1 | Studies of homeostatic eating behaviors in mouse models with genetic alterations in ghrelin-signaling system.

Model	Summary	References
Ghrelin overexpression (driven by various promoters)	Elevated desacyl-ghrelin levels with conflicting food intake and body weight results	Ariyasu et al., 2005; Asakawa et al., 2005; Iwakura et al., 2005; Wei et al., 2006; Zhang et al., 2008
	Elevated ghrelin levels with no change in food intake (Reed et al.) or increase in food intake (Bewick et al.)	Reed et al., 2008; Bewick et al., 2009
Ghrelin overexpression (SV40 T-antigen)	Increased ghrelin levels, with food intake and body weights indistinguishable from wild-type littermates; Older transgenic mice lose weight, likely due to large gastric tumors	Iwakura et al., 2009; Zhao et al., 2010b
Ghrelin analog overexpression	Increase of ghrelin-like activity, no change in food intake, body weight, energy expenditure or fat mass	Matsumoto et al., 2001; Yamada et al., 2010; Ariyasu et al., 2012
Human ghrelin and GOAT overexpression	Elevated human ghrelin only when fed diet containing medium-chain triglycerides. No change in food intake but increase in body weight	Kirchner et al., 2009
Ghrelin knockout	No changes in eating behaviors under CHD. Inconsistent results in HFD feeding studies	Sun et al., 2003; Wortley et al., 2004, 2005; De Smet et al., 2006; Dezaki et al., 2006; Pfluger et al., 2008; Sato et al., 2008
GHSR knockout	Attenuated anticipatory locomotor response. Similar food intake, but subtle decrease in body weight under CHD feeding	Sun et al., 2004, 2008; Abizaid et al., 2006; Blum et al., 2009; Lin et al., 2011; Ma et al., 2011
GHSR null (removable transcriptional blocking cassette)	Female mice on CHD weigh less than wild-type controls. Consume less food and are resistant to HFD-induced body weight gain upon early HFD exposure	Zigman et al., 2005; Perello et al., 2012
Ghrelin and GHSR knockout	Lower body weight, but no change in food intake	Pfluger et al., 2008
Site-selective GHSR Expression (GHSR null crossed with cell-specific Cre)	TH-promoter driven Cre: partially restores ghrelin-stimulated food intake Phox2b-promoter driven Cre: lack ghrelin-induced food intake	Chuang et al., 2011; Scott et al., 2012
GHSR overexpression in GHRH neurons	Increase in post-weaning growth rate. Increase in growth rate not maintained in adulthood. No change in body weight on HFD, although fat pad mass trended lower. Possible increase in HFD intake	Lall et al., 2004
GOAT deficiency	Normal body weight and fat mass under CHD feeding. Increase in food intake but lower body weights under medium-chain triglyceride rich diet	Gutierrez et al., 2008; Kirchner et al., 2009; Zhao et al., 2010a
Ghrelin knockout (*Ob/Ob* background)	Similar food intake and a modest increase in body weight as compared to ob/ob mice	Sun et al., 2006
GHSR knockout (*Ob/Ob* background)	Similar food intake and body weights to ob/ob mice	Ma et al., 2012

Table 2 | Studies of hedonic eating behaviors in mouse models with genetic alterations in ghrelin-signaling system.

Model		References
GHSR knockout	Suppressed intake of rewarding food in a free choice (CHD/rewarding food) paradigm	Blum et al., 2009; Egecioglu et al., 2010; Disse et al., 2011; Verhagen et al., 2011; Lamont et al., 2012
GHSR null	Lack cue potentiated feeding behavior. Do demonstrate CPP for HFD post-CSDS. Fail to increase intake of CHD in response to chronic stress	Perello et al., 2010; Chuang et al., 2011; Walker et al., 2012
Site-selective GHSR expression (GHSR null crossed with cell-specific Cre)	TH-promoter driven Cre: sufficient to restore CSDS-induced CPP for HFD	Chuang et al., 2011
GOAT deficiency	Attenuated motivation for food in an operant responding model. Decreased hedonic feeding response as examined in a "dessert effect" protocol	Davis et al., 2012

that stimulates food intake (Cummings, 2006). Ghrelin triggers eating even at times of minimal spontaneous food intake, and its orexigenic actions are rapid, short-lived and potent (Nakazato et al., 2001; Wren et al., 2001; Cummings, 2006). It has been suggested that ghrelin is a physiologic meal initiator, since its levels increase preprandially and decrease post-prandially (Cummings et al., 2001). Ghrelin levels, in individuals under restricted feeding schedules, increase approximately 1 h prior to food presentation. In animals, this preprandial rise in ghrelin occurs simultaneously or possibly precedes the increased locomotion in anticipation of a scheduled meal (Drazen et al., 2006). Indeed, ghrelin administration increases locomotor activity in rats and foraging-like activities in hamsters (Keen-Rhinehart and Bartness, 2005; Jerlhag et al., 2007). Thus, Blum et al. hypothesized that ghrelin is secreted in anticipation of a meal and correlates with anticipatory locomotor activity (Blum et al., 2009).

The mechanism for ghrelin's action on stimulating food intake was initially thought to be through homeostatic hypothalamic circuits originating in the arcuate nucleus (ARC) (Willesen et al., 1999; Nakazato et al., 2001; Kageyama et al., 2010). The expression of GHSR, the only known receptor for ghrelin, has been confirmed within the ARC by many techniques, including *in situ* hybridization histochemistry, RT-PCR, immunohistochemistry and Western blot analysis (Howard et al., 1996; Guan et al., 1997; Willesen et al., 1999; Zigman et al., 2006). Several studies have demonstrated that ghrelin has the capacity to bind its receptor on arcuate NPY/AgRP neurons, resulting in their activation and ensuing orexigenic behaviors (Willesen et al., 1999; Nakazato et al., 2001; Wren et al., 2001; Kageyama et al., 2010; Schaeffer et al., 2013). Interaction of ghrelin with several other neuronal subtypes and brain sites also has been shown to increase intake of freely-available food. These include, among others, orexin-producing neurons of the lateral hypothalamus (Olszewski et al., 2003; Toshinai et al., 2003). Additionally, some evidence indicates that vagus nerve integrity is required for ghrelin-induced food intake as well (Date et al., 2002; Date, 2012).

HOMEOSTATIC EATING IN MICE OVER EXPRESSING GHRELIN

In order to confirm the effects of ghrelin on homeostatic eating, several groups have attempted to produce transgenic mice with chronically elevated ghrelin. These initial transgenes contained the ghrelin gene driven by various promoters—including those for CAG (Ariyasu et al., 2005; Asakawa et al., 2005), rat insulin II (Iwakura et al., 2005), rat glucagon (Iwakura et al., 2005), CMV (Wei et al., 2006) and FABP4 (Zhang et al., 2008)—resulting in elevated ghrelin gene expression mainly in cell types in which ghrelin gene expression does not usually occur. These mouse models resulted in elevated desacyl-ghrelin rather than elevations of ghrelin, presumably because this overexpression of the ghrelin gene was targeted mostly to tissues lacking the ghrelin-acylating enzyme, GOAT. Indeed, GOAT was not discovered until 2008. Most mouse models overproducing desacyl-ghrelin exhibited similar food intake and body weight as compared to wild-type mice (Iwakura et al., 2005; Wei et al., 2006); nonetheless, some studies found reduced body weight, fat mass, nose-to-anus length and/or food intake in their transgenic mice overexpressing desacyl-ghrelin (Ariyasu et al., 2005; Asakawa et al., 2005; Zhang et al., 2008). These findings may suggest that desacyl-ghrelin has metabolic effects opposite to those of ghrelin.

Two groups successfully produced standard transgenic mice with ghrelin overexpression. Reed et al. generated a transgenic mouse model overexpressing ghrelin in neurons by using a neuron-specific enolase promoter. These mice showed increased ghrelin expression in brain and increased circulating ghrelin (∼5-fold), although they did not differ from wild-type controls in body weight, food intake, energy expenditure or fat mass (Reed et al., 2008). Likewise, Bewick et al. generated a transgenic mouse model overexpressing ghrelin, using a bacterial artificial chromosome containing the ghrelin gene and its promoter (Bewick et al., 2009). These mice exhibited increased circulating ghrelin (∼1.5-fold) and increased food intake without long-term body weight gain, perhaps due to a paradoxical increase in energy expenditure (Bewick et al., 2009). Bewick et al. proposed that the differences between their model and the Reed et al. mouse model could be due to differential peripheral vs. central nervous system ghrelin

concentrations (Bewick et al., 2009). Of note, results regarding the existence, sites of production and physiological significance of endogenous central ghrelin production in non-genetically-manipulated models have been inconsistent (Furness et al., 2011), although ghrelin from the periphery does make its way to the central nervous system.

After GOAT was identified, Kirchner et al. generated a double-transgenic mouse model overexpressing both human ghrelin and MBOAT4 (the membrane-bound O-acyltransferase domain containing 4 or human variant of GOAT) genes in the liver via the human apo-lipoprotein E promoter (Kirchner et al., 2009). These transgenic mice, which exhibited elevated concentrations of human ghrelin only when fed on a diet containing medium-chain triglycerides, showed decreased energy expenditure and increased rate of body weight gain without changes in food intake (Kirchner et al., 2009).

Other unique techniques have been employed to increase ghrelin expression. Yamada and colleagues generated transgenic mice overexpressing a ghrelin analog, which replaced Ser^3 with Trp^3, under the control of human serum amyloid-P promoter (Yamada et al., 2010). Trp^3-ghrelin possesses lower levels of ghrelin-like activity without the need for post-transcriptional modification (Matsumoto et al., 2001). Although mice overexpressing Trp^3-ghrelin displayed a ∼6-fold increase of ghrelin-like activity, neither body weight, food intake, energy expenditure or fat mass differed from wild-type controls (Ariyasu et al., 2012). Our group generated transgenic mice that express the immortalizing SV40 large T-antigen in ghrelin cells (Zhao et al., 2010b). These mice develop ghrelin-secreting tumors, which result in ∼25-fold higher plasma ghrelin concentrations and ∼37-fold higher plasma desacyl-ghrelin levels by the time they reach 20 weeks of age (Zhao et al., 2010b). Despite the sustained increase in plasma ghrelin levels, food intake and body weights in these transgenic mice were indistinguishable from those in wild-type mice; older transgenic mice lost weight, presumably due to the development of large gastric tumors (Zhao et al., 2010b). Iwakura et al. also created ghrelin promoter SV40 large T-antigen transgenic mice (Iwakura et al., 2009). These mice showed no difference in body weights as compared to wild-type mice before 12 weeks of age, after which body weight and food intake declined, likely due to tumor growth (Iwakura et al., 2009).

HOMEOSTATIC EATING IN MICE WITH GHRELIN DEFICIENCY

To our knowledge, four different ghrelin knockout mouse models have been reported (Sun et al., 2003; Wortley et al., 2004; De Smet et al., 2006; Dezaki et al., 2006). Regardless of the complete absence of ghrelin in circulation in these models, under *ad libitum* standard rodent chow diet (CHD) feeding, food intake and body weights in these ghrelin knockout mice were indistinguishable from those in wild-type mice. In addition, no differences were observed when other parameters of food intake were measured, including post-fasting hyperphagia (Sun et al., 2003; Wortley et al., 2004; Pfluger et al., 2008), forced dark cycle-induced eating (De Smet et al., 2006) or eating memory (Sato et al., 2008). These findings suggest that genetic ghrelin deficiencies fail to cause significant alterations in homeostatic aspects of eating behaviors.

Furthermore, three of the four ghrelin knockout models have been studied under chronic high fat diet (HFD) feeding, but yielded inconsistent results (Wortley et al., 2005; Dezaki et al., 2006; Sun et al., 2008). Surprisingly, none of the studies exhibited the expected reduction of HFD intake. Wortley et al. did demonstrate that ghrelin deficiency resulted in reduced body weight and fat mass, but these results were not recapitulated in the other mouse ghrelin knockout mouse models. Differences in the age at which the mice were first exposed to HFD diet are thought to account for the inconsistencies in the body weight phenotype among the different ghrelin knockout models. In particular, resistance to the development of diet-induced obesity may manifest in ghrelin knockout mice upon early exposure to HFD, but not when HFD challenge is initiated later in life (Wortley et al., 2004, 2005; Sun et al., 2008).

HOMEOSTATIC EATING IN MICE WITH GHSR DEFICIENCY

To our knowledge, three different mouse models with GHSR deficiency have been reported in the literature (Sun et al., 2004; Zigman et al., 2005; Abizaid et al., 2006). Two are traditional GHSR knockout models, in which the GHSR gene has been removed (Sun et al., 2004; Abizaid et al., 2006), and the other is a GHSR-null model in which the GHSR locus has been modified by the insertion of a loxP-flanked transcriptional blocking cassette (Zigman et al., 2005). More specifically, this transcriptional blocking cassette sequence disrupts GHSR gene expression and, as a consequence, the GHSR-null mice (harboring 2 copies of the recombinant GHSR allele) have no detectable functional GHSR expression. Cre recombinase mediates reactivation of GHSR gene expression by removal of the transcriptional blocking cassette sequence.

GHSR-deficient mice show a subtle but significant decrease in body weight. Sun et al. reported that GHSR knockout mice fed on CHD show a lower body weight than wild-type mice from 16 to 24 weeks of age (Sun et al., 2004). GHSR-deficient mice do not differ from wild-type controls in food intake under CHD (Sun et al., 2004; Zigman et al., 2005). Sun et al. found that GHSR knockout mice have a similar susceptibility to diet-induced obesity compared to wild-type mice upon exposure to HFD as adults (Sun et al., 2008); however, GHSR knockout mice manifest reduced age-associated obesity mainly due to reduced adiposity and increased thermogenesis (Lin et al., 2011; Ma et al., 2011). Using a restrictive eating paradigm, Abizaid et al. showed that GHSR knockout mice did not increase their food intake in response to repeated fasts as seen in wild-type mice. This was also true in ghrelin knockout mice (Abizaid et al., 2006).

In the GHSR-null mice from our group, female GHSR-null mice on CHD were significantly lighter than wild-type controls starting at 12 weeks of age (Zigman et al., 2005). Also, in two independent studies, GHSR-null mice were resistant to HFD-induced body weight gain upon early HFD exposure (Zigman et al., 2005; Perello et al., 2012).

As previously mentioned, in animals, a preprandial rise in ghrelin occurs simultaneously or possibly precedes the increased locomotion exhibited in anticipation of a scheduled meal (Drazen et al., 2006). In preliminary studies, Gooley et al. examined food-entrainable circadian rhythms in a very small cohort of

female GHSR-null mice (Gooley et al., 2006). Similar to wild-type littermates, the GHSR-null mice continued to show a preprandial rise in locomotor activity and body temperature, suggesting that intact ghrelin signaling was not required for the expression of food-entrainable circadian rhythms that are likely related to finding food (Gooley et al., 2006). Furthermore, Blum et al. also demonstrated increased activity prior to scheduled mealtime in both wild-type and GHSR knockout mice, although in their study, the response in GHSR knockout mice was attenuated (Blum et al., 2009). Others have also similarly reported that GHSR knockout mice under a restricted feeding paradigm demonstrate attenuated anticipatory locomotor responses and reduced expression of the marker of cellular activation c-fos in the mesolimbic dopamine pathway (Blum et al., 2009; Lamont et al., 2012). Using a different paradigm, Verhagen et al. have shown that GHSR knockout mice did not anticipate food when exposed to an activity-based anorexia model, in which mice are given free access to a running wheel and fed once per day for 2 h (Verhagen et al., 2011). Thus, most of these studies suggest that a disruption in the ghrelin signaling results in the lack of food anticipation behavior.

HOMEOSTATIC EATING IN MICE WITH BOTH GHRELIN AND GHSR DEFICIENCY

In another study, significant differences in body weight (decreased), energy expenditure (increased) and locomotor activity (increased) were observed when both ghrelin and GHSR were deficient in mice (double knockout mice), whereas neither ghrelin deficiency nor GHSR deficiency independently affected energy balance under CHD feeding conditions (Pfluger et al., 2008). Thus, the authors speculated that additional components of the ghrelin signaling system might be present.

HOMEOSTATIC EATING IN MICE WITH TISSUE-SPECIFIC GHSR EXPRESSION

The GHSR-null mouse model with Cre recombinase-dependent reactivation of GHSR has been valuable in establishing the physiological roles of certain of ghrelin's brain targets (Zigman et al., 2005). In one study, we have used GHSR-null mice to assess the role of direct action of ghrelin on the ventral tegmental area (VTA) and other catecholaminergic neurons. We achieved this by crossing GHSR-null mice with mice containing Cre recombinase expression driven by the tyrosine hydroxylase (TH) promoter, resulting in mice expressing GHSR selectively in TH-containing cells normally programmed to express this receptor (Chuang et al., 2011). This selective GHSR expression did not affect body weight, but partially restored ghrelin-stimulated food intake to levels achievable in wild-type littermates (Chuang et al., 2011). In another study, we crossed GHSR-null mice with mice containing expression driven by the paired-like homeobox 2b (Phox2b) promoter, resulting in mice expressing GHSR selectively in specific hindbrain nuclei, including the nucleus of the solitary tract, dorsomotor nucleus of the vagus, area postrema, nucleus ambiguus and facial motor nucleus (Scott et al., 2012). In contrast to mice with catecholaminergic neuron-selective GHSR expression, mice with hindbrain-selective GHSR expression lacked ghrelin-induced acute food intake (Scott et al., 2012). Thus, as opposed to GHSR-expressing catecholaminergic neurons, GHSR-expressing hindbrain neurons are not sufficient to mediate the acute orexigenic effects of ghrelin.

HOMEOSTATIC EATING IN MICE WITH GHSR OVER EXPRESSION IN GROWTH HORMONE RELEASING HORMONE NEURONS

Lall et al. generated a mouse model overexpressing GHSR in GH releasing hormone (GHRH) neurons using a rat GHRH cosmid promoter (GHRH-GHSR) (Lall et al., 2004). GHRH-GHSR mice had an increased post-weaning growth rate compared to wild-type littermates. When placed on HFD at 2 months of age, weight gain of GHRH-GHSR mice was similar to that of their wild-type counterparts, although fat pad mass trended lower. Food intake was not measured for individually-housed mice, however groups of GHRH-GHSR mice ate significantly more calories when fed HFD than when fed CHD, while groups of wild-type mice ate similar caloric amounts of HFD and CHD (Lall et al., 2004).

HOMEOSTATIC EATING IN MICE WITH GOAT DEFICIENCY

Two GOAT knockout mouse lines have been reported (Gutierrez et al., 2008; Zhao et al., 2010a). Using one of these lines, GOAT knockout mice demonstrated higher levels of desacyl-ghrelin compared to their wild-type littermates, and under CHD feeding had normal body weight and fat mass (Kirchner et al., 2009). However, when fed HFD, these GOAT knockout mice displayed significantly lower body weights compared to wild-type animals, despite not having significant changes in body composition. Challenging GOAT knockout mice with HFD rich in medium-chain triglycerides significantly reduced body weight and fat mass compared to similarly-treated wild-type littermates (Kirchner et al., 2009). Such was likely due to increased energy expenditure, since food intake in the GOAT knockout mice was higher (Kirchner et al., 2009). In contrast, the GOAT knockout mice generated by Zhao et al. had similar body weight, body composition and food intake as wild-type littermates, both on CHD and HFD (Zhao et al., 2010a). They also demonstrated similar weight loss curves when subjected to a severe caloric restriction protocol (Zhao et al., 2010a).

HOMEOSTATIC EATING IN MICE WITH DEFICIENCIES IN GHRELIN SIGNALING AND LEPTIN

Ghrelin and leptin are both regulators of feeding, but have opposite effects on food intake. Unlike ghrelin, leptin increases with positive energy balance and inhibits food intake. Furthermore, leptin- and ghrelin-responsive central targets partially overlap (Zigman and Elmquist, 2003; Perello et al., 2012). To investigate the physiological roles of ghrelin and leptin signaling, and their potential interaction in regulating energy balance, Sun et al. crossed ghrelin knockout mice with leptin deficient (ob/ob) mice to generate mice lacking both ghrelin and leptin (ghrelin knockout ob/ob). Although they predicted that the ghrelin knockout ob/ob mice would have a leaner and relatively hypophagic phenotype, the double knockout mice instead displayed similar food intake and a modest increase in body weight as compared to ob/ob mice (Sun et al., 2006). Ma and colleagues generated mice with GHSR deletion on the ob/ob background (GHSR knockout ob/ob) (Ma et al., 2012). They found that food intake and body

weights of GHSR knockout ob/ob mice were similar to those of ob/ob mice, possibly suggesting that the obesity in ob/ob mice is unrelated to unopposed ghrelin action.

GHRELIN AND HEDONIC EATING

Hedonic eating is defined as food intake motivated primarily by pleasure. Hedonic eating also encompasses behaviors aimed at facilitating access to pleasurable food. Ghrelin is thought to increase the rewarding value of palatable foods. Recent studies show that ghrelin is able to regulate different aspects of hedonic eating. The two-food choice test is a conventional behavioral method for determining preference. In this test, rodents are offered a pair of different types of food simultaneously, and the consumed amount is measured for a defined time. Using this test, it has been shown that ghrelin administration shifts food preference toward diets rich in fat (Shimbara et al., 2004). Ghrelin administration also increases intake of palatable saccharin solution and preference for saccharin-flavored foods in mice (Disse et al., 2010). Similarly, rats treated with a GHSR antagonist consume less peanut butter and Ensure®, but do not change intake of CHD in a free choice protocol (Egecioglu et al., 2010). As will be discussed below, ghrelin administration also affects other models of hedonic eating, including the conditioned place preference (CPP) test and operant responding.

The central distribution of GHSRs within mesolimbic centers supports the likelihood of ghrelin playing a major role in hedonic eating. In particular, GHSR is highly expressed in dopaminergic VTA neurons, which project to the nucleus accumbens (NAc) and strongly drive reward behaviors (Perello and Zigman, 2012). Mesolimbic circuitries also may be indirectly regulated by cholinergic neurons located in the laterodorsal tegmental area (LDTg), which express GHSR and innervate the VTA (Dickson et al., 2010). In addition, we have recently shown that ghrelin action on food reward requires intact orexin signaling, although the neuronal circuits linking orexin neurons and ghrelin action remain unclear (Perello et al., 2010).

HEDONIC EATING IN MICE WITH GENETIC DELETION OF GHSR

The use of mouse models deficient in GHSR have been instrumental in demonstrating an obligatory role for intact ghrelin signaling in various hedonic aspects of eating. Egecioglu et al. showed that GHSR knockout mice have a suppressed intake of the rewarding food in a free choice (CHD/rewarding food) paradigm and a reduced accumbal dopamine release induced by rewarding foods (Egecioglu et al., 2010). We recently subjected GHSR-null mice to a cue-potentiated feeding protocol, in which cue-potentiated feeding was demonstrated by enhanced intake of grain-based pellets selectively upon presentation of a positive conditioned stimulus (Walker et al., 2012). While wild-type mice demonstrated cue-potentiated feeding behavior, GHSR-null mice lacked this behavior. More specifically, GHSR-null mice displayed enhanced food intake in response to both positive and negative conditioned stimuli (Walker et al., 2012). This study supports pharmacologic studies suggesting a key role for intact ghrelin signaling pathways in establishing a specific positive cue-food association (Kanoski et al., 2013).

Altered eating behaviors are observed under stress as well as depression. In order to study the role of ghrelin in stress-associated hedonic eating, our group subjected both GHSR-null and wild-type littermates to a mouse behavioral model of chronic psychosocial stress and major depression, known as chronic social defeat stress (CSDS). Mice were challenged with ten daily bouts of social defeat by aggressive CD1 male mice, a protocol which results in an increase in plasma ghrelin (Lutter et al., 2008). Despite an increase in plasma ghrelin levels, GHSR-null mice failed to increase intake of CHD in response to chronic stress, unlike wild-type controls which increased their food intake. This suggests that activation of GHSR signaling is required for a stress-induced increase in caloric intake. In addition, stressed GHSR-null and wild-type mice were also tested in a food CPP test, which models a more complex, reward-related type of eating behavior. In the food CPP test, animals are conditioned to associate one chamber of the CPP apparatus with CHD and a second, visually and texturally distinct chamber with an equal calorie amount of a more pleasurable food, such as HFD. After conditioning, animals are given free access to both chambers in the absence of the food, and CPP is demonstrated when more time is spent in the chamber previously paired to the food reward. The CSDS-CPP protocol seems to model stress-based comfort food eating. Following exposure to the CSDS protocol, GHSR-null mice did not demonstrate CPP for HFD, whereas wild-type mice did (Chuang et al., 2011).

The above findings in GHSR-null mice confirm pharmacological studies; as administration of ghrelin and other means of achieving increases in plasma ghrelin (caloric restriction) both enable acquisition of food CPP (Perello et al., 2010; Disse et al., 2011), while administration of GHSR antagonists blocks food CPP (Egecioglu et al., 2010; Perello et al., 2010). Likewise, in our studies using mice subjected to a caloric restriction in which mice were limited to 80% of their usual daily food intake, GHSR-null mice did not demonstrate CPP for HFD, whereas wild-type mice did (Perello et al., 2010). Interestingly, when the study was repeated in mice exposed to a more intense calorie restriction protocol, in which mice were limited to *ad libitum* access to CHD for 4 h per day, no differences between genotypes were detected in the food CPP test. Thus, endogenous increases in GHSR signaling following stress as modeled with the CSDS protocol or under mild food restrictive conditions enable acquisition of CPP for HFD (Perello et al., 2010).

HEDONIC EATING IN MICE WITH TISSUE-SPECIFIC REACTIVATION OF GHSR

We also assessed food CPP performance following CSDS or as induced by administered ghrelin in mice with catecholaminergic selective GHSR expression, using the mice described above in section Homeostatic Eating in Mice with Tissue Specific GHSR Expression (Chuang et al., 2011). Such selective GHSR expression in TH-expressing cells was sufficient to mediate the ability of administered ghrelin or chronic stress to induce CPP for HFD.

HEDONIC EATING IN MICE WITH GOAT DEFICIENCY

Recently, it was determined that GOAT-mediated acylation of ghrelin is a critical modulator of food reward. Following a

24-h food deprivation period, GOAT-deficient mice displayed an attenuated motivation for food in an operant responding model (Davis et al., 2012). This supports several pharmacologic studies in which ghrelin administration increased operant lever pressing for sucrose, peanut butter-flavored sucrose or HFD pellets in rodents, and in which GHSR antagonist reduced operant responding for sucrose solution (Perello et al., 2010; Finger et al., 2012; Landgren et al., 2012; Skibicka et al., 2012). GOAT knockout mice also showed a decreased hedonic feeding response as examined in a "dessert effect" protocol, wherein the intake of a palatable HFD "dessert" was assessed in calorically-sated mice (Davis et al., 2012). Furthermore, GOAT knockout mice displayed atypical transcriptional changes in orexin 1 receptor gene expression in the NAc, potentially implying the involvement of orexin signaling in these effects of ghrelin (Davis et al., 2012). Taken together, these results suggest that the motivation to obtain food is regulated by endogenous GOAT activity.

DISCUSSION

In this review, we have summarized the findings of ghrelin action on eating behavior as determined or confirmed using different mouse models with genetic manipulations of the ghrelin signaling system. Of note, the various transgenic mouse models more consistently demonstrated alterations in hedonic eating, while changes to homeostatic eating and body weight changes were more variable and subtle.

An important consideration when evaluating the eating-related phenotypes of the various transgenic models relates to differences between manipulations of ghrelin vs. GHSR vs. GOAT. For example, variable degrees of altered homeostatic eating, hedonic eating and body weight have been observed in ghrelin-deficient, GHSR-deficient, and GOAT-deficient models. Although the exact reasons for these differences are unclear, they could result from differences inherent to each of those molecules. For instance, GHSR possesses strong ligand-independent constitutive activity when studied *in vitro* (Holst et al., 2004; Holst and Schwartz, 2004), and thus phenotypic changes in GHSR-null mice could result from the combined loss of ghrelin binding and of baseline GHSR "tone." Additionally, it has been proposed that ghrelin has other receptors besides GHSR, which could explain why simultaneous deletion of both ghrelin and GHSR result in a more severe phenotype than deletion of either alone (Holst et al., 2004; Pfluger et al., 2008). Furthermore, GOAT may have other substrates besides ghrelin, and thus its loss could directly affect more than just ghrelin bioactivity.

Another concern when evaluating these mouse models, as with any transgenic mouse models, is the possibility of functional redundancy or developmental compensation by another related system, which may mask the true effect of manipulation of the ghrelin system. While this has not yet been demonstrated specifically for the ghrelin system, it has been demonstrated for AgRP neurons that are thought to be crucial for ghrelin's effects on eating. Importantly, unlike the lack of significant body weight or food intake phenotypes in mice with genetic AgRP ablation achieved since inception or in neonates, rapid starvation ensues when AgRP expression is deleted in adult mice (Qian et al., 2002; Gropp et al., 2005; Luquet et al., 2005). Such a time-dependent effect of genetic AgRP deletion may be influenced by a time-sensitive capacity for the development of compensatory neurocircuits (Bouret et al., 2004; Luquet et al., 2005). Indeed, leptin's neurotrophic effects on hypothalamic development are restricted to a critical neonatal period (Bouret et al., 2004). Similar time-sensitive neurotrophic actions have been predicted for ghrelin (Steculorum and Bouret, 2011).

Further considerations when evaluating the effects of genetic engineering—in particular, those changes induced in standard transgenic models in which the transgene is randomly inserted into the genome—include the potential for the site of integration to affect tissue specificity, levels of transgene expression as well as expression of other genes present in the nearby genomic vicinity. For all these reasons, the use of the multiple mouse models generated using different genetic strategies and comparison of these mice to mice treated pharmacologically with agents targeting the ghrelin system is important for best determining the importance of the ghrelin system in eating behavior

It should also be noted that these various genetically-engineered mouse models targeting the ghrelin system additionally have been used to assess the importance of ghrelin action in domains other than those related to eating and body weight. For instance, GHSR-null mice have been used to demonstrate a key role for ghrelin action in minimizing depressive-like behavior following chronic stress (Lutter et al., 2008). Also, ghrelin-overexpressing, ghrelin-knockout, GHSR-knockout, GHSR-null and GOAT-knockout mice all demonstrate alterations in glucose homeostasis, with the most extreme phenotype—namely marked hypoglycemia and near death—appearing upon severe caloric restriction of mice with genetic deletion of either GOAT or ghrelin (Zhao et al., 2010a; Li et al., 2012).

These studies predict eating- and body weight-related phenotypes for people if they were to carry a genetic mutation engendering either ghrelin and/or GOAT overexpression or ghrelin, GHSR or GOAT deletion. Indeed, the extreme hyperphagia, early-onset obesity and debilitating food seeking behaviors associated with Prader-Willi Syndrome, in which ghrelin levels are found to elevated and abnormally regulated, represent much more extreme phenotypes than the mouse models of ghrelin over expression would predict (Feigerlova et al., 2008; Hinton et al., 2010). As of yet, no definitive examples of ghrelin, GHSR or GOAT deletion have been identified in people. However, other types of mutations—in the ghrelin gene and the GHSR gene—have been identified. One, the Leu72Met ghrelin polymorphism has been associated with binge eating disorder in small cohorts (Monteleone et al., 2007). Several GHSR polymorphisms have been found in individuals with short stature, which makes sense given ghrelin's ability to potently stimulate GH secretion. Using *in vitro* methods, these mutations result in loss of endogenous constitutive activity of GHSR, altered binding of ghrelin, decreased ghrelin-stimulated signal transduction, and/or decrease cell surface expression of GHSR (Wang et al., 2004; Pantel et al., 2006; Inoue et al., 2011). Interestingly, carriers of some of these GHSR mutations demonstrate an incomplete penetrance of overweight and obesity, although the relationship of the mutation and the body weight phenotypes have not been confirmed (Wang et al., 2004).

Given the crucial insight regarding ghrelin action on feeding and other domains provided by these mouse models, we would encourage further investigations using not only the currently available mouse models, but also novel genetically modified mouse models. For instance, a GHSR-conditional knockout mouse in which GHSR is eliminated exclusively from a single cell type or a single brain region by using the Cre-loxP technology is an exciting alternative. Additionally, the Cre-loxP system could be used to investigate the role of GHSR at specific stages of development by breeding GHSR floxed mouse lines with transgenic mice bearing a tamoxifen-dependent Cre recombinase expressed under the control of specific promoters, thus allowing the manipulation of GHSR to be controlled in a spatiotemporal-specific manner. In addition, mouse models containing some of the above-described ghrelin and GHSR polymorphisms described in individuals with binge eating disorder and families with short stature ± overweight/obesity would help clarify and causative role for the mutations in those conditions.

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REFERENCES

Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., et al. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J. Clin. Invest.* 116, 3229–3239. doi: 10.1172/JCI29867

Ariyasu, H., Takaya, K., Iwakura, H., Hosoda, H., Akamizu, T., Arai, Y., et al. (2005). Transgenic mice overexpressing des-acyl ghrelin show small phenotype. *Endocrinology* 146, 355–364. doi: 10.1210/en.2004-0629

Ariyasu, H., Yamada, G., Iwakura, H., Akamizu, T., Kangawa, K., and Nakao, K. (2012). Transgenic mice overexpressing ghrelin or ghrelin analog. *Meth. Enzymol.* 514, 371–377. doi: 10.1016/B978-0-12-381272-8.00023-4

Asakawa, A., Inui, A., Fujimiya, M., Sakamaki, R., Shinfuku, N., Ueta, Y., et al. (2005). Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut* 54, 18–24. doi: 10.1136/gut.2004.038737

Bewick, G. A., Kent, A., Campbell, D., Patterson, M., Ghatei, M. A., Bloom, S. R., et al. (2009). Mice with hyperghrelinemia are hyperphagic and glucose intolerant and have reduced leptin sensitivity. *Diabetes* 58, 840–846. doi: 10.2337/db08-1428

Blum, I. D., Patterson, Z., Khazall, R., Lamont, E. W., Sleeman, M. W., Horvath, T. L., et al. (2009). Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. *Neuroscience* 164, 351–359. doi: 10.1016/j.neuroscience.2009.08.009

Bouret, S. G., Draper, S. J., and Simerly, R. B. (2004). Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science* 304, 108–110. doi: 10.1126/science.1095004

Chuang, J. C., Perello, M., Sakata, I., Osborne-Lawrence, S., Savitt, J. M., Lutter, M., et al. (2011). Ghrelin mediates stress-induced food-reward behavior in mice. *J. Clin. Invest.* 121, 2684–2692. doi: 10.1172/JCI57660

Cruz, C. R., and Smith, R. G. (2008). The growth hormone secretagogue receptor. *Vitam. Horm.* 77, 47–88. doi: 10.1016/S0083-6729(06)77004-2

Cummings, D. E. (2006). Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiol. Behav.* 89, 71–84. doi: 10.1016/j.physbeh.2006.05.022

Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., and Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50, 1714–1719. doi: 10.2337/diabetes.50.8.1714

Damian, M., Marie, J., Leyris, J. P., Fehrentz, J. A., Verdie, P., Martinez, J., et al. (2012). High constitutive activity is an intrinsic feature of Ghrelin receptor protein: a study with a functional monomeric GHS-R1a receptor reconstituted in lipid discs. *J. Biol. Chem.* 287, 3630–3641. doi: 10.1074/jbc.M111.288324

Date, Y. (2012). Ghrelin and the vagus nerve. *Methods Enzymol.* 514, 261–269. doi: 10.1016/B978-0-12-381272-8.00016-7

Date, Y., Murakami, N., Toshinai, K., Matsukura, S., Niijima, A., Matsuo, H., et al. (2002). The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 123, 1120–1128. doi: 10.1053/gast.2002.35954

Davey, R. A., and Maclean, H. E. (2006). Current and future approaches using genetically modified mice in endocrine research. *Am. J. Physiol. Endocrinol. Metab.* 291, E429–E438. doi: 10.1152/ajpendo.00124.2006

Davis, J. F., Perello, M., Choi, D. L., Magrisso, I. J., Kirchner, H., Pfluger, P. T., et al. (2012). GOAT induced ghrelin acylation regulates hedonic feeding. *Horm. Behav.* 62, 598–604. doi: 10.1016/j.yhbeh.2012.08.009

Delhanty, P. J., Neggers, S. J., and Van Der Lely, A. J. (2012). Mechanisms in endocrinology: ghrelin: the differences between acyl- and des-acyl ghrelin. *Eur. J. Endocrinol.* 167, 601–608. doi: 10.1530/EJE-12-0456

De Smet, B., Depoortere, I., Moechars, D., Swennen, Q., Moreaux, B., Cryns, K., et al. (2006). Energy homeostasis and gastric emptying in ghrelin knockout mice. *J. Pharmacol. Exp. Ther.* 316, 431–439. doi: 10.1124/jpet.105.091504

Dezaki, K., Sone, H., Koizumi, M., Nakata, M., Kakei, M., Nagai, H., et al. (2006). Blockade of pancreatic islet-derived ghrelin enhances insulin secretion to prevent high-fat diet-induced glucose intolerance. *Diabetes* 55, 3486–3493. doi: 10.2337/db06-0878

Dickson, S. L., Hrabovszky, E., Hansson, C., Jerlhag, E., Alvarez-Crespo, M., Skibicka, K. P., et al. (2010). Blockade of central nicotine acetylcholine receptor signaling attenuate ghrelin-induced food intake in rodents. *Neuroscience* 171, 1180–1186. doi: 10.1016/j.neuroscience.2010.10.005

Disse, E., Bussier, A. L., Deblon, N., Pfluger, P. T., Tschop, M. H., Laville, M., et al. (2011). Systemic ghrelin and reward: effect of cholinergic blockade. *Physiol. Behav.* 102, 481–484. doi: 10.1016/j.physbeh.2010.12.006

Disse, E., Bussier, A. L., Veyrat-Durebex, C., Deblon, N., Pfluger, P. T., Tschop, M. H., et al. (2010). Peripheral ghrelin enhances sweet taste food consumption and preference, regardless of its caloric content. *Physiol. Behav.* 101, 277–281. doi: 10.1016/j.physbeh.2010.05.017

Drazen, D. L., Vahl, T. P., D'Alessio, D. A., Seeley, R. J., and Woods, S. C. (2006). Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status. *Endocrinology* 147, 23–30. doi: 10.1210/en.2005-0973

Egecioglu, E., Jerlhag, E., Salome, N., Skibicka, K. P., Haage, D., Bohlooly, Y. M., et al. (2010). Ghrelin increases intake of rewarding food in rodents. *Addict Biol.* 15, 304–311. doi: 10.1111/j.1369-1600.2010.00216.x

Feigerlova, E., Diene, G., Conte-Auriol, F., Molinas, C., Gennero, I., Salles, J. P., et al. (2008). Hyperghrelinemia precedes obesity in Prader-Willi syndrome. *J. Clin. Endocrinol. Metab.* 93, 2800–2805. doi: 10.1210/jc.2007-2138

Finger, B. C., Dinan, T. G., and Cryan, J. F. (2012). Diet-induced obesity blunts the behavioural effects of ghrelin: studies in a mouse-progressive ratio task. *Psychopharmacology (Berl.)* 220, 173–181. doi: 10.1007/s00213-011-2468-0

Furness, J. B., Hunne, B., Matsuda, N., Yin, L., Russo, D., Kato, I., et al. (2011). Investigation of the presence of ghrelin in the central nervous system of the rat and mouse. *Neuroscience* 193, 1–9. doi: 10.1016/j.neuroscience.2011.07.063

Goldstein, J. L., Zhao, T. J., Li, R. L., Sherbet, D. P., Liang, G., and Brown, M. S. (2011). Surviving starvation: essential role of the ghrelin-growth hormone axis. *Cold Spring Harb. Symp. Quant. Biol.* 76, 121–127. doi: 10.1101/sqb.2011.76.010447

Gooley, J. J., Schomer, A., and Saper, C. B. (2006). The dorsomedial hypothalamic nucleus is critical for

the expression of food- entrainable circadian rhythms. *Nat. Neurosci.* 9, 398–407. doi: 10.1038/nn1651

Gropp, E., Shanabrough, M., Borok, E., Xu, A. W., Janoschek, R., Buch, T., et al. (2005). Agouti-related peptide-expressing neurons are mandatory for feeding. *Nat. Neurosci.* 8, 1289–1291. doi: 10.1038/nn1548

Guan, X. M., Yu, H., Palyha, O. C., McKee, K. K., Feighner, S. D., Sirinathsinghji, D. J., et al. (1997). Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res. Mol. Brain Res.* 48, 23–29. doi: 10.1016/S0169-328X(97)00071-5

Gutierrez, J. A., Solenberg, P. J., Perkins, D. R., Willency, J. A., Knierman, M. D., Jin, Z., et al. (2008). Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6320–6325. doi: 10.1073/pnas.0800708105

Hinton, E. C., Isles, A. R., Williams, N. M., and Parkinson, J. A. (2010). Excessive appetitive arousal in Prader-Willi syndrome. *Appetite* 54, 225–228. doi: 10.1016/j.appet.2009.12.002

Holst, B., Holliday, N. D., Bach, A., Elling, C. E., Cox, H. M., and Schwartz, T. W. (2004). Common structural basis for constitutive activity of the ghrelin receptor family. *J. Biol. Chem.* 279, 53806–53817. doi: 10.1074/jbc.M407676200

Holst, B., and Schwartz, T. W. (2004). Constitutive ghrelin receptor activity as a signaling set-point in appetite regulation. *Trends Pharmacol. Sci.* 25, 113–117. doi: 10.1016/j.tips.2004.01.010

Howard, A. D., Feighner, S. D., Cully, D. F., Arena, J. P., Liberator, P. A., Rosenblum, C. I., et al. (1996). A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 273, 974–977. doi: 10.1126/science.273.5277.974

Inoue, H., Kangawa, N., Kinouchi, A., Sakamoto, Y., Kimura, C., Horikawa, R., et al. (2011). Identification and functional analysis of novel human growth hormone secretagogue receptor (GHSR) gene mutations in Japanese subjects with short stature. *J. Clin. Endocrinol. Metab.* 96, E373–E378. doi: 10.1210/jc.2010-1570

Iwakura, H., Ariyasu, H., Li, Y., Kanamoto, N., Bando, M., Yamada, G., et al. (2009). A mouse model of ghrelinoma exhibited activated growth hormone-insulin-like growth factor I axis and glucose intolerance. *Am. J. Physiol. Endocrinol. Metab.* 297, E802–E811. doi: 10.1152/ajpendo.00205.2009

Iwakura, H., Hosoda, K., Son, C., Fujikura, J., Tomita, T., Noguchi, M., et al. (2005). Analysis of rat insulin II promoter-ghrelin transgenic mice and rat glucagon promoter-ghrelin transgenic mice. *J. Biol. Chem.* 280, 15247–15256. doi: 10.1074/jbc.M411358200

Jerlhag, E., Egecioglu, E., Dickson, S. L., Douhan, A., Svensson, L., and Engel, J. A. (2007). Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addict. Biol.* 12, 6–16. doi: 10.1111/j.1369-1600.2006.00041.x

Kageyama, H., Takenoya, F., Shiba, K., and Shioda, S. (2010). Neuronal circuits involving ghrelin in the hypothalamus-mediated regulation of feeding. *Neuropeptides* 44, 133–138. doi: 10.1016/j.npep.2009.11.010

Kanoski, S. E., Fortin, S. M., Ricks, K. M., and Grill, H. J. (2013). Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. *Biol. Psychiatry* 73, 915–923. doi: 10.1016/j.biopsych.2012.07.002

Keen-Rhinehart, E., and Bartness, T. J. (2005). Peripheral ghrelin injections stimulate food intake, foraging, and food hoarding in Siberian hamsters. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, R716–R722. doi: 10.1152/ajpregu.00705.2004

Kirchner, H., Gutierrez, J. A., Solenberg, P. J., Pfluger, P. T., Czyzyk, T. A., Willency, J. A., et al. (2009). GOAT links dietary lipids with the endocrine control of energy balance. *Nat. Med.* 15, 741–745. doi: 10.1038/nm.1997

Kojima, M., and Kangawa, K. (2010). Ghrelin: from gene to physiological function. *Results Probl. Cell Differ.* 50, 185–205.

Lall, S., Balthasar, N., Carmignac, D., Magoulas, C., Sesay, A., Houston, P., et al. (2004). Physiological studies of transgenic mice over-expressing growth hormone (GH) secretagogue receptor 1A in GH-releasing hormone neurons. *Endocrinology* 145, 1602–1611. doi: 10.1210/en.2003-1509

Lamont, E. W., Patterson, Z., Rodrigues, T., Vallejos, O., Blum, I. D., and Abizaid, A. (2012). Ghrelin-deficient mice have fewer orexin cells and reduced cFOS expression in the mesolimbic dopamine pathway under a restricted feeding paradigm. *Neuroscience* 218, 12–19. doi: 10.1016/j.neuroscience.2012.05.046

Landgren, S., Simms, J. A., Hyytia, P., Engel, J. A., Bartlett, S. E., and Jerlhag, E. (2012). Ghrelin receptor (GHS-R1A) antagonism suppresses both operant alcohol self-administration and high alcohol consumption in rats. *Addict. Biol.* 17, 86–94. doi: 10.1111/j.1369-1600.2010.00280.x

Li, R. L., Sherbet, D. P., Elsbernd, B. L., Goldstein, J. L., Brown, M. S., and Zhao, T. J. (2012). Profound hypoglycemia in starved, ghrelin-deficient mice is caused by decreased gluconeogenesis and reversed by lactate or fatty acids. *J. Biol. Chem.* 287, 17942–17950. doi: 10.1074/jbc.M112.358051

Lin, L., Saha, P. K., Ma, X., Henshaw, I. O., Shao, L., Chang, B. H., et al. (2011). Ablation of ghrelin receptor reduces adiposity and improves insulin sensitivity during aging by regulating fat metabolism in white and brown adipose tissues. *Aging Cell* 10, 996–1010. doi: 10.1111/j.1474-9726.2011.00740.x

Luquet, S., Perez, F. A., Hnasko, T. S., and Palmiter, R. D. (2005). NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* 310, 683–685. doi: 10.1126/science.1115524

Lutter, M., Sakata, I., Osborne-Lawrence, S., Rovinsky, S. A., Anderson, J. G., Jung, S., et al. (2008). The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat. Neurosci.* 11, 752–753. doi: 10.1038/nn.2139

Ma, X., Lin, L., Qin, G., Lu, X., Fiorotto, M., Dixit, V. D., et al. (2011). Ablations of ghrelin and ghrelin receptor exhibit differential metabolic phenotypes and thermogenic capacity during aging. *PLoS ONE* 6:e16391. doi: 10.1371/journal.pone.0016391

Ma, X., Lin, Y., Lin, L., Qin, G., Pereira, F. A., Haymond, M. W., et al. (2012). Ablation of ghrelin receptor in leptin-deficient ob/ob mice has paradoxical effects on glucose homeostasis when compared with ablation of ghrelin in ob/ob mice. *Am. J. Physiol. Endocrinol. Metab.* 303, E422–E431. doi: 10.1152/ajpendo.00576.2011

Matsumoto, M., Hosoda, H., Kitajima, Y., Morozumi, N., Minamitake, Y., Tanaka, S., et al. (2001). Structure-activity relationship of ghrelin: pharmacological study of ghrelin peptides. *Biochem. Biophys. Res. Commun.* 287, 142–146. doi: 10.1006/bbrc.2001.5553

Monteleone, P., Tortorella, A., Castaldo, E., Di Filippo, C., and Maj, M. (2007). The Leu72Met polymorphism of the ghrelin gene is significantly associated with binge eating disorder. *Psychiatr. Genet.* 17, 13–16. doi: 10.1097/YPG.0b013e328010e2c3

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., et al. (2001). A role for ghrelin in the central regulation of feeding. *Nature* 409, 194–198. doi: 10.1038/35051587

Olszewski, P. K., Li, D., Grace, M. K., Billington, C. J., Kotz, C. M., and Levine, A. S. (2003). Neural basis of orexigenic effects of ghrelin acting within lateral hypothalamus. *Peptides* 24, 597–602. doi: 10.1016/S0196-9781(03)00105-0

Pantel, J., Legendre, M., Cabrol, S., Hilal, L., Hajaji, Y., Morisset, S., et al. (2006). Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J. Clin. Invest.* 116, 760–768. doi: 10.1172/JCI25303

Peeters, T. L. (2003). Central and peripheral mechanisms by which ghrelin regulates gut motility. *J. Physiol. Pharmacol.* 54 (Suppl. 4), 95–103.

Perello, M., Sakata, I., Birnbaum, S., Chuang, J. C., Osborne-Lawrence, S., Rovinsky, S. A., et al. (2010). Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. *Biol Psychiatry* 67, 880–886. doi: 10.1016/j.biopsych.2009.10.030

Perello, M., Scott, M. M., Sakata, I., Lee, C. E., Chuang, J. C., Osborne-Lawrence, S., et al. (2012). Functional implications of limited leptin receptor and ghrelin receptor coexpression in the brain. *J. Comp. Neurol.* 520, 281–294. doi: 10.1002/cne.22690

Perello, M., and Zigman, J. M. (2012). The role of ghrelin in reward-based eating. *Biol. Psychiatry* 72, 347–353. doi: 10.1016/j.biopsych.2012.02.016

Pfluger, P. T., Kirchner, H., Gunnel, S., Schrott, B., Perez-Tilve, D., Fu, S., et al. (2008). Simultaneous deletion of ghrelin and its receptor increases motor activity and energy expenditure. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294, G610–G618. doi: 10.1152/ajpgi.00321.2007

Qian, S., Chen, H., Weingarth, D., Trumbauer, M. E., Novi, D. E., Guan, X., et al. (2002). Neither agouti-related protein nor neuropeptide Y is critically required for the regulation of

energy homeostasis in mice. *Mol. Cell. Biol.* 22, 5027–5035. doi: 10.1128/MCB.22.14.5027-5035.2002

Reed, J. A., Benoit, S. C., Pfluger, P. T., Tschop, M. H., D'Alessio, D. A., and Seeley, R. J. (2008). Mice with chronically increased circulating ghrelin develop age-related glucose intolerance. *Am. J. Physiol. Endocrinol. Metab.* 294, E752–E760. doi: 10.1152/ajpendo.00463.2007

Sato, T., Kurokawa, M., Nakashima, Y., Ida, T., Takahashi, T., Fukue, Y., et al. (2008). Ghrelin deficiency does not influence feeding performance. *Regul. Pept.* 145, 7–11. doi: 10.1016/j.regpep.2007.09.010

Schaeffer, M., Langlet, F., Lafont, C., Molino, F., Hodson, D. J., Roux, T., et al. (2013). Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1512–1517. doi: 10.1073/pnas.1212137110

Scott, M. M., Perello, M., Chuang, J. C., Sakata, I., Gautron, L., Lee, C. E., et al. (2012). Hindbrain ghrelin receptor signaling is sufficient to maintain fasting glucose. *PLoS ONE* 7:e44089. doi: 10.1371/journal.pone.0044089

Shimbara, T., Mondal, M. S., Kawagoe, T., Toshinai, K., Koda, S., Yamaguchi, H., et al. (2004). Central administration of ghrelin preferentially enhances fat ingestion. *Neurosci. Lett.* 369, 75–79. doi: 10.1016/j.neulet.2004.07.060

Skibicka, K. P., Hansson, C., Egecioglu, E., and Dickson, S. L. (2012). Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. *Addict. Biol.* 17, 95–107. doi: 10.1111/j.1369-1600.2010.00294.x

Steculorum, S. M., and Bouret, S. G. (2011). Developmental effects of ghrelin. *Peptides* 32, 2362–2366. doi: 10.1016/j.peptides.2011.06.021

Sun, Y., Ahmed, S., and Smith, R. G. (2003). Deletion of ghrelin impairs neither growth nor appetite. *Mol. Cell. Biol.* 23, 7973–7981. doi: 10.1128/MCB.23.22.7973-7981.2003

Sun, Y., Asnicar, M., Saha, P. K., Chan, L., and Smith, R. G. (2006). Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. *Cell. Metab.* 3, 379–386. doi: 10.1016/j.cmet.2006.04.004

Sun, Y., Butte, N. F., Garcia, J. M., and Smith, R. G. (2008). Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. *Endocrinology* 149, 843–850. doi: 10.1210/en.2007-0271

Sun, Y., Wang, P., Zheng, H., and Smith, R. G. (2004). Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. *Proc. Natl. Acad. Sci. U.S.A.* 101, 4679–4684. doi: 10.1073/pnas.0305930101

Toshinai, K., Date, Y., Murakami, N., Shimada, M., Mondal, M. S., Shimbara, T., et al. (2003). Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology* 144, 1506–1512. doi: 10.1210/en.2002-220788

Verhagen, L. A., Egecioglu, E., Luijendijk, M. C., Hillebrand, J. J., Adan, R. A., and Dickson, S. L. (2011). Acute and chronic suppression of the central ghrelin signaling system reveals a role in food anticipatory activity. *Eur. Neuropsychopharmacol.* 21, 384–392. doi: 10.1016/j.euroneuro.2010.06.005

Walker, A. K., Ibia, I. E., and Zigman, J. M. (2012). Disruption of cue-potentiated feeding in mice with blocked ghrelin signaling. *Physiol. Behav.* 108, 34–43. doi: 10.1016/j.physbeh.2012.10.003

Wang, H. J., Geller, F., Dempfle, A., Schauble, N., Friedel, S., Lichtner, P., et al. (2004). Ghrelin receptor gene: Identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature. *J. Clin. Endocrinol. Metabol.* 89, 157–162. doi: 10.1210/jc.2003-031395

Wei, W., Qi, X., Reed, J., Ceci, J., Wang, H. Q., Wang, G., et al. (2006). Effect of chronic hyperghrelinemia on ingestive action of ghrelin. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290, R803–R808. doi: 10.1152/ajpregu.00331.2005

Willesen, M. G., Kristensen, P., and Romer, J. (1999). Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* 70, 306–316. doi: 10.1159/000054491

Wortley, K. E., Anderson, K. D., Garcia, K., Murray, J. D., Malinova, L., Liu, R., et al. (2004). Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. *Proc. Natl. Acad. Sci. U.S.A.* 101, 8227–8232. doi: 10.1073/pnas.0402763101

Wortley, K. E., Del Rincon, J. P., Murray, J. D., Garcia, K., Iida, K., Thorner, M. O., et al. (2005). Absence of ghrelin protects against early-onset obesity. *J. Clin. Invest.* 115, 3573-3578. doi: 10.1172/JCI26003

Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., et al. (2001). Ghrelin enhances appetite and increases food intake in humans. *J. Clin. Endocrinol. Metab.* 86, 5992. doi: 10.1210/jc.86.12.5992

Yamada, G., Ariyasu, H., Iwakura, H., Hosoda, H., Akamizu, T., Nakao, K., et al. (2010). Generation of transgenic mice overexpressing a ghrelin analog. *Endocrinology* 151, 5935–5940. doi: 10.1210/en.2010-0635

Yang, J., Brown, M. S., Liang, G., Grishin, N. V., and Goldstein, J. L. (2008). Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 132, 387–396. doi: 10.1016/j.cell.2008.01.017

Zhang, W., Chai, B., Li, J. Y., Wang, H., and Mulholland, M. W. (2008). Effect of des-acyl ghrelin on adiposity and glucose metabolism. *Endocrinology* 149, 4710–4716. doi: 10.1210/en.2008-0263

Zhao, T. J., Liang, G., Li, R. L., Xie, X., Sleeman, M. W., Murphy, A. J., et al. (2010a). Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proc. Natl. Acad. Sci. U.S.A.* 107, 7467–7472. doi: 10.1073/pnas.1002271107

Zhao, T. J., Sakata, I., Li, R. L., Liang, G., Richardson, J. A., Brown, M. S., et al. (2010b). Ghrelin secretion stimulated by {beta}1-adrenergic receptors in cultured ghrelinoma cells and in fasted mice. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15868–15873. doi: 10.1073/pnas.1011116107

Zigman, J. M., and Elmquist, J. K. (2003). Minireview: from anorexia to obesity–the yin and yang of body weight control. *Endocrinology* 144, 3749–3756. doi: 10.1210/en.2003-0241

Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B., and Elmquist, J. K. (2006). Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J. Comp. Neurol.* 494, 528–548. doi: 10.1002/cne.20823

Zigman, J. M., Nakano, Y., Coppari, R., Balthasar, N., Marcus, J. N., Lee, C. E., et al. (2005). Mice lacking ghrelin receptors resist the development of diet-induced obesity. *J. Clin. Invest.* 115, 3564–3572. doi: 10.1172/JCI26002

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Taking two to tango: a role for ghrelin receptor heterodimerization in stress and reward

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The gut hormone, ghrelin, is the only known peripherally derived orexigenic signal. It activates its centrally expressed receptor, the growth hormone secretagogue receptor (GHS-R1a), to stimulate food intake. The ghrelin signaling system has recently been suggested to play a key role at the interface of homeostatic control of appetite and the hedonic aspects of food intake, as a critical role for ghrelin in dopaminergic mesolimbic circuits involved in reward signaling has emerged. Moreover, enhanced plasma ghrelin levels are associated with conditions of physiological stress, which may underline the drive to eat calorie-dense "comfort-foods" and signifies a role for ghrelin in stress-induced food reward behaviors. These complex and diverse functionalities of the ghrelinergic system are not yet fully elucidated and likely involve crosstalk with additional signaling systems. Interestingly, accumulating data over the last few years has shown the GHS-R1a receptor to dimerize with several additional G-protein coupled receptors (GPCRs) involved in appetite signaling and reward, including the GHS-R1b receptor, the melanocortin 3 receptor (MC_3), dopamine receptors (D_1 and D_2), and more recently, the serotonin 2C receptor (5-HT_{2C}). GHS-R1a dimerization was shown to affect downstream signaling and receptor trafficking suggesting a potential novel mechanism for fine-tuning GHS-R1a receptor mediated activity. This review summarizes ghrelin's role in food reward and stress and outlines the GHS-R1a dimer pairs identified to date. In addition, the downstream signaling and potential functional consequences of dimerization of the GHS-R1a receptor in appetite and stress-induced food reward behavior are discussed. The existence of multiple GHS-R1a heterodimers has important consequences for future pharmacotherapies as it significantly increases the pharmacological diversity of the GHS-R1a receptor and has the potential to enhance specificity of novel ghrelin-targeted drugs.

Keywords: ghrelin, dimerization, obesity, stress, food reward

INTRODUCTION

Appetite regulation, food intake and diet are closely intertwined with mood regulation and stress perception and stress response (Oliver and Wardle, 1999; Gibson, 2006; Morrison, 2009; Dallman, 2010). Obesity and the metabolic syndrome, which can be defined as a combination of comorbid medical disorders, including atherosclerosis, hypertension, insulin resistance or diabetes mellitus type II, glucose intolerance, dyslipidemia, and a general pro-inflammatory phenotype (Cheng and Leiter, 2006; Mikhail, 2009), have been identified as environmental risk factors for affective psychiatric disorders, including anxiety and depression (McElroy et al., 2004; Goldbacher and Matthews, 2007; Kloiber et al., 2007; Gariepy et al., 2010; Marijnissen et al., 2011). Epidemiologic data even suggests that obesity is associated with a 25% increased incidence of anxiety and mood disorders (Simon et al., 2006). In addition, major depression in adolescence is linked with a higher risk for obesity in adulthood (Richardson et al., 2003). Moreover, metabolic conditions may be exacerbated in depression and *vice versa*, which indicates a reciprocal link (McElroy et al., 2004; Simon et al., 2006; de Wit et al., 2010; Luppino et al., 2010; Marijnissen et al., 2011). Likewise, stress significantly impacts on food intake

Abbreviations: ACTH, adrenocorticotropic hormone; AgRP, agouti-related peptide; ARC, arcuate nucleus; BRET, bioluminescence resonance energy transfer; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin; CPP, conditioned place preference; CSDS, chronic social defeat stress; CRF, corticotrophin-releasing factor; DA, dopamine; GH, growth hormone; GHRH, growth hormone-releasing hormone; D_1, dopamine 1 receptor; D_2, dopamine 2 receptor; FRAP, fluorescence recovery after photobleaching; GHS-R1a, growth-hormone secretagogue receptor; GOAT, ghrelin O-acyl transferase; GPCR, G-protein coupled receptors; Hek, human embryonic kidney cells; HPA, hypothalamic-pituitary-adrenal; MC3, melanocortin 3 receptor; NAcc, nucleus accumbens; NPY, neuropeptide Y; NTS, nucleus of the solitary tract (nucleus tractus solitarii); PVN, paraventricular nucleus; POMC, proopiomelanocortin; SN, substantia nigra; TIRF, total internal reflection fluorescence imaging; UPC2/3, uncoupling proteins 2 and 3; VTA, ventral tegmentum; 5-HT, serotonin (5-hydroxytryptamine); 5-HT_{2C}, serotonin 2C receptor; 6-OHDA, 6-hydroxydopamine.

in humans and animals and may promote metabolic disturbances (Block et al., 2009; Dallman, 2010; Maniam and Morris, 2012). Interestingly, stress-induced hyperphagia and subsequent increases in body weight and obesity are also associated with major depressive disorders in humans (Novick et al., 2005; Simon et al., 2006; Kloiber et al., 2007). Moreover, acute stress responses are reduced following intake of palatable rewarding foods, potentially explaining the phenomenon of "comfort eating" observed in stressed individuals as self-medication for stress relief (Dallman et al., 2003).

Ghrelin, a 28 amino acid stomach-derived peptide (Kojima et al., 1999), is currently the only described orexigenic hormone from the periphery, acting centrally to increase food intake and modulates the body's metabolism via centrally activated mechanisms (Tschop et al., 2000; Nakazato et al., 2001; Kojima et al., 2004; Andrews, 2011). Peripheral ghrelin mediates its appetite-inducing effects centrally, after passing through the blood brain barrier (Banks et al., 2002, 2008). Recent experiments by Schaeffer and colleagues have confirmed that the hypothalamus directly senses circulating ghrelin from the periphery to modify energy status (Schaeffer et al., 2013). This study demonstrated that circulating ghrelin rapidly binds neurons in the vicinity of fenestrated capillaries using *in vivo* multiphoton microscopy together with fluorescently labeled ligands. Interestingly, it was also shown that the number of labeled cell bodies varies with feeding status, suggesting a potential differential ability to sense ghrelin under altered metabolic conditions. In addition, in both rodents and humans, ghrelin also reaches the brain via vagal afferents to the nucleus of the solitary tract (NTS) in the brain stem with further projections to the arcuate nucleus (ARC) of the hypothalamus (Asakawa et al., 2001b; Date et al., 2002; Williams et al., 2003; le Roux et al., 2005). However, contradictory results exists in rats, disputing the role of vagal afferents in the acute eating-stimulatory effect of peripheral ghrelin (Arnold et al., 2006).

When the orexigenic hormone ghrelin activates its receptor, the growth-hormone secretagogue receptor (GHS-R1a), it stimulates appetite and food intake but also mediates a multitude of additional biological activities, including the secretion of growth hormone (GH), glucose and lipid metabolism and gastrointestinal motility, which together maintain the body's energy homeostasis (for review see Schellekens et al., 2010). Moreover, ghrelin contributes to the accumulation of adipose tissue via the promotion of carbohydrates over fat as energy substrate (Tschop et al., 2000). The ghrelinergic system has therefore received considerable attention in the pharmaceutical industry as a promising target in obesity treatment and other eating disorders (Horvath et al., 2003; Zorrilla et al., 2006; Leite-Moreira and Soares, 2007; Moulin et al., 2007; Soares et al., 2008; Chollet et al., 2009; Lu et al., 2009; Schellekens et al., 2010; Patterson et al., 2011; Yi et al., 2011). In addition, accumulating data has revealed that the ghrelinergic system has an important function in other behaviors related to food intake and plays a pivotal role in the mesolimbic dopaminergic circuitry, which is responsible for various non-homeostatic, hedonic rewarding and motivational aspects of food intake (for review see Dickson et al., 2011; Egecioglu et al., 2011; Skibicka and Dickson, 2011; Perello and Zigman, 2012; Schellekens et al., 2012b; Skibicka et al., 2012). More recently, ghrelin has been shown to be involved in mediating a stress response and to mediate stress-induced food reward behavior (Lutter et al., 2008; Chuang and Zigman, 2010; Patterson et al., 2010, 2013; Chuang et al., 2011; Diz-Chaves, 2011; Schellekens et al., 2012b; Spencer et al., 2012).

Thus, current research indicates a potential link between ghrelin and affective disorders, such as anxiety and depression. In line with this hypothesis are the decreased plasma ghrelin levels often observed in depressed patients (Barim et al., 2009) (but also see Schanze et al., 2008; Kluge et al., 2009). Furthermore, recent data also demonstrates that ghrelin administration in patients with major depression has some antidepressant effects (Kluge et al., 2011), which is in support of the involvement of ghrelin in the etiology of depressive disorders. Interestingly, a ghrelin gene polymorphism has also been linked with the symptomatology of depression (Nakashima et al., 2008). It is clear, that the ghrelin signaling system is involved in a multitude of centrally regulated functionalities, which exceeds far beyond appetite regulation, energy homeostasis and GH secretion. This is reinforced by the ubiquitous expression of the GHS-R1a receptor in both the periphery as well as the brain (Howard et al., 1996; Guan et al., 1997; Zigman et al., 2006). In particular, the extra-hypothalamic expression of the GHS-R1a receptor, including in the ventral tegmental area (VTA) and nucleus accumbens (NAcc), hippocampus and amygdala, reinforces a function for ghrelin signaling in the hedonic regulation of food intake (Guan et al., 1997; Zigman et al., 2006). Together, this data suggests that the role of the ghrelinergic system in obesity, appetite and food intake now extends toward the reward and motivation pathways as well as to the signaling pathways involved in stress, psychiatric disposition (i.e., mood) and affective disorders such as anxiety and depression. However, it is currently unclear what the exact molecular mechanisms are that mediate this biological diversity of the GHS-R1a receptor. We hypothesize that the differential GHS-R1a receptor signaling events can be explained through crosstalk with additional neuropeptide systems and dimerization with other G-protein coupled receptors (GPCRs) (Schellekens et al., 2013a,b).

This review outlines ghrelin's role in food reward signaling and stress, highlights the identified GHS-R1a dimer pairs to date, and discusses the potential functional consequences of dimerization of the GHS-R1a receptor, with particular focus on its key role in appetite and stress-induced food reward behavior. The ability of the GHS-R1a receptor to form heterodimers with multiple GPCRs involved in the homeostatic or hedonic regulation of food intake and potentially also in the stress response significantly increases its pharmacological diversity (**Figure 1**). Further understanding of the biological significance of GHS-R1a receptor dimers in the neuroendocrine system may pertain to potential new molecular targets and novel strategies for the treatment of stress-associated psychiatric disorders of anxiety as well as eating disorders and metabolic disturbances leading to obesity.

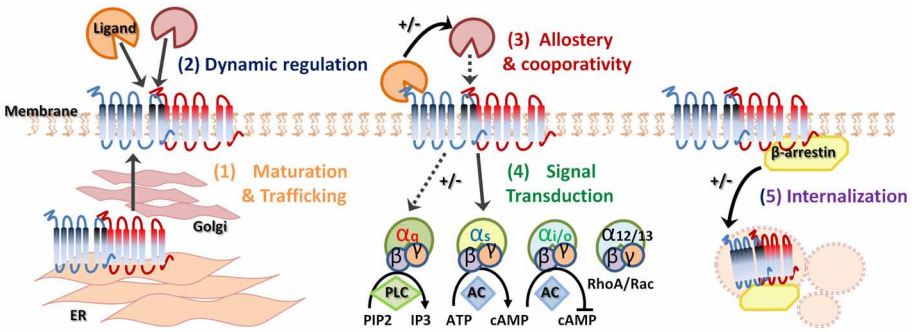

FIGURE 1 | Heterodimerization of G-protein coupled receptors. G-protein coupled receptor (GPCR) oligomerization has several biological functions and consequences. Receptor dimerization can play a role in receptor maturation and correct trafficking **(1)**. Specific ligand binding can dynamically regulate heterodimerization **(2)** and allostery can enhance or suppress downstream signaling **(3)**. In addition, GPCR heterodimerization may demonstrate preferential G protein coupling **(4)**. Finally, agonist-promoted GPCR endocytosis and co-internalization may lead to signal attenuation **(5)**. +/− indicates increase or decrease, respectively.

GHRELIN SIGNALING IN STRESS AND REWARD

The hedonic signaling following ingestion of palatable and calorie-rich food is increasingly being recognized as an important underlying cause for the increase in obesity worldwide, as the overconsumption of calorie-dense foods extends far beyond the individual's nutritional needs (Berthoud, 2006). Obesity-associated habitual overconsumption is mediated by rewarding feelings associated with eating as well as by a natural sensitivity to food stimuli. Stress also affects feeding behavior in humans (Oliver and Wardle, 1999; Gibson, 2006; Dallman, 2010). Interestingly, while some individuals display a stress-induced hyperphagia, others display hypophagia, but an overall increased consumption of highly palatable and caloric-dense foods compared to non-stressed controls is reported (Gibson, 2006; Dallman, 2010). Stress-related psychiatric disorders, including depression, are also linked with extremes in eating behavior according to criteria within the diagnostic and statistical manual of mental disorders (DSM-IV) (American Psychiatric Association, 1994). In addition, the experience of intense stress during childhood represents a strong risk factor to develop depression later in life (O'Mahony et al., 2009; Heim and Binder, 2012). Moreover, these same early life stress events have been associated with metabolic abnormalities later in life (Kaufman et al., 2007). The complex bi-directional relationship between stress, mood and feeding behavior are regulated by converging neuronal pathways mediating appetite and subsequent food intake and circuitries that modulate stress via the hypothalamic-pituitary-adrenal (HPA) axis (Kyrou and Tsigos, 2007; Ulrich-Lai and Herman, 2009; Dallman, 2010; Finger et al., 2011, 2012b; Maniam and Morris, 2012; Schellekens et al., 2012b; Scott et al., 2012). The current knowledge on the role of the metabolic peptide ghrelin in this non-homeostatic food intake behavior is discussed below.

FEEDING BEHAVIOR AND STRESS RESPONSE

Stress response in mammals is mediated via the sympathetic nervous system and involves activation of the HPA axis, immunological changes as well as changes in neural and endocrine mechanisms (McEwen, 2007; Ulrich-Lai and Herman, 2009). The behavioral and physiological responses following stress are initiated with the release of corticotrophin-releasing factor (CRF) from specialized neurons in the paraventricular nucleus (PVN) of the hypothalamus, which subsequently induces the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland into the general circulation. This ultimately stimulates the release of the glucocorticoid corticosterone (or cortisol in primates) from the adrenal cortex. A negative feedback loop exists, which is activated after the elevation in circulating glucocorticoids and prevents further stimulation of the HPA axis via activation of glucocorticoid receptors expressed on the hypothalamus, hippocampus, medial prefrontal cortex, and pituitary gland (Jones et al., 1977; Mahmoud et al., 1984; Sapolsky et al., 1984, 2000; Diorio et al., 1993; Radley et al., 2008; Ulrich-Lai and Herman, 2009). The acute activation of the HPA axis following stress promotes survival via physiological and behavioral adaptations and aims to re-establish the challenged body equilibrium. However, exposure to chronic stress and overstimulation of the HPA-axis can result in physiological alterations with detrimental effects and metabolic disturbances, including obesity (Black, 2006; McEwen, 2007). *Vice versa*, obesity constitutes a chronic stressful state that may cause HPA axis dysfunction (Kyrou and Tsigos, 2007). Interestingly, and in line with the above, the HPA axis has been linked with the control of metabolism and neurotransmitter release in several brain regions, facilitating the appropriate channeling of energy resources to promote survival following stress (Kyrou and Tsigos, 2007; McEwen, 2007; Dallman, 2010; Bowers et al., 2012).

It is likely that the neurobiological mechanisms at the heart of the relationship between food intake and stress responses and the neuronal circuitry of fear, underlying anxiety, are evolutionary selected to function as defensive survival mechanism regulating our response to environmental threats (Siervo et al., 2009). The link between stress and feeding behavior is reinforced by the overlapping neuronal circuitry between the two and involves the same neuropeptides (Bowers et al., 2012; Maniam and Morris, 2012). The stress response following HPA axis activation, and the central mechanisms that regulate appetite and subsequent food intake, proopiomelanocortin (POMC) or neuropeptide Y and

agouti-related peptide (NPY/AgRP), both converge on the PVN and on the CRF-producing neurons within this region. In addition, several peripherally derived hormones and neuropeptides regulating appetite and satiety also play a key role in anxiety-like behavior, highlighting the importance of a bi-directional relationship in anxiety-related mechanisms and eating disorders. For example, the orexigenic neuropeptide NPY (Stanley and Leibowitz, 1985; Heilig et al., 1989) has been shown to exert anxiolytic effects and the anorexigenic peptide cholecystokinin (CCK) (Gibbs et al., 1973) induces panic-like effects (Strohle et al., 2000), impacting on general anxiety and stress-related behavior. Moreover, cocaine- and amphetamine-regulated transcript (CART) is both satiety inducing as well as a mediator of anxiety-like behaviors (Kristensen et al., 1998; Stanek, 2006) and neuropeptide S has both anorexigenic (Smith et al., 2006) and anxiolytic (Xu et al., 2004) responses. Furthermore, neuropeptide expression has been shown to be altered following acute and chronic stressors (Bowers et al., 2012). Likewise, the CRF system is implicated in the regulation of energy balance and in the pathophysiology of obesity and eating disorders. The corticostriatal-hypothalamic circuitry mediates the motivation to obtain food rewards and to promote the overconsumption of palatable foods beyond acute homeostatic needs (Kelley et al., 2005). However, the precise effects of chronic psychosocial stress on neuropeptide and gut hormone expression under conditions of obesity as well as under conditions of malnutrition or anorexia nervosa have not been extensively explored. However, as most neuropeptides convey onto centrally expressed GPCRs, we hypothesize a crucial role for GPCR dimerization and oligomerization.

GHRELIN SIGNALING IN STRESS

Recently, a key role for ghrelin in anxiety and in stress-induced food intake behavior has been suggested (Chuang and Zigman, 2010; Chuang et al., 2011; Schellekens et al., 2012b; Spencer et al., 2012; Patterson et al., 2013). Preproghrelin mRNA levels have been shown to be up-regulated in conditions of stress in both rodents and humans (Asakawa et al., 2001a; Kristenssson et al., 2006; Rouach et al., 2007; Ochi et al., 2008). In addition, circulating levels of plasma ghrelin increase in parallel with corticosterone following both acute and chronic stressors and remain elevated after cessation of the stressor (Asakawa et al., 2001a; Kristenssson et al., 2006; Ochi et al., 2008; Zheng et al., 2009). This may suggest that ghrelin functions as a potential defence mechanism against the consequences of stress and to prevent the manifestation of anxiety and depressive-like symptoms following chronic stress (Lutter et al., 2008). In line with this hypothesis was the observed ghrelin-mediated activation of CRF-producing neurons and enhanced CRF gene expression in the PVN of the hypothalamus (Cabral et al., 2012). In addition, recent findings suggest that CRF type 2 receptors may mediate some metabolic actions of ghrelin (Gershon and Vale, 2013). In this study, a CRF type 2 receptor selective antagonist, anti-sauvagine-30, blocked the ghrelin-mediated increase of glucose uptake as well as the ghrelin-induced enhanced expression of mitochondrial uncoupling proteins 2 and 3 (UCP2 and UCP3) in C2C12 cells, a mouse myoblast cell line. Moreover, both female and male cortistatin (CST) knockout mice were associated with enhanced levels of corticosterone compared to control as well as enhanced ACTH levels in female CST knockout mice. Furthermore, the observed stimulatory effects on prolactin secretion in the CST knockout mice were blocked by a GHS-R1a receptor antagonists and CST knockouts were also correlated with an enhanced level of circulating acyl-ghrelin and increased expression of stomach ghrelin O-acyl transferase (GOAT) (Cordoba-Chacon et al., 2011). The neuropeptide CST is able to bind the GHS-R1a receptor and these findings may further suggest a potential role for CST and ghrelin in the dysregulation of the HPA axis.

Interestingly, ghrelin knockout mice have been found to be more anxious in behavioral tests after acute restraint stress compared with wild-type mice but exhibited a reduced ACTH response and a lower corticosterone response, suggesting that in absence of ghrelin the glucocorticoid negative feedback may be dysregulated (Spencer et al., 2012). Thus, ghrelin may act to reduce anxiety following an acute stressor by stimulating the HPA axis at the level of the anterior pituitary and releasing glucocorticoids (Spencer et al., 2012). Interestingly, glucocorticoids have also been shown to increase the motivation for certain foods (Dallman et al., 2006; Zimmermann et al., 2007; Kageyama et al., 2012) as well as to further activate the ghrelinergic system (Kageyama et al., 2012), reinforcing the feedback theory between chronic stress and food intake and the involvement of ghrelin. Together, we can conclude that under normal physiology ghrelin may contribute to the stress-induced rise in glucocorticoids, activating the negative feedback loop in an attempt to prevent HPA-axis overstimulation.

In line with these findings, increases in ghrelin levels following subcutaneous injections or calorie restriction produced anxiolytic- and antidepressant-like responses in the elevated plus maze and forced swim test (Lutter et al., 2008). In the same study, chronic social defeat stress (CSDS), a model of prolonged psychosocial stress, enhanced acylated ghrelin levels in mice. Moreover, social avoidance was increased in GHS-R1a null mice, suggesting ghrelin to regulate social isolation in response to CSDS (Lutter et al., 2008). Another study, showed a decreased immobility of mice in the forced swim test and increased time spent in the open arms of the elevated plus maze following caloric restriction or subcutaneous ghrelin injection, reinforcing the anxiolytic and antidepressant-like effects of elevated plasma ghrelin (Patterson et al., 2010). In contrast, enhanced ghrelin levels have also been implicated in the induction of anxiety-like and depressive-like behavior (Asakawa et al., 2001a; Carlini et al., 2002, 2004; Kanehisa et al., 2006). These opposing findings are not easily reconciled but have been attributed to the type and duration of stressor used (Stengel et al., 2011) and to differential experimental conditions (for review see Chuang and Zigman, 2010). In these studies, different types of stress yielded differential circulating levels of ghrelin, (Stengel et al., 2011). Circulating ghrelin was elevated following metabolic stressors, including caloric restriction, acute fasting as well as cold exposure, and psychological stressors, such as unpredictable or CSDS. Ghrelin secretion following these stressors is suggested to be mediated via a sympathoadrenal response after sympathetic nervous system activation and catecholamine release (Mundinger et al., 2006; Zhao et al., 2010). However, physical stressors, such as immunological/endotoxin

injection, abdominal surgery and exercise reduced plasma ghrelin levels. Further research is needed to delineate the divergent pathways underlying the changes in circulating ghrelin levels following differential stressors.

Only a few clinical studies are available on ghrelin's role in stress, anxiety and depression. Despite the limited number of human studies reported, these also have found that administration of ghrelin induces cortisol and ACTH secretion (Takaya et al., 2000; Arvat et al., 2001). Moreover, elevated circulating plasma ghrelin levels are equally observed in humans in response to stress exposure, which were found to correlate with cortisol levels following the standardized Trier-Social-Stress test (Rouach et al., 2007). In this study, stress perception and anxiety were enhanced and plasma serum cortisol levels were acutely increased. Interestingly, cortisol levels correlated with an increase in ghrelin but not to BMI or eating scores. Interestingly, while in non-emotional eaters baseline ghrelin exceeded that of high emotional eaters, ghrelin levels declined in the low emotional eaters following food intake, but not in emotional eaters, which may explain sustained eating in high emotional eaters (Raspopow et al., 2010). Moreover, ghrelin is able to induce an exaggerated ACTH response, independent of ghrelin-mediated GH response in Cushing's disease, a disorder characterized by major weight gain and chronic hypercortisolism (Monsonego et al., 2001; Moon et al., 2011). Interestingly, monoaminergic neurotransmitters are both activated by stressors and modulated by ghrelin (Brunetti et al., 2002; Date et al., 2006; Nonogaki et al., 2006; Kawakami et al., 2008). For example, dopaminergic cells in the VTA are sensitive to ghrelin, as well as noradrenergic cells in the NTS in the brainstem and serotinergic cells in the midbrain raphe nuclei (Carlini et al., 2004; Abizaid et al., 2006; Date et al., 2006). In addition, these ghrelin-sensitive monoamines mediate the activity of limbic and hypothalamic structures associated with stress-associated dysfunction (Anisman and Zacharko, 1990; Bremner et al., 1996). This ghrelin-induced activity may be mediated via specific GHS-R1a heterodimers present on these monoaminergic cells.

GHRELIN SIGNALING IN FOOD REWARD BEHAVIOR

Ghrelin can promote the predisposition to overeat when presented with pleasurable energy dense food sources, highlighting the key role for ghrelin signaling in the motivation to obtain palatable food rewards (for review see Skibicka and Dickson, 2011; Perello and Zigman, 2012; Schellekens et al., 2012b). Indeed, both peripheral and central ghrelin were shown to enhance hedonic feeding associated with food palatability (Shimbara et al., 2004; Disse et al., 2010). The expression of the GHS-R1a receptor on midbrain dopaminergic neurons in the VTA and NAcc is in line with ghrelin's role in hedonic eating behavior mediated in the mesolimbic reward system (Guan et al., 1997; Abizaid, 2009; Skibicka et al., 2011). Indeed, administration of ghrelin into the VTA and NAcc of rats directly activated these regions and stimulated chow hyperphagia (Naleid et al., 2005). In addition, direct injection of ghrelin into the VTA of mice significantly increased the preference for rewarding foods (Egecioglu et al., 2010).

The ghrelin-mediated enhanced preference for rewarding foods was absent in GHS-R1a knock-out mice (Disse et al., 2010) and following GHS-R1a antagonist treatment in rats (Egecioglu et al., 2010), demonstrating GHS-R1a receptor dependence. In addition, several studies, using conditioned place preference (CPP) in rodents, have demonstrated that increases in ghrelin, following peripheral or central administration and caloric restriction, enhance the CPP response for HFD but not chow (Chuang and Zigman, 2010; Egecioglu et al., 2010; Perello et al., 2010; Disse et al., 2011). Moreover, this was dependent on the GHS-R1a receptor and an intact VTA region. Furthermore, the ability of ghrelin to alter food-associated reward has been assessed using operant conditioning paradigms (Perello et al., 2010; Skibicka et al., 2011, 2012; Finger et al., 2012a). In these studies, intra-VTA microinjection of ghrelin significantly stimulated free feeding of chow and increased operant responding to palatable rewards in rodents (Skibicka et al., 2011), while this did not occur when ghrelin was directly microinjected into the NAcc (Dickson et al., 2011; Skibicka et al., 2011). Interestingly, these studies also demonstrated that ghrelin enhances incentive motivation for sucrose rewards in satiated rats but when ghrelin signaling is blocked operant responding for sugar in hungry rats returns to the level of satiated rats (Skibicka et al., 2012). Moreover, GHS-R1a blockade in the VTA decreased the motivation to obtain sucrose rewards but did not affect fasting induced chow hyperphagia (Skibicka et al., 2011). This suggests the selection of rewarding foods and food-motivated behavior is attributed more specifically to ghrelin's action on the VTA and not the NAcc.

Finally, data is accumulating identifying the ghrelinergic system to mediate food reward following exposure to stress (Asakawa et al., 2001a; Chuang and Zigman, 2010; Chuang et al., 2011; Schellekens et al., 2012b; Spencer et al., 2012; Patterson et al., 2013). For example, intake of high-fat diet and the CPP response are increased after exposure to CSDS and absent in GHS-R1a knockout mice, demonstrating again dependence on intact ghrelin signaling (Chuang et al., 2011).

Ghrelin's ability to modify the rewarding properties of palatable foods is mediated via connections to dopamine (DA) neurons and DA release from mesolimbic dopaminergic neurons in the VTA, which project to the NAcc (Jerlhag et al., 2007; Dickson et al., 2011; Skibicka and Dickson, 2011). The direct activation of dopaminergic neurons in the VTA following peripheral and central ghrelin administration is in support of this notion (Abizaid et al., 2006). Moreover, direct injection of ghrelin in the VTA increases appetitive motivation in rats as shown by an increase in breakpoint in a progressive ratio schedule and this was shown to be dependent on intact dopamine signaling (Weinberg et al., 2011). The ghrelin-mediated increase in food-reinforced behavior was absent and breakpoints were stabilized following administration of the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA), which significantly depleted striatal dopamine. These findings demonstrate that activation of dopamine neurons is crucial for the ghrelin-mediated potentiation of food reward in the mesolimbic reward pathway.

We suggest the involvement of specific heterodimers of the GHS-R1a receptor with dopamine D_1 and D_2 receptors, which are involved in the rewarding aspects of food. These specific GHS-R1a dimers may mediate ghrelin's function in the dopaminergic

reward-signaling pathway within the VTA. Indeed, evidence for heterodimerization of the GHS-R1a and D_1 and D_2 receptor was recently demonstrated (Jiang et al., 2006; Kern et al., 2012) and is further discussed in this review in the next paragraphs.

POTENTIAL MECHANISM OF STRESS-INDUCED GHRELIN SIGNALING IN FOOD REWARD BEHAVIOR

It is clear that stress perception and response are modulated by food intake and diet (Oliver and Wardle, 1999; Gibson, 2006; Morrison, 2009; Dallman, 2010). The hormone ghrelin is poised to be involved in the association between stress, food intake and diet, considering the strong impact of ghrelin on both physiological processes. Interestingly, typical stress-induced metabolic changes in caloric intake, caloric efficiency and body-weight gain are absent in GHS-R1a null mice, suggesting that ghrelin is important for the metabolic shift required to deal with the energetic challenge of chronic stress (Patterson et al., 2010). Moreover, the stress-induced rise in circulating ghrelin may be behind the phenomenon of "comfort eating" observed in conditions of stress. This hypothesis is in line with the suggested function of ghrelin as an energy deficit signal, which may have evolved to favor consumption of calorie-dense palatable foods and to protect the storage of fat in times of energy insufficiency (Wells, 2009). Indeed, a continuous peripheral or central ghrelin infusion decreases fat utilization as a fuel substrate without significantly changing energy expenditure or locomotor activity, ultimately leading to increased adiposity (Tschop et al., 2000). This may be beneficial under conditions of stress as fat stores are maintained while energy from carbohydrates is available as a rapid fuel source, to maintain the defensive response (Kyrou and Tsigos, 2007). Thus, given that, both stress and ghrelin favor the use of carbohydrate substrates, which are rapidly oxidized, and the enhanced secretion of ghrelin following stress, it is likely that ghrelin contributes to the stress-induced metabolic switch that favors carbohydrate utilization and the accumulation of fat stores. Indeed, elevated ghrelin levels following chronic exposure to social stress increase caloric intake, preserve fat stores and stimulate body weight gain in mice, while under the same social stress paradigm these metabolic responses are attenuated or absent in GHS-R1a knockout mice and following pharmacological blockade of the GHS-R1a receptor (Patterson et al., 2013). Thus, enhanced ghrelin levels subsequently increase the hedonic and rewarding value of food, which further stimulates the intake of palatable and caloric dense "comfort" foods, which elicits activation of central reward pathways and increases dopamine signaling. This may mediate metabolic adaptations in response to the psychosocial stressors to meet the higher energetic demands and may act to reduce the detrimental effects of these stressors to promote survival and may ultimately protect against anxiety- and depression-like behaviors (Schellekens et al., 2012b).

However, chronic stress can lead to metabolic dysfunction and aberrant ghrelin signaling, which may ultimately lead to obesity and a downregulated reward signaling, which may be causal to the increased sensitivity for the development of psychiatric disorders including depression and anxiety. Thus, ghrelin and its receptor, the GHS-R1a receptor, likely represent key metabolic regulators required to cope with stress and to prevent excessive anxiety under conditions of chronic stress (Patterson et al., 2010). However, prolonged exposure to stress and stress-induced elevations in ghrelinergic signaling may unmask negative metabolic consequences, leading to long-lasting metabolic dysfunction and ultimately obesity as well as an associated sensitivity to the stress-induced psychiatric disorders of anxiety and depression (Patterson et al., 2010; Schellekens et al., 2012b).

In conclusion, it is clear that classical feeding peptides, including ghrelin, are strongly linked in the regulation of appetite and food intake, the rewarding and motivational drive to eat and the ability to cope with stress. The precise molecular mechanism linking ghrelin in appetite, reward and stress are not fully elucidated but it is clear that connections to DA neurons in the mesolimbic circuitry system are important. We hypothesize that direct interactions of the GHS-R1a receptor with other GPCRs, which have recently been described, are poised to play a key role in the association of these ghrelin-mediated physiological responses in reward signaling and stress. This review will further discuss the potential dynamic role for GHS-R1a receptor dimerization in the regulation of ghrelin-specific functions within stress response and food intake.

MODULATION OF G-PROTEIN COUPLED RECEPTORS VIA OLIGOMERIZATION

The GHS-R1a receptor belongs to the class A GPCRs, which are seven transmembrane domain proteins in the plasma membrane of cells. Conformational changes of the receptor are mediated via interactions of the GPCR with extracellular small-molecule ligands, which subsequently results in interaction and activation of hetero-trimeric intracellular G proteins, which further transduce the downstream signaling events necessary for the cell to respond to changes in the environment (Pierce et al., 2002). GPCRs represent the largest family of proteins (>800 members) in the human genome and are involved in nearly all physiological processes, mediating signal transductions across the cellular membrane. Many GPCRs have been implicated in the pathogenesis of human disease and thus, represent attractive targets for the development of drugs in the pharmaceutical industry. However, GPCR-targeting drugs are often associated with side effects due to non-specific activation of other GPCR and non-GPCR targets and novel pharmacotherapies with enhanced specificity are urgently sought after.

The concept of the monomeric existence and functioning of GPCRs, including the GHS-R1a receptor, is firmly established in literature and has been confirmed by several lines of evidence from recent studies (Whorton et al., 2007, 2008; Rasmussen et al., 2011; Damian et al., 2012). However, a large accumulating body of biochemical and biophysical work now supports the notion that GPCRs, including class A GPCRs, to which the GHS-R1a receptor also belongs, do not exclusively exist as monomeric entities, but extensively crosstalk with each other and form physiologically relevant oligomers (for review see George et al., 2002; Franco et al., 2008; Birdsall, 2010; Rozenfeld and Devi, 2010; Gonzalez-Maeso, 2011; Kamal and Jockers, 2011; Rozenfeld and Devi, 2011; Goddard and Watts, 2012; Herrick-Davis, 2013; Milligan, 2013; Ward et al., 2013). Indeed, many GPCRs have been recently found to function as oligomeric complexes, whereby receptors of the same or different families combine

to generate homo- or heterodimers or even higher-structure multimeric complexes (Gupta et al., 2010; Rivero-Muller et al., 2010; Teitler and Klein, 2012; Jastrzebska et al., 2013). Moreover, elaborate methods such as fluorescence imaging studies, including fluorescence recovery after photobleaching (FRAP) and total internal reflection fluorescence imaging (TIRF) of single molecules, as well as intracellular trafficking experiments, have been explored to confirm both monomeric GPCRs as well as stable and transient GPCR oligomers (Dorsch et al., 2009; Hern et al., 2010; Calebiro et al., 2013; Gavalas et al., 2013). Thus, GPCR oligomerization can now be considered as a fundamental process in receptor signaling. These GPCR oligomers exhibit unique pharmacological, biochemical and functional characteristics including specific signaling cascades, binding cooperativity, altered receptor internalization as well as changes in recycling properties (**Figure 1**) (Hebert and Bouvier, 1998; Terrillon and Bouvier, 2004; Springael et al., 2005; Urizar et al., 2005; Kent et al., 2007; Smith and Milligan, 2010; Urizar et al., 2011; de Poorter et al., 2013). Dimerization of GPCRs may explain the high constitutive activity of some GPCRs and heterodimerization may be obligatory for GPCR maturation and trafficking from the endoplasmic reticulum (ER) to the cell surface to ensure full receptor functionality [**Figure 1(1)**], as has been seen for the $GABA_B$ receptors, which is a class C GPCR (Kaupmann et al., 1998; Cryan and Kaupmann, 2005). The metabotropic $GABA_B$ receptor requires both $GABA_BR1$ and $GABA_BR2$ receptors for functional receptor expression on the cell membrane (White et al., 1998; Galvez et al., 2000, 2001; Duthey et al., 2002). Heterologous expression of the $GABA_BR1$ subunit without the $GABA_BR2$ leads to retention of the subunit in the ER, while the $GABA_BR2$ subunit alone is expressed on the membrane but not functional. Heterodimerization of both GABA subunits causes masking of a retention signal and functional receptor expression (Margeta-Mitrovic et al., 2000). Similarly, homodimerization of two β2-adrenergic receptor (β2-AR) subunits is critical for cell surface expression (Salahpour et al., 2004) and the α1δ-AR is only fully functional and rescued from the ER when heterodimerized with a α1β-ARs or β2-AR subunit (Uberti et al., 2005; Hague et al., 2006). Secondly, receptor dimerization may be dynamically regulated following ligand-mediated receptor activation [**Figure 1(2)**] and specific ligand binding may promote or inhibit dimerization (Horvat et al., 2001; Patel et al., 2002). Alternatively, dimerization may alter the ligand-binding properties of the receptor complex. In addition, heterodimerization can have consequences on allosteric potentiating or attenuating of downstream signaling [**Figure 1(3)**] as well as influencing positive or negative co-operativity of ligand binding (Terrillon and Bouvier, 2004). Moreover, heterodimerization may have the potential to change G-protein selectivity, alter G-protein coupling [**Figure 1(4)**] and alter subsequent downstream signaling and GPCR specificity (Rozenfeld and Devi, 2011). Interestingly, oligomerized receptor complexes have been shown to couple to multiple G-proteins, depending on their cellular environment, thereby conferring the ability to mediate different intracellular responses to the same ligand (Rashid et al., 2004). Heterodimerization may also promote co-internalization of two receptors after the stimulation of only one protomer. Alternatively, differential desensitization and β-arrestin recruitment may occur and the presence of a protomer that is resistant to agonist-promoted endocytosis, within a heterodimer, can inhibit the internalization of the complex [**Figure 1(5)**]. Heterodimer GPCR expression will likely not be the same in all tissue as not all GPCRs are equally expressed across various cell types. Therefore, tissue specific expression of GPCRs will directly regulate dimerization and may explain the tissue specificity of current GPCR-targeting drugs. In addition, heterodimerization of GPCRs will likely be different under pathological human conditions, including obesity and depression, which may be exploited in the development of novel ligands with increased specificity and selectivity.

However, current evidence for GPCR interactions are limited by the fact that the vast majority of studies to date have been performed in model systems only, which may explain why GPCR oligomerization is still met with a certain degree of controversy. In addition, during the interpretation of results it has proved to be very difficult to exclude cross-talk. Therefore, conclusions on GPCR oligomerization should be taken with caution. Although in-depth discussion of the limitations of previous studies is beyond the scope of this manuscript, several other reviews discussing this topic exists (Ferre et al., 2009; Birdsall, 2010; Teitler and Klein, 2012; Milligan, 2013).

While the concept of GPCR is certainly interesting, further validation at the physiological level is required to convincingly show that GPCRs indeed form physiologically relevant dimers. However, demonstrating dimerization in native tissue will be challenging as GPCRs are not always very highly expressed endogenously and many GPCRs, including the GHS-R1a receptor, are often associated with high constitutive activity (Holst et al., 2003; Petersen et al., 2009; Els et al., 2010). Previously, the acceptance of the functioning of GPCR dimers *in vivo* was dependent on co-expression of both protomers for functionality or the abolishment of heteromeric receptor function following genetic deletion of one protomer (Pin et al., 2007; Ferre et al., 2009; Teitler and Klein, 2012). Moreover, the complex and dynamic nature of GPCR dimerization needs to be considered, as dimerization has been suggested to be both reversible as well as ligand and cell type dependent (Smith and Milligan, 2010). Thus, additional evidence demonstrating dimers *in vivo* is warranted as well as more defined criteria. The advancement of the field will be complicated by the fact that many class A GPCRs, including the GHS-R1a receptor are most likely functional as both monomeric units as well as in heterodimers or oligomeric complexes. The next steps forward for the field will include the development and utilization of advanced techniques with the capability to extrapolate dimerization from overexpressing model systems to wild-type GPCRs expressed in native tissues using for example fluorescent ligands in FRET-based binding assays (Albizu et al., 2010; Cottet et al., 2012, 2013).

Nevertheless, the implications of enhanced diversity of GPCRs pharmacology following GPCR oligomerization fundamentally changes our current knowledge on the structure, activation and desensitization processes of GPCRs. Ultimately, GPCR dimerization is poised to have a dramatic impact on drug development and screening as it opens up new avenues for the development of potential novel therapeutics targeting GPCRs, including the

GHS-R1a receptor (George et al., 2002; Waldhoer et al., 2005; Panetta and Greenwood, 2008; Casado et al., 2009; Valant et al., 2009; Rozenfeld and Devi, 2010, 2011).

GHS-R1a RECEPTOR DIMERIZATION AND IMPLICATIONS IN STRESS AND REWARD SIGNALING

Evidence is accumulating demonstrating dimerization of the GHS-R1a receptor (Chan and Cheng, 2004; Holst et al., 2005; Jiang et al., 2006; Takahashi et al., 2006; Chu et al., 2007; Leung et al., 2007; Chow et al., 2008, 2012; Rediger et al., 2009, 2011; Kern et al., 2012; Park et al., 2012; Schellekens et al., 2013b). Dimerization of the GHS-R1a receptor into homo- and/or heterodimers has the potential to significantly alter downstream signaling. Indeed, evidence from mostly heterologous expression systems and also some native cells has demonstrated that the GHS-R1a receptor can both traffic and signal as higher-order oligomeric-complexes dimerized with additional GPCRs involved in appetite regulation, food reward and stress (Jiang et al., 2006; Rediger et al., 2009, 2011; Kern et al., 2012; Schellekens et al., 2013b). This promiscuous dimerization of the GHS-R1a receptor may function to fine-tune receptor mediated activity via differential downstream signaling and altered GHS-R1a receptor trafficking. This paragraph describes GHS-R1a receptor dimers known to date and discusses their potential functional significance. GHS-R1a dimerization is poised to impact on the regulation of food intake, hedonic appetite signaling and stress-induced food intake, in which the ghrelinergic system has been shown to play a major role.

HOMODIMERS AND HETERODIMERS OF THE GHS-R1a RECEPTOR

Recent research has identified that the GHS-R1a receptor also has the ability to form homodimers as well as to dimerize with other GPCRs, forming heterodimers (**Table 1**). In 2005, Holst and et al. were the first to propose a model in which the GHS-R1a receptor functions as a homodimer (Holst et al., 2005). In this study it was shown that coadministration of a non-endogenous agonist can act as a neutral (MK-677), positive (L-692,429), or negative (GHRP-6) modulator of the GHS-R1a receptor in the presence of the endogenous GHS-R1a agonist ghrelin. The hypothesized homodimeric model of the GHS-R1a receptor was supported by the potentiated response of ghrelin upon binding of growth hormone-releasing hormone (GHRH) to the GHS-R1a receptor (Casanueva et al., 2008). Co-administration of GHRH dose-dependently potentiated ghrelin-induced cellular calcium mobilization and inositol phosphate turnover via Gq-associated signal transduction without competing with ghrelin for binding but enhancing ghrelin's binding capacity. This positive binding co-operativity was suggested to occur following the interaction of GHRH on an allosteric binding site of the GHS-R1a receptor, acting as a co-agonist in presence of the endogenous ligand ghrelin. This allows for an increased affinity of ghrelin for the GHS-R1a receptor and the simultaneous binding of two ghrelin molecules, one to each GHS-R1a monomer subunit within the homodimer, while in the absence of ghrelin GHRH binds to the orthosteric binding site of GHS-R1a (Casanueva et al., 2008). However, it cannot be ruled out that a direct interaction between the GHS-R1a and GHRH receptor is responsible for the synergistic interaction, which is reinforced by the observed potentiation of GHRH-mediated cAMP production when the GHS-R1a receptor is co-expressed (Cunha and Mayo, 2002). The homodimeric GHS-R1a model was confirmed in human embryonic kidney cells (Hek), using bioluminescence resonance energy transfer (BRET) and co-immunoprecipitation (Leung et al., 2007). Further evidence for a direct physical interaction of two GHS-R1a subunits in a homodimeric complex or a heterodimer between the GHS-R1a and the GHRH receptor and the existence of such dimers *in vivo* is warranted.

In addition, evidence is accumulating suggesting that the GHS-R1a receptor has the ability to form a heterodimer with its non-signaling truncated splice variant, the GHS-R1b receptor. The GHS-R1b receptor is a splice variant with only 5 transmembrane domains, lacking the last 2 transmembrane domains typical for GPCRs, and is primarily localized in the ER (Chan and Cheng, 2004; Schellekens et al., 2010; Chow et al., 2012). This dimerization has been shown to attenuate the GHS-R1a/GHS-R1b receptor pair in the in the ER, which consequently reduces ghrelin responsiveness and suggests the GHS-R1b receptor to act as a dominant-negative mutant of the full-length GHS-R1a receptor (Chan and Cheng, 2004; Chu et al., 2007; Leung et al., 2007; Chow et al., 2012). The existence of the GHS-R1a/GHS-R1b heterodimer was reinforced using BRET and in co-immunoprecipitation experiments and a decreased cell surface expression and decreased constitutive GHS-R1a receptor activity were observed with increasing expression of GHS-R1b (Leung et al., 2007; Chow et al., 2012). Interestingly, dimerization of the mu-opioid with truncated splice variants was recently shown to increase total membrane receptor expression (Pasternak, 2013; Xu et al., 2013). In addition, a recent study using purified GHS-R monomers and dimers reconstituted into lipid discs revealed that the dominant negative effect of the truncated GHS-R1b receptor is exerted via a conformational restriction of the full-length GHS-R1a protein, which blocks subsequent G protein activation and β-arrestin recruitment. This clearly demonstrates heteromer-directed selectivity whereby the specific dimerization impacts on the functional and structural behavior of the ghrelin receptor (Mary et al., 2013).

Moreover, the GHS-R1a receptor was found to dimerize with members of the prostanoid receptor family, which are involved in modulating vascular activity and inflammatory responses (Chow et al., 2008). Heterodimers of the GHS-R1a receptor with the vasodilator prostacyclin (IP) receptor, the vasoconstrictor prostaglandin E2 receptor subtype EP3-I (EP3-1) and the thromboxane A2 (TPα) receptor were demonstrated using co-immunoprecipitations and BRET following transient co-expression in Hek cells. Decreased GHS-R1a receptor expression, increase intracellular GHS-R1a receptor localization and attenuated constitutive GHS-R1a receptor activation, were observed upon co-expression of prostanoid receptors, suggesting a dynamic regulation of GHS-R1a receptor activity under conditions of increased prostanoid receptors expression, including vascular inflammation and in atherosclerotic plaques.

Table 1 | Homo- and heterodimerization of the GHS-R1a receptor.

Dimer	Physical interaction *in vitro*	Cell lines	Physical interaction *in/ex vivo*	Functional interaction *in vivo*	References
GHS-R1a/GHS-R1a	Binding and signal transduction assays demonstrate allosteric modulation of ghrelin signaling: calcium mobilization, inositol phosphate turnover, CRE and SRE transcription assay, β-arrestin mobilization, BRET, co-IP	Hek, COS-7	nd	nd	Holst et al., 2005; Leung et al., 2007
GHS-R1a/GHS-R1b	Subcellular co-localization using immunocytochemistry; BRET; ghrelin binding assay; cell surface expression ELISA; receptor downstream signaling; co-IP	Hek, CHO	nd	nd	Chan and Cheng, 2004; Chu et al., 2007; Leung et al., 2007; Chow et al., 2012; Mary et al., 2013
GHS-R1a/EP3-1	Co-IP; BRET	Hek	nd	nd	Chow et al., 2008
GHS-R1a/IP	Co-IP; BRET	Hek	nd	nd	Chow et al., 2008
GHS-R1a/TPα	Co-IP; BRET	Hek	nd	nd	Chow et al., 2008
GHS-R1a/SST5	Glucose-stimulated insulin secretion assay; tr-FRET; BRET; downstream Gq (calcium assay) and Gs (cAMP) signaling	Hek, INS-1SJ	nd	nd	Park et al., 2012
GHS-R1b/NTS_1	Co-IP; Co-localization and receptor trafficking	Cos-7, LC319	nd	nd	Takahashi et al., 2006
GHS-R1a/MC_3	FRET; ELISA; Co-localization; binding assay; receptor trafficking and downstream Gq (NFAT-luciferase reporter or calcium assay) and Gs (cAMP) signaling	Hek, COS-7	Co-expression of MC_3 with lacZ-immunoreactive cells, representing GHS-R1a in Arc of GHS-R1a knock-out mice	nd	Rediger et al., 2009, 2011; Schellekens et al., 2013b
GHS-R1a/D_1	Co-IP; Co-localization; BRET; receptor trafficking and downstream Gq (calcium assay) and Gs (cAMP) signaling	Hek, SK-N-SH	Co-expression of D_1 with GHS-R1a in the VTA of GHSR-IRES-tauGFP knock-in homozygous mice	nd	Jiang et al., 2006; Schellekens et al., 2013b
GHS-R1a/D_2	Co-localization; downstream signaling Gq and Gβγ subunit of Gi (calcium mobilization assay and imaging); tr-FRET	Hek, SH-SY5Y, Primary hypothalamic neurons	Co-expression of D_2/GHS-R1a in hippocampus, striatum and hypothalamus of GHSR-IRES-tauGFP knock-in homozygous mice; Tr-FRET of striatum and hypothalamic tissue in wt and $GHSR^{-/-}$ mice	Allosteric function for GHS-R1a on D_2-mediated inhibition of food intake. D_2 agonist cabergoline reduces food intake in mice, which is absent when GHS-R1a is pharmacologically blocked or knocked out in GHS-$R1a^{-/-}$ mice	Kern et al., 2012
GHS-R1a/5-HT_{2C}	Co-localization; receptor trafficking and downstream Gq (calcium assay) signaling	Hek	nd	nd	Schellekens et al., 2013b

Abbreviations: BRET, bioluminescence resonance energy transfer; co-IP, co-immunoprecipitation; CRE, cAMP-responsive element; ELISA, Enzyme-linked immunosorbent assay; $D_{1/2}$, dopamine receptor 1/2; GHS-R, growth hormone secretagogue receptor; IP, prostaglandin (prostacyclin) receptor; EP, prostanoid receptor; MC_3, melanocortin receptor 3; nd, not done; NFAT, nuclear factor of activated T cells; NTS1, neurotensin 1 receptor; SRE, serum-responsive element; TPα, thromboxane A2; tr-FRET, time-resolved fluorescence resonance energy transfer.

Furthermore, the GHS-R1b receptor was shown to physically interact with the neurotensin receptor 1 (NTS1) and this GHS-R1b/NTS1 heterodimer was suggested to play a role in the autocrine growth-promoting pathway of non–small cell lung cancers (Takahashi et al., 2006).

Finally, a recent study indicates that the GHS-R1a receptor also interacts with the somatostatin receptor family, specifically with the somatostatin receptor-5 (SST5) (Park et al., 2012). Constitutive formation of a GHS-R1a/SST5 heterodimer was shown using time-resolved FRET and BRET assays, in which endogenous ghrelin rather than SST, suppressed glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells. This was shown to involve a noncanonical ghrelin receptor (GHS-R1a)–G-protein coupling to Gαi/o and subsequent increased cAMP production, instead of coupling to the Gαq11 subunit. Moreover, the formation of GHS-R1a/SST5 heterodimer demonstrated to be dependent on a high ratio of ghrelin to SST, suggesting a dependence on energy balance, with enhanced dimer formation following fasting-induced peak ghrelin levels. This model predicts that under conditions of low energy balance the formation of a physiologically relevant GHS-R1a/SST5 heterodimer establishes an inhibitory tone on β-cells via ghrelin signaling. This may potentially explain the differential regulation of islet function by ghrelin and SST and reinforces the potential dynamic nature of GPCR dimerization.

A GHS-R1a/MC_3 RECEPTOR HETERODIMER IN APPETITE SIGNALING

Evidence for other GHS-R1a heterodimerization pairs is accumulating and to date heterodimerization of the GHS-R1a receptor with several other hypothalamic GPCRs involved in appetite signaling has been demonstrated (Rediger et al., 2009, 2011; Schellekens et al., 2013b). Evidence for a physical interaction between the MC_3 and GHS-R1a receptor was demonstrated, using enzyme-linked immuno sorbent assay (ELISA) and fluorescence resonance energy transfer (FRET) approaches in Hek cells (Rediger et al., 2009). A more recent study, confirmed dimerization of the GHS-R1a receptor in the ARC of the hypothalamus with the MC_3 receptor (Rediger et al., 2011), which is an important downstream signaling receptor in the homeostatic control of food intake and energy balance (Cone et al., 1996; Kishi et al., 2003; Adan et al., 2006; Yang, 2011). The study demonstrated a mutual signaling interference upon receptor dimerization. Ghrelin-induced Gq-mediated GHS-R1a receptor signaling was significantly reduced upon co-expression of the MC3 receptor in COS-7 cells, while MC_3-mediated cAMP signaling upon administration of alpha-MSH was enhanced in Hek cells co-expressing both receptors (Rediger et al., 2011). In addition, a strong attenuation of GHS-R1a mediated downstream signaling was shown upon co-expression of both the MC_3 and the GHS-R1a receptors in Hek cells, which has been suggested to be most likely due to an attenuation of the dimer pair in the cytosol of Hek cells (Rediger et al., 2009; Schellekens et al., 2013b). However, no decrease of membrane receptor expression was observed when both GHS-R1a and MC_3 were expressed in COS-7 cells, as investigated by ELISA and binding studies, which may indicate cell-specific effects (Rediger et al., 2011).

DIMERIZATION OF THE GHS-R1a RECEPTOR WITH DOPAMINE D_1 AND D_2 RECEPTORS

Considering the involvement of ghrelin signaling in other physiological pathways besides appetite, evidence of heterodimers of the GHS-R1a with extra-hypothalamic GPCRs involved in hedonic and rewarding aspects of food intake may be able to explain crosstalk between the ghrelinergic and mesolimbic dopaminergic systems. In line with this concept, is the recent discovery that the GHS-R1a receptor can form dimers with dopamine D_1 and D_2 receptors (Jiang et al., 2006; Kern et al., 2012). The formation of GHS-R1a/D_1 or GHS-R1a/D_2 receptor heterodimers are poised to have important functional consequences for the role of ghrelin in the regulation of rewarding and motivational eating behavior. The GHS-R1a receptor was first reported to be able to dimerize with the dopamine D_1 receptor (Jiang et al., 2006). In this study, Hek cells expressing both the GHS-R1a and the D_1 receptor displayed a ghrelin-mediated potentiation of dopamine induced c-AMP accumulation in a GHS-R dependent manner. Additional evidence for a dimerization of the GHS-R1a receptor with the D_1 receptor was recently demonstrated by the attenuation of GHS-R1a-mediated calcium signaling in Hek cells coexpressing both receptors (Schellekens et al., 2013b). Together, this may suggest a dimer-induced switch in GHS-R1a receptor G-protein coupling from Gq to Gs mediated signaling, which has been previously suggested for neuronal GHS-R1a receptors expressed in NPY cells of the ARC (Kohno et al., 2003). In addition, co-internalization of the GHS-R1a/D_1 receptor pair was also demonstrated following agonist treatment, further supporting the existence of a GHS-R1/D_1 heterodimer (Schellekens et al., 2013b).

Recently, the D_2 receptor has also been found to dimerize with the GHS-R1a receptor in hypothalamic neurons and to alter canonical D_2 signal transduction resulting in dopamine-induced calcium mobilization (Kern et al., 2012). Coexpression of the D_2 and the GHS-R1a receptor in mouse neuronal hypothalamic neurons was shown as well as in hippocampal and striatal neurons. In addition, a D_2-dependent increase in calcium was observed in SH-SY5Y neuroblastoma cells and Hek cells overexpressing both receptors as well as in primary hypothalamic cells using calcium mobilization assay or calcium imaging to monitor calcium influxes in live cells. A significant increase in intracellular calcium was observed following treatment with dopamine or a selective D_2 agonist, quinpirole, only in cells co-expressing the Gi/o-coupled D_2 receptor and the Gq-coupled GHS-R1a receptor. Moreover, the dopamine-induced calcium mobilization was demonstrated to be independent of GHS-R1a-Gq mediated signaling or basal GHS-R1a receptor activity, as Gq siRNA was unable to inhibit dopamine-induced calcium release in Hek cells, but the involvement of Gβγ subunits in the calcium mobilization from intracellular stores and Gβγ-mediated activation of PLC was shown. Finally, the functional interaction between the D_2 and GHS-R1a receptor was demonstrated *in vitro* in Hek cells as well as *in vivo* in native hypothalamic mouse tissue using time resolved fluorescence energy transfer (tr-FRET) and confocal FRET. This study by Kern and colleagues is one of the very few studies to extrapolate the evidence from overexpressing model system to naïve tissue with endogenously expressed GHS-R1a

receptor. Most importantly, the GHS-R1a/D_2 heterodimer was shown to allosterically modify D_2-mediated calcium mobilization, as the modification of D_2-mediated signaling was observed in the absence of the endogenous GHS-R1a ligand ghrelin, but was blocked by both D_2 and GHS-R1a antagonism. This highlights a potential function of the GHS-R1a receptor expressed in areas of the brain without ghrelin immunoreactivity and which are considered inaccessible to peripherally produced ghrelin. Finally, the functional relevance of the GHS-R1a/D_2 dimer was investigated and it was demonstrated that D_2/GHS-R1a pairing attenuates food intake (Kern et al., 2012). Evidence for the allosteric function for GHS-R1a on D_2-mediated inhibition of food intake was shown using the D_2 agonist cabergoline, which reduced food intake in mice. The cabergoline-induced anorexia did not require ghrelin but was dependent on GHS-R1a signaling and the GHS-R1a-D_2 interaction as it was blocked when the GHS-R1a receptor was pharmacologically inhibited or knocked out in GHS-R1a$^{-/-}$ mice. Ghrelin has previously been suggested to interact with dopamine and to modulate appetitive behavior within the dopaminergic reward circuitry (Abizaid et al., 2006; Abizaid, 2009; Weinberg et al., 2011). Indeed, the ghrelinergic system has been shown to alter the rewarding value of foods and to elicit a preference for palatable foods rich in sugar or fats via modulation of dopamine release (Egecioglu et al., 2010). This may suggest a role for GHS-R1a receptor dimerization in the regulation of rewarding qualities of food, independent of homeostatic regulation of food intake, and the function of GHS-R1a heterodimers in the hedonic appetite signaling warrants further investigation.

EVIDENCE FOR A HETERODIMER BETWEEN THE GHS-R1a RECEPTOR AND THE 5-HT$_{2C}$ RECEPTOR

Finally, in a recent study evidence was presented for a novel heterodimer between the GHS-R1a receptor and the 5-HT$_{2C}$ receptor (Schellekens et al., 2013b). Here it was shown that GHS-R1a-mediated calcium mobilization following ghrelin or MK0677 treatment, was attenuated following interaction with the fully active unedited form of the 5-HT$_{2C}$ receptor but not following co-expression of a partially edited isoform of the 5-HT$_{2C}$ receptor. Editing of the 5-HT$_{2C}$ receptor has been demonstrated to lead to a decreased receptor functioning (Burns et al., 1997; Niswender et al., 1999; Berg et al., 2008; Olaghere da Silva et al., 2010; Schellekens et al., 2012a). This may suggest the possibility that alteration of the 5-HT$_{2C}$ receptor editing profile can equally impact on GHS-R1a receptor signaling *in vivo*. The attenuated GHS-R1a receptor signaling was completely restored following pharmacological blockade of the 5-HT$_{2C}$ receptor. Moreover, ligand-mediated co-internalization of the GHS-R1a/5-HT$_{2C}$ receptor pair was demonstrated in Hek cells generated to express the receptor pair. Further evidence for a GHS-R1a/5-HT$_{2C}$ receptor dimer was shown when a potent specific inverse agonist of the GHS-R1a receptor, [D-Arg1, D-Phe5,D-Trp7,9, Leu11]-substance P, was able to increase signaling in heterologous cells co-expressing the GHS-R1a/5-HT$_{2C}$ receptor dimer following serotonin (5-HT, 5-hydroxytryptamine) treatment, while no potentiation of Gq-mediated calcium mobilization was observed in cells solely expressing the 5-HT$_{2C}$ receptor. The inverse GHS-R1a agonist suppresses ligand-independent basal activity of the GHS-R1a receptor (Holst et al., 2003) and is known to increase GHS-R1a receptor membrane expression (Liu et al., 2007). This data demonstrates a co-recruitment of the 5-HT$_{2C}$ receptor with the GHS-R1a receptor to the cellular membrane, upon treatment with the inverse GHS-R1a receptor agonist, indicating a direct physical interaction. Together, this suggests a potential novel mechanism of 5-HT$_{2C}$ mediated attenuation of the GHS-R1a receptor. Both the GHS-R1a receptor and the 5-HT$_{2C}$ receptor are involved in feeding behavior. Activation of the 5-HT$_{2C}$ receptor signaling mediates hypophagia (Lam et al., 2008). Interestingly the 5-HT$_{2C}$ receptor also mediates anxiety like behavior (Heisler et al., 2007b). It is therefore tempting to speculate that GHS-R1a/5-HT$_{2C}$ receptor heterodimerization may play a role in homeostatic appetite signaling and in stress-induced food intake. A recent study demonstrated an attenuation of ghrelin's orexigenic effect following direct PVN administration of 5-HT in rats, which supports a potential significant role for the GHS-R1a/5-HT$_{2C}$ receptor dimer pair in appetite regulation *in vivo* (Currie et al., 2010). Further investigations into the extent and functional relevance of GHS-R1a/5-HT$_{2C}$ dimerization *in vivo* are now warranted.

CONCLUSION

Obesity is a growing concern increasing in prevalence worldwide. In addition, humans are increasingly faced with excessive psychological stress in modern day society, which appears to have synergistic effects with the obesity epidemic. The orexigenic gastric-derived hormone ghrelin plays a key role in the homeostatic control of appetite, rewarding and motivational aspects of food intake as well as stress-induced food reward behaviors, linking eating behavior with both stress and hedonic signaling (for review see Chuang and Zigman, 2010; Diz-Chaves, 2011; Perello and Zigman, 2012; Schellekens et al., 2012b). Ghrelin signals via the peripheral and central expressed GHS-R1a receptor, which has recently shown to form heterodimers with several additional GPCRs involved in appetite signaling and reward, including the truncated GHS-R1b receptor, the MC_3 receptor, D_1 and D_2 receptors, and more recently, the 5-HT$_{2C}$ receptor (Jiang et al., 2006; Rediger et al., 2009, 2011; Kern et al., 2012; Schellekens et al., 2013b). Heterodimerization of the GHS-R1a receptor may function to fine-tune GHS-R1a mediated signaling and could potentially explain crosstalk between ghrelin and monoaminergic neurotransmission in the brain. Heterodimerization may regulate GHS-R1a receptor function via stabilization of specific receptor conformations, altering preferential G-protein coupling and lead to heteromer-specific signal transduction and receptor trafficking.

Overall, GHS-R1a heterodimerization may modulate specific signaling pathways and GHS-R1a receptor functionalities or serve as an allosteric mechanism able to regulate signaling pathways of the other receptor, independently of ghrelin binding. The latter has been demonstrated recently for the GHS-R1a/D_2 heterodimer by the observation that GHS-R1a receptor expression alone was able to allosterically mediate D_2 receptor signaling (Kern et al., 2012). In addition, this study also demonstrated that dopamine-mediated anorexia was inhibited following the pharmacological

blockade of the GHS-R1a receptor. Interestingly, D_2 receptor mutations have demonstrated to attenuate dopamine signaling and to be associated with human obesity (Tataranni et al., 2001). Moreover, since central ghrelin signaling has been shown to be involved in the control of reward seeking behaviors for food, alcohol, and drugs of abuse by modulating mesolimbic dopaminergic reward signaling, the existence of GHS-R1a/D_2 heterodimers would offer a potential mechanism to explain the effects of ghrelin on reward. Moreover, dopamine signaling has been shown to be crucial for ghrelin-mediated increase in food reward. Thus, the GHS-R1a/D_2 dimer would represent a new molecular target and rational for the development of ghrelin-based therapeutic interventions in conditions associated with abnormal reward-seeking behavior. Moreover, circulating ghrelin levels are enhanced following acute stress and interactions of the GHS-R1a receptor with other GPCRs mediating the rewarding and motivational drive to eat, such as the D_1 and D_2 receptor, may activate pathways necessary to cope with stress.

The stress-induced increase in ghrelin has been suggested to be able to reduce anxiety under conditions of acute stress following the stimulation of the HPA and the neurobiology of stress and appetite regulation overlap significantly. The 5-HT_{2C} receptor is expressed in the ARC of the hypothalamus mediating hypophagia (Lam et al., 2008) as well as on CRF neurons in the PVN and similar to ghrelin, 5-HT_{2C} receptor activation here, mediates secretion of CRF (Heisler et al., 2007a). Moreover, 5-HT_{2C} receptor knockdown decreases anxiety-like behavior (Heisler et al., 2007b), while mice with genetic ablation of ghrelin exhibit a more anxious phenotype (Spencer et al., 2012). Together this may suggest a functional role for the recently identified GHS-R1a/5-HT_{2C} dimer in HPA axis activation, which warrants further investigations.

Targeting GHS-R1a receptor dimers within the metabolic regulation of food intake and food reward is poised to impact on the dopamine-related reward systems and stress response and therefore not only provides exiting opportunities for future pharmacotherapies in the treatment of metabolic and eating disorders, but also in psychiatric disorders with dysregulated DA signaling. The recent findings demonstrating heterodimerization of the GHS-R1a under certain conditions and reinforces the concept that GPCR signaling is most likely a dynamic process, which is dependent on physiological context. Understanding the contributions of the interactions between the serotinergic and ghrelinergic system on the dopaminergic output following stress and maladaptive food intake and the role of the recently identified GHS-R1a heterodimer complexes herein, will increase our knowledge of the pathology of obesity and anxiety and its bidirectional relationship. While the GHS-R1a receptor is expressed ubiquitously in the brain at multiple sites involved in both food intake behavior and stress, the MC_3, 5-HT_{2C}, D_1 and D_2 receptors have a more localized expression. For example the MC_3 is expressed in the hypothalamus only, which means that the GHS-R1a/MC_3 dimer potentially only has an impact on homeostatic regulation of appetite. The exact central locations of GHS-R1a complex formation remain to be determined.

Noteworthy, evidence supporting a physiologically relevant interaction and physiological role for GHS-R1a heterodimers is limited. It has been notoriously difficult to establish the existence of the vast majority of GPCR dimers *in vivo* due to the experimental challenge of differentiating a direct physical interaction from receptor crosstalk and this remain somewhat controversial. Nevertheless, the conceptually important premise of promiscuous GHS-R1a heterodimerization confers unique pharmacological and functional properties to the receptor, significantly enhancing the pharmacological diversity and with it the potential for novel therapies. Additional research is warranted to further investigate the physiological relevance of GHS-R1a dimers in metabolic eating disorders, such as obesity, and under conditions of stress. Further studies are required to determine whether the dimerization in the *in vitro* biochemical assays translates to the functionally relevant dimers *in vivo*. Nevertheless, the oligomerization characteristic of GPCRs, including the GHS-R1a receptor, creates a broader regulatory complexity and has therapeutically important implication as heterodimers provide novel pharmacological targets and thus may be exploited to create more specific therapeutic drugs (Prinster et al., 2005). Thus, although more evidence and functional relevance for GPCR dimerization *in vivo* as well as methods to deal with the most likely dynamic nature of physical receptor interaction is warranted, the achievements of the last few years on GPCR dimerization provide the foundation of what promises to be very exciting times for GPCR-targeted molecular pharmacology. In addition, the field of heterodimer specific pharmacology and drug discovery is set to change significantly with these new findings.

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REFERENCES

Abizaid, A. (2009). Ghrelin and dopamine: new insights on the peripheral regulation of appetite. *J. Neuroendocrinol.* 21, 787–793. doi: 10.1111/j.1365-2826.2009.01896.x

Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., et al. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J. Clin. Invest.* 116, 3229–3239. doi: 10.1172/JCI29867

Adan, R. A., Tiesjema, B., Hillebrand, J. J., la Fleur, S. E., Kas, M. J., and de Krom, M. (2006). The MC4 receptor and control of appetite. *Br. J. Pharmacol.* 149, 815–827. doi: 10.1038/sj.bjp.0706929

Albizu, L., Cottet, M., Kralikova, M., Stoev, S., Seyer, R., Brabet, I., et al. (2010). Time-resolved FRET between GPCR ligands reveals oligomers in native tissues. *Nat. Chem. Biol.* 6, 587–594. doi: 10.1038/nchembio.396

American Psychiatric Association. (1994). *Diagnostic and Statistical Manual of Mental Disorders, 4th Edn.* Washington, DC: American Psychiatric Press.

Andrews, Z. B. (2011). Central mechanisms involved in the orexigenic actions of ghrelin. *Peptides* 32, 2248–2255. doi: 10.1016/j.peptides.2011.05.014

Anisman, H., and Zacharko, R. M. (1990). Multiple neurochemical and behavioral consequences of stressors: implications for depression.

Pharmacol. Ther. 46, 119–136. doi: 10.1016/0163-7258(90)90039-5

Arnold, M., Mura, A., Langhans, W., and Geary, N. (2006). Gut vagal afferents are not necessary for the eating-stimulatory effect of intraperitoneally injected ghrelin in the rat. *J. Neurosci.* 26, 11052–11060. doi: 10.1523/JNEUROSCI.2606-06.2006

Arvat, E., Maccario, M., Di Vito, L., Broglio, F., Benso, A., Gottero, C., et al. (2001). Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. *J. Clin. Endocrinol. Metab.* 86, 1169–1174. doi: 10.1210/jc.86.3.1169

Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Fujimiya, M., et al. (2001a). A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. *Neuroendocrinology* 74, 143–147. doi: 10.1159/000054680

Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Ueno, N., et al. (2001b). Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 120, 337–345. doi: 10.1053/gast.2001.22158

Banks, W. A., Burney, B. O., and Robinson, S. M. (2008). Effects of triglycerides, obesity, and starvation on ghrelin transport across the blood-brain barrier. *Peptides* 29, 2061–2065. doi: 10.1016/j.peptides.2008.07.001

Banks, W. A., Tschop, M., Robinson, S. M., and Heiman, M. L. (2002). Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J. Pharmacol. Exp. Ther.* 302, 822–827. doi: 10.1124/jpet.102.034827

Barim, A. O., Aydin, S., Colak, R., Dag, E., Deniz, O., and Sahin, I. (2009). Ghrelin, paraoxonase and arylesterase levels in depressive patients before and after citalopram treatment. *Clin. Biochem.* 42, 1076–1081. doi: 10.1016/j.clinbiochem.2009.02.020

Berg, K. A., Clarke, W. P., Cunningham, K. A., and Spampinato, U. (2008). Fine-tuning serotonin2c receptor function in the brain: molecular and functional implications. *Neuropharmacology* 55, 969–976. doi: 10.1016/j.neuropharm.2008.06.014

Berthoud, H. R. (2006). Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. *Obesity (Silver Spring)* 14(Suppl. 5), 197S–200S. doi: 10.1038/oby.2006.308

Birdsall, N. J. (2010). Class A GPCR heterodimers: evidence from binding studies. *Trends Pharmacol. Sci.* 31, 499–508. doi: 10.1016/j.tips.2010.08.003

Black, P. H. (2006). The inflammatory consequences of psychologic stress: relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. *Med. Hypotheses* 67, 879–891. doi: 10.1016/j.mehy.2006.04.008

Block, J. P., He, Y., Zaslavsky, A. M., Ding, L., and Ayanian, J. Z. (2009). Psychosocial stress and change in weight among US adults. *Am. J. Epidemiol.* 170, 181–192. doi: 10.1093/aje/kwp104

Bowers, M. E., Choi, D. C., and Ressler, K. J. (2012). Neuropeptide regulation of fear and anxiety: implications of cholecystokinin, endogenous opioids, and neuropeptide Y. *Physiol. Behav.* 107, 699–710. doi: 10.1016/j.physbeh.2012.03.004

Bremner, J. D., Krystal, J. H., Southwick, S. M., and Charney, D. S. (1996). Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. *Synapse* 23, 28–38.

Brunetti, L., Recinella, L., Orlando, G., Michelotto, B., Di Nisio, C., and Vacca, M. (2002). Effects of ghrelin and amylin on dopamine, norepinephrine and serotonin release in the hypothalamus. *Eur. J. Pharmacol.* 454, 189–192. doi: 10.1016/S0014-2999(02)02552-9

Burns, C. M., Chu, H., Rueter, S. M., Hutchinson, L. K., Canton, H., Sanders-Bush, E., et al. (1997). Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature* 387, 303–308. doi: 10.1038/387303a0

Cabral, A., Suescun, O., Zigman, J. M., and Perello, M. (2012). Ghrelin indirectly activates hypophysiotropic CRF neurons in rodents. *PLoS ONE* 7:e31462. doi: 10.1371/journal.pone.0031462

Calebiro, D., Rieken, F., Wagner, J., Sungkaworn, T., Zabel, U., Borzi, A., et al. (2013). Single-molecule analysis of fluorescently labeled G-protein-coupled receptors reveals complexes with distinct dynamics and organization. *Proc. Natl. Acad. Sci. U.S.A.* 110, 743–748. doi: 10.1073/pnas.1205798110

Carlini, V. P., Monzon, M. E., Varas, M. M., Cragnolini, A. B., Schioth, H. B., Scimonelli, T., et al. (2002). Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochem. Biophys. Res. Commun.* 299, 739–743. doi: 10.1016/S0006-291X(02)02740-7

Carlini, V. P., Varas, M. M., Cragnolini, A. B., Schioth, H. B., Scimonelli, T. N., and de Barioglio, S. R. (2004). Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. *Biochem. Biophys. Res. Commun.* 313, 635–641. doi: 10.1016/j.bbrc.2003.11.150

Casado, V., Cortes, A., Mallol, J., Perez-Capote, K., Ferre, S., Lluis, C., et al. (2009). GPCR homomers and heteromers: a better choice as targets for drug development than GPCR monomers? *Pharmacol. Ther.* 124, 248–257. doi: 10.1016/j.pharmthera.2009.07.005

Casanueva, F. F., Camina, J. P., Carreira, M. C., Pazos, Y., Varga, J. L., and Schally, A. V. (2008). Growth hormone-releasing hormone as an agonist of the ghrelin receptor GHS-R1a. *Proc. Natl. Acad. Sci. U.S.A.* 105, 20452–20457. doi: 10.1073/pnas.0811680106

Chan, C. B., and Cheng, C. H. (2004). Identification and functional characterization of two alternatively spliced growth hormone secretagogue receptor transcripts from the pituitary of black seabream *Acanthopagrus schlegeli*. *Mol. Cell. Endocrinol.* 214, 81–95. doi: 10.1016/j.mce.2003.11.020

Cheng, A. Y., and Leiter, L. A. (2006). Metabolic syndrome under fire: weighing in on the truth. *Can. J. Cardiol.* 22, 379–382. doi: 10.1016/S0828-282X(06)70921-7

Chollet, C., Meyer, K., and Beck-Sickinger, A. G. (2009). Ghrelin-a novel generation of anti-obesity drug: design, pharmacomodulation and biological activity of ghrelin analogues. *J. Pept. Sci.* 15, 711–730. doi: 10.1002/psc.1177

Chow, K. B., Leung, P. K., Cheng, C. H., Cheung, W. T., and Wise, H. (2008). The constitutive activity of ghrelin receptors is decreased by co-expression with vasoactive prostanoid receptors when overexpressed in human embryonic kidney 293 cells. *Int. J. Biochem. Cell Biol.* 40, 2627–2637. doi: 10.1016/j.biocel.2008.05.008

Chow, K. B., Sun, J., Chu, K. M., Tai Cheung, W., Cheng, C. H., and Wise, H. (2012). The truncated ghrelin receptor polypeptide (GHS-R1b) is localized in the endoplasmic reticulum where it forms heterodimers with ghrelin receptors (GHS-R1a) to attenuate their cell surface expression. *Mol. Cell. Endocrinol.* 348, 247–254. doi: 10.1016/j.mce.2011.08.034

Chu, K. M., Chow, K. B., Leung, P. K., Lau, P. N., Chan, C. B., and Cheng, C. H. (2007). Overexpression of the truncated ghrelin receptor polypeptide attenuates the constitutive activation of phosphatidylinositol-specific phospholipase C by ghrelin receptors but has no effect on ghrelin-stimulated extracellular signal-regulated kinase 1/2 activity. *Int. J. Biochem. Cell Biol.* 39, 752–764. doi: 10.1016/j.biocel.2006.11.007

Chuang, J. C., Perello, M., Sakata, I., Osborne-Lawrence, S., Savitt, J. M., Lutter, M., et al. (2011). Ghrelin mediates stress-induced food-reward behavior in mice. *J. Clin. Invest.* 121, 2684–2692. doi: 10.1172/JCI57660

Chuang, J. C., and Zigman, J. M. (2010). Ghrelin's roles in stress, mood, and anxiety regulation. *Int. J. Pept.* 2010:460549. doi: 10.1155/2010/460549

Cone, R. D., Lu, D., Koppula, S., Vage, D. I., Klungland, H., Boston, B., et al. (1996). The melanocortin receptors: agonists, antagonists, and the hormonal control of pigmentation. *Recent Prog. Horm. Res.* 51, 287–317. discussion: 318.

Cunha, S. R. and Mayo, K. E. (2002). Ghrelin and growth hormone (GH) secretagogues potentiate GH-releasing hormone (GHRH)-induced cyclic adenosine 3′,5′-monophosphate production in cells expressing transfected GHRH and GH secretagogue receptors. *Endocrinology* 143, 4570–4582. doi: 10.1210/en.2002-220670

Cordoba-Chacon, J., Gahete, M. D., Pozo-Salas, A. I., Martinez-Fuentes, A. J., de Lecea, L., Gracia-Navarro, F., et al. (2011). Cortistatin is not a somatostatin analogue but stimulates prolactin release and inhibits GH and ACTH in a gender-dependent fashion: potential role of ghrelin. *Endocrinology* 152, 4800–4812. doi: 10.1210/en.2011-1542

Cottet, M., Faklaris, O., Falco, A., Trinquet, E., Pin, J. P., Mouillac, B., et al. (2013). Fluorescent ligands to investigate GPCR binding properties and oligomerization. *Biochem. Soc. Trans.* 41, 148–153. doi: 10.1042/BST20120237

Cottet, M., Faklaris, O., Maurel, D., Scholler, P., Doumazane, E., Trinquet, E., et al. (2012). BRET and Time-resolved FRET strategy

to study GPCR oligomerization: from cell lines toward native tissues. *Front. Endocrinol. (Lausanne)* 3:92. doi: 10.3389/fendo.2012.00092

Cryan, J. F., and Kaupmann, K. (2005). Don't worry 'B' happy!: a role for GABA(B) receptors in anxiety and depression. *Trends Pharmacol. Sci.* 26, 36–43. doi: 10.1016/j.tips.2004.11.004

Currie, P. J., John, C. S., Nicholson, M. L., Chapman, C. D., and Loera, K. E. (2010). Hypothalamic paraventricular 5-hydroxytryptamine inhibits the effects of ghrelin on eating and energy substrate utilization. *Pharmacol. Biochem. Behav.* 97, 152–155. doi: 10.1016/j.pbb.2010.05.027

Dallman, M. F. (2010). Stress-induced obesity and the emotional nervous system. *Trends Endocrinol. Metab.* 21, 159–165. doi: 10.1016/j.tem.2009.10.004

Dallman, M. F., Pecoraro, N., Akana, S. F., La Fleur, S. E., Gomez, F., Houshyar, H., et al. (2003). Chronic stress and obesity: a new view of comfort food. *Proc. Natl. Acad. Sci. U.S.A.* 100, 11696–11701. doi: 10.1073/pnas.1934666100

Dallman, M. F., Pecoraro, N. C., La Fleur, S. E., Warne, J. P., Ginsberg, A. B., Akana, S. F., et al. (2006). Glucocorticoids, chronic stress, and obesity. *Prog. Brain Res.* 153, 75–105. doi: 10.1016/S0079-6123(06)53004-3

Damian, M., Marie, J., Leyris, J. P., Fehrentz, J. A., Verdie, P., Martinez, J., et al. (2012). High constitutive activity is an intrinsic feature of ghrelin receptor protein: a study with a functional monomeric GHS-R1a receptor reconstituted in lipid discs. *J. Biol. Chem.* 287, 3630–3641. doi: 10.1074/jbc.M111.288324

Date, Y., Murakami, N., Toshinai, K., Matsukura, S., Niijima, A., Matsuo, H., et al. (2002). The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 123, 1120–1128. doi: 10.1053/gast.2002.35954

Date, Y., Shimbara, T., Koda, S., Toshinai, K., Ida, T., Murakami, N., et al. (2006). Peripheral ghrelin transmits orexigenic signals through the noradrenergic pathway from the hindbrain to the hypothalamus. *Cell Metab.* 4, 323–331. doi: 10.1016/j.cmet.2006.09.004

de Poorter, C., Baertsoen, K., Lannoy, V., Parmentier, M., and Springael, J. Y. (2013). Consequences of ChemR23 heteromerization with the chemokine receptors CXCR4 and CCR7. *PLoS ONE* 8:e58075. doi: 10.1371/journal.pone.0058075

de Wit, L., Luppino, F., van Straten, A., Penninx, B., Zitman, F., and Cuijpers, P. (2010). Depression and obesity: a meta-analysis of community-based studies. *Psychiatry Res.* 178, 230–235. doi: 10.1016/j.psychres.2009.04.015

Dickson, S. L., Egecioglu, E., Landgren, S., Skibicka, K. P., Engel, J. A., and Jerlhag, E. (2011). The role of the central ghrelin system in reward from food and chemical drugs. *Mol. Cell. Endocrinol.* 340, 80–87. doi: 10.1016/j.mce.2011.02.017

Diorio, D., Viau, V., and Meaney, M. J. (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J. Neurosci.* 13, 3839–3847.

Disse, E., Bussier, A. L., Deblon, N., Pfluger, P. T., Tschop, M. H., Laville, M., et al. (2011). Systemic ghrelin and reward: effect of cholinergic blockade. *Physiol. Behav.* 102, 481–484. doi: 10.1016/j.physbeh.2010.12.006

Disse, E., Bussier, A. L., Veyrat-Durebex, C., Deblon, N., Pfluger, P. T., Tschop, M. H., et al. (2010). Peripheral ghrelin enhances sweet taste food consumption and preference, regardless of its caloric content. *Physiol. Behav.* 101, 277–281. doi: 10.1016/j.physbeh.2010.05.017

Diz-Chaves, Y. (2011). Ghrelin, appetite regulation, and food reward: interaction with chronic stress. *Int. J. Pept.* 2011, 898450. doi: 10.1155/2011/898450

Dorsch, S., Klotz, K. N., Engelhardt, S., Lohse, M. J., and Bunemann, M. (2009). Analysis of receptor oligomerization by FRAP microscopy. *Nat. Methods* 6, 225–230. doi: 10.1038/nmeth.1304

Duthey, B., Caudron, S., Perroy, J., Bettler, B., Fagni, L., Pin, J. P., et al. (2002). A single subunit (GB2) is required for G-protein activation by the heterodimeric GABA(B) receptor. *J. Biol. Chem.* 277, 3236–3241. doi: 10.1074/jbc.M108900200

Egecioglu, E., Jerlhag, E., Salome, N., Skibicka, K. P., Haage, D., Bohlooly, Y. M., et al. (2010). Ghrelin increases intake of rewarding food in rodents. *Addict. Biol.* 15, 304–311. doi: 10.1111/j.1369-1600.2010.00216.x

Egecioglu, E., Skibicka, K. P., Hansson, C., Alvarez-Crespo, M., Friberg, P. A., Jerlhag, E., et al. (2011). Hedonic and incentive signals for body weight control. *Rev. Endocr. Metab. Disord.* 12, 141–151. doi: 10.1007/s11154-011-9166-4

Els, S., Beck-Sickinger, A. G., and Chollet, C. (2010). Ghrelin receptor: high constitutive activity and methods for developing inverse agonists. *Meth. Enzymol.* 485, 103–121. doi: 10.1016/B978-0-12-381296-4.00006-3

Ferre, S., Baler, R., Bouvier, M., Caron, M. G., Devi, L. A., Durroux, T., et al. (2009). Building a new conceptual framework for receptor heteromers. *Nat. Chem. Biol.* 5, 131–134. doi: 10.1038/nchembio0309-131

Finger, B. C., Dinan, T. G., and Cryan, J. F. (2011). High-fat diet selectively protects against the effects of chronic social stress in the mouse. *Neuroscience* 192, 351–360. doi: 10.1016/j.neuroscience.2011.06.072

Finger, B. C., Dinan, T. G., and Cryan, J. F. (2012a). Diet-induced obesity blunts the behavioural effects of ghrelin: studies in a mouse-progressive ratio task. *Psychopharmacology* 220, 173–181. doi: 10.1007/s00213-011-2468-0

Finger, B. C., Dinan, T. G., and Cryan, J. F. (2012b). The temporal impact of chronic intermittent psychosocial stress on high-fat diet-induced alterations in body weight. *Psychoneuroendocrinology* 37, 729–741. doi: 10.1016/j.psyneuen.2011.06.015

Franco, R., Casado, V., Cortes, A., Mallol, J., Ciruela, F., Ferre, S., et al. (2008). G-protein-coupled receptor heteromers: function and ligand pharmacology. *Br. J. Pharmacol.* 153(Suppl. 1), S90–S98. doi: 10.1038/sj.bjp.0707571

Galvez, T., Duthey, B., Kniazeff, J., Blahos, J., Rovelli, G., Bettler, B., et al. (2001). Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. *EMBO J.* 20, 2152–2159. doi: 10.1093/emboj/20.9.2152

Galvez, T., Prezeau, L., Milioti, G., Franek, M., Joly, C., Froestl, W., et al. (2000). Mapping the agonist-binding site of GABAB type 1 subunit sheds light on the activation process of GABAB receptors. *J. Biol. Chem.* 275, 41166–41174. doi: 10.1074/jbc.M007848200

Gariepy, G., Nitka, D., and Schmitz, N. (2010). The association between obesity and anxiety disorders in the population: a systematic review and meta-analysis. *Int. J. Obes. (Lond.)* 34, 407–419. doi: 10.1038/ijo.2009.252

Gavalas, A., Lan, T. H., Liu, Q., Correa, I. R., Javitch, J. A., and Lambert, N. A. (2013). Segregation of family A G Protein Coupled Receptor protomers in the plasma membrane. *Mol. Pharmacol.* 84, 346–352. doi: 10.1124/mol.113.086868

George, S. R., O'Dowd, B. F., and Lee, S. P. (2002). G-protein-coupled receptor oligomerization and its potential for drug discovery. *Nat. Rev. Drug Discov.* 1, 808–820. doi: 10.1038/nrd913

Gershon, E., and Vale, W. W. (2013). CRF type 2 receptors mediate the metabolic effects of ghrelin in C2C12 cells. *Obesity (Silver Spring)* doi: 10.1002/oby.20535. [Epub ahead of print].

Gibbs, J., Young, R. C., and Smith, G. P. (1973). Cholecystokinin elicits satiety in rats with open gastric fistulas. *Nature* 245, 323–325. doi: 10.1038/245323a0

Gibson, E. L. (2006). Emotional influences on food choice: sensory, physiological and psychological pathways. *Physiol. Behav.* 89, 53–61. doi: 10.1016/j.physbeh.2006.01.024

Goddard, A., and Watts, A. (2012). Contributions of fluorescence techniques to understanding G protein-coupled receptor dimerisation. *Biophys. Rev.* 4, 291–298. doi: 10.1007/s12551-012-0073-z

Goldbacher, E. M., and Matthews, K. A. (2007). Are psychological characteristics related to risk of the metabolic syndrome? A review of the literature. *Ann. Behav. Med.* 34, 240–252. doi: 10.1007/BF02874549

Gonzalez-Maeso, J. (2011). GPCR oligomers in pharmacology and signaling. *Mol. Brain* 4, 20. doi: 10.1186/1756-6606-4-20

Guan, X. M., Yu, H., Palyha, O. C., McKee, K. K., Feighner, S. D., Sirinathsinghji, D. J., et al. (1997). Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res. Mol. Brain Res.* 48, 23–29. doi: 10.1016/S0169-328X(97)00071-5

Gupta, A., Mulder, J., Gomes, I., Rozenfeld, R., Bushlin, I., Ong, E., et al. (2010). Increased abundance of opioid receptor heteromers after chronic morphine administration. *Sci. Signal.* 3, ra54. doi: 10.1126/scisignal.2000807

Hague, C., Lee, S. E., Chen, Z., Prinster, S. C., Hall, R. A., and Minneman, K. P. (2006). Heterodimers of alpha1B- and alpha1D-adrenergic receptors form a single functional entity. *Mol. Pharmacol.* 69, 45–55. doi: 10.1124/mol.105.014985

Hebert, T. E., and Bouvier, M. (1998). Structural and functional aspects of G protein-coupled receptor

oligomerization. *Biochem. Cell Biol.* 76, 1–11. doi: 10.1139/o98-012

Heilig, M., Soderpalm, B., Engel, J. A., and Widerlov, E. (1989). Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. *Psychopharmacology (Berl.)* 98, 524–529. doi: 10.1007/BF00441953

Heim, C., and Binder, E. B. (2012). Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics. *Exp. Neurol.* 233, 102–111. doi: 10.1016/j.expneurol.2011.10.032

Heisler, L. K., Pronchuk, N., Nonogaki, K., Zhou, L., Raber, J., Tung, L., et al. (2007a). Serotonin activates the hypothalamic-pituitary-adrenal axis via serotonin 2C receptor stimulation. *J. Neurosci.* 27, 6956–6964. doi: 10.1523/JNEUROSCI.2584-06.2007

Heisler, L. K., Zhou, L., Bajwa, P., Hsu, J., and Tecott, L. H. (2007b). Serotonin 5-HT(2C) receptors regulate anxiety-like behavior. *Genes Brain Behav.* 6, 491–496. doi: 10.1111/j.1601-183X.2007.00316.x

Hern, J. A., Baig, A. H., Mashanov, G. I., Birdsall, B., Corrie, J. E., Lazareno, S., et al. (2010). Formation and dissociation of M1 muscarinic receptor dimers seen by total internal reflection fluorescence imaging of single molecules. *Proc. Natl. Acad. Sci. U.S.A.* 107, 2693–2698. doi: 10.1073/pnas.0907915107

Herrick-Davis, K. (2013). Functional significance of serotonin receptor dimerization. *Exp. Brain Res.* doi: 10.1007/s00221-013-3622-1. [Epub ahead of print].

Holst, B., Brandt, E., Bach, A., Heding, A., and Schwartz, T. W. (2005). Nonpeptide and peptide growth hormone secretagogues act both as ghrelin receptor agonist and as positive or negative allosteric modulators of ghrelin signaling. *Mol. Endocrinol.* 19, 2400–2411. doi: 10.1210/me.2005-0059

Holst, B., Cygankiewicz, A., Jensen, T. H., Ankersen, M., and Schwartz, T. W. (2003). High constitutive signaling of the ghrelin receptor-identification of a potent inverse agonist. *Mol. Endocrinol.* 17, 2201–2210. doi: 10.1210/me.2003-0069

Horvat, R. D., Roess, D. A., Nelson, S. E., Barisas, B. G., and Clay, C. M. (2001). Binding of agonist but not antagonist leads to fluorescence resonance energy transfer between intrinsically fluorescent gonadotropin-releasing hormone receptors. *Mol. Endocrinol.* 15, 695–703. doi: 10.1210/me.15.5.695

Horvath, T. L., Castaneda, T., Tang-Christensen, M., Pagotto, U., and Tschop, M. H. (2003). Ghrelin as a potential anti-obesity target. *Curr. Pharm. Des.* 9, 1383–1395. doi: 10.2174/1381612033454748

Howard, A. D., Feighner, S. D., Cully, D. F., Arena, J. P., Liberator, P. A., Rosenblum, C. I., et al. (1996). A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 273, 974–977. doi: 10.1126/science.273.5277.974

Jastrzebska, B., Ringler, P., Palczewski, K., and Engel, A. (2013). The rhodopsin-transducin complex houses two distinct rhodopsin molecules. *J. Struct. Biol.* 182, 164–172. doi: 10.1016/j.jsb.2013.02.014

Jerlhag, E., Egecioglu, E., Dickson, S. L., Douhan, A., Svensson, L., and Engel, J. A. (2007). Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addict. Biol.* 12, 6–16. doi: 10.1111/j.1369-1600.2006.00041.x

Jiang, H., Betancourt, L., and Smith, R. G. (2006). Ghrelin amplifies dopamine signaling by cross talk involving formation of growth hormone secretagogue receptor/dopamine receptor subtype 1 heterodimers. *Mol. Endocrinol.* 20, 1772–1785. doi: 10.1210/me.2005-0084

Jones, M. T., Hillhouse, E. W., and Burden, J. L. (1977). Structure-activity relationships of corticosteroid feedback at the hypothalamic level. *J. Endocrinol.* 74, 415–424. doi: 10.1677/joe.0.0740415

Kageyama, K., Akimoto, K., Yamagata, S., Sugiyama, A., Murasawa, S., Watanuki, Y., et al. (2012). Dexamethasone stimulates the expression of ghrelin and its receptor in rat hypothalamic 4B cells. *Regul. Pept.* 174, 12–17. doi: 10.1016/j.regpep.2011.11.003

Kamal, M., and Jockers, R. (2011). Biological significance of GPCR heteromerization in the neuro-endocrine system. *Front. Endocrinol. (Lausanne)* 2:2. doi: 10.3389/fendo.2011.00002

Kanehisa, M., Akiyoshi, J., Kitaichi, T., Matsushita, H., Tanaka, E., Kodama, K., et al. (2006). Administration of antisense DNA for ghrelin causes an antidepressant and anxiolytic response in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 30, 1403–1407. doi: 10.1016/j.pnpbp.2006.05.005

Kaufman, D., Banerji, M. A., Shorman, I., Smith, E. L., Coplan, J. D., Rosenblum, L. A., et al. (2007). Early-life stress and the development of obesity and insulin resistance in juvenile bonnet macaques. *Diabetes* 56, 1382–1386. doi: 10.2337/db06-1409

Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., et al. (1998). GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature* 396, 683–687. doi: 10.1038/25360

Kawakami, A., Okada, N., Rokkaku, K., Honda, K., Ishibashi, S., and Onaka, T. (2008). Leptin inhibits and ghrelin augments hypothalamic noradrenaline release after stress. *Stress* 11, 363–369. doi: 10.1080/10253890701820257

Kelley, A. E., Baldo, B. A., Pratt, W. E., and Will, M. J. (2005). Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol. Behav.* 86, 773–795. doi: 10.1016/j.physbeh.2005.08.066

Kent, T., McAlpine, C., Sabetnia, S., and Presland, J. (2007). G-protein-coupled receptor heterodimerization: assay technologies to clinical significance. *Curr. Opin. Drug Discov. Dev.* 10, 580–589.

Kern, A., Albarran-Zeckler, R., Walsh, H. E., and Smith, R. G. (2012). Apo-ghrelin receptor forms heteromers with DRD2 in hypothalamic neurons and is essential for anorexigenic effects of DRD2 agonism. *Neuron* 73, 317–332. doi: 10.1016/j.neuron.2011.10.038

Kishi, T., Aschkenasi, C. J., Lee, C. E., Mountjoy, K. G., Saper, C. B., and Elmquist, J. K. (2003). Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. *J. Comp. Neurol.* 457, 213–235. doi: 10.1002/cne.10454

Kloiber, S., Ising, M., Reppermund, S., Horstmann, S., Dose, T., Majer, M., et al. (2007). Overweight and obesity affect treatment response in major depression. *Biol. Psychiatry* 62, 321–326. doi: 10.1016/j.biopsych.2006.10.001

Kluge, M., Schussler, P., Dresler, M., Schmidt, D., Yassouridis, A., Uhr, M., et al. (2011). Effects of ghrelin on psychopathology, sleep and secretion of cortisol and growth hormone in patients with major depression. *J. Psychiatr. Res.* 45, 421–426. doi: 10.1016/j.jpsychires.2010.09.002

Kluge, M., Schussler, P., Schmid, D., Uhr, M., Kleyer, S., Yassouridis, A., et al. (2009). Ghrelin plasma levels are not altered in major depression. *Neuropsychobiology* 59, 199–204. doi: 10.1159/000223731

Kohno, D., Gao, H. Z., Muroya, S., Kikuyama, S., and Yada, T. (2003). Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus: Ca^{2+} signaling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes* 52, 948–956. doi: 10.2337/diabetes.52.4.948

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402, 656–660. doi: 10.1038/45230

Kojima, M., Hosoda, H., and Kangawa, K. (2004). Clinical endocrinology and metabolism. Ghrelin, a novel growth-hormone-releasing and appetite-stimulating peptide from stomach. *Best Pract. Res. Clin. Endocrinol. Metab.* 18, 517–530. doi: 10.1016/j.beem.2004.07.001

Kristensen, P., Judge, M. E., Thim, L., Ribel, U., Christjansen, K. N., Wulff, B. S., et al. (1998). Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* 393, 72–76. doi: 10.1038/29993

Kristenssson, E., Sundqvist, M., Astin, M., Kjerling, M., Mattsson, H., Dornonville de la Cour, C., et al. (2006). Acute psychological stress raises plasma ghrelin in the rat. *Regul. Pept.* 134, 114–117. doi: 10.1016/j.regpep.2006.02.003

Kyrou, I., and Tsigos, C. (2007). Stress mechanisms and metabolic complications. *Horm. Metab. Res.* 39, 430–438. doi: 10.1055/s-2007-981462

Lam, D. D., Przydzial, M. J., Ridley, S. H., Yeo, G. S., Rochford, J. J., O'Rahilly, S., et al. (2008). Serotonin 5-HT2C receptor agonist promotes hypophagia via downstream activation of melanocortin 4 receptors. *Endocrinology* 149, 1323–1328. doi: 10.1210/en.2007-1321

Leite-Moreira, A. F., and Soares, J. B. (2007). Physiological, pathological and potential therapeutic roles of ghrelin. *Drug Discov. Today* 12, 276–288. doi: 10.1016/j.drudis.2007.02.009

le Roux, C. W., Neary, N. M., Halsey, T. J., Small, C. J., Martinez-Isla, A. M., Ghatei, M. A., et al. (2005). Ghrelin does not stimulate food intake in patients with surgical procedures involving vagotomy. *J. Clin.*

Endocrinol. Metab. 90, 4521–4524. doi: 10.1210/jc.2004-2537

Leung, P. K., Chow, K. B., Lau, P. N., Chu, K. M., Chan, C. B., Cheng, C. H., et al. (2007). The truncated ghrelin receptor polypeptide (GHS-R1b) acts as a dominant-negative mutant of the ghrelin receptor. *Cell. Signal.* 19, 1011–1022. doi: 10.1016/j.cellsig.2006.11.011

Liu, G., Fortin, J. P., Beinborn, M., and Kopin, A. S. (2007). Four missense mutations in the ghrelin receptor result in distinct pharmacological abnormalities. *J. Pharmacol. Exp. Ther.* 322, 1036–1043. doi: 10.1124/jpet.107.123141

Lu, S. C., Xu, J., Chinookoswong, N., Liu, S., Steavenson, S., Gegg, C., et al. (2009). An acyl-ghrelin-specific neutralizing antibody inhibits the acute ghrelin-mediated orexigenic effects in mice. *Mol. Pharmacol.* 75, 901–907. doi: 10.1124/mol.108.052852

Luppino, F. S., de Wit, L. M., Bouvy, P. F., Stijnen, T., Cuijpers, P., Penninx, B. W., et al. (2010). Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch. Gen. Psychiatry* 67, 220–229. doi: 10.1001/archgenpsychiatry.2010.2

Lutter, M., Sakata, I., Osborne-Lawrence, S., Rovinsky, S. A., Anderson, J. G., Jung, S., et al. (2008). The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat. Neurosci.* 11, 752–753. doi: 10.1038/nn.2139

Mahmoud, S. N., Scaccianoce, S., Scraggs, P. R., Nicholson, S. A., Gillham, B., and Jones, M. T. (1984). Characteristics of corticosteroid inhibition of adrenocorticotrophin release from the anterior pituitary gland of the rat. *J. Endocrinol.* 102, 33–42. doi: 10.1677/joe.0.1020033

Maniam, J., and Morris, M. J. (2012). The link between stress and feeding behaviour. *Neuropharmacology* 63, 97–110. doi: 10.1016/j.neuropharm.2012.04.017

Margeta-Mitrovic, M., Jan, Y. N., and Jan, L. Y. (2000). A trafficking checkpoint controls GABA(B) receptor heterodimerization. *Neuron* 27, 97–106. doi: 10.1016/S0896-6273(00)00012-X

Marijnissen, R. M., Bus, B. A., Holewijn, S., Franke, B., Purandare, N., de Graaf, J., et al. (2011). Depressive symptom clusters are differentially associated with general and visceral obesity. *J. Am. Geriatr. Soc.* 59, 67–72. doi: 10.1111/j.1532-5415.2010.03228.x

Mary, S., Fehrentz, J. A., Damian, M., Gaibelet, G., Orcel, H., Verdie, P., et al. (2013). Heterodimerization with its splice variant blocks the ghrelin receptor 1a in a nonsignaling conformation. A study with a purified heterodimer assembled into lipid discs. *J. Biol. Chem.* doi: 10.1074/jbc.M113.453423. [Epub ahead of print].

McElroy, S. L., Kotwal, R., Malhotra, S., Nelson, E. B., Keck, P. E., and Nemeroff, C. B. (2004). Are mood disorders and obesity related? A review for the mental health professional. *J. Clin. Psychiatry* 65, 634–651. doi: 10.4088/JCP.v65n0507

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* 87, 873–904. doi: 10.1152/physrev.00041.2006

Mikhail, N. (2009). The metabolic syndrome: insulin resistance. *Curr. Hypertens. Rep.* 11, 156–158. doi: 10.1007/s11906-009-0027-4

Milligan, G. (2013). The prevalence, maintenance, and relevance of g protein-coupled receptor oligomerization. *Mol. Pharmacol.* 84, 158–169. doi: 10.1124/mol.113.084780

Monsonego, A., Maron, R., Zota, V., Selkoe, D. J., and Weiner, H. L. (2001). Immune hyporesponsiveness to amyloid beta-peptide in amyloid precursor protein transgenic mice: implications for the pathogenesis and treatment of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 98, 10273–10278. doi: 10.1073/pnas.191118298

Moon, M., Choi, J. G., Nam, D. W., Hong, H. S., Choi, Y. J., Oh, M. S., et al. (2011). Ghrelin ameliorates cognitive dysfunction and neurodegeneration in intrahippocampal amyloid-beta1-42 oligomer-injected mice. *J. Alzheimers Dis.* 23, 147–159.

Morrison, C. D. (2009). Leptin signaling in brain: A link between nutrition and cognition? *Biochim. Biophys. Acta* 1792, 401–408. doi: 10.1016/j.bbadis.2008.12.004

Moulin, A., Ryan, J., Martinez, J., and Fehrentz, J. A. (2007). Recent developments in ghrelin receptor ligands. *ChemMedChem* 2, 1242–1259. doi: 10.1002/cmdc.200700015

Mundinger, T. O., Cummings, D. E., and Taborsky, G. J. Jr. (2006). Direct stimulation of ghrelin secretion by sympathetic nerves. *Endocrinology* 147, 2893–2901. doi: 10.1210/en.2005-1182

Nakashima, K., Akiyoshi, J., Hatano, K., Hanada, H., Tanaka, Y., Tsuru, J., et al. (2008). Ghrelin gene polymorphism is associated with depression, but not panic disorder. *Psychiatr. Genet.* 18, 257. doi: 10.1097/YPG.0b013e328306c979

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., et al. (2001). A role for ghrelin in the central regulation of feeding. *Nature* 409, 194–198. doi: 10.1038/35051587

Naleid, A. M., Grace, M. K., Cummings, D. E., and Levine, A. S. (2005). Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. *Peptides* 26, 2274–2279. doi: 10.1016/j.peptides.2005.04.025

Niswender, C. M., Copeland, S. C., Herrick-Davis, K., Emeson, R. B., and Sanders-Bush, E. (1999). RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *J. Biol. Chem.* 274, 9472–9478. doi: 10.1074/jbc.274.14.9472

Nonogaki, K., Ohashi-Nozue, K., and Oka, Y. (2006). A negative feedback system between brain serotonin systems and plasma active ghrelin levels in mice. *Biochem. Biophys. Res. Commun.* 341, 703–707. doi: 10.1016/j.bbrc.2006.01.021

Novick, J. S., Stewart, J. W., Wisniewski, S. R., Cook, I. A., Manev, R., Nierenberg, A. A., et al. (2005). Clinical and demographic features of atypical depression in outpatients with major depressive disorder: preliminary findings from STAR*D. *J. Clin. Psychiatry* 66, 1002–1011. doi: 10.4088/JCP.v66n0807

Ochi, M., Tominaga, K., Tanaka, F., Tanigawa, T., Shiba, M., Watanabe, T., et al. (2008). Effect of chronic stress on gastric emptying and plasma ghrelin levels in rats. *Life Sci.* 82, 862–868. doi: 10.1016/j.lfs.2008.01.020

Olaghere da Silva, U. B., Morabito, M. V., Canal, C. E., Airey, D. C., Emeson, R. B., and Sanders-Bush, E. (2010). Impact of RNA editing on functions of the serotonin 2C receptor *in vivo*. *Front. Neuropharmacol.* 4:26. doi: 10.3389/neuro.23.001.2010

Oliver, G., and Wardle, J. (1999). Perceived effects of stress on food choice. *Physiol. Behav.* 66, 511–515. doi: 10.1016/S0031-9384(98)00322-9

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A. M., Quigley, E. M., et al. (2009). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biol. Psychiatry* 65, 263–267. doi: 10.1016/j.biopsych.2008.06.026

Panetta, R., and Greenwood, M. T. (2008). Physiological relevance of GPCR oligomerization and its impact on drug discovery. *Drug Discov. Today* 13, 1059–1066. doi: 10.1016/j.drudis.2008.09.002

Park, S., Jiang, H., Zhang, H., and Smith, R. G. (2012). Modification of ghrelin receptor signaling by somatostatin receptor-5 regulates insulin release. *Proc. Natl. Acad. Sci. U.S.A.* 109, 19003–19008. doi: 10.1073/pnas.1209590109

Pasternak, G. W. (2013). Opioids and their receptors: are we there yet? *Neuropharmacology* doi: 10.1016/j.neuropharm.2013.03.039. [Epub ahead of print].

Patel, R. C., Kumar, U., Lamb, D. C., Eid, J. S., Rocheville, M., Grant, M., et al. (2002). Ligand binding to somatostatin receptors induces receptor-specific oligomer formation in live cells. *Proc. Natl. Acad. Sci. U.S.A.* 99, 3294–3299. doi: 10.1073/pnas.042705099

Patterson, M., Bloom, S. R., and Gardiner, J. V. (2011). Ghrelin and appetite control in humans-potential application in the treatment of obesity. *Peptides* 32, 2290–2294. doi: 10.1016/j.peptides.2011.07.021

Patterson, Z. R., Ducharme, R., Anisman, H., and Abizaid, A. (2010). Altered metabolic and neurochemical responses to chronic unpredictable stressors in ghrelin receptor-deficient mice. *Eur. J. Neurosci.* 32, 632–639. doi: 10.1111/j.1460-9568.2010.07310.x

Patterson, Z. R., Khazall, R., Mackay, H., Anisman, H., and Abizaid, A. (2013). Central ghrelin signaling mediates the metabolic response of C57BL/6 male mice to chronic social defeat stress. *Endocrinology* 154, 1080–1091. doi: 10.1210/en.2012-1834

Perello, M., Sakata, I., Birnbaum, S., Chuang, J. C., Osborne-Lawrence, S., Rovinsky, S. A., et al. (2010). Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. *Biol. Psychiatry* 67, 880–886. doi: 10.1016/j.biopsych.2009.10.030

Perello, M., and Zigman, J. M. (2012). The role of ghrelin in reward-based eating. *Biol. Psychiatry* 72, 347–353. doi: 10.1016/j.biopsych.2012.02.016

Petersen, P. S., Woldbye, D. P., Madsen, A. N., Egerod, K. L., Jin, C., Lang, M., et al. (2009). *In vivo*

characterization of high basal signaling from the ghrelin receptor. *Endocrinology* 150, 4920–4930. doi: 10.1210/en.2008-1638

Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. (2002). Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* 3, 639–650. doi: 10.1038/nrm908

Pin, J. P., Neubig, R., Bouvier, M., Devi, L., Filizola, M., Javitch, J. A., et al. (2007). International Union of Basic and Clinical Pharmacology. LXVII. Recommendations for the recognition and nomenclature of G protein-coupled receptor heteromultimers. *Pharmacol. Rev.* 59, 5–13. doi: 10.1124/pr.59.1.5

Prinster, S. C., Hague, C., and Hall, R. A. (2005). Heterodimerization of g protein-coupled receptors: specificity and functional significance. *Pharmacol. Rev.* 57, 289–298. doi: 10.1124/pr.57.3.1

Radley, J. J., Williams, B., and Sawchenko, P. E. (2008). Noradrenergic innervation of the dorsal medial prefrontal cortex modulates hypothalamo-pituitary-adrenal responses to acute emotional stress. *J. Neurosci.* 28, 5806–5816. doi: 10.1523/JNEUROSCI.0552-08.2008

Rashid, A. J., O'Dowd, B. F., and George, S. R. (2004). Minireview: diversity and complexity of signaling through peptidergic G protein-coupled receptors. *Endocrinology* 145, 2645–2652. doi: 10.1210/en.2004-0052

Rasmussen, S. G., DeVree, B. T., Zou, Y., Kruse, A. C., Chung, K. Y., Kobilka, T. S., et al. (2011). Crystal structure of the beta2 adrenergic receptor-Gs protein complex. *Nature* 477, 549–555. doi: 10.1038/nature10361

Raspopow, K., Abizaid, A., Matheson, K., and Anisman, H. (2010). Psychosocial stressor effects on cortisol and ghrelin in emotional and non-emotional eaters: influence of anger and shame. *Horm. Behav.* 58, 677–684. doi: 10.1016/j.yhbeh.2010.06.003

Rediger, A., Piechowski, C. L., Yi, C. X., Tarnow, P., Strotmann, R., Gruters, A., et al. (2011). Mutually opposite signal modulation by hypothalamic heterodimerization of ghrelin and melanocortin-3 receptors. *J. Biol. Chem.* 286, 39623–39631. doi: 10.1074/jbc.M111.287607

Rediger, A., Tarnow, P., Bickenbach, A., Schaefer, M., Krude, H., Grüters, A., et al. (2009). Heterodimerization of hypothalamic G-protein-coupled receptors involved in weight regulation. *Obes. Facts* 2, 80–86. doi: 10.1159/000209862

Richardson, L. P., Davis, R., Poulton, R., McCauley, E., Moffitt, T. E., Caspi, A., et al. (2003). A longitudinal evaluation of adolescent depression and adult obesity. *Arch. Pediatr. Adolesc. Med.* 157, 739–745. doi: 10.1001/archpedi.157.8.739

Rivero-Muller, A., Chou, Y. Y., Ji, I., Lajic, S., Hanyaloglu, A. C., Jonas, K., et al. (2010). Rescue of defective G protein-coupled receptor function *in vivo* by intermolecular cooperation. *Proc. Natl. Acad. Sci. U.S.A.* 107, 2319–2324. doi: 10.1073/pnas.0906695106

Rouach, V., Bloch, M., Rosenberg, N., Gilad, S., Limor, R., Stern, N., et al. (2007). The acute ghrelin response to a psychological stress challenge does not predict the post-stress urge to eat. *Psychoneuroendocrinology* 32, 693–702. doi: 10.1016/j.psyneuen.2007.04.010

Rozenfeld, R., and Devi, L. A. (2010). Receptor heteromerization and drug discovery. *Trends Pharmacol. Sci.* 31, 124–130. doi: 10.1016/j.tips.2009.11.008

Rozenfeld, R., and Devi, L. A. (2011). Exploring a role for heteromerization in GPCR signalling specificity. *Biochem. J.* 433, 11–18. doi: 10.1042/BJ20100458

Salahpour, A., Angers, S., Mercier, J. F., Lagace, M., Marullo, S., and Bouvier, M. (2004). Homodimerization of the beta2-adrenergic receptor as a prerequisite for cell surface targeting. *J. Biol. Chem.* 279, 33390–33397. doi: 10.1074/jbc.M403363200

Sapolsky, R. M., Krey, L. C., and McEwen, B. S. (1984). Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. *Proc. Natl. Acad. Sci. U.S.A.* 81, 6174–6177. doi: 10.1073/pnas.81.19.6174

Sapolsky, R. M., Romero, L. M., and Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21, 55–89. doi: 10.1210/er.21.1.55

Schaeffer, M., Langlet, F., Lafont, C., Molino, F., Hodson, D. J., Roux, T., et al. (2013). Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1512–1517. doi: 10.1073/pnas.1212137110

Schanze, A., Reulbach, U., Scheuchenzuber, M., Groschl, M., Kornhuber, J., and Kraus, T. (2008). Ghrelin and eating disturbances in psychiatric disorders. *Neuropsychobiology* 57, 126–130. doi: 10.1159/000138915

Schellekens, H., Clarke, G., Jeffery, I. B., Dinan, T. G., and Cryan, J. F. (2012a). Dynamic 5-HT(2C) receptor editing in a mouse model of obesity. *PLoS ONE* 7:e32266. doi: 10.1371/journal.pone.0032266

Schellekens, H., Finger, B. C., Dinan, T. G., and Cryan, J. F. (2012b). Ghrelin signalling and obesity: at the interface of stress, mood and food reward. *Pharmacol. Ther.* 135, 316–326. doi: 10.1016/j.pharmthera.2012.06.004

Schellekens, H., Dinan, T. G., and Cryan, J. F. (2010). Lean mean fat reducing ghrelin machine: hypothalamic ghrelin and ghrelin receptors as therapeutic targets in obesity. *Neuropharmacology* 58, 2–16. doi: 10.1016/j.neuropharm.2009.06.024

Schellekens, H., Dinan, T. G., and Cryan, J. F. (2013a). Ghrelin at the interface of obesity and reward. *Vitam. Horm.* 91, 285–323. doi: 10.1016/B978-0-12-407766-9.00013-4

Schellekens, H., van Oeffelen, W. E., Dinan, T. G., and Cryan, J. F. (2013b). Promiscuous dimerization of the Growth Hormone Secretagogue Receptor (GHS-R1a) attenuates ghrelin-mediated signaling. *J. Biol. Chem.* 288, 181–191. doi: 10.1074/jbc.M112.382473

Scott, K. A., Melhorn, S. J., and Sakai, R. R. (2012). Effects of chronic social stress on obesity. *Curr. Obes. Rep.* 1, 16–25. doi: 10.1007/s13679-011-0006-3

Shimbara, T., Mondal, M. S., Kawagoe, T., Toshinai, K., Koda, S., Yamaguchi, H., et al. (2004). Central administration of ghrelin preferentially enhances fat ingestion. *Neurosci. Lett.* 369, 75–79. doi: 10.1016/j.neulet.2004.07.060

Siervo, M., Wells, J. C., and Cizza, G. (2009). The contribution of psychosocial stress to the obesity epidemic: an evolutionary approach. *Horm. Metab. Res.* 41, 261–270. doi: 10.1055/s-0028-1119377

Simon, G. E., Von Korff, M., Saunders, K., Miglioretti, D. L., Crane, P. K., van Belle, G., et al. (2006). Association between obesity and psychiatric disorders in the US adult population. *Arch. Gen. Psychiatry* 63, 824–830. doi: 10.1001/archpsyc.63.7.824

Skibicka, K. P., and Dickson, S. L. (2011). Ghrelin and food reward: the story of potential underlying substrates. *Peptides* 32, 2265–2273. doi: 10.1016/j.peptides.2011.05.016

Skibicka, K. P., Hansson, C., Alvarez-Crespo, M., Friberg, P. A., and Dickson, S. L. (2011). Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* 180, 129–137. doi: 10.1016/j.neuroscience.2011.02.016

Skibicka, K. P., Hansson, C., Egecioglu, E., and Dickson, S. L. (2012). Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. *Addict. Biol.* 17, 95–107. doi: 10.1111/j.1369-1600.2010.00294.x

Smith, K. L., Patterson, M., Dhillo, W. S., Patel, S. R., Semjonous, N. M., Gardiner, J. V., et al. (2006). Neuropeptide S stimulates the hypothalamo-pituitary-adrenal axis and inhibits food intake. *Endocrinology* 147, 3510–3518. doi: 10.1210/en.2005-1280

Smith, N. J., and Milligan, G. (2010). Allostery at G protein-coupled receptor homo- and heteromers: uncharted pharmacological landscapes. *Pharmacol. Rev.* 62, 701–725. doi: 10.1124/pr.110.002667

Soares, J. B., Roncon-Albuquerque, R. Jr., and Leite-Moreira, A. (2008). Ghrelin and ghrelin receptor inhibitors: agents in the treatment of obesity. *Expert Opin. Ther. Targets* 12, 1177–1189. doi: 10.1517/14728222.12.9.1177

Spencer, S. J., Xu, L., Clarke, M. A., Lemus, M., Reichenbach, A., Geenen, B., et al. (2012). Ghrelin regulates the hypothalamic-pituitary-adrenal axis and restricts anxiety after acute stress. *Biol. Psychiatry* 72, 457–465. doi: 10.1016/j.biopsych.2012.03.010

Springael, J. Y., Urizar, E., and Parmentier, M. (2005). Dimerization of chemokine receptors and its functional consequences. *Cytokine Growth Factor Rev.* 16, 611–623. doi: 10.1016/j.cytogfr.2005.05.005

Stanek, L. M. (2006). Cocaine- and amphetamine related transcript (CART) and anxiety. *Peptides* 27, 2005–2011. doi: 10.1016/j.peptides.2006.01.027

Stanley, B. G., and Leibowitz, S. F. (1985). Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. *Proc. Natl. Acad. Sci. U.S.A.* 82, 3940–3943. doi: 10.1073/pnas.82.11.3940

Stengel, A., Wang, L., and Taché, Y. (2011). Stress-related alterations of acyl and desacyl ghrelin circulating levels: mechanisms and functional implications. *Peptides* 32, 2208–2217. doi: 10.1016/j.peptides.2011.07.002

Strohle, A., Holsboer, F., and Rupprecht, R. (2000). Increased ACTH concentrations associated with cholecystokinin tetrapeptide-induced panic attacks in patients with panic disorder. *Neuropsychopharmacology* 22, 251–256. doi: 10.1016/S0893-133X(99)00115-3

Takahashi, K., Furukawa, C., Takano, A., Ishikawa, N., Kato, T., Hayama, S., et al. (2006). The neuromedin U-growth hormone secretagogue receptor 1b/neurotensin receptor 1 oncogenic signaling pathway as a therapeutic target for lung cancer. *Cancer Res.* 66, 9408–9419. doi: 10.1158/0008-5472.CAN-06-1349

Takaya, K., Ariyasu, H., Kanamoto, N., Iwakura, H., Yoshimoto, A., Harada, M., et al. (2000). Ghrelin strongly stimulates growth hormone release in humans. *J. Clin. Endocrinol. Metab.* 85, 4908–4911. doi: 10.1210/jc.85.12.4908

Tataranni, P. A., Baier, L., Jenkinson, C., Harper, I., Del Parigi, A., and Bogardus, C. (2001). A Ser311Cys mutation in the human dopamine receptor D2 gene is associated with reduced energy expenditure. *Diabetes* 50, 901–904. doi: 10.2337/diabetes.50.4.901

Teitler, M., and Klein, M. T. (2012). A new approach for studying GPCR dimers: drug-induced inactivation and reactivation to reveal GPCR dimer function *in vitro*, in primary culture, and *in vivo*. *Pharmacol. Ther.* 133, 205–217. doi: 10.1016/j.pharmthera.2011.10.007

Terrillon, S., and Bouvier, M. (2004). Roles of G-protein-coupled receptor dimerization. *EMBO Rep.* 5, 30–34. doi: 10.1038/sj.embor.7400052

Tschop, M., Smiley, D. L., and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* 407, 908–913. doi: 10.1038/35038090

Uberti, M. A., Hague, C., Oller, H., Minneman, K. P., and Hall, R. A. (2005). Heterodimerization with beta2-adrenergic receptors promotes surface expression and functional activity of alpha1D-adrenergic receptors. *J. Pharmacol. Exp. Ther.* 313, 16–23. doi: 10.1124/jpet.104.079541

Ulrich-Lai, Y. M., and Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nat. Rev. Neurosci.* 10, 397–409. doi: 10.1038/nrn2647

Urizar, E., Montanelli, L., Loy, T., Bonomi, M., Swillens, S., Gales, C., et al. (2005). Glycoprotein hormone receptors: link between receptor homodimerization and negative cooperativity. *EMBO J.* 24, 1954–1964. doi: 10.1038/sj.emboj.7600686

Urizar, E., Yano, H., Kolster, R., Gales, C., Lambert, N., and Javitch, J. A. (2011). CODA-RET reveals functional selectivity as a result of GPCR heteromerization. *Nat. Chem. Biol.* 7, 624–630. doi: 10.1038/nchembio.623

Valant, C., Sexton, P. M., and Christopoulos, A. (2009). Orthosteric/allosteric bitopic ligands: going hybrid at GPCRs. *Mol. Interv.* 9, 125–135. doi: 10.1124/mi.9.3.6

Waldhoer, M., Fong, J., Jones, R. M., Lunzer, M. M., Sharma, S. K., Kostenis, E., et al. (2005). A heterodimer-selective agonist shows *in vivo* relevance of G protein-coupled receptor dimers. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9050–9055. doi: 10.1073/pnas.0501112102

Ward, R. J., Xu, T. R., and Milligan, G. (2013). GPCR oligomerization and receptor trafficking. *Methods Enzymol.* 521, 69–90. doi: 10.1016/B978-0-12-391862-8.00004-1

Weinberg, Z. Y., Nicholson, M. L., and Currie, P. J. (2011). 6-Hydroxydopamine lesions of the ventral tegmental area suppress ghrelin's ability to elicit food-reinforced behavior. *Neurosci. Lett.* 499, 70–73. doi: 10.1016/j.neulet.2011.05.034

Wells, T. (2009). Ghrelin-Defender of fat. *Prog. Lipid Res.* 48, 257–274. doi: 10.1016/j.plipres.2009.04.002

White, J. H., Wise, A., Main, M. J., Green, A., Fraser, N. J., Disney, G. H., et al. (1998). Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature* 396, 679–682. doi: 10.1038/25354

Whorton, M. R., Bokoch, M. P., Rasmussen, S. G., Huang, B., Zare, R. N., Kobilka, B., et al. (2007). A monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein. *Proc. Natl. Acad. Sci. U.S.A.* 104, 7682–7687. doi: 10.1073/pnas.0611448104

Whorton, M. R., Jastrzebska, B., Park, P. S., Fotiadis, D., Engel, A., Palczewski, K., et al. (2008). Efficient coupling of transducin to monomeric rhodopsin in a phospholipid bilayer. *J. Biol. Chem.* 283, 4387–4394. doi: 10.1074/jbc.M703346200

Williams, D. L., Grill, H. J., Cummings, D. E., and Kaplan, J. M. (2003). Vagotomy dissociates short- and long-term controls of circulating ghrelin. *Endocrinology* 144, 5184–5187. doi: 10.1210/en.2003-1059

Xu, J., Xu, M., Brown, T., Rossi, G. C., Hurd, Y. L., Inturrisi, C. E., et al. (2013). Stabilization of the μ-opioid receptor by truncated single transmembrane splice variants through a chaperone-like action. *J. Biol. Chem.* 288, 21211–21227. doi: 10.1074/jbc.M113.458687

Xu, Y. L., Reinscheid, R. K., Huitron-Resendiz, S., Clark, S. D., Wang, Z., Lin, S. H., et al. (2004). Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. *Neuron* 43, 487–497. doi: 10.1016/j.neuron.2004.08.005

Yang, Y. (2011). Structure, function and regulation of the melanocortin receptors. *Eur. J. Pharmacol.* 660, 125–130. doi: 10.1016/j.ejphar.2010.12.020

Yi, C. X., Heppner, K., and Tschop, M. H. (2011). Ghrelin in eating disorders. *Mol. Cell. Endocrinol.* 340, 29–34. doi: 10.1016/j.mce.2011.03.001

Zhao, T. J., Sakata, I., Li, R. L., Liang, G., Richardson, J. A., Brown, M. S., et al. (2010). Ghrelin secretion stimulated by {beta}1-adrenergic receptors in cultured ghrelinoma cells and in fasted mice. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15868–15873. doi: 10.1073/pnas.1011116107

Zheng, J., Dobner, A., Babygirija, R., Ludwig, K., and Takahashi, T. (2009). Effects of repeated restraint stress on gastric motility in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1358–R1365. doi: 10.1152/ajpregu.90928.2008

Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B., and Elmquist, J. K. (2006). Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J. Comp. Neurol.* 494, 528–548. doi: 10.1002/cne.20823

Zimmermann, U. S., Buchmann, A., Steffin, B., Dieterle, C., and Uhr, M. (2007). Alcohol administration acutely inhibits ghrelin secretion in an experiment involving psychosocial stress. *Addict. Biol.* 12, 17–21. doi: 10.1111/j.1369-1600.2006.00026.x

Zorrilla, E. P., Iwasaki, S., Moss, J. A., Chang, J., Otsuji, J., Inoue, K., et al. (2006). Vaccination against weight gain. *Proc. Natl. Acad. Sci. U.S.A.* 103, 13226–13231. doi: 10.1073/pnas.0605376103

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Ghrelin and ghrelin receptor modulation of psychostimulant action

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Ghrelin (GHR) is an orexigenic gut peptide that modulates multiple homeostatic functions including gastric emptying, anxiety, stress, memory, feeding, and reinforcement. GHR is known to bind and activate growth-hormone secretagogue receptors (termed GHR-Rs). Of interest to our laboratory has been the assessment of the impact of GHR modulation of the locomotor activation and reward/reinforcement properties of psychostimulants such as cocaine and nicotine. Systemic GHR infusions augment cocaine stimulated locomotion and conditioned place preference (CPP) in rats, as does food restriction (FR) which elevates plasma ghrelin levels. Ghrelin enhancement of psychostimulant function may occur owing to a direct action on mesolimbic dopamine function or may reflect an indirect action of ghrelin on glucocorticoid pathways. Genomic or pharmacological ablation of GHR-Rs attenuates the acute locomotor-enhancing effects of nicotine, cocaine, amphetamine and alcohol and blunts the CPP induced by food, alcohol, amphetamine and cocaine in mice. The stimulant nicotine can induce CPP and like amphetamine and cocaine, repeated administration of nicotine induces locomotor sensitization in rats. Inactivation of ghrelin circuit function in rats by injection of a ghrelin receptor antagonist (e.g., JMV 2959) diminishes the development of nicotine-induced locomotor sensitization. These results suggest a key permissive role for GHR-R activity for the induction of locomotor sensitization to nicotine. Our finding that GHR-R null rats exhibit diminished patterns of responding for intracranial self-stimulation complements an emerging literature implicating central GHR circuits in drug reward/reinforcement. Finally, antagonism of GHR-Rs may represent a smoking cessation modality that not only blocks nicotine-induced reward but that also may limit weight gain after smoking cessation.

Keywords: ghrelin, ghrelin receptors, JMV 2959, locomotion, sensitization, self-administration, feeding

INTRODUCTION

The accumulation of excess body fat in obesity is a key etiological factor in current health disorders and early mortality (Olshansky et al., 2005). In spite of efforts to identify the underlying metabolic, hormonal, and behavioral underpinnings of this disorder, there is evidence that the prevalence of obesity is increasing in American children, adults and the elderly (Fakhouri et al., 2012; Flegal et al., 2012; Ogden et al., 2012). In recent years, considerable effort sought to determine effective treatments for obesity including drugs that either alter metabolism, diminish nutrient absorption or suppress appetite (Bray, 2000). One approach has focused on targeting physiological factors that control eating (Bray, 2000) with the hope that strategies might be developed to block orexigenic circuits or to facilitate the activity of circuits that suppress eating. A key example of the latter was the identification of leptin, a peptide that functions to suppress eating. Although there was initial enthusiasm that leptin-modulating drugs might reduce appetite and normalize body weight in obese subjects (Zhang et al., 1994; Nogueiras et al., 2008), leptin treatments are not effective as antiobesity agents (Hukshorn and Saris, 2004). With regard to modulation of orexigenic circuits, a recent candidate peptide has been the gastric peptide ghrelin (GHR), which is the only peripheral orexigenic peptide identified to date (Kojima et al., 1999; Tschop et al., 2000; Ariyasu et al., 2001; Asakawa et al., 2005). Antagonism of ghrelin receptors presumably would suppress appetite and thus represent a novel therapeutic approach for obesity (Asakawa et al., 2003).

GHRELIN AND GHRELIN RECEPTORS

In 1999, the Kojima group identified GHR as a 28 amino acid peptide secreted from the endocrine cells of the stomach and gut (Kojima et al., 1999). GHR is unique in that this peptide undergoes a posttranslational modification in which an octanoate group is added to the third serine group to form acylated-GHR. (Hosoda et al., 2000; Sakata et al., 2009; Davis et al., 2012). Acyl-GHR (hereafter referred to as GHR) is the endogenous ligand for the growth hormone (GH) secretagogue 1a receptor (GHS-R1a or GHR-R) (Kojima et al., 1999). Unlike GHR, the non-acylated form of ghrelin (des-acyl-ghrelin or DAG) does not activate GHR-Rs (Kojima et al., 1999; Delhanty et al., 2012). Normally, circulating DAG levels are higher than those of GHR levels (Ferrini et al., 2009). Acylation of ghrelin occurs via activation of ghrelin-O-acyl-transferase (GOAT) (Yang et al., 2008) whereas de-acylation of ghrelin can be induced by the enzyme acyl-protein thioesterase

1/lysophospholipase 1 (Satou et al., 2010; Stengel et al., 2011). Diminished GHR signaling would be expected to occur after inhibition of GHR or by activation of de-acylation of GHR. Facilitation of GOAT function should enhance GHR signaling.

The ghrelin peptide is widely distributed throughout the gut and the brain. Ghrelin is primarily secreted from the fundus of the stomach and is transported into the brain across the blood brain barrier (BBB) (Wren et al., 2001; Banks et al., 2002, 2008; Diano et al., 2006). Ghrelin is expressed primarily within the arcuate nucleus (ARC), the lateral hypothalamus (LH), the paraventricular hypothalamic nucleus (PVN), portions of cortex, and the dorsal vagal complex (Cowley et al., 2003; Cowley and Grove, 2004; Hou et al., 2006; Ferrini et al., 2009). Ghrelin receptors appear to modulate a diverse set of physiological and behavioral functions. Ghrelin receptors located within the pituitary (Kojima et al., 1999) and the arcuate nucleus of the hypothalamus (Wren et al., 2000; Mano-Otagiri et al., 2010) play a key role in the release of growth hormone. In the hippocampus, ghrelin receptors may promote long term potentiation (Diano et al., 2006; Banks et al., 2008) and enhance memory consolidation (Carlini et al., 2002; Hansson et al., 2011). Ghrelin receptors have been localized on neurons within the ventral tegmental area (Guan et al., 1997; Abizaid, 2009), which in turn project via the mesolimbic dopamine (DA) pathway to multiple brain regions including the nucleus accumbens (NACc), the amygdala, prefrontal cortex and hippocampus (Fields et al., 2007). Modulation of the mesolimbic dopamine system by ghrelin is likely involved in the capacity of ghrelin to elicit eating and food-related reinforcement (Dickson et al., 2011; Egecioglu et al., 2011; Skibicka and Dickson, 2011) and also plays a key role in the behavioral activating and reward/reinforcement properties of drugs of abuse such as cocaine and nicotine (Jerlhag et al., 2010; Dickson et al., 2011; Wellman et al., 2011, 2012). Finally, ghrelin receptors located on cells of the PVN may play a key role in activation of the hypothalamic-pituitary-adrenal (HPA) axis (Asakawa et al., 2001; Patterson et al., 2010; Cabral et al., 2012), which suggests a role for ghrelin in stress.

Systemic ghrelin can rapidly exert changes in neuronal signaling within the brain. Abizaid reported that systemic infusion of GHR produced synaptic reorganization of neurons within the VTA within 60 min of exposure. VTA cells exposed to ghrelin expressed more excitatory inputs and diminished inhibitory inputs (Abizaid et al., 2006), an outcome similar to that noted in the arcuate nucleus (Cowley et al., 2003; Cowley and Grove, 2004), Systemic infusion of GHR can induce overflow of dopamine in the NACc on a time scale of minutes (Jerlhag et al., 2006a,b; Quarta et al., 2009). These effects of ghrelin on brain neuron function may occur through multiple pathways. One pathway involves a potential activation of GHR-Rs located on afferent fibers of the vagus (Date et al., 2002; Arnold et al., 2006; De Lartigue et al., 2007; Date, 2012) that in turn project to the brainstem. Another potential pathway involves permeation of systemic GHR across the BBB (Banks et al., 2002, 2008; Diano et al., 2006) in a bi-directional fashion (Banks et al., 2002). There is evidence that nutritional state can modulate ghrelin transport into brain with greater transport evident in fasted animals (Banks et al., 2008).

PSYCHOSTIMULANTS AND GHRELIN

Psychostimulant drugs generally induce behavioral arousal, suppress eating, activate the reward system and, at high doses, may induce symptoms of psychosis (Leibowitz, 1975; Wise and Bozarth, 1987; Kalivas, 2000; Wellman et al., 2009). These effects are often attributed to the capacity of these drugs to activate dopamine-secreting neurons in brain, including neurons of the mesolimbic and corticolimbic dopamine systems (Woolverton and Kleven, 1988; Rothman and Baumann, 2003; Vezina, 2004; Wise, 2004; Di Chiara and Bassareo, 2007). GHR-Rs are located on neurons within the VTA (Guan et al., 1997; Naleid et al., 2005; Abizaid et al., 2006; Diano et al., 2006; Zigman et al., 2006; Abizaid, 2009). The VTA, in turn sends projections to multiple regions including the NACc, amygdala, and prefrontal cortex (Fields et al., 2007) and is thus positioned so as to modulate reinforcement to addictive drugs and natural reinforcers that act via modulation of brain dopamine circuits (Jerlhag et al., 2006a; Abizaid, 2009; Perello et al., 2010; Dickson et al., 2011). In the subsequent sections, we consider strategies for induction of hyperghrelinemia and hypoghrelinemia in relation to psychostimulant function.

STRATEGIES FOR MODULATION OF GHRELIN CIRCUITS

GHR acts as a neuromodulator at brain synapses—either increasing or decreasing neuron activity. One strategy to assess GHR function is to produce variation in plasma GHR levels. There are endogenous rhythms of GHR secretion and these can be temporally related to behavior and other motivated states. Feeding, for example, is known to covary with the peaks and troughs of GHR secretion (Cummings et al., 2001). A more common means to induce hyperghrelinemia would involve exogenous administration of ghrelin or of drugs that act as GHR-R agonists. Systemic or central injections of ghrelin or of GHR agonists are known to induce eating as well as to activate brain mesolimbic circuits involved in reinforcement (Abizaid et al., 2006; Jerlhag et al., 2007; Jerlhag, 2008; Dickson et al., 2011; Skibicka and Dickson, 2011). However, a potential difficulty in such manipulations of the GHR system is that GHR-Rs have a degree of constitutive activity—that is, GHR-Rs may exhibit significant biological activity in the absence of ghrelin (Holst et al., 2003, 2004; Chollet et al., 2009; Petersen et al., 2009) which may limit the magnitude of changes induced by GHR administration or manipulation of the GHR-R.

An alternative strategy to determine the importance of GHR signaling for reinforcement involves inactivation of either GHR or GHR-Rs. A variety of GHR inactivation strategies have been developed (Allas and Abribat, 2013) including inhibition of the enzyme (GOAT) that creates ghrelin (Yang et al., 2008; Al Massadi et al., 2011; Davis et al., 2012; Allas and Abribat, 2013). One recent study reported that knockout of GOAT function (Davis et al., 2012), reduced ghrelin levels and resulted in diminished intake of a palatable high-fat diet "dessert," a measure of hedonic eating. Other approaches include immunosuppression of ghrelin (Lu et al., 2009) and RNA silencing (Shrestha et al., 2009). Spiegelmers are enantiomers of natural oligonucleotides that have the capacity to bind a peptide such as ghrelin. Spiegelmers may be resistant to degradation and thus may have a long duration

of action (Kobelt et al., 2006). Ghrelin spiegelmers have been used to block the orexigenic action of systemic ghrelin and to block the induction by ghrelin of Fos within the arcuate nucleus (Kobelt et al., 2006; Becskei et al., 2008). No study to date has used this method to assess the role of ghrelin in reinforcement. Genomic ablation of the protein that codes for ghrelin has also been accomplished in mice (Sun et al., 2003, 2008) which would allow for an assessment of ghrelin signaling in reinforcement.

A key method for blocking the activation of GHR-Rs by ghrelin involves the use of drugs that function as GHR-R antagonists (Asakawa et al., 2003; Halem et al., 2005; Salome et al., 2009a; Jerlhag and Engel, 2011; Allas and Abribat, 2013; Moulin et al., 2013). The impetus to develop pharmacological antagonists of GHR-Rs is, in part, due to the linkage of GHR-R activation to the induction of feeding (Nakazato et al., 2001; Depoortere, 2009), with the hope that GHR-R inactivation might represent a therapeutic approach for the treatment of obesity. One such drug is the triazole derivative JMV 2959, a selective competitive GHR-R antagonist (Moulin et al., 2007; Salome et al., 2009a,b). JMV 2959 binds to GHR-Rs with low nanomolar affinity (Salome et al., 2009a). As would be expected of a GHR-R antagonist, systemic administration of JMV 2959 dose-dependently blocked the feeding response induced by the synthetic GHR agonist hexarelin (Moulin et al., 2007). JMV 2959 thus represents an important tool for the study of the role of GHR-Rs in drug abuse and other functions.

Another GHR-R inactivation strategy involves genomic manipulation (gene knockout), primarily in mice (Sun et al., 2003, 2008). GHR-R null mice exhibit diminished behavioral activation and reinforcement/reward in response to cocaine, amphetamine and nicotine (Jerlhag et al., 2010; Abizaid et al., 2011; Jerlhag and Engel, 2011; Wellman et al., 2011). GHR-R ablation has also been accomplished in rats using N-ethyl-N-nitrosourea (ENU)-driven target-selected mutagenesis (Zan et al., 2003; Till et al., 2007). GHR-R null rats do not overeat in response to systemic injection (i.p.: 15 nmol) of GHR and importantly, these rats exhibit diminished induction of locomotor sensitization (relative to WT rats) when injected daily with 10 mg/kg cocaine HCl (Clifford et al., 2012). As discussed below, such rats exhibit diminished reinforcement to low-level electrical stimulation of the brain (Wellman et al., 2012).

In the subsequent sections, we consider a variety of issues relating to the interplay of ghrelin and of ghrelin receptors for the impact of psychostimulant drugs such as cocaine, amphetamine or nicotine on behavioral activation and reward/reinforcement.

GHRELIN MODULATES THE ACUTE HYPERLOCOMOTOR EFFECTS OF COCAINE

To examine the potential role of ghrelin in psychostimulant action, our preliminary studies focused on the impact of systemic injections of ghrelin on cocaine hyper-locomotion and cocaine-induced conditioned place preference (CPP). The systemic injection route was chosen primarily because the gut is the major source of body ghrelin. In our first study, we examined the impact of 5 nmol ghrelin on cocaine-induced hyperlocomotion (Wellman et al., 2005). The 5 nmol ghrelin dose was chosen, in part, because this dose induced a significant interoceptive cue similar to that produced by food restriction (FR) (Davidson et al., 2005) and secondly because this dose was noted to induce Fos expression in the hypothalamus, but not in the area postrema (Ruter et al., 2003). Male Sprague–Dawley rats were pretreated at −60 min with either 0 (vehicle) or 5 nmol rat ghrelin (i.p.) and then injected (i.p.) at time 0 with 0, 2.5, 5.0, or 10.0 mg/kg cocaine. Locomotor activity was monitored over a 45-min post-cocaine period. Rats received the same ghrelin dose, but a different cocaine dose (in random order) on each of the four drug trials, with each drug trial separated by at least 2 days. Administration of 5 nmol ghrelin-0 mg/kg cocaine slightly increased locomotion relative to that of 0 nmol ghrelin-0 mg/kg cocaine. Cocaine increased locomotion as a function of dose in the 0 nmol ghrelin group, but the effect of cocaine was even greater when preceded by 5 nmol ghrelin. These results indicate that acute injection of ghrelin, at a feeding-relevant dose, augments the acute effects of cocaine on locomotion in rats. A recent study by Jang and colleagues noted a similar outcomes in that injections of ghrelin directly into the NACc enhanced the hyperlocomotor effect of systemic cocaine (15 mg/kg, i.p.), whereas intra-NACc injections of a ghrelin receptor antagonist prevented this effect (Jang et al., 2013). Although GHR-R mRNA is sparse within the NACc, it is detectable and ghrelin infusions into this region can elicit feeding (Naleid et al., 2005; Skibicka et al., 2011a). These studies strongly suggest that acute ghrelin can facilitate the hyperlocomotor effects of a psychostimulant such as cocaine.

GHRELIN INDUCES CROSS-SENSITIZATION OF HYPERLOCOMOTION TO COCAINE

The capacity for repeated administration of a psychostimulant drug to produce behavioral sensitization (i.e., enhanced hyperlocomotion) has been considered as a useful model for understanding the neural underpinnings of drug addiction (Wise and Leeb, 1993; Miller et al., 1999; Davidson et al., 2002; Schoffelmeer et al., 2002; Steketee and Kalivas, 2011). Given that ghrelin alone can induce CPP (Bell et al., 1997) and that a single ghrelin injection can reorganize the inputs of the VTA (Abizaid et al., 2006), we considered the possibility that elevated levels of ghrelin could induce a form of central sensitization in which such treated rats are more reactive to cocaine (Wellman et al., 2008b). Male Sprague–Dawley rats were pretreated daily for 7 days with 0, 5, or 10 nmol rat ghrelin (i.p.) in their home cage. On the 8th day, rats were transported to a testing room, placed in a locomotion chamber for 15 min, and then injected (i.p.) with either 0, 7.5, or 15 mg/kg cocaine with locomotor activity monitored over a 45 min post-cocaine period. Pretreatment with 5 or 10 nmol ghrelin alone did not significantly increase basal locomotion relative to that of the 0 nmol ghrelin group. Rats pretreated with 5 or 10 nmol ghrelin showed an enhanced locomotor response after treatment with 15 mg/kg cocaine relative to rats treated with 0 nmol ghrelin (Wellman et al., 2008b). These results extend our acute locomotion data and suggest that hyperghrelinemia can result in a form of sensitization that facilitates the behavioral activating effects of cocaine.

GHRELIN ENHANCES COCAINE- AND FOOD-INDUCED CPP

CPP is a measure of the hedonic quality of a treatment in which a treatment is paired repeatedly with a non-preferred spatial location (Bardo and Bevins, 2000). After multiple pairings, subjects are noted to spend more time in the initially non-preferred location, an outcome which is taken as an indication of the rewarding property of the treatment. Notably, systemic and central administration of GHR alone can induce CPP (Jerlhag, 2008; Jerlhag et al., 2010) and can enhance the CPP induced by cocaine and by food (Davis et al., 2007; Egecioglu et al., 2010; Perello et al., 2010). In a subsequent study, we determined that systemically administered ghrelin (5 nmol/rat) can enhance the rewarding properties of 0.3125 and 0.625 mg/kg cocaine, as indexed by a CPP procedure (Davis et al., 2007). The observation that systemic ghrelin can facilitate the acquisition of CPP to low doses of cocaine suggests that the facilitatory effect of increased ghrelin level is not limited to locomotion and suggests that the effect is not one of a general non-specific enhancement of locomotion, inasmuch as reward in the CPP paradigm is assessed long after the ghrelin-drug pairing (Bardo and Bevins, 2000; Fields et al., 2007).

The capacity of ghrelin to facilitate reward is not limited to psychostimulant-induced reward. Perello et al. (2010) reported that the rewarding effect of exposure to a high-fat pellet in a specific environment was enhanced by pretreatment with 2 ug/g (∼18 nmol) ghrelin, but this effect was absent in mice pretreated with a GHR-R antagonist. These latter results strongly implicate ghrelin signaling in reward processes.

GHRELIN RECEPTOR ANTAGONISM ATTENUATES PSYCHOSTIMULANT-INDUCED HYPERLOCOMOTION AND REWARD

Our early experiments involving ghrelin and psychostimulants focused on systemic administration of ghrelin, in part, because of a lack of a readily available method of GHR-R antagonism. Subsequently, the ghrelin receptor antagonist JMV 2959, was made available to our research group by Drs. Fehrentz and Martinez (Salome et al., 2009a; Moulin et al., 2013). Pharmacological inactivation of GHR-Rs by JMV 2959 has been noted to attenuate or to ablate the acute hyperlocomotor and CPP properties of amphetamine, cocaine, ethanol, and most recently that of nicotine (Jerlhag et al., 2009, 2010, 2011; Jerlhag and Engel, 2011). These findings indicate that ghrelin receptors exert a permissive function for the activation of dopamine circuits by psychostimulant drugs (and other drugs of abuse).

Of specific interest to us was the potential impact of GHR-R antagonism on the development of sensitized hyper-locomotion to psychostimulants such as cocaine or nicotine. As noted earlier, repeated injections of ghrelin *per se* can produce a degree of cross-sensitization to cocaine (Wellman et al., 2008b). Drug sensitization reflects a form of neuronal plasticity in which repeated drug administration leads to long-lasting increases in behavioral activation and dopamine overflow within the NACc and it is thought that these effects can lead to enhanced drug taking and addiction (Robinson and Berridge, 2003; Vezina, 2004, 2007). As such, manipulations that attenuate drug sensitization might be of value for the conceptualization and perhaps treatment of addiction either to block the acquisition of sensitization or to block the expression of an already established drug sensitization. Psychostimulants act via the mesolimbic pathway from the VTA to NACc to induce behavioral activation and there is data to suggest that the induction of behavioral induction of sensitization most likely involves the VTA rather than the NACc (Cador et al., 1995). Given an emerging literature linking GHR-Rs to the activation of the VTA (Guan et al., 1997; Cowley et al., 2003; Abizaid et al., 2006; Zigman et al., 2006; Dickson et al., 2011; Skibicka et al., 2011a), we sought to determine the impact of pharmacological blockade of GHR-Rs using the GHR-R antagonist JMV 2959 (Moulin et al., 2007; Salome et al., 2009b) on the development of locomotor sensitization induced by repeated exposure to nicotine (Wellman et al., 2011) as well as to cocaine (Clifford et al., 2012).

In the nicotine study (Wellman et al., 2011), rats were treated daily for 7 days with 0.4 mg/kg nicotine (s.c), a dose sufficient to result in behavioral sensitization. Half of the rats were pretreated each day with either vehicle or 3 mg/kg JMV 2959. Whereas rats pretreated with vehicle and treated with nicotine developed a robust hyperlocomotor sensitization (**Figure 1A**), pretreatment with JMV 2959 abolished the development of nicotine sensitization (**Figure 1B**). The impact of JMV on nicotine sensitization is unlikely to be an artifact of the capacity of JMV 2959 to reduce baseline locomotion. At this dose, there were no baseline differences attributable to JMV 2959 (compare the vehicle-vehicle group in **Figure 1A** with the JMV 3-vehicle group in **Figure 1B**). In our cocaine study (Clifford et al., 2012), daily pretreatment with JMV 2959 attenuated the development of locomotor sensitization to 10 mg/kg cocaine, but the magnitude of this effect was less than that noted for nicotine. It should be noted that Abizaid and colleagues have shown that genetic ablation of ghrelin diminished the development of locomotor sensitization of cocaine-induced hyperlocomotion in mice (Abizaid et al., 2011), an outcome which further supports our view that ghrelin and ghrelin receptors may play a permissive role for the induction of psychostimulant-induced locomotor sensitization.

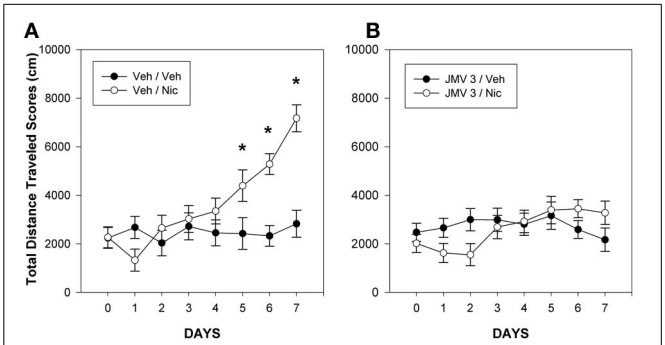

FIGURE 1 | Mean group total changes in total distance traveled scores (cm/45 min). On day 0, the rats were injected with Veh at −5 min prior to the 15 min baseline period and then again with Veh just prior to the 45 min test period. During days 1–7, the rats were injected with either Veh, (panel **A**) or 3 mg/kg JMV 2959 (JMV 3: panel **B**) at −5 min prior to the 15 min baseline period and then injected with either vehicle or 0.4 mg/kg nicotine (Nic) just prior to the 45 min test period on days 1–7. The lines above and below each symbol represent the SEM. Figure reprinted with permission from Regulatory Peptides (Wellman et al., 2011). $*p < 0.05$.

Although the aforementioned findings suggest a permissive role for GHR-Rs for the induction of psychostimulant-induced locomotor sensitization, no study to date has assessed whether antagonism of GHR-Rs would diminish the expression of an already established nicotine locomotor sensitization. In our JMV 2959-nicotine study, JMV 2959 was used throughout the course of nicotine exposure (Wellman et al., 2011). What is needed is an experiment in which nicotine sensitization is produced and then animals are treated with either vehicle or JMV 2959. If JMV 2959 has the capacity to block expression as well as induction of sensitization, this drug may be of value for the treatment of smoking and perhaps other drugs of addiction (Kim et al., 2011).

DOES GHRELIN CONTRIBUTE TO THE CAPACITY OF FOOD RESTRICTION TO ENHANCE PSYCHOSTIMULANT ACTION?

FR can increase the rate of acquisition of learned responses for many reinforcers, including food as well as the psychostimulant drugs cocaine or amphetamine (Carroll and Meisch, 1981; Carroll, 1985; Carr, 2002). A link between caloric homeostasis and psychostimulant action in rats is further supported by studies in which FR augments the capacity of psychostimulants to enhance locomotion and to induce CPP (Bell et al., 1997; Zheng et al., 2012). FR also augments the rewarding effects of intracranial self-stimulation of the lateral hypothalamus (LH-ICSS) (Cabeza De Vaca et al., 1998; Fulton et al., 2000); a model system used to explore mechanisms that modulate reinforcement function in brain (Olds and Milner, 1954; Wise, 1996). FR is routinely used by addiction researchers employing intravenous self-administration paradigms to enhance psychostimulant-based reinforcement (Carroll et al., 1979; Carroll and Meisch, 1980, 1981; Carroll, 1985; Comer et al., 1995). Prior studies suggested that signals related to the acute availability of metabolic fuels (e.g., glucose, free fatty acids) are unlikely to wholly account for FR-associated changes in psychostimulant action, inasmuch as short-term glucoprivation or lipoprivation does not alter LH-ICSS in FR rats (Cabeza De Vaca et al., 1998; Fulton et al., 2000; Carr, 2002). Prolonged negative energy balance results in increased expression of neuropeptide Y (NPY) in the hypothalamus; however, administration of NPY does not alter LH-ICSS (Cabeza De Vaca et al., 1998). Thus, the mechanism by which FR enhances psychostimulant effects has remained elusive.

A variety of studies suggest that the orexigenic peptide ghrelin may be the substrate through which FR acts to enhance psychostimulant action. Human plasma ghrelin levels are at a nadir after a meal and then peak prior to the next meal (Cummings et al., 2001). Plasma ghrelin levels increase during periods of FR, and decrease after eating (Toshinai et al., 2001). Importantly, Davidson and colleagues (Davidson et al., 2005) noted that a systemic infusion of ghrelin can generate an interoceptive food cue that resembles that induced by food deprivation (for example, 6 nmol GHR was noted to be comparable to the cue induced by 24 h food deprivation). Collectively, these data suggested that changes in peripheral ghrelin levels occasioned by FR could result in changes in dopamine signaling in brain reinforcement systems that in turn may enhance psychostimulant action. Indeed, our early studies which showed facilitation by ghrelin of acute locomotion to cocaine (Wellman et al., 2005) and enhanced CPP to cocaine (Davis et al., 2007) were generally consistent with the notion that FR may act on a ghrelin-dependent substrate to facilitate psychostimulant action.

To further examine the interaction of FR, ghrelin and psychostimulant function, we examined the capacity of FR to enhance cocaine-induced hyper-locomotion in wild-type (WT) mice vs. GHR-null or GHR-R null mice (Clifford et al., 2011). WT, GHR null, and GHR-R null mice were either restricted to 60% of baseline caloric intake (FR) or allowed to free-feed (FF). Mice were treated with 0, 1.25, 2.5, and 5.0 mg/kg cocaine on separate test days (in random dose order) and forward locomotion was recorded on each drug day for 45 min after drug dosing. FR increased locomotion scores in vehicle treated WT mice, but these increases were diminished in GHR-R and GHR null mice. The doses of cocaine chosen in this study were low (0–5 mg/kg) and did not increase locomotion in FF mice, but produced similar and significant increases in FR mice irrespective of ghrelin status. These preliminary results did not support the contention that GHR pathways are required for the capacity of FR to augment the acute effect of cocaine on locomotion.

Zheng and colleagues conducted psychostimulant testing in the ICSS paradigm during preprandial and postprandial periods in rats fed a single meal per day that represented 40% of their normal daily food intake (Zheng et al., 2013). In such a situation, ghrelin levels are entrained to the feeding period such that ghrelin levels are high during the preprandial period, but low during the postprandial period. Rats received vehicle or amphetamine microinjections into the NACc shell region. All rats were food restricted, but some were tested prior to a daily meal and others tested after that meal. Amphetamine injections shifted the ICSS rate-frequency curve to the left (an outcome indicating enhanced reinforcement) but this effect did not vary by time of feeding. These data suggest that endogenous variation of ghrelin levels of the magnitude noted under these testing conditions was not sufficient to modify psychostimulant reactivity in an ICSS paradigm.

The relatively few studies that have examined the notion that FR may act through a ghrelin-dependent substrate to modify reinforcement are inconclusive. A firm conclusion as to whether ghrelin plays a causal role in enhancing psychostimulant function requires additional study. A key issue is whether conditional GHR-R knockouts would exert an effect on FR activation of psychostimulant locomotion, as opposed to conventional knockouts for which there may be compensations that develop over time. Another issue is the relatively few methods employed to diminish GHR-R function. Additional studies should be conducted using other methods of GHR-R antagonism such as pretreatment with JMV 2959 or de-acylation of GOAT, as specific examples. Additional endpoints (other than locomotion) need to be assessed including CCP as well as intravenous self-administration of cocaine or nicotine. A final issue of concern is the fact that GHR-Rs exhibit a degree of constitutive activity and thus may modulate drug reinforcement, independent of FR and/or ghrelin levels (Holst et al., 2003; Petersen et al., 2009; Steketee and Kalivas, 2011).

STRESS, GHRELIN, AND FOOD RESTRICTION INTERACTIONS WITH PSYCHOSTIMULANTS

Another issue regarding FR and enhancement of psychostimulant function is that ghrelin and GHR-Rs may play an indirect role via activation of the HPA axis. Stressors result in increased CRF secretion from the PVN, secretion of ACTH and enhanced levels of plasma glucocorticoids (GC) such as cortisol (Patterson et al., 2010; Cabral et al., 2012). FR is well-known to enhance circulating levels of ghrelin (Stengel et al., 2011). Interestingly, various forms of psychosocial stress can increase circulating ghrelin levels in rodents (Asakawa et al., 2001; Patterson et al., 2010) as well as humans (Rouach et al., 2007). Moreover, it appears that ghrelin can activate the HPA axis, depending on dose and route of administration. Doses of ghrelin that induce eating (30 nmol) may not alter plasma corticosterone levels when given systemically (Wren et al., 2002). ICV infusion of ghrelin, however, elicits increased plasma ACTH and corticosterone levels (Wren et al., 2002; Stevanovic et al., 2007). Systemic ghrelin (∼18 nmol) indirectly activates hypothalamic CRF neurons in mice (Cabral et al., 2012). Another linkage between stress and GHR-R is that GHR-R null mice show diminished HPA response to acute restraint stress (Spencer et al., 2012). In a related vein, stress and enhanced GC signaling has been found to facilitate psychostimulant function. Stress can enhance the magnitude of locomotor activation induced by psychostimulant drugs (Piazza and Le Moal, 1996) and can modify the acquisition (Campbell and Carroll, 2000), maintenance (Carroll, 1985) and reinstatement (De Wit and Stewart, 1981; Carroll, 1985) of self-administration patterns of cocaine in rats (Goeders, 2002, 2003) as does FR (Carroll et al., 1979; Carroll, 1985; Bell et al., 1997). In contrast, blockade of corticosterone signaling can diminish the psychoactive effect of cocaine (Piazza and Le Moal, 1996; Piazza et al., 1996; Marinelli et al., 1997a,b).

These aforementioned studies raise the possibility that FR may act indirectly by increasing GHR signaling which in turn acts at the level of the hypothalamus to enhance the HPA axis modulation of psychostimulant function, perhaps at the level of the VTA (Piazza and Le Moal, 1996; Piazza et al., 1996; Goeders, 2002, 2003). Few studies, however, are available by which to evaluate this hypothesis. One negative finding is that generated by Tessari et al. (2007) in which circulating corticosterone levels were not positively related to the reinstatement of responding for intravenous cocaine in rats. In this same study, plasma levels of ghrelin were related to reinstatement of responding for cocaine. Another negative finding is that acute changes in corticosterone availability does not alter the capacity of FR to sensitize LH-ICSS patterns (Abrahamsen et al., 1995). The same laboratory, however, has generated parallel data in which variation of ghrelin levels does not alter ICSS patterns (Zheng et al., 2013).

Additional experiments are needed to evaluate the potential interaction of stress, GHR signaling and psychostimulant-based reinforcement. A key issue is the need to examine the impact of GHR-R antagonists on self-administration end-points for cocaine and for nicotine. This would include studies of maintenance levels of drug self-administration as well as reinstatement of self-administration (Carroll, 1985; Goeders, 2002, 2003).

ROLE OF GHRELIN RECEPTORS IN ICSS AND DRUG SELF-ADMINISTRATION

Although psychostimulant-induced locomotor sensitization is thought to contribute to the development of addiction, locomotion is but a proxy for the processes that underlie reinforcement. A number of behavioral paradigms provide models of reinforcement including intracranial self-stimulation (ICSS) (Olds and Milner, 1954; Stein, 1964) and intravenous drug self-administration (IVSA) (Brady and Griffiths, 1976; Carroll and Meisch, 1981; De Wit and Stewart, 1981). In the following sections, we consider published studies in which agonism/antagonism of GHR-Rs alters either ICSS rate-frequency profiles or alters IVSA responding for cocaine and for heroin.

Ablation of the GHR-R in rats has been accomplished using N-ethyl-N-nitrosourea (ENU)-driven target-selected mutagenesis (Zan et al., 2003; Till et al., 2007). GHR-R null rats do not overeat in response to systemic injection (i.p.: 15 nmol) of GHR and importantly, these rats exhibit diminished induction of locomotor sensitization (relative to WT rats) when injected daily with 10 mg/kg cocaine (Clifford et al., 2011). A similar attenuation has been noted in ghrelin null mice (Abizaid et al., 2011). Thus, the lack of GHR or of GHR-Rs compromises the ability of a psychostimulant such as cocaine to induce hyperlocomotor sensitization. To address the issue of whether GHR-null rats exhibit diminished brain reinforcement, we examined the reinforcing action of ICSS in WT and GHR-R null rats. ICSS is a model of brain reinforcement in which animals can press a lever in order to deliver a series of constant-current pulses via an electrode implanted into brain reinforcement circuits (Wise, 1996; Kenny et al., 2003; Carlezon and Chartoff, 2007; Wellman et al., 2008a). Adult male WT and GHR-R null rats were implanted with a single electrode aimed at the LH and then trained, after recovery, to lever-press for ICSS (Wellman et al., 2012). In the rate-frequency procedure, ICSS stimulation intensity is set at a minimum-to-moderate value (75–120 uA) and responding is then recorded as stimulation frequency is reduced in 0.05 log steps from 128 Hz in each minute over 15 min (Carlezon and Chartoff, 2007; Zheng et al., 2013). Responding usually decreases with lower stimulation frequencies. In this study, WT littermates showed normal acquisition of ICSS responding at an intensity of ∼70 uA. The lab technician running these rats during acquisition reported that the GHR-R null rats failed to acquire until current intensity was raised 4-fold to 300 uA (see **Figure 2** from Wellman et al., 2012). When current intensity was dropped back to 75–100 uA, the GHR-R null did not respond at any stimulation frequency. These results indicate a permissive function for GHR-Rs in ICSS-based reinforcement and generally support the proposition of ghrelin involvement in central reinforcement.

There are multiple papers that demonstrate that GHR-R inactivation attenuates the CPP induced by cocaine, amphetamine, nicotine, and ethanol (Jerlhag et al., 2009, 2010, 2011; Dickson et al., 2010; Jerlhag and Engel, 2011) as well as by food and by novelty (Egecioglu et al., 2010; Hansson et al., 2012). Few studies, however, have more directly examined the role of GHR-Rs in drug abuse using the IVSA method. In this procedure, rats are trained to lever-press to deliver a drug bolus into their venous system. One variation of the IVSA method involves assessment of the

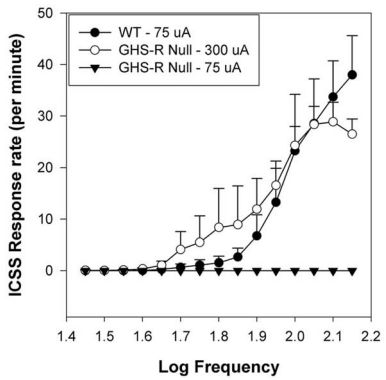

FIGURE 2 | Mean group Intracranial Self-Stimulation (ICSS) rate-frequency scores for wild-type (WT: $n = 5$) and GHR-R null rats ($n = 6$) as a function of stimulation intensity. WT rats tested at 75 uA and GHR-R null rats tested at 300 uA exhibit overlapping rate-frequency curves. When GHR-R null rats were tested at 75 uA, their rate-frequency curves dropped to 0. Figure reprinted with permission from Addiction Biology (Wellman et al., 2012).

impact of a particular pretreatment on responding for a particular dose of a drug such as cocaine or nicotine (Carroll, 1985). Tessari et al. (2007) reported that circulating GHR levels (but not plasma levels of corticosterone) were positively related to the reinstatement of responding for intravenous cocaine in rats. This study indicates that augmenting GHR levels can facilitate the reinforcing actions of cocaine. An implication of this study is that high plasma ghrelin levels may predispose to relapse in persons formerly addicted to cocaine. A similar effect was noted in a report from Shalev's group in which intracerebroventricular infusions of ghrelin (0.0, 1.5, and 3.0 ug/rat) produced increases in breakpoints on a progressive ratio schedule of heroin reinforcement (Maric et al., 2011).

Another variation of the IVSA method is to examine the potential impact of GHR-R antagonist on reinstatement of previously extinguished drug responding (De Wit and Stewart, 1981; Carroll, 1985). To date, no published study has considered the impact of pretreatment with a GHR-R antagonist on maintenance patterns of responding for cocaine, nicotine or other psychostimulants. With regard to opiate drug self-administration, central administration of the ghrelin receptor antagonist, [D-Lys-3]-GHRP-6 (0.0 or 20.0 ug/rat) had no effect on ongoing heroin self-administration and did not alter reinstatement of heroin responding. To date, this latter work is the only published report to examine the interaction of ghrelin systems and opiate reinforcement. Additional studies of IVSA for cocaine, amphetamine, nicotine and heroin are in order using GHR agonists as well as multiple GHR-R antagonist methods.

CONCLUSIONS

The current literature strongly supports a key role for GHR-Rs within the VTA for the induction of reinforcement for multiple classes of reinforcers including food and perhaps other natural rewards, drugs of abuse and novelty (Jerlhag et al., 2009, 2010; Perello et al., 2010; Dickson et al., 2011; Egecioglu et al., 2011; Jerlhag and Engel, 2011; Skibicka and Dickson, 2011; Hansson et al., 2012; Wellman et al., 2012). The prominent role of GHR-Rs in reinforcement suggests that therapeutic interventions that target these receptors may be of value. Of particular interest for our laboratory is the extent to which GHR-R modulation may offer a valuable route for the treatment of smoking addiction. This disorder remains intractable with few effective therapies available (Laniado-Laborin, 2010). Acute nicotine induces hyperlocomotion, CPP and overflow of DA within the NACc (Jerlhag and Engel, 2011). GHR-Rs are located on cholinergic afferents arising from the laterodorsal tegmental area (LDTg) that project to the VTA, which in turn activate dopamine overflow within the NACc (Jerlhag et al., 2006a). Intra-LDTg infusion of ghrelin enhances locomotion and induces NACc dopamine release (Jerlhag et al., 2007). Within the VTA, GHR-Rs and cholinergic receptors are located on DA neurons that project to the VTA (Dickson et al., 2011: cf. Figure 1, page 82)—thus nicotine may directly alter activity of VTA neurons. In contrast, acute pharmacological blockade of GHR-Rs blocks nicotine-induced locomotion, the induction of CPP by nicotine, and dopamine overflow within the NAcc (Jerlhag and Engel, 2011). Our preliminary behavioral studies (Wellman et al., 2011, 2012) indicate that a drug such as JMV 2959 may offer a route to block nicotine-induced reinforcement, which would be of value for smoking cessation. Additional studies are required to confirm these observations and to demonstrate that GHR-R antagonists can promote smoking abstinence. It is likely that modulation of GHR-Rs may be of use for other drugs of abuse, such as cocaine, alcohol and heroin (Jerlhag et al., 2009, 2010; Maric et al., 2011).

It seems likely that ghrelin acts via multiple circuits to elicit eating (Cowley and Grove, 2004; Naleid et al., 2005; Dickson et al., 2011). A dense concentration of ghrelin receptor protein is localized within the arcuate hypothalamus (Harrold et al., 2008). Ghrelin infusion into the ARC can trigger the release of feeding-relevant peptides and neurotransmitters, which in turn influence food intake and play a role in controlling energy homeostasis (Bagnasco et al., 2003; Cowley et al., 2003; Sato et al., 2005). Ghrelin can alter arcuate nucleus inputs by augmenting NPY signaling, which stimulates food intake, and diminishing proopiomelanocortin (POMC) signaling, which plays a role in the induction of satiety (Cowley et al., 2003). Ghrelin neurons project from the hypothalamus to the brainstem—these fibers likely interact with the cells of the dorsal vagal complex that can also modulate eating (Hou et al., 2006; Hori et al., 2008). In addition to the arcuate nucleus, acute and chronic injections of ghrelin into the VTA as well as the NACc can induce eating (Naleid et al., 2005; King et al., 2011; Skibicka and Dickson, 2011; Skibicka et al., 2011a). Additionally, VTA neurons receive inputs from orexin neurons in the LH, which are also sensitive to ghrelin. What is unknown at the present time is whether these are truly independent circuits or whether these reflect interconnections between these circuits (i.e., involving arcuate or lateral hypothalamic to VTA projections) or interconnections between the NACc and the hypothalamus (Kelley et al., 2005; Dickson et al., 2011). The rewarding effects of palatable food consumption appears to be ghrelin-dependent for both sweets and fats (Egecioglu et al., 2010; Perello et al., 2010; Skibicka and Dickson, 2011; Skibicka et al., 2011b). The involvement of ghrelin for palatable food reward is

fascinating since this suggests that antagonism of GHR-Rs may be of value in helping persons to control their overconsumption of palatable foods, which would be of assistance for the treatment of being overweight. Another therapeutic issue for the use of GHR-R antagonists follows from the observation that persons smoke for multiple reasons. Some persons smoke because they enjoy the reinforcement elicited by nicotine but another factor is that smoking can allow people to overeat fat, calories and alcohol (Dallongeville et al., 1998) all while weighing less than normal (Klesges et al., 1989; Zoli and Picciotto, 2012). Cessation of smoking can result in weight gain which can be viewed as unacceptable and therefore diminish treatment compliance (Pomerleau and Saules, 2007). To date, we have few effective interventions by which to limit smoking cessation weight gain (Farley et al., 2012). GHR-R drug antagonists may therefore have a dual utility for the treatment of nicotine addiction: the first being antagonism of the rewarding action of nicotine; the second being obviation of the weight gain often noted following smoking cessation.

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REFERENCES

Abizaid, A. (2009). Ghrelin and dopamine: new insights on the peripheral regulation of appetite. *J. Neuroendocrinol.* 21, 787–793. doi: 10.1111/j.1365-2826.2009.01896.x

Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., et al. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J. Clin. Invest.* 116, 3229–3239. doi: 10.1172/JCI29867

Abizaid, A., Mineur, Y. S., Roth, R. H., Elsworth, J. D., Sleeman, M. W., Picciotto, M. R., et al. (2011). Reduced locomotor responses to cocaine in ghrelin-deficient mice. *Neuroscience* 192, 500–506. doi: 10.1016/j.neuroscience.2011.06.001

Abrahamsen, G. C., Berman, Y., and Carr, K. D. (1995). Curve-shift analysis of self-stimulation in food-restricted rats: relationship between daily meal, plasma corticosterone and reward sensitization. *Brain Res.* 695, 186–194. doi: 10.1016/0006-8993(95)00764-H

Al Massadi, O., Tschop, M. H., and Tong, J. (2011). Ghrelin acylation and metabolic control. *Peptides* 32, 2301–2308. doi: 10.1016/j.peptides.2011.08.020

Allas, S., and Abribat, T. (2013). Clinical perspectives for ghrelin-derived therapeutic products. *Endocr. Dev.* 25, 157–166. doi: 10.1159/000346065

Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., et al. (2001). Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J. Clin. Endocrinol. Metab.* 86, 4753–4758. doi: 10.1210/jc.86.10.4753

Arnold, M., Mura, A., Langhans, W., and Geary, N. (2006). Gut vagal afferents are not necessary for the eating-stimulatory effect of intraperitoneally injected ghrelin in the rat. *J. Neurosci.* 26, 11052–11060. doi: 10.1523/JNEUROSCI.2606-06.2006

Asakawa, A., Inui, A., Fujimiya, M., Sakamaki, R., Shinfuku, N., Ueta, Y., et al. (2005). Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut* 54, 18–24. doi: 10.1136/gut.2004.038737

Asakawa, A., Inui, A., Kaga, T., Katsuura, G., Fujimiya, M., Fujino, M. A., et al. (2003). Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut* 52, 947–952. doi: 10.1136/gut.52.7.947

Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Fujimiya, M., et al. (2001). A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. *Neuroendocrinology* 74, 143–147. doi: 10.1159/000054680

Bagnasco, M., Tulipano, G., Melis, M. R., Argiolas, A., Cocchi, D., and Muller, E. E. (2003). Endogenous ghrelin is an orexigenic peptide acting in the arcuate nucleus in response to fasting. *Regul. Pept.* 111, 161–167. doi: 10.1016/S0167-0115(02)00283-5

Banks, W. A., Burney, B. O., and Robinson, S. M. (2008). Effects of triglycerides, obesity, and starvation on ghrelin transport across the blood-brain barrier. *Peptides* 29, 2061–2065. doi: 10.1016/j.peptides.2008.07.001

Banks, W. A., Tschop, M., Robinson, S. M., and Heiman, M. L. (2002). Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J. Pharmacol. Exp. Ther.* 302, 822–827. doi: 10.1124/jpet.102.034827

Bardo, M. T., and Bevins, R. A. (2000). Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology* 153, 31–43. doi: 10.1007/s002130000569

Becskei, C., Bilik, K. U., Klussmann, S., Jarosch, F., Lutz, T. A., and Riediger, T. (2008). The anti-ghrelin Spiegelmer NOX-B11-13 blocks ghrelin- but not fasting-induced neuronal activation in the hypothalamic arcuate nucleus. *J. Neuroendocrinol.* 20, 85–92.

Bell, S. M., Stewart, R. B., Thompson, S. C., and Meisch, R. A. (1997). Food-deprivation increases cocaine-induced conditioned place preference and locomotor activity in rats. *Psychopharmacology* 131, 1–8. doi: 10.1007/s002130050258

Brady, J. V., and Griffiths, R. R. (1976). Behavioral procedures for evaluating the relative abuse potential of CNS drugs in primate. *Fed. Proc.* 35, 2245–2253.

Bray, G. A. (2000). A concise review on the therapeutics of obesity. *Nutrition* 16, 953–960. doi: 10.1016/S0899-9007(00)00424-X

Cabeza De Vaca, S., Holiman, S., and Carr, K. D. (1998). A search for the metabolic signal that sensitizes lateral hypothalamic self-stimulation in food-restricted rats. *Physiol. Behav.* 64, 251–260. doi: 10.1016/S0031-9384(98)00050-X

Cabral, A., Suescun, O., Zigman, J. M., and Perello, M. (2012). Ghrelin indirectly activates hypophysiotropic CRF neurons in rodents. *PLoS ONE* 7:e31462. doi: 10.1371/journal.pone.0031462

Cador, M., Bjijou, Y., and Stinus, L. (1995). Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine. *Neuroscience* 65, 385–395. doi: 10.1016/0306-4522(94)00524-9

Campbell, U. C., and Carroll, M. E. (2000). Acquisition of drug self-administration: environmental and pharmacological interventions. *Exp. Clin. Psychopharmacol.* 8, 312–325. doi: 10.1037/1064-1297.8.3.312

Carlezon, W. A. Jr., and Chartoff, E. H. (2007). Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. *Nat. Protoc.* 2, 2987–2995. doi: 10.1038/nprot.2007.441

Carlini, V. P., Monzon, M. E., Varas, M. M., Cragnolini, A. B., Schioth, H. B., Scimonelli, T. N., et al. (2002). Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochem. Biophys. Res. Commun.* 299, 739–743. doi: 10.1016/S0006-291X(02)02740-7

Carr, K. D. (2002). Augmentation of drug reward by chronic food restriction: behavioral evidence and underlying mechanisms. *Physiol. Behav.* 76, 353–364. doi: 10.1016/S0031-9384(02)00759-X

Carroll, M. E. (1985). The role of food deprivation in the maintenance and reinstatement of cocaine-seeking behavior in rats. *Drug Alcohol Depend.* 16, 95–109. doi: 10.1016/0376-8716(85)90109-7

Carroll, M. E., France, C. P., and Meisch, R. A. (1979). Food deprivation increases oral and intravenous drug intake in rats. *Science* 205, 319–321. doi: 10.1126/science.36665

Carroll, M. E., and Meisch, R. A. (1980). The effects of feeding conditions on drug-reinforced behavior: maintenance at reduced body weight versus availability of food. *Psychopharmacology* 68, 121–124. doi: 10.1007/BF00432128

Carroll, M. E., and Meisch, R. A. (1981). Determinants of increased drug self-administration due to food deprivation. *Psychopharmacology* 74, 197–200. doi: 10.1007/BF00427092

Chollet, C., Meyer, K., and Beck-Sickinger, A. G. (2009). Ghrelin-a novel generation of anti-obesity drug: design, pharmacomodulation and biological activity of ghrelin analogues. *J. Pept. Sci.* 15, 711–730. doi: 10.1002/psc.1177

Clifford, P. S., Rodriguez, J., Schul, D., Hughes, S., Kniffin, T., Hart, N., et al. (2012). Attenuation of cocaine-induced locomotor sensitization in rats sustaining genetic or pharmacologic antagonism of ghrelin receptors. *Addict. Biol.* 17, 956–963. doi: 10.1111/j.1369-1600.2011.00339.x

Clifford, S., Zeckler, R. A., Buckman, S., Thompson, J., Hart, N., Wellman, P. J., et al. (2011). Impact of food restriction and cocaine on locomotion in ghrelin- and ghrelin-receptor knockout mice. *Addict. Biol.* 16, 386–392. doi: 10.1111/j.1369-1600.2010.00253.x

Comer, S. D., Lac, S. T., Wyvell, C. L., Curtis, L. K., and Carroll, M. E. (1995). Food deprivation affects extinction and reinstatement of responding in rats. *Psychopharmacology (Berl.)* 121, 150–157. doi: 10.1007/BF02245624

Cowley, M. A., and Grove, K. L. (2004). Ghrelin–satisfying a hunger for the mechanism. *Endocrinology* 145, 2604–2606. doi: 10.1210/en.2004-0346

Cowley, M. A., Smith, R. G., Diano, S., Tschop, M., Pronchuk, N., Grove, K. L., et al. (2003). The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37, 649–661. doi: 10.1016/S0896-6273(03)00063-1

Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., and Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50, 1714–1719. doi: 10.2337/diabetes.50.8.1714

Dallongeville, J., Marecaux, N., Fruchart, J. C., and Amouyel, P. (1998). Cigarette smoking is associated with unhealthy patterns of nutrient intake: a meta-analysis. *J. Nutr.* 128, 1450–1457.

Date, Y. (2012). Ghrelin and the vagus nerve. *Meth. Enzymol.* 514, 261–269. doi: 10.1016/B978-0-12-381272-8.00016-7

Date, Y., Murakami, N., Toshinai, K., Matsukura, S., Niijima, A., Matsuo, H., et al. (2002). The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 123, 1120–1128. doi: 10.1053/gast.2002.35954

Davidson, C., Lazarus, C., Lee, T. H., and Ellinwood, E. H. (2002). Behavioral sensitization is greater after repeated versus single chronic cocaine dosing regimens. *Eur. J. Pharmacol.* 441, 75–78. doi: 10.1016/S0014-2999(02)01437-1

Davidson, T. L., Kanoski, S. E., Tracy, A. L., Walls, E. K., Clegg, D., and Benoit, S. C. (2005). The interoceptive cue properties of ghrelin generalize to cues produced by food deprivation. *Peptides* 26, 1602–1610. doi: 10.1016/j.peptides.2005.02.014

Davis, J. F., Perello, M., Choi, D. L., Magrisso, I. J., Kirchner, H., Pfluger, P. T., et al. (2012). GOAT induced ghrelin acylation regulates hedonic feeding. *Horm. Behav.* 62, 598–604. doi: 10.1016/j.yhbeh.2012.08.009

Davis, K. W., Wellman, P. J., and Clifford, P. S. (2007). Augmented cocaine conditioned place preference in rats pretreated with systemic ghrelin. *Regul. Pept.* 140, 148–152. doi: 10.1016/j.regpep.2006.12.003

De Lartigue, G., Dimaline, R., Varro, A., and Dockray, G. J. (2007). Cocaine- and amphetamine-regulated transcript: stimulation of expression in rat vagal afferent neurons by cholecystokinin and suppression by ghrelin. *J. Neurosci.* 27, 2876–2882. doi: 10.1523/JNEUROSCI.5508-06.2007

Delhanty, P. J., Neggers, S. J., and Van Der Lely, A. J. (2012). Mechanisms in endocrinology: Ghrelin: the differences between acyl- and des-acyl ghrelin. *Eur. J. Endocrinol.* 167, 601–608. doi: 10.1530/EJE-12-0456

Depoortere, I. (2009). Targeting the ghrelin receptor to regulate food intake. *Regul. Pept.* 156, 13–23. doi: 10.1016/j.regpep.2009.04.002

De Wit, H., and Stewart, J. (1981). Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology (Berl.)* 75, 134–143. doi: 10.1007/BF00432175

Diano, S., Farr, S. A., Benoit, S. C., Mcnay, E. C., Da Silva, I., Horvath, B., et al. (2006). Ghrelin controls hippocampal spine synapse density and memory performance. *Nat. Neurosci.* 9, 381–388. doi: 10.1038/nn1656

Di Chiara, G., and Bassareo, V. (2007). Reward system and addiction: what dopamine does and doesn't do. *Curr. Opin. Pharmacol.* 7, 69–76. doi: 10.1016/j.coph.2006.11.003

Dickson, S. L., Egecioglu, E., Landgren, S., Skibicka, K. P., Engel, J. A., and Jerlhag, E. (2011). The role of the central ghrelin system in reward from food and chemical drugs. *Mol. Cell. Endocrinol.* 340, 80–87. doi: 10.1016/j.mce.2011.02.017

Dickson, S. L., Hrabovszky, E., Hansson, C., Jerlhag, E., Alvarez-Crespo, M., Skibicka, K. P., et al. (2010). Blockade of central nicotine acetylcholine receptor signaling attenuate ghrelin-induced food intake in rodents. *Neuroscience* 171, 1180–1186. doi: 10.1016/j.neuroscience.2010.10.005

Egecioglu, E., Jerlhag, E., Salome, N., Skibicka, K. P., Haage, D., Bohlooly, Y. M., et al. (2010). Ghrelin increases intake of rewarding food in rodents. *Addict. Biol.* 15, 304–311. doi: 10.1111/j.1369-1600.2010.00216.x

Egecioglu, E., Skibicka, K. P., Hansson, C., Alvarez-Crespo, M., Friberg, P. A., Jerlhag, E., et al. (2011). Hedonic and incentive signals for body weight control. *Rev. Endocr. Metab. Disord.* 12, 141–151. doi: 10.1007/s11154-011-9166-4

Fakhouri, T. H., Ogden, C. L., Carroll, M. D., Kit, B. K., and Flegal, K. M. (2012). Prevalence of obesity among older adults in the United States, 2007–2010. *NCHS Data Brief* 106, 1–8.

Farley, A. C., Hajek, P., Lycett, D., and Aveyard, P. (2012). Interventions for preventing weight gain after smoking cessation. *Cochrane Database Syst. Rev.* 1:CD006219. doi: 10.1002/14651858.CD006219.pub3

Ferrini, F., Salio, C., Lossi, L., and Merighi, A. (2009). Ghrelin in central neurons. *Curr. Neuropharmacol.* 7, 37–49. doi: 10.2174/157015909787602779

Fields, H. L., Hjelmstad, G. O., Margolis, E. B., and Nicola, S. M. (2007). Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. *Annu. Rev. Neurosci.* 30, 289–316. doi: 10.1146/annurev.neuro.30.051606.094341

Flegal, K. M., Carroll, M. D., Kit, B. K., and Ogden, C. L. (2012). Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010. *JAMA* 307, 491–497. doi: 10.1001/jama.2012.39

Fulton, S., Woodside, B., and Shizgal, P. (2000). Modulation of brain reward circuitry by leptin. *Science* 287, 125–128. doi: 10.1126/science.287.5450.125

Goeders, N. E. (2002). The HPA axis and cocaine reinforcement. *Psychoneuroendocrinology* 27, 13–33. doi: 10.1016/S0306-4530(01)00034-8

Goeders, N. E. (2003). The impact of stress on addiction. *Eur. Neuropsychopharmacol.* 13, 435–441. doi: 10.1016/j.euroneuro.2003.08.004

Guan, X. M., Yu, H., Palyha, O. C., Mckee, K. K., Feighner, S. D., Sirinathsinghji, D. J., et al. (1997). Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res. Mol. Brain Res.* 48, 23–29. doi: 10.1016/S0169-328X(97)00071-5

Halem, H. A., Taylor, J. E., Dong, J. Z., Shen, Y., Datta, R., Abizaid, A., et al. (2005). A novel growth hormone secretagogue-1a receptor antagonist that blocks ghrelin-induced growth hormone secretion but induces increased body weight gain. *Neuroendocrinology* 81, 339–349. doi: 10.1159/000088796

Hansson, C., Haage, D., Taube, M., Egecioglu, E., Salome, N., and Dickson, S. L. (2011). Central administration of ghrelin alters emotional responses in rats: behavioural, electrophysiological and molecular evidence. *Neuroscience* 180, 201–211. doi: 10.1016/j.neuroscience.2011.02.002

Hansson, C., Shirazi, R. H., Naslund, J., Vogel, H., Neuber, C., Holm, G., et al. (2012). Ghrelin influences novelty seeking behavior in rodents and men. *PLoS ONE* 7:e50409. doi: 10.1371/journal.pone.0050409

Harrold, J. A., Dovey, T., Cai, X. J., Halford, J. C., and Pinkney, J. (2008). Autoradiographic analysis of ghrelin receptors in the rat hypothalamus. *Brain Res.* 1196, 59–64. doi: 10.1016/j.brainres.2007.12.055

Holst, B., Cygankiewicz, A., Jensen, T. H., Ankersen, M., and Schwartz, T. W. (2003). High constitutive signaling of the ghrelin receptor–identification of a potent inverse agonist. *Mol. Endocrinol.* 17, 2201–2210. doi: 10.1210/me.2003-0069

Holst, B., Holliday, N. D., Bach, A., Elling, C. E., Cox, H. M., and Schwartz, T. W. (2004). Common structural basis for constitutive

activity of the ghrelin receptor family. *J. Biol. Chem.* 279, 53806–53817. doi: 10.1074/jbc.M407676200

Hori, Y., Kageyama, H., Guan, J. L., Kohno, D., Yada, T., Takenoya, F., et al. (2008). Synaptic interaction between ghrelin- and ghrelin-containing neurons in the rat hypothalamus. *Regul. Pept.* 145, 122–127. doi: 10.1016/j.regpep.2007.09.027

Hosoda, H., Kojima, M., Matsuo, H., and Kangawa, K. (2000). Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochem. Biophys. Res. Commun.* 279, 909–913. doi: 10.1006/bbrc.2000.4039

Hou, Z., Miao, Y., Gao, L., Pan, H., and Zhu, S. (2006). Ghrelin-containing neuron in cerebral cortex and hypothalamus linked with the DVC of brainstem in rat. *Regul. Pept.* 134, 126–131. doi: 10.1016/j.regpep.2006.02.005

Hukshorn, C. J., and Saris, W. H. (2004). Leptin and energy expenditure. *Curr. Opin. Clin. Nutr. Metab. Care* 7, 629–633. doi: 10.1097/00075197-200411000-00007

Jang, J. K., Kim, W. Y., Cho, B. R., Lee, J. W., and Kim, J. H. (2013). Microinjection of ghrelin in the nucleus accumbens core enhances locomotor activity induced by cocaine. *Behav. Brain Res.* 248C, 7–11. doi: 10.1016/j.bbr.2013.03.049

Jerlhag, E. (2008). Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. *Addict. Biol.* 13, 358–363. doi: 10.1111/j.1369-1600.2008.00125.x

Jerlhag, E., Egecioglu, E., Dickson, S. L., Andersson, M., Svensson, L., and Engel, J. A. (2006a). Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. *Addict. Biol.* 11, 45–54. doi: 10.1111/j.1369-1600.2006.00002.x

Jerlhag, E., Grotli, M., Luthman, K., Svensson, L., and Engel, J. A. (2006b). Role of the subunit composition of central nicotinic acetylcholine receptors for the stimulatory and dopamine-enhancing effects of ethanol. *Alcohol Alcohol.* 41, 486–493. doi: 10.1093/alcalc/agl049

Jerlhag, E., Egecioglu, E., Dickson, S. L., Douhan, A., Svensson, L., and Engel, J. A. (2007). Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addict. Biol.* 12, 6–16. doi: 10.1111/j.1369-1600.2006.00041.x

Jerlhag, E., Egecioglu, E., Dickson, S. L., and Engel, J. A. (2010). Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference. *Psychopharmacology (Berl.)* 211, 45–422. doi: 10.1007/s00213-010-1907-7

Jerlhag, E., Egecioglu, E., Landgren, S., Salome, N., Heilig, M., Moechars, D., et al. (2009). Requirement of central ghrelin signaling for alcohol reward. *Proc. Natl. Acad. Sci. U.S.A.* 106, 11318–11323. doi: 10.1073/pnas.0812809106

Jerlhag, E., and Engel, J. A. (2011). Ghrelin receptor antagonism attenuates nicotine-induced locomotor stimulation, accumbal dopamine release and conditioned place preference in mice. *Drug Alcohol Depend.* 117, 126–131. doi: 10.1016/j.drugalcdep.2011.01.010

Jerlhag, E., Landgren, S., Egecioglu, E., Dickson, S. L., and Engel, J. A. (2011). The alcohol-induced locomotor stimulation and accumbal dopamine release is suppressed in ghrelin knockout mice. *Alcohol* 45, 341–347. doi: 10.1016/j.alcohol.2010.10.002

Kalivas, P. W. (2000). A role for glutamate transmission in addiction to psychostimulants. *Addict. Biol.* 5, 325–329. doi: 10.1111/j.1369-1600.2000.tb00199.x

Kelley, A. E., Baldo, B. A., Pratt, W. E., and Will, M. J. (2005). Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol. Behav.* 86, 773–795. doi: 10.1016/j.physbeh.2005.08.066

Kenny, P. J., Koob, G. F., and Markou, A. (2003). Conditioned facilitation of brain reward function after repeated cocaine administration. *Behav. Neurosci.* 117, 1103–1107. doi: 10.1037/0735-7044.117.5.1103

Kim, S., Shin, J. K., Yoon, H. S., and Kim, J. H. (2011). Blockade of ERK phosphorylation in the nucleus accumbens inhibits the expression of cocaine-induced behavioral sensitization in rats. *Korean J. Physiol. Pharmacol.* 15, 389–395. doi: 10.4196/kjpp.2011.15.6.389

King, S. J., Isaacs, A. M., O'farrell, E., and Abizaid, A. (2011). Motivation to obtain preferred foods is enhanced by ghrelin in the ventral tegmental area. *Horm. Behav.* 60, 572–580. doi: 10.1016/j.yhbeh.2011.08.006

Klesges, R. C., Meyers, A. W., Klesges, L. M., and La Vasque, M. E. (1989). Smoking, body weight, and their effects on smoking behavior: a comprehensive review of the literature. *Psychol. Bull.* 106, 204–230. doi: 10.1037/0033-2909.106.2.204

Kobelt, P., Helmling, S., Stengel, A., Wlotzka, B., Andresen, V., Klapp, B. F., et al. (2006). Anti-ghrelin Spiegelmer NOX-B11 inhibits neurostimulatory and orexigenic effects of peripheral ghrelin in rats. *Gut* 55, 788–792. doi: 10.1136/gut.2004.061010

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402, 656–660. doi: 10.1038/45230

Laniado-Laborin, R. (2010). Smoking cessation intervention: an evidence-based approach. *Postgrad. Med.* 122, 74–82. doi: 10.3810/pgm.2010.03.2124

Leibowitz, S. F. (1975). Amphetamine: possible site and mode of action for producing anorexia in the rat. *Brain Res.* 84, 160–167. doi: 10.1016/0006-8993(75)90811-2

Lu, S. C., Xu, J., Chinookoswong, N., Liu, S., Steavenson, S., Gegg, C., et al. (2009). An acyl-ghrelin-specific neutralizing antibody inhibits the acute ghrelin-mediated orexigenic effects in mice. *Mol. Pharmacol.* 75, 901–907. doi: 10.1124/mol.108.052852

Mano-Otagiri, A., Iwasaki-Sekino, A., Nemoto, T., Ohata, H., Shuto, Y., Nakabayashi, H., et al. (2010). Genetic suppression of ghrelin receptors activates brown adipocyte function and decreases fat storage in rats. *Regul. Pept.* 160, 81–90. doi: 10.1016/j.regpep.2009.11.010

Maric, T., Sedki, F., Ronfard, B., Chafetz, D., and Shalev, U. (2011). A limited role for ghrelin in heroin self-administration and food deprivation-induced reinstatement of heroin seeking in rats. *Addict. Biol.* 17, 613–622. doi: 10.1111/j.1369-1600.2011.00396.x

Marinelli, M., Rouge-Pont, F., De Jesus-Oliveira, C., Le Moal, M., and Piazza, P. V. (1997a). Acute blockade of corticosterone secretion decreases the psychomotor stimulant effects of cocaine. *Neuropsychopharmacology* 16, 156–161. doi: 10.1016/S0893-133X(96)00169-8

Marinelli, M., Rouge-Pont, F., Deroche, V., Barrot, M., De Jesus-Oliveira, C., Le Moal, M., et al. (1997b). Glucocorticoids and behavioral effects of psychostimulants. I: locomotor response to cocaine depends on basal levels of glucocorticoids. *J. Pharmacol. Exp. Ther.* 281, 1392–1400.

Miller, D. K., Nation, J. R., and Wellman, P. J. (1999). Sensitization of anorexia and locomotion induced by chronic administration of ephedrine in rats. *Life Sci.* 65, 501–511. doi: 10.1016/S0024-3205(99)00271-4

Moulin, A., Brunel, L., Boeglin, D., Demange, L., Ryan, J., M'kadmi, C., et al. (2013). The 1,2,4-triazole as a scaffold for the design of ghrelin receptor ligands: development of JMV 2959, a potent antagonist. *Amino Acids* 44, 301–314. doi: 10.1007/s00726-012-1355-2

Moulin, A., Demange, L., Berge, G., Gagne, D., Ryan, J., Mousseaux, D., et al. (2007). Toward potent ghrelin receptor ligands based on trisubstituted 1,2,4-triazole structure. 2. Synthesis and pharmacological *in vitro* and *in vivo* evaluations. *J. Med. Chem.* 50, 5790–5806. doi: 10.1021/jm0704550

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., et al. (2001). A role for ghrelin in the central regulation of feeding. *Nature* 409, 194–198. doi: 10.1038/35051587

Naleid, A. M., Grace, M. K., Cummings, D. E., and Levine, A. S. (2005). Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. *Peptides* 26, 2274–2279. doi: 10.1016/j.peptides.2005.04.025

Nogueiras, R., Tschop, M. H., and Zigman, J. M. (2008). Central nervous system regulation of energy metabolism: ghrelin versus leptin. *Ann. N.Y. Acad. Sci.* 1126, 14–19. doi: 10.1196/annals.1433.054

Ogden, C. L., Carroll, M. D., Kit, B. K., and Flegal, K. M. (2012). Prevalence of obesity in the United States, 2009–2010. *NCHS Data Brief* 82, 1–8.

Olds, J., and Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J. Comp. Physiol. Psychol.* 47, 419–427. doi: 10.1037/h0058775

Olshansky, S. J., Passaro, D. J., Hershow, R. C., Layden, J., Carnes, B. A., Brody, J., et al. (2005). A potential decline in life expectancy in the United States in the 21st

century. *N. Engl. J. Med.* 352, 1138–1145. doi: 10.1056/NEJMsr043743

Patterson, Z. R., Ducharme, R., Anisman, H., and Abizaid, A. (2010). Altered metabolic and neurochemical responses to chronic unpredictable stressors in ghrelin receptor-deficient mice. *Eur. J. Neurosci.* 32, 632–639. doi: 10.1111/j.1460-9568.2010.07310.x

Perello, M., Sakata, I., Birnbaum, S., Chuang, J. C., Osborne-Lawrence, S., Rovinsky, S. A., et al. (2010). Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. *Biol. Psychiatry* 67, 880–886. doi: 10.1016/j.biopsych.2009.10.030

Petersen, P. S., Woldbye, D. P., Madsen, A. N., Egerod, K. L., Jin, C., Lang, M., et al. (2009). *In vivo* characterization of high basal signaling from the ghrelin receptor. *Endocrinology* 150, 4920–4930. doi: 10.1210/en.2008-1638

Piazza, P. V., and Le Moal, M. L. (1996). Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annu. Rev. Pharmacol. Toxicol.* 36, 359–378. doi: 10.1146/annurev.pa.36.040196.002043

Piazza, P. V., Marinelli, M., Rouge-Pont, F., Deroche, V., Maccari, S., Simon, H., et al. (1996). Stress, glucocorticoids, and mesencephalic dopaminergic neurons: a pathophysiological chain determining vulnerability to psychostimulant abuse. *NIDA Res. Monogr.* 163, 277–299.

Pomerleau, C. S., and Saules, K. (2007). Body image, body satisfaction, and eating patterns in normal-weight and overweight/obese women current smokers and never-smokers. *Addict. Behav.* 32, 2329–2334. doi: 10.1016/j.addbeh.2007.01.027

Quarta, D., Di Francesco, C., Melotto, S., Mangiarini, L., Heidbreder, C., and Hedou, G. (2009). Systemic administration of ghrelin increases extracellular dopamine in the shell but not the core subdivision of the nucleus accumbens. *Neurochem. Int.* 54, 89–94. doi: 10.1016/j.neuint.2008.12.006

Robinson, T. E., and Berridge, K. C. (2003). Addiction. *Annu. Rev. Psychol.* 54, 25–53. doi: 10.1146/annurev.psych.54.101601.145237

Rothman, R. B., and Baumann, M. H. (2003). Monoamine transporters and psychostimulant drugs. *Eur. J. Pharmacol.* 479, 23–40. doi: 10.1016/j.ejphar.2003.08.054

Rouach, V., Bloch, M., Rosenberg, N., Gilad, S., Limor, R., Stern, N., et al. (2007). The acute ghrelin response to a psychological stress challenge does not predict the post-stress urge to eat. *Psychoneuroendocrinology* 32, 693–702. doi: 10.1016/j.psyneuen.2007.04.010

Ruter, J., Kobelt, P., Tebbe, J. J., Avsar, Y., Veh, R., Wang, L., et al. (2003). Intraperitoneal injection of ghrelin induces Fos expression in the paraventricular nucleus of the hypothalamus in rats. *Brain Res.* 991, 26–33. doi: 10.1016/j.brainres.2003.07.005

Sakata, I., Yang, J., Lee, C. E., Osborne-Lawrence, S., Rovinsky, S. A., Elmquist, J. K., et al. (2009). Colocalization of ghrelin O-acyltransferase and ghrelin in gastric mucosal cells. *Am. J. Physiol. Endocrinol. Metab.* 297, E134–E141. doi: 10.1152/ajpendo.90859.2008

Salome, N., Haage, D., Perrissoud, D., Moulin, A., Demange, L., Egecioglu, E., et al. (2009a). Anorexigenic and electrophysiological actions of novel ghrelin receptor (GHS-R1A) antagonists in rats. *Eur. J. Pharmacol.* 612, 167–173. doi: 10.1016/j.ejphar.2009.03.066

Salome, N., Hansson, C., Taube, M., Gustafsson-Ericson, L., Egecioglu, E., Karlsson-Lindahl, L., et al. (2009b). On the central mechanism underlying ghrelin's chronic pro-obesity effects in rats: new insights from studies exploiting a potent ghrelin receptor antagonist. *J. Neuroendocrinol.* 21, 777–785. doi: 10.1111/j.1365-2826.2009.01895.x

Sato, T., Fukue, Y., Teranishi, H., Yoshida, Y., and Kojima, M. (2005). Molecular forms of hypothalamic ghrelin and its regulation by fasting and 2-deoxy-d-glucose administration. *Endocrinology* 146, 2510–2516. doi: 10.1210/en.2005-0174

Satou, M., Nishi, Y., Yoh, J., Hattori, Y., and Sugimoto, H. (2010). Identification and characterization of acyl-protein thioesterase 1/lysophospholipase I as a ghrelin deacylation/lysophospholipid hydrolyzing enzyme in fetal bovine serum and conditioned medium. *Endocrinology* 151, 4765–4775. doi: 10.1210/en.2010-0412

Schoffelmeer, A. N., De Vries, T. J., Wardeh, G., Van De Ven, H. W., and Vanderschuren, L. J. (2002). Psychostimulant-induced behavioral sensitization depends on nicotinic receptor activation. *J. Neurosci.* 22, 3269–3276.

Shrestha, Y. B., Wickwire, K., and Giraudo, S. (2009). Effect of reducing hypothalamic ghrelin receptor gene expression on energy balance. *Peptides* 30, 1336–1341. doi: 10.1016/j.peptides.2009.03.013

Skibicka, K. P., and Dickson, S. L. (2011). Ghrelin and food reward: the story of potential underlying substrates. *Peptides* 32, 2265–2273. doi: 10.1016/j.peptides.2011.05.016

Skibicka, K. P., Hansson, C., Alvarez-Crespo, M., Friberg, P. A., and Dickson, S. L. (2011a). Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* 180, 129–137. doi: 10.1016/j.neuroscience.2011.02.016

Skibicka, K. P., Hansson, C., Egecioglu, E., and Dickson, S. L. (2011b). Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. *Addict. Biol.* 17, 95–107. doi: 10.1111/j.1369-1600.2010.00294.x

Spencer, S. J., Xu, L., Clarke, M. A., Lemus, M., Reichenbach, A., Geenen, B., et al. (2012). Ghrelin regulates the hypothalamic-pituitary-adrenal axis and restricts anxiety after acute stress. *Biol. Psychiatry* 72, 457–465. doi: 10.1016/j.biopsych.2012.03.010

Stein, L. (1964). Self-stimulation of the brain and the central stimulant action of amphetamine. *Fed. Proc.* 23, 836–850.

Steketee, J. D., and Kalivas, P. W. (2011). Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. *Pharmacol. Rev.* 63, 348–365. doi: 10.1124/pr.109.001933

Stengel, A., Wang, L., and Tache, Y. (2011). Stress-related alterations of acyl and desacyl ghrelin circulating levels: mechanisms and functional implications. *Peptides* 32, 2208–2217. doi: 10.1016/j.peptides.2011.07.002

Stevanovic, D., Milosevic, V., Starcevic, V. P., and Severs, W. B. (2007). The effect of centrally administered ghrelin on pituitary ACTH cells and circulating ACTH and corticosterone in rats. *Life Sci.* 80, 867–872. doi: 10.1016/j.lfs.2006.11.018

Sun, Y., Ahmed, S., and Smith, R. G. (2003). Deletion of ghrelin impairs neither growth nor appetite. *Mol. Cell. Biol.* 23, 7973–7981. doi: 10.1128/MCB.23.22.7973-7981.2003

Sun, Y., Butte, N. F., Garcia, J. M., and Smith, R. G. (2008). Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. *Endocrinology* 149, 843–850. doi: 10.1210/en.2007-0271

Tessari, M., Catalano, A., Pellitteri, M., Di Francesco, C., Marini, F., Gerrard, P. A., et al. (2007). Correlation between serum ghrelin levels and cocaine-seeking behaviour triggered by cocaine-associated conditioned stimuli in rats. *Addict. Biol.* 12, 22–29. doi: 10.1111/j.1369-1600.2007.00052.x

Till, B. J., Cooper, J., Tai, T. H., Colowit, P., Greene, E. A., Henikoff, S., et al. (2007). Discovery of chemically induced mutations in rice by TILLING. *BMC Plant Biol.* 7:19. doi: 10.1186/1471-2229-7-19

Toshinai, K., Mondal, M. S., Nakazato, M., Date, Y., Murakami, N., Kojima, M., et al. (2001). Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem. Biophys. Res. Commun.* 281, 1220–1225. doi: 10.1006/bbrc.2001.4518

Tschop, M., Smiley, D. L., and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* 407, 908–913. doi: 10.1038/35038090

Vezina, P. (2004). Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. *Neurosci. Biobehav. Rev.* 27, 827–839. doi: 10.1016/j.neubiorev.2003.11.001

Vezina, P. (2007). Sensitization, drug addiction and psychopathology in animals and humans. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31, 1553–1555. doi: 10.1016/j.pnpbp.2007.08.030

Wellman, P. J., Clifford, P. S., Rodriguez, J., Hughes, S., Eitan, S., Brunel, L., et al. (2011). Pharmacologic antagonism of ghrelin receptors attenuates development of nicotine induced locomotor sensitization in rats. *Regul. Pept.* 172, 77–80. doi: 10.1016/j.regpep.2011.08.014

Wellman, P. J., Clifford, P. S., Rodriguez, J. A., Hughes, S., Di Francesco, C., Melotto, S., et al. (2012). Brain reinforcement system function is ghrelin dependent: studies in the rat using pharmacological fMRI and intracranial self-stimulation. *Addict. Biol.* 17, 908–919. doi: 10.1111/j.1369-1600.2011.00392.x

Wellman, P. J., Davis, K. W., Clifford, P. S., Rothman, R. B., and Blough, B. E. (2009). Changes in feeding and locomotion induced

by amphetamine analogs in rats. *Drug Alcohol Depend.* 100, 234–239. doi: 10.1016/j.drugalcdep.2008.10.005

Wellman, P. J., Davis, K. W., and Nation, J. R. (2005). Augmentation of cocaine hyperactivity in rats by systemic ghrelin. *Regul. Pept.* 125, 151–154. doi: 10.1016/j.regpep.2004.08.013

Wellman, P. J., Elliott, A. E., Barbee, S., Hollas, C. N., Clifford, P. S., and Nation, J. R. (2008a). Lobeline attenuates progressive ratio breakpoint scores for intracranial self-stimulation in rats. *Physiol. Behav.* 93, 952–957. doi: 10.1016/j.physbeh.2007.12.018

Wellman, P. J., Hollas, C. N., and Elliott, A. E. (2008b). Systemic ghrelin sensitizes cocaine-induced hyperlocomotion in rats. *Regul. Pept.* 146, 33–37. doi: 10.1016/j.regpep.2007.07.007

Wise, R. A. (1996). Addictive drugs and brain stimulation reward. *Annu. Rev. Neurosci.* 19, 319–340. doi: 10.1146/annurev.ne.19.030196.001535

Wise, R. A. (2004). Dopamine, learning and motivation. *Nat. Rev. Neurosci.* 5, 483–494. doi: 10.1038/nrn1406

Wise, R. A., and Bozarth, M. A. (1987). A psychomotor stimulant theory of addiction. *Psychol. Rev.* 94, 469–492. doi: 10.1037/0033-295X.94.4.469

Wise, R. A., and Leeb, K. (1993). Psychomotor-stimulant sensitization: a unitary phenomenon? *Behav. Pharmacol.* 4, 339–349. doi: 10.1097/00008877-199308000-00007

Woolverton, W. L., and Kleven, M. S. (1988). Multiple dopamine receptors and the behavioral effects of cocaine. *NIDA Res. Monogr.* 88, 160–184.

Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., et al. (2001). Ghrelin enhances appetite and increases food intake in humans. *J. Clin. Endocrinol. Metab.* 86, 5992. doi: 10.1210/jc.86.12.5992

Wren, A. M., Small, C. J., Fribbens, C. V., Neary, N. M., Ward, H. L., Seal, L. J., et al. (2002). The hypothalamic mechanisms of the hypophysiotropic action of ghrelin. *Neuroendocrinology* 76, 316–324. doi: 10.1159/000066629

Wren, A. M., Small, C. J., Ward, H. L., Murphy, K. G., Dakin, C. L., Taheri, S., et al. (2000). The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 141, 4325–4328. doi: 10.1210/en.141.11.4325

Yang, J., Brown, M. S., Liang, G., Grishin, N. V., and Goldstein, J. L. (2008). Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 132, 387–396. doi: 10.1016/j.cell.2008.01.017

Zan, Y., Haag, J. D., Chen, K. S., Shepel, L. A., Wigington, D., Wang, Y. R., et al. (2003). Production of knockout rats using ENU mutagenesis and a yeast-based screening assay. *Nat. Biotechnol.* 21, 645–651. doi: 10.1038/nbt830

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* 372, 425–432. doi: 10.1038/372425a0

Zheng, D., Cabeza De Vaca, S., and Carr, K. D. (2012). Food restriction increases acquisition, persistence and drug prime-induced expression of a cocaine-conditioned place preference in rats. *Pharmacol. Biochem. Behav.* 100, 538–544. doi: 10.1016/j.pbb.2011.10.021

Zheng, D., Liu, S., Cabeza De Vaca, S., and Carr, K. D. (2013). Effects of time of feeding on psychostimulant reward, conditioned place preference, metabolic hormone levels, and nucleus accumbens biochemical measures in food-restricted rats. *Psychopharmacology (Berl.)* 227, 307–320. doi: 10.1007/s00213-013-2981-4

Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B., and Elmquist, J. K. (2006). Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J. Comp. Neurol.* 494, 528–548. doi: 10.1002/cne.20823

Zoli, M., and Picciotto, M. R. (2012). Nicotinic regulation of energy homeostasis. *Nicotine Tob. Res.* 14, 1270–1290. doi: 10.1093/ntr/nts159

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Is it stress? The role of stress related systems in chronic food restriction-induced augmentation of heroin seeking in the rat

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Drug addiction is a chronic disease characterized by recurring episodes of abstinence and relapse. The precise mechanisms underlying this pattern are yet to be elucidated, but stress is thought to be a major factor in relapse. Recently, we reported that rats under withdrawal and exposed to a mild chronic stressor, prolonged food restriction, show increased heroin seeking compared to sated controls. Previous studies demonstrated a critical role for corticotropin-releasing factor (CRF) and corticosterone, hormones involved in the stress response, in acute food deprivation-induced reinstatement of extinguished drug seeking. However, the role of CRF and corticosterone in chronic food restriction-induced augmentation of drug seeking remains unknown. Here, male Long-Evans rats were trained to self-administer heroin for 10 days in operant conditioning chambers. Rats were then removed from the training chambers, and subjected to 14 days of unrestricted (sated rats) or a mildly restricted (FDR rats) access to food, which maintained their body weight (BW) at 90% of their baseline weight. On day 14, different groups of rats were administered a selective CRF_1 receptor antagonist (R121919; 0.0, 20.0 mg/kg; s.c.), a non-selective CRF receptor antagonist (α-helical CRF; 0.0, 10.0, 25.0 μg/rat; i.c.v.) or a glucocorticoid receptor antagonist (RU486; 0.0, 30.0 mg/kg; i.p.), and underwent a 1 h drug seeking test under extinction conditions. An additional group of rats was tested following adrenalectomy. All FDR rats showed a statistically significant increase in heroin seeking compared to the sated rats. No statistically significant effects for treatment with α-helical CRF, R121919, RU486 or adrenalectomy were observed. These findings suggest that stress may not be a critical factor in the augmentation of heroin seeking in food-restricted rats.

Keywords: self-administration, chronic food restriction, corticotropin-releasing factor, corticosterone, adrenalectomy, R121919, α-helical CRF, RU486

INTRODUCTION

Stress is consistently reported by drug users as a factor in subjective craving as well as in the initiation, maintenance, and relapse of drug use (Brewer et al., 1998; Matheny and Weatherman, 1998; Sinha and O'Malley, 1999; Sinha, 2001, 2008). A role for stress in triggering relapse to drugs has also been identified in retrospective studies in which interviews and questionnaires were given to addicts, as well as in controlled laboratory studies (Kosten et al., 1983, 1986; Sinha et al., 2006). Of many potential stressful life events, a particularly interesting one is exposure to caloric restriction, which precipitates negative affective states, as well as physiological changes including increases in circulating glucocorticoid levels (Dallman et al., 1999; Tomiyama et al., 2010). Studies showing an increased risk for relapse among calorically restricted abstinent smokers (Hall et al., 1992), and a positive correlation between the severity of diet and the risk of drug taking in young women (Krahn et al., 1992), suggest a strong link between caloric restriction and drug intake. Moreover, in humans, only prolonged food restriction, and not acute food deprivation, is associated with increased drug taking (Zacny and de Wit, 1991; Cheskin et al., 2005, also see D'Cunha et al., 2013).

In laboratory animals, the effects of restricting food availability on drug-associated behaviors have been demonstrated unequivocally. The initiation and maintenance of drug intake are reliably enhanced following periods of caloric restriction (Piazza and Le Moal, 1998; Lu et al., 2003). Acute food deprivation (FD: 24–48 h), can induce reinstatement to drug seeking in rats with a history of heroin or cocaine self-administration (Shalev et al., 2000, 2003). This effect is attenuated by antagonism of the stress neuropeptide corticotropin-releasing factor (CRF) receptor, but not the removal of corticosterone (Shalev et al., 2003, 2006), a pattern of results similar to those obtained by Shaham et al. (1997) in a study utilizing footshock as a stressor.

Interestingly, although both acute FD and chronic food restriction (days to weeks) decrease BW and augment drug seeking in rodents, they can have quite different metabolic and behavioral effects on the organism. For example, Fulton et al. (2000)

demonstrated a decreased threshold for electrical brain stimulation reward in chronically food restricted but not acutely food deprived rats. Similarly, increased cigarette smoking following prolonged food restriction, but not acute food deprivation, has been reported in human subjects (Zacny and de Wit, 1992; Cheskin et al., 2005). In response to these findings, we have recently developed a clinically relevant model, in which drug seeking in drug-free rats with a history of heroin self-administration is tested following prolonged food restriction. Using this model, our laboratory has reported a dramatic enhancement of heroin seeking in food restricted rats under going withdrawal (>250%) compared to sated controls (D'Cunha et al., 2013).

Although the extrahypothalamic CRF system is critically involved in acute FD-induced reinstatement of heroin seeking (Shalev et al., 2006), its role in chronic food restriction-induced augmentation of drug seeking is not known. In addition, while a key role for a stress-induced increase in corticosterone in acute FD-induced reinstatement was ruled out (Shalev et al., 2003, 2006), previous studies demonstrated that pharmacological blockade of corticosterone synthesis or complete removal of corticosterone attenuates chronic food restriction-induced augmentation of drug-related behaviors (Deroche et al., 1995; Marinelli et al., 1996). Thus, the aim of this study was to investigate the involvement of CRF and corticosterone, in the augmentation of heroin seeking following prolonged food restriction in rats under withdrawal.

MATERIALS AND METHODS

SUBJECTS

One hundred and thirty-three male, Long-Evans rats (Charles River, St. Constant, Quebec, Canada; 300–350 g) were used. Before surgery, animals were pair-housed for 1 week in the animal care facility (ACF) under reverse light/dark conditions (lights OFF at 0930). Following intravenous (IV) catheterization, and 2 days of recovery, rats were single-housed in plastic shoebox cages before being transferred to operant conditioning chambers for drug self-administration. Following self-administration training, rats were returned to the ACF and single-housed in shoebox cages for the drug withdrawal phase. Except for the withdrawal phase, all rats were given unrestricted access to food and water. Rats were treated according to the Canadian Council on Animal Care guidelines, and approval was granted by the Concordia University Animal Research Ethics Committee.

SURGICAL PROCEDURES

Intravenous catheterization and intracerebroventricular (I.C.V.) cannulation

Rats were implanted with IV silastic catheters (Dow Corning, Midland, MI, USA) under xylazine/ketamine (13.0 + 90.0 mg/kg; i.p.). Three centimeters of silastic catheter was inserted through a small incision on the right jugular vein, and secured using silk sutures. The remainder of the catheter was passed subcutaneously to the skull, attached to a modified 22-gauge cannula (Plastics One, Roanoke, VA) and anchored to the skull using dental cement and 5 jeweler's screws. Some rats (Experiment 1A) were also implanted with a 22-gauge guide cannula (Plastics One) aimed 2 mm above the right or left lateral ventricle (AP, ±0.5; ML, +1.4; DV, −3.0; relative to bregma) to allow for i.c.v. injections.

Following surgery, rats were administered buprenorphine (600.0 μg/rat; s.c.; Schering-Plough Ltd., Welwyn Garden City Hertfordshire, UK) and penicillin (450,000 IU/rat; s.c.) to reduce pain and prevent infection. Catheters were flushed daily with heparin/gentamicin (7.5 IU +12.0 mg/rat) to prevent blockage and infection.

Adrenalectomy

Bilateral adrenalectomy (ADX) surgeries were performed on withdrawal day 10 on rats in Experiment 2B, under isoflurane USP anesthetic. The adrenal glands were rapidly removed via the dorsal approach, and the rats were given ketoprofen injections (5.0 mg/kg; s.c.) following surgery to reduce pain. Sham-operated rats were exposed to the same procedure as the ADX rats, with the exception that the adrenal glands were not removed. After surgery, the ADX rats were given physiological saline (0.9% NaCl) in their drinking bottles.

APPARATUS

Rats were housed individually in operant conditioning chambers (Coulbourn Instruments, Allentown, PA, USA; 29.0 × 29.0 × 25.5 cm) enclosed in sound attenuating wooden compartments equipped with a fan. Each chamber was fitted with two retractable levers (Coulbourn Instruments) mounted 9 cm above the floor of the right sidewall. Responses on the "active" lever activated the infusion pump and a cue-light/tone (Coulbourn Instruments, Sonalert, 2.9 KHz) located above the lever. Responses on the "inactive" lever had no programmable consequences. Rats were attached to the infusion pump via a liquid swivel (Instec Laboratories Inc., Boulder, CO, USA) and Tygon tubing shielded with a metal spring.

DRUGS

Heroin HCl (a contribution from the National Institute for Drug Abuse, Research Triangle Park, NC, USA) was dissolved in sterile saline (5.0 mg/ml) and then further diluted with saline, for each rat according to BW to yield 0.1 mg/kg/infusion.

R121919, the selective CRF_1 receptor antagonist, was kindly supplied by Dr. Kenner Rice (National Institute on Drug Abuse, NIH, Baltimore, MD, USA). R121919 was dissolved in a 20% β-Cyclodextrin (Sigma-Aldrich) sterile saline solution to a concentration of 10.0 mg/ml and adjusted to a pH of 4.5. The antagonist was injected (s.c.) at a final dose of 20.0 mg/kg. A solution of 20% β-Cyclodextrin mixed in sterile saline solution was used as a vehicle injected at a volume of 2.0 ml/kg. A similar dose of R121919 reduced both drug self-administration and anxiety-like behaviors (Greenwell et al., 2009; Gutman et al., 2010).

The non-selective CRF antagonist, α-helical CRF (Sigma-Aldrich), was dissolved in sterile water to a concentration of 5.0 μg/μl or 12.5 μg/μl. α-helical CRF was injected i.c.v. over 2 min at a rate of 1.0 μl/min for a final dose of 10.0 or 25.0 μg/rat. Sterile water was used as a vehicle. Injections were made using

a syringe pump (Harvard Apparatus, Holliston, MA, USA) connected to a 10 μl Hamilton syringe. This syringe was attached via polyethylene-20 tubing to a 28-gauge injector (Plastics One) that extended 2 mm below the guide cannulae. The injector was kept in place for 1 min after the injections to allow for proper diffusion of the drug. The doses for α-helical CRF were based on previous reports (Baldwin et al., 1991; Krahn et al., 1986; Shalev et al., 2006).

RU486, a glucocorticoid receptor antagonist, was dissolved using a 25% β-Cyclodextrin (Sigma-Aldrich), 1% Tween-80 (Sigma-Aldrich), 2–3 drops of 1N HCl, and sterile water mixture which also served as the vehicle. RU486 and vehicle solutions were adjusted to a pH of ∼5.6 and injected (i.p.) at a dose of 30 mg/kg (RU486), or a volume of 1 ml/kg (vehicle). A similar dose was shown to attenuate stress-induced reinstatement of alcohol seeking (Simms et al., 2012).

PROCEDURE

Self-administration

Following a 24-h habituation period in the chamber, rats were trained to self-administer heroin in daily three 3-h sessions separated by 3-h intervals for 10 days. The first daily session began shortly after the onset of the dark phase with the extension of the active and inactive levers into the conditioning chamber, illumination of a house-light and activation of the cue-light/tone complex for 30 s. Responses on the active lever, which was armed with a fixed ratio-1 schedule (FR-1), resulted in activation of the drug pump (5 s, 0.13 ml/infusion) and the initiation of a 20 s timeout during which the house-light was turned off and the cue light/tone complex above the active lever was activated. During the timeout period, active lever responses were recorded but not reinforced. Following each 3-h session, the active lever was retracted whereas the inactive lever was not retracted until 1 h before the first session of the following day. Inactive lever responses were recorded but had no programmable consequences.

DRUG WITHDRAWAL PHASE

Experiments 1A, 1B, and 2A

Following self-administration training, rats were individually housed in the ACF, and given unrestricted access to food and water for one drug-washout day. Rats were then divided into two groups: food restricted (FDR) or Sated that were matched according to BW, number of infusions, and active lever responses across the last 5 days of training. Following the washout day, FDR rats had their food removed and were fed ∼15 g of rat chow at 1330. The amount of food was adjusted through 14 days of food restriction to maintain the food restricted rats' BW at ∼75–80% of the Sated rats and 90% of their baseline BW.

Experiment 2B

Rats were treated as in Experiment 2A, except for the ADX surgery that was performed on day 10 of withdrawal. Following surgery, rats were given 25 g of food and allowed 1 day of recovery, after which they were re-restricted to the previously described regimen and allowed 2 extra days of withdrawal to ensure their BWs reached criteria.

DRUG-SEEKING TEST

Experiment 1A: the effect of treatment with the selective CRF_1 receptor antagonist, R121919

On withdrawal day 14, rats were returned to the operant conditioning chambers and attached to the metal spring. The drug-seeking test consisted of a 1-h session during which active lever responses had the same consequences as in training excluding the availability of the drug. A subcutaneous injection of R121919 (0.0, 20.0 mg/kg, s.c.) was administered 30 min before the test session.

Experiment 1B: the effects of treatment with the non-selective CRF receptor antagonist, α-helical CRF, on chronic food restriction-induced augmentation of heroin seeking in the rat

The testing procedure was similar to the one described for Experiment 1A, except that the rats were given an ICV injection of α-helical CRF (0.0, 10.0 or 25.0 μg/rat, i.c.v.) 10 min before the test session.

Experiment 1C: the effects of treatment with the non-selective CRF receptor antagonist, α-helical CRF, on open-field behavior

This experiment was designed to verify the efficiency of the α-helical CRF treatment used in Experiment 1B. Previously, α-helical CRF has been shown to have anxiolytic properties (Koob and Heinrichs, 1999). To ensure that the antagonist had similar effects in our hands, eight rats that participated in Experiment 1B were given a test for anxiety. These rats were food restricted for an additional 8 days following the drug-seeking test and on day 8 at 1330, were brought into a novel, brightly lit, environment and placed in a white circular arena (diameter 137 cm; height 46 cm) with one food pellet in the center. Rats were placed at either the north, south, west, or east positions of the arena and allowed to explore the environment for 10 min. The rats' behavior was recorded by a video camera. Two variables were then scored from the video recordings. The first, latency to consumption, was defined as the time for the rat to first consume a portion of the food pellet. The second, number of approaches, was defined as the number of times a rat approached the food pellet until first consumption.

Experiment 2A: the effect of treatment with the glucocorticoid receptor antagonist, RU486

Testing procedure was similar to the one described for Experiment 1A, except that the rats were given an injection of RU486 (30.0 mg/kg, i.p.) or vehicle in the ACF 45 min before the test session.

Experiment 2B: the effect of ADX

Testing procedure was similar to the one described for Experiment 1A, except that, other then the ADX surgery, no further treatment was given before the test.

Plasma corticosterone determination

Immediately following the drug-seeking test (1030), tail blood was collected, and plasma was separated by centrifugation at 10,000 rpm for 10 min. Samples were stored at −80°C. Plasma samples were analyzed for corticosterone levels using a corticosterone specific enzyme-linked immunosorbent assay (ELISA) kit

(Enzo Life Sciences: Cedarlane, Burlington, ON, Canada). The reported detection sensitivity for the kit is 27.0 pg/ml.

STATISTICAL ANALYSIS

All analyses were conducted using SPSS software (IBM, SPSS Statistics, version 20). Training data for all rats were analyzed using a repeated measures ANOVA, with training day (1–10) as a within-subjects factor and the number of active lever responses, inactive lever responses or number of infusions as the dependent variables.

Experiment 1

Number of responses on the active and inactive levers during the drug-seeking test sessions were analyzed separately using Two-Way ANOVAs. Antagonist dose (Experiment 1A: 0.0, 20.0 mg/kg; Experiment 1B: 0.0, 10.0, 25.0 μg/rat) and feeding condition (FDR, Sated) served as between subject factors.

The effect of treatment with α-helical CRF (0.0, 25.0 μg/rat) on the open field behavior was analyzed using two independent samples *t*-tests. Latency to consumption and number of approaches were the dependent variables.

Experiment 2

Number of responses on the active and inactive levers during the test session were analyzed separately using Two-Way ANOVAs. Antagonist dose (Experiment 2A: 0.0, 30.0 mg/kg) or surgery condition (Experiment 2B: ADX, Sham) and feeding condition (FDR, Sated) served as the between subject factors.

Plasma corticosterone levels (ng/ml), sampled following the test sessions in Experiment 2, were analyzed using a Two-Way ANOVA. Antagonist dose (Experiment 2A: 0.0, 30.0 mg/kg) or surgery condition (Experiment 2B: ADX, Sham) and feeding condition (FDR, Sated) served as the between subject factors.

In all analyses, statistically significant interactions were investigated with the appropriate *post-hoc* tests and the critical threshold for statistically significant results was set at $p \leq 0.05$.

RESULTS

All rats acquired reliable heroin self-administration behavior. Mean ± SEM number of infusions and number of active and inactive lever responses made on the last day of heroin self-administration training, for each experiment, are shown in **Table 1**. There were no statistically significant differences in any of the above parameters between the different experimental groups within each experiment.

Table 1 | Mean ± SEM number of heroin infusions and active and inactive lever responses made on the last day of training (9 h) in each experiment.

	Infusions	Active lever	Inactive lever
Experiment 1A	42.08 ± 4.25	155.72 ± 18.97	13.28 ± 3.36
Experiment 1B	35.06 ± 1.91	99.62 ± 13.40	6.90 ± 0.84
Experiment 2A	40.23 ± 4.60	141.62 ± 23.45	18.00 ± 8.27
Experiment 2B	36.72 ± 2.84	116.66 ± 17.77	25.03 ± 6.01

EXPERIMENT 1A: THE EFFECTS OF TREATMENT WITH THE SELECTIVE CRF_1 RECEPTOR ANTAGONIST, R121919, ON CHRONIC FOOD RESTRICTION-INDUCED AUGMENTATION OF HEROIN SEEKING IN THE RAT

Five rats were removed due to catheter leakage, failure to train, or detached head-cap. Thus, the final analysis included 25 rats in 4 experimental groups: FDR-0.0 ($n = 5$), FDR-20.0 ($n = 7$), Sated-0.0 ($n = 6$), Sated-20.0 ($n = 7$). On the test day, average BW of the rats in the Sated group ($n = 13$; 452.92 ± 9.65 g) was statistically significantly greater than that of the rats in the FDR group ($n = 12$; 333.50 ± 6.47 g; $t_{(23)} = -10.12$, $p < 0.001$).

The mean number of active lever responses performed by the rats in the FDR group was almost three times higher than the lever responses made by the Sated group during the drug-seeking test. The robust effect was confirmed by a statistically significant main effect of feeding condition [$F_{(1, 21)} = 6.91$, $p = 0.016$, $\eta^2 = 0.24$; **Figure 1A**]. No statistically significant effects were found for

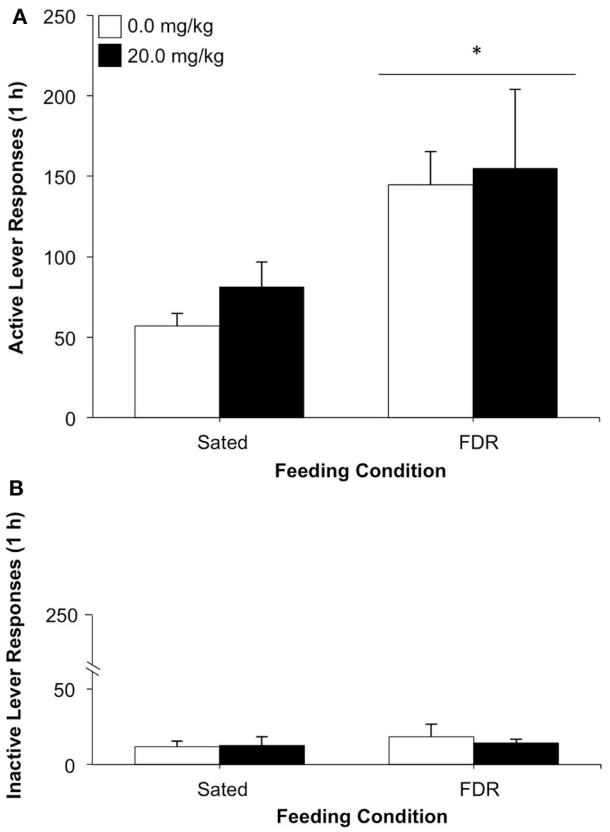

FIGURE 1 | The effect of treatment with the selective CRF_1 receptor antagonist, R121919 (0.0, 20.0 mg/kg, s.c.) on heroin seeking in food-restricted (FDR) and sated rats under withdrawal; Experiment 1A. Data shown are the mean (+SEM) numbers of active **(A)** and inactive **(B)** lever responses made on test day by rats in the FDR (*n*'s: FDR-0.0 = 6, FDR-20.0 = 7) and Sated (*n*'s: Sated-0.0 = 5, Sated-20.0 = 7) groups. Test day consisted of one 1-h drug-seeking session under extinction conditions, following heroin self-administration training and 14 days of withdrawal under FDR or sated conditions. *Significantly different from the sated condition, $p = 0.016$.

antagonist dose or for the interaction feeding condition × antagonist dose. No statistically significant effects were observed for inactive lever responding on the test day (**Figure 1B**).

EXPERIMENT 1B: THE EFFECTS OF TREATMENT WITH THE NON-SELECTIVE CRF RECEPTOR ANTAGONIST, α-HELICAL CRF, ON CHRONIC FOOD RESTRICTION-INDUCED AUGMENTATION OF HEROIN SEEKING IN THE RAT

Eight rats were removed due to catheter leakage, failure to train or detached head-caps. Therefore, the final analysis included 52 rats in 6 experimental groups: FDR-0.0 ($n = 8$), FDR-10.0 ($n = 11$), FDR-25.0 ($n = 7$), Sated-0.0 ($n = 10$), Sated-10.0 ($n = 11$), Sated-25.0 ($n = 5$). On test day, the average BWs of rats in the Sated group ($n = 26$; 439.62 ± 9.27 g) was statistically significantly greater than that of rats in the FDR groups ($n = 26$; 333.08 ± 4.17 g; $t_{(50)} = -10.48$, $p < 0.001$).

Rats in the FDR group made a higher number of responses on the active lever during the drug-seeking test, compared to the Sated group (see **Figure 2A**). This finding was supported by a statistically significant main effect of feeding condition, [$F_{(1, 46)} = 17.68$, $p < 0.001$, $\eta^2 = 0.27$]. No statistically significant effects were found for either the antagonist dose or for the feeding condition × antagonist dose interaction. No significant effects were observed for inactive lever responding on test day (**Figure 2B**).

EXPERIMENT 1C: THE EFFECT OF TREATMENT WITH THE NON-SELECTIVE CRF RECEPTOR ANTAGONIST, α-HELICAL CRF ON OPEN-FIELD BEHAVIOR

Rats in the α-helical CRF-treated group demonstrated a statistically significant decrease in latency to first food consumption and the number of approaches prior to first consumption, compared to vehicle controls [$t_{(5)} = 2.80$, $p = 0.038$; **Figure 3A**; $t_{(5)} = 3.13$, $p = 0.021$; **Figure 3B**, respectively].

EXPERIMENT 2A: THE EFFECT OF TREATMENT WITH THE GLUCOCORTICOID RECEPTOR ANTAGONIST, RU486, ON CHRONIC FOOD RESTRICTION-INDUCED AUGMENTATION OF HEROIN SEEKING IN THE RAT

Three rats were removed due to catheter leakage, failure to train or being an outlier. Therefore, the final analysis included 27 rats in 4 experimental groups: FDR-0.0 ($n = 7$), FDR-30.0 ($n = 7$), Sated-0.0 ($n = 8$), Sated-30.0 ($n = 5$). On test day, average BW of the rats in the Sated group ($n = 13$; 426.23 ± 8.02 g) was statistically significantly greater than that of the rats in the FDR group ($n = 14$; 317.36 ± 5.21 g; $t_{(25)} = -11.54$, $p < 0.001$).

As can be seen in **Figure 4A**, the FDR group showed more active lever responding than the Sated group, [feeding condition effect: $F_{(1, 23)} = 8.46$, $p = 0.008$, $\eta^2 = 0.26$] during the drug-seeking test. No statistically significant effects for antagonist dose or feeding condition × antagonist dose interaction were observed. The FDR group also responded more on the inactive lever than the Sated group [feeding condition effect: $F_{(1, 23)} =$

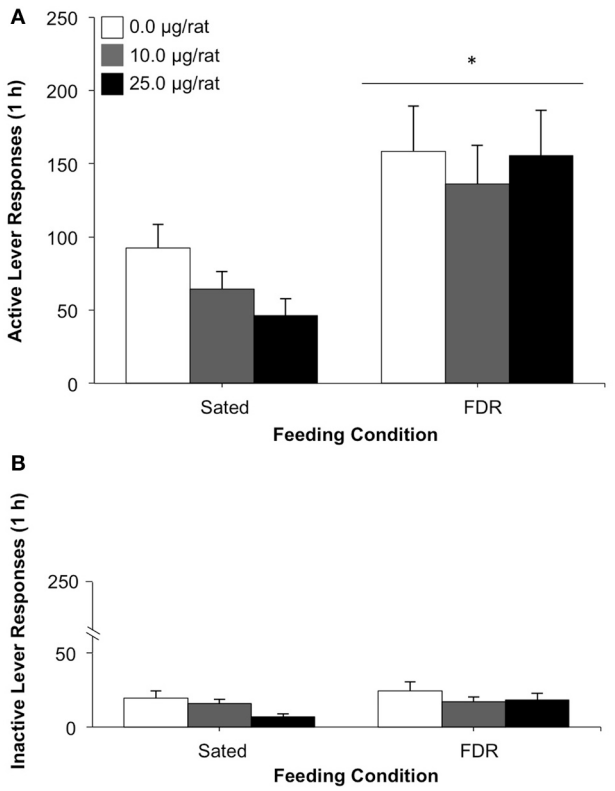

FIGURE 2 | The effect of treatment with the non-specific CRF receptor antagonist, α-Helical CRF (0.0, 10.0, 25.0 µg/rat, i.c.v.) on heroin seeking in food-restricted (FDR) and sated rats under withdrawal; Experiment 1B. Data shown are the mean (+SEM) numbers of active **(A)** and inactive **(B)** lever responses made on test day by rats in the FDR (*n*'s: FDR-0.0 = 8, FDR-10.0 = 11, FDR-25.0 = 7) and Sated (*n*'s: Sated-0.0 = 10, Sated-10.0 = 11, Sated-20.0 = 5) groups. Test day consisted of one 1-h drug-seeking session under extinction conditions, following heroin self-administration training and 14 days of withdrawal under FDR or sated conditions. *Significantly different from the sated condition, $p < 0.001$.

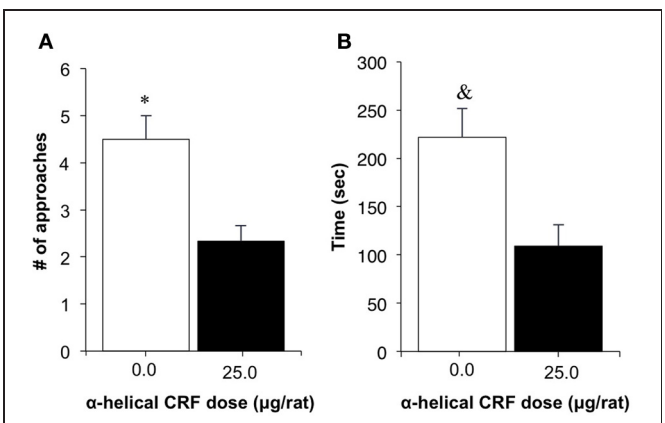

FIGURE 3 | The effect of treatment with the non-specific CRF receptor antagonist α-Helical CRF (0.0, 25.0 µg/rat, i.c.v.) on behavior in an open field, following an 8-day food restriction period; Experiment 1C. Data shown are the mean (+SEM) latencies to first consumption of **(A)** and number of approaches to **(B)** a food pellet placed at the center of an open arena following injections of α-Helical CRF in food restricted rats (*n*'s: 0.0 = 4, 25.0 = 3). *$p = 0.038$; & $p = 0.021$.

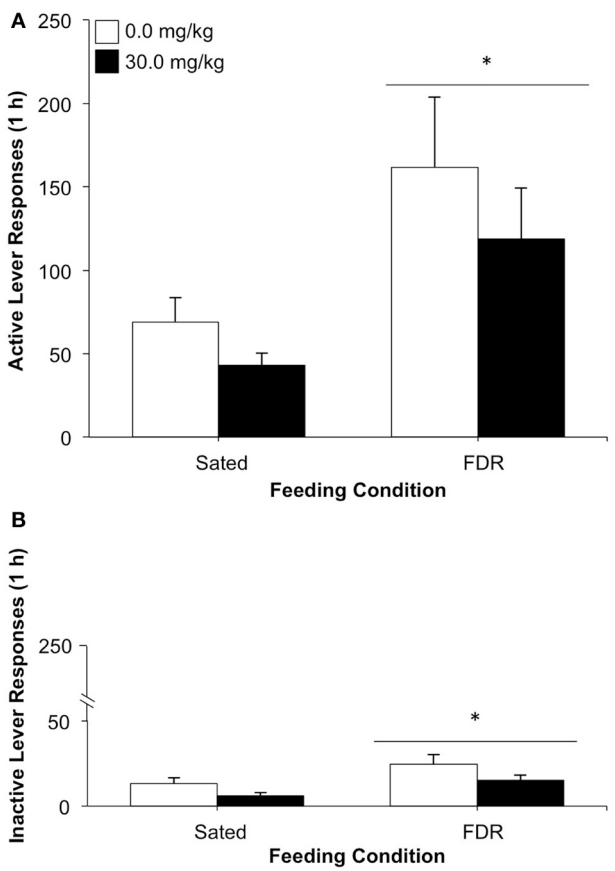

FIGURE 4 | The effect of treatment with the glucocorticoid receptor antagonist, RU486 (0.0, 30.0 mg/kg, i.p.) on heroin seeking in food-restricted (FDR) and sated rats under withdrawal; Experiment 2A. Data shown are the mean (+SEM) numbers of active **(A)** and inactive **(B)** lever responses made on test day by rats in the FDR (*n*'s: FDR-0.0 = 7, FDR-30.0 = 7) and Sated (*n*'s: Sated-0.0 = 8, Sated-30.0 = 5) groups. Test day consisted of one 1-h drug-seeking session under extinction conditions, following heroin self-administration training and 16 days of withdrawal under FDR or sated conditions. *Significantly different from the sated condition, $p < 0.02$.

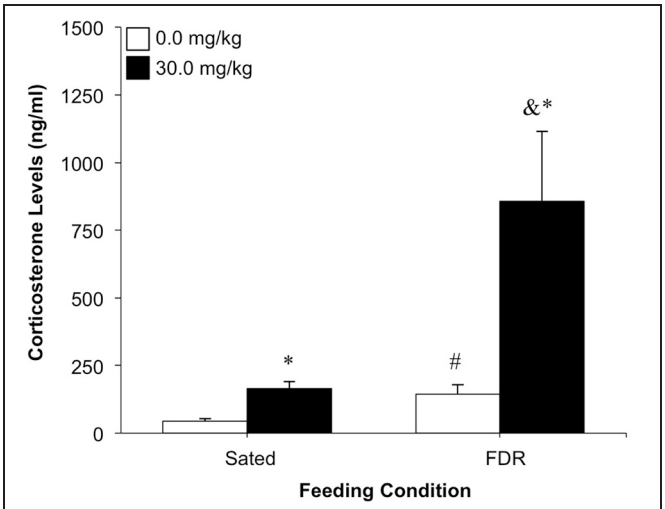

FIGURE 5 | The effect of treatment with the glucocorticoid receptor antagonist, RU486 (0.0, 30.0 mg/kg, i.p.) on corticosterone levels; Experiment 2A. Data shown are the mean (+SEM) corticosterone levels (ng/ml) immediately following a drug-seeking test session under extinction conditions, in food restricted rats (*n*'s: 0.0 = 6, 30.0 = 3) and Sated rats (*n*'s: 0.0 = 6, 30.0 = 4). *Significantly different from the 0.0 mg/kg group, $p < 0.020$; #Significantly different from the Sated-0.0 group, $p = 0.03$; & $p = 0.07$ compared to the Sated-30.0 group.

6.42, $p = 0.019$, $\eta^2 = 0.19$; **Figure 4B**]. However, the number of inactive lever responses was very low (<25) and the rats clearly preferred the active lever.

Some corticosterone samples that were collected immediately following the test session were not included in the final analysis due to an unacceptable variability between the duplicates in the ELISA kit (>30%). Administration of RU486 resulted in increased levels of corticosterone in both the sated and FDR rats. However, treatment with RU486 resulted in considerably higher corticosterone levels in the FDR group (**Figure 5**). ANOVA revealed statistically significant main effects of feeding condition [$F_{(1, 15)} = 12.80$, $p = 0.003$, $\eta^2 = 0.30$] and antagonist dose [$F_{(1, 15)} = 14.13$, $p = 0.002$, $\eta^2 = 0.34$], and a significant feeding condition × antagonist dose interaction effect [$F_{(1, 15)} = 7.15$, $p = 0.017$, $\eta^2 = 0.17$]. Although this significant interaction effect was clearly driven by the dramatic increase in corticosterone levels induced by the RU486 treatment in the FDR rats, corticosterone levels in this group were not statistically significantly different from the sated, RU486-treated rats [$t_{(5)} = 2.33$, $p = 0.07$, Cohen's $d = 2.08$], probably due to the sample size in the FDR, RU486-treated group ($n = 3$). Further *post-hoc* tests are described in **Figure 5**.

EXPERIMENT 2B: THE EFFECT OF ADRENALECTOMY ON CHRONIC FOOD RESTRICTION-INDUCED AUGMENTATION OF HEROIN SEEKING IN THE RAT

Three rats were removed due to catheter leakage, failure to train or detached head-caps. Thus, the final analysis included 29 rats in 4 experimental groups: FDR-Sham = 6, FDR-ADX = 7, Sated-Sham = 7, Sated-ADX = 9.

On test day, the average BW of the Sated group (Sham: 391.57 ± 10.41 g; ADX: 396.89 ± 4.37 g) was statistically significantly greater [feeding condition: $F_{(1, 25)} = 108.85$, $p < 0.0001$] than the FDR group's BW (Sham: 310.17 ± 8.63 g; ADX: 312.00 ± 9.19 g). No statistically significant effects on BW for surgery group or the feeding condition × surgery group interaction were observed.

As can be seen in **Figure 6A**, the FDR group pressed the active lever more than the Sated group [feeding condition effect: $F_{(1, 25)} = 19.19$, $p < 0.001$, $\eta^2 = 0.40$] during the drug-seeking test. No statistically significant effects for surgery group or the feeding condition × surgery group interaction were observed. No significant effects were observed for inactive lever responding on test day (**Figure 6B**).

As expected, corticosterone levels in adrenalectomized rats were very low (<5 ng/ml), and were not affected by food restriction (data not shown).

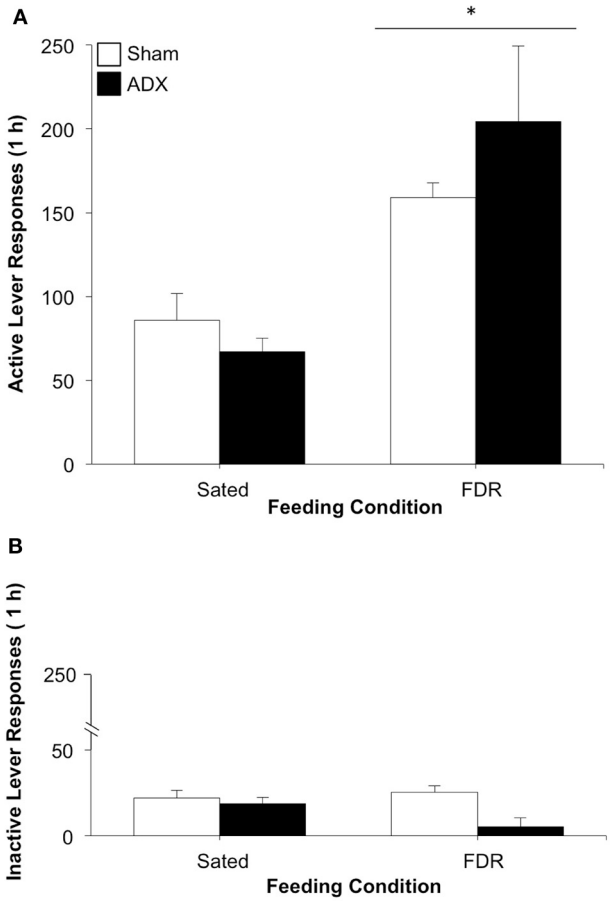

FIGURE 6 | The effect of adrenalectomy on heroin seeking in food-restricted (FDR) and sated rats under withdrawal; Experiment 2B. Data shown are the mean (+SEM) numbers of active **(A)** and inactive **(B)** lever responses made on test day by rats in the FDR (*n*'s: FDR-SHAM = 6, FDR-ADX = 7) and Sated (*n*'s: Sated-SHAM = 7, Sated-ADX = 9). Test day consisted of 1-h drug-seeking session under extinction conditions, following heroin self-administration training and 16 days of withdrawal under FDR or sated conditions. *Significantly different from the sated condition, $p < 0.001$.

DISCUSSION

Recent work in our laboratory has demonstrated an augmentation of heroin seeking in chronically food restricted rats, under withdrawal (D'Cunha et al., 2013). Thus, as expected, a prolonged period of food restriction resulted in a robust increase in heroin seeking, compared to sated rats, across all experimental groups in the current study. In contrast to the robust attenuation of acute food deprivation-induced reinstatement of drug seeking following treatment with CRF-receptor antagonists, treatment with R121919, a selective CRF_1-R antagonist, or α-helical CRF, a non-specific CRF-R antagonist, did not result in a statistically significant reduction in heroin seeking in food restricted rats under withdrawal. Similarly, neither treatment with RU486, a glucocorticoid receptor antagonist, nor adrenalectomy affected heroin seeking in this model.

Our findings are consistent with considerable evidence supporting a modulatory role for food restriction on drug-related behaviors in humans (Hall et al., 1992; Krahn et al., 1992; Cheskin et al., 2005) and in laboratory animals, where food deficiency drastically influences drug taking and the reinforcing properties of abused drugs (Carroll and Meisch, 1984; Stuber et al., 2002; Carr, 2007).

Despite previous evidence demonstrating that CRF_1-R antagonists attenuate acute food deprivation-induced reinstatement of extinguished cocaine and heroin seeking (Shalev et al., 2003, 2006), the experiments described here suggest that these findings do not extend to chronic food restriction-induced augmentation of heroin seeking in rats under withdrawal. The observed lack of effect for CRF-R antagonists in chronically food-restricted rats may be due to the differential effects of food restriction and deprivation on metabolism and behavior (Fulton et al., 2000) or differences between the reinstatement model and the withdrawal procedure used here. First, rats in the present study did not undergo a period of extinction. Extinction training and withdrawal (without extinction) result in activation of distinct neural circuits during drug-seeking tests (Fuchs et al., 2008), which might be differentially modulated by the CRF system. Second, our current study employed a prolonged period of mild stress induced by food restriction (Deroche et al., 1995) compared to the acute 24–48 h food deprivation we have used previously. Alterations in gene expression suggest that different neural adaptations occur following exposure to acute and chronic stress. For example, increased CRF_1-R and c-fos mRNA in the paraventricular nucleus of the hypothalamus (PVN) are observed following acute, but not chronic stress. In contrast, chronic stress results in lowered levels of CRF_1-R and c-fos mRNA in the PVN (Bonaz and Rivest, 1998). However, other reports have demonstrated the opposite result, where increased levels of CRF_1-R mRNA in the PVN were reported following chronic but not acute stress (Imaki et al., 1991). Notwithstanding these inconsistencies, there appear to be distinct adaptations in the CRF system following exposure to acute or chronic stress.

In the present study, rats were exposed to a 14-day food restriction stress, which may have resulted in progressive CRF-induced adaptations in critical neuronal circuits over the withdrawal period. For example, greater dopamine (DA) tissue levels in the nucleus accumbens (NAc), and reduced levels in the prefrontal cortex (PFC), were found 1 week following the completion of a chronic treatment (13 days) with CRF (Izzo et al., 2005). Moreover, differential effects for acute vs. chronic treatment with CRF antagonists are suggested by findings of Mällo et al. (2004) who reported a reduction in anxiety (as defined by increased exploration) in an elevated-zero-plus-maze test following chronic, but not acute treatment with a selective CRF_1-R antagonist. Consequently, acute CRF-R activation during the test may no longer be necessary to demonstrate the augmentation of heroin seeking in food restricted rats. Future studies should investigate the effects of chronic CRF-R antagonist treatment, over the withdrawal period, on the augmentation of heroin seeking induced by chronic food restriction.

An interesting, albeit not statistically significant, trend for a dose dependent reduction in responding on the previously heroin paired (active) lever on the test day was observed in the α-helical CRF-treated sated group. Recently, CRF-R antagonism was shown

to reduce cue-induced reinstatement of drug seeking (Moffett and Goeders, 2006), which could provide a possible explanation for the reduction of active lever responding observed in sated rats in the current experiments, following exposure to the drug-associated environment and cues. However, a similar pattern was found for inactive lever responding in the food restricted and sated groups, suggesting that the reduced lever seeking in the α-helical CRF-treated rats was not due exclusively to changes in the motivational value of the drug-associated stimuli. Furthermore, administration of R121919 did not reduce active or inactive lever responding in the drug treated groups, further supporting a lack of motivational effects for CRF-R antagonists in the current procedure. It is possible that treatment with α-helical CRF resulted in an overall reduction of locomotor responding, which was obscured by the increased drug-seeking behavior in the food restricted rats; yet, we found no indication for such an effect in the previous studies conducted in our laboratory (Shalev et al., 2006).

To ensure the efficiency of the treatment with α-helical CRF, its effect on anxiety-related behaviors was investigated. In this test a reduction in anxiety was assessed by the latency to consume a food pellet placed in the center of an open field, and the number of approaches made prior to finally consuming the food. We found shorter latencies and reduced number of approaches made before food consumption in food restricted rats that received α-helical CRF treatment. Rats that did not receive the drug treatment approached the food multiple times with no attempt at consumption and would instead continue to explore the environment.

To gain a better understanding of the involvement of the physiological stress response in food restriction-induced augmentation of heroin seeking, we investigated the role of corticosterone, the major stress-associated hormone. Chronically food restricted rats exhibit greater levels of corticosterone compared to controls (Carr, 1996). Furthermore, the elevated concentrations observed following food restriction are positively associated with the proclivity to self-administer cocaine. Additionally, the removal of corticosterone via adrenalectomy can also decrease the psychostimulant challenge-induced heightened locomotor activity observed in food restricted rats (Deroche et al., 1995; Piazza and Le Moal, 1996). It can be argued that the lack of effect following treatment with CRF-R antagonists indicates that the complete HPA-axis is not involved in food restriction-induced augmentation of heroin seeking. However, plasma levels of adrenocorticotropic hormone (ACTH) and the subsequent production of corticosterone can be affected by mechanisms independent of CRF's actions in the HPA axis (Tsigos and Chrousos, 2002).

In the current study, neither acute treatment with a glucocorticoid antagonist, RU486, nor adrenalectomy reduced the increased heroin seeking observed in chronically food-restricted rats under withdrawal. These results are consistent with past studies in the literature on stress- and reward-related behaviors. In CRF deficient mice, activity in an anxiety provoking situation (e.g., elevated plus maze) remains unaffected, in spite of a blunted HPA axis response and lowered concentrations of corticosterone (Dunn and Swiergiel, 1999). Therefore, a heightened physiological stress response may not always be necessary for the expression of stress-related behaviors. Abrahamsen and Carr (1996) demonstrated that in a lateral hypothalamus self-stimulation procedure, the sensitization of the rewarding effects of the stimulation by food restriction is unaltered following a treatment with a corticosterone synthesis inhibitor or an acute feeding-induced decrease in plasma corticosterone (Abrahamsen et al., 1995). The aforementioned studies argue against a modulatory role for corticosterone in rewarding-seeking. As Carr (2002) suggests, however, the most comprehensive test of corticosterone's involvement in food restriction would be to maintain corticosterone concentrations in the food restricted group at similar concentrations as those reported in the sated controls over the full period of restriction. A recent study from DiLeone's group (Guarnieri et al., 2012) offers further support for a mediating role for corticosterone in the effects of food restriction on motivation. Guarnieri et al. (2012) report that 5 days of mild food restriction in mice resulted in an upregulation of genes in the mesocorticolimbic system that are associated with the stress response. These changes in gene expression were critically dependent on food restriction-induced increases in corticosterone levels, and the hormone was also shown to be important for the potentiation of food seeking in food restricted mice. The authors suggest that the identified genes are the molecular signals that drive food restriction-induced behavioral plasticity (Guarnieri et al., 2012). It is important to note, however, that some of these corticosterone-dependent changes in gene expression were triggered following only 1 day of food restriction. In contrast, we have reported that a short-term food restriction period (3–5 days) is not sufficient to induced augmentation of heroin seeking (D'Cunha et al., 2013), suggesting that different, slower to develop, adaptations underlie this phenomenon.

Concentrations of corticosterone were statistically significantly greater in food restricted and sated rats after treatment with RU486 compared to the vehicle pretreatment. Interestingly, the magnitude of increase in the food-restricted group (∼500%) was greater than that in the sated group (∼74%) following RU486 treatment. We speculate that these differences in magnitude can be explained by the lack of negative feedback from circulating corticosterone, which resulted in amplification of the increased corticosterone levels that are typically found in food restricted rats.

There is evidence that the augmentation of drug seeking following food restriction can be modulated by homeostatic mechanisms that are triggered by the hunger state (Cabeza de Vaca and Carr, 1998). For example, infusions of ghrelin, an orexigenic gut hormone, can increase extracellular DA concentrations in the NAc (Jerlhag et al., 2007), and cocaine-induced increases of extracellular DA in the NAc are attenuated by ghrelin receptor antagonism (Jerlhag et al., 2010). Importantly, increased serum concentrations of ghrelin have been observed in response to cue-induced reinstatement of cocaine seeking (Tessari et al., 2007), suggesting an involvement of the peptide in the conditioned reinforcing effects of cocaine. However, the involvement of ghrelin in cocaine seeking and taking might not be easily generalized to other drugs. Treatment with a ghrelin antagonist did not impair food deprivation-induced reinstatement of heroin seeking, although central infusions of ghrelin did increase the breakpoints on a

progressive ratio schedule of heroin reinforcement (Maric et al., 2011). It is critical to note that the aforementioned study used acute food deprivation in a reinstatement of extinguished drug seeking procedure, which as mentioned above, might involve different brain mechanisms than prolonged food restriction in rats under withdrawal.

An additional peripheral signal that is involved in energy balance is leptin, an anorexigenic hormone that is secreted by peripheral adipocytes (Friedman and Halaas, 1998). Leptin can regulate activity in the mesocorticolimbic circuitry through its actions on ventral tegmental area (VTA) DA neurons, and has been implicated in reward processes (Fulton et al., 2000). Interestingly, leptin was shown to attenuate acute food deprivation-induced reinstatement of heroin seeking (Shalev et al., 2001). However, this effect was not observed with footshock stress- or heroin priming-induced reinstatement (Shalev et al., 2001), suggesting that leptin's effect on acute food deprivation-induced reinstatement was not mediated by DA or stress-related pathways. In contrast, it is possible that in chronically food-restricted rats, as in the current study, the decrease in leptin signal contributed to the sensitized response to the drug-associated cues through disinhibition of the mesocorticolimbic DA system (Hommel et al., 2006).

In conclusion, we suggest that pathways involved in the acute stress response are not critical for the expression of augmented drug seeking in food-restricted rats under withdrawal. Our findings, however, do not exclude a role for food restriction-induced prolonged increases in CRF and corticosterone in the induction of downstream brain adaptations, which in turn may drive the augmented drug seeking observed in rats under withdrawal from heroin.

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REFERENCES

Abrahamsen, G. C., Berman, Y., and Carr, K. D. (1995). Curve-shift analysis of self-stimulation in food-restricted rats: relationship between daily meal, plasma corticosterone and reward sensitization. *Brain Res.* 695, 186–194. doi: 10.1016/0006-8993(95)00764-H

Abrahamsen, G., and Carr, K. (1996). Effects of corticosteroid synthesis inhibitors on the sensitization of reward by food restriction. *Brain Res.* 726, 39–48. doi: 10.1016/0006-8993(96)00289-2

Baldwin, H. A., Rassnick, S., Rivier, J., Koob, G. F., and Britton, K. T. (1991). CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. *Psychopharmacology* 103, 227–232. doi: 10.1007/BF02244208

Bonaz, B., and Rivest, S. (1998). Effect of a chronic stress on CRF neuronal activity and expression of its type 1 receptor in the rat brain. *Am. J. Physiol.* 275(5 Pt 2), R1438–R1449.

Brewer, D. D., Catalano, R. F., Haggerty, K., Gainey, R. R., and Fleming, C. B. (1998). A meta-analysis of predictors of continued drug use during and after treatment for opiate addiction. *Addiction* 93, 73–92. doi: 10.1016/0031-9384(90)90301-J

Cabeza de Vaca, S., and Carr, K. D. (1998). Food restriction enhances the central rewarding effect of abused drugs. *J. Neurosci.* 18, 7502–7510.

Carr, K. D. (1996). Feeding, drug abuse, and the sensitization of reward by metabolic need. *Neurochem. Res.* 21, 1455–1467. doi: 10.1007/BF02532386

Carr, K. D. (2002). Augmentation of drug reward by chronic food restriction: behavioral evidence and underlying mechanisms. *Physiol. Behav.* 76, 353–364. doi: 10.1016/S0031-9384(02)00759-X

Carr, K. D. (2007). Chronic food restriction: Enhancing effects on drug reward and striatal cell signaling. *Physiol. Behav.* 91, 459–472. doi: 10.1016/j.physbeh.2006.09.021

Carroll, M. E., and Meisch, R. A. (1984). "Increased drug-reinforced behavior due to food-deprivation" in *Advances in Behavioral Pharmacology*, eds T. Thompson, P. W. Dews, and J. E. Barrett (New York, NY: Academic Press), 47–88.

Cheskin, L. J., Hess, J. M., Henningfield, J., and Gorelick, D. A. (2005). Calorie restriction increases cigarette use in adult smokers. *Psychopharmacology* 179, 430–436. doi: 10.1007/s00213-004-2037-x

D'Cunha, T. M., Sedki, F., Macri, J., Casola, C., and Shalev, U. (2013). The effects of chronic food restriction on cue-induced heroin seeking in abstinent male rats. *Psychopharmacology* 225, 241–250. doi: 10.1007/s00213-012-2810-1

Dallman, M. F., Akana, S. F., Bhatnagar, S., Bell, M. E., Choi, S., Chu, A., et al. (1999). Starvation: early signals, sensors, and sequelae. *Endocrinology* 140, 4015–4023. doi: 10.1210/en.140.9.4015

Deroche, V., Marinelli, M., Maccari, S., Le Moal, M., Simon, H., and Piazza, P. V. (1995). Stress-induced sensitization and glucocorticoids. I. Sensitization of dopamine-dependent locomotor effects of amphetamine and morphine depends on stress-induced corticosterone secretion. *J. Neurosci.* 15, 7181–7188.

Dunn, A. J., and Swiergiel, A. H. (1999). Behavioral responses to stress are intact in CRF-deficient mice. *Brain Res.* 845, 14–20. doi: 10.1016/S0006-8993(99)01912-5

Friedman, J. M., and Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature* 395, 763–770. doi: 10.1038/27376

Fuchs, R. A., Lasseter, H. C., Ramirez, D. R., and Xie, X. (2008). Relapse to drug seeking following prolonged abstinence: the role of environmental stimuli. *Drug Discov. Today Dis. Models* 5, 251–258. doi: 10.1016/j.ddmod.2009.03.001

Fulton, S., Woodside, B., and Shizgal, P. (2000). Modulation of brain reward circuitry by leptin. *Science* 287, 125–128. doi: 10.1126/science.287.5450.125

Greenwell, T. N., Funk, C. K., Cottone, P., Richardson, H. N., Chen, S. A., Rice, K. C., et al. (2009). Corticotropin-releasing factor-1 receptor antagonists decrease heroin self-administration in long- but not short-access rats. *Addict. Biol.* 14, 130–143. doi: 10.1111/j.1369-1600.2008.00142.x

Guarnieri, D. J., Brayton, C. E., Richards, S. M., Maldonado-Aviles, J., Trinko, J. R., Nelson, J., et al. (2012). Gene profiling reveals a role for stress hormones in the molecular and behavioral response to food restriction. *Biol. Psychiatry* 71, 358–365. doi: 10.1016/j.biopsych.2011.06.028

Gutman, D. A., Owens, M. J., Thrivikraman, K. V., and Nemeroff, C. B. (2010). Persistent anxiolytic affects after chronic administration of the CRF1 receptor antagonist R121919 in rats. *Neuropharmacology* 60, 1135–1141. doi: 10.1016/j.neuropharm.2010.10.004

Hall, S. M., Tunstall, C. D., Vila, K. L., and Duffy, J. (1992). Weight gain prevention and smoking cessation: cautionary findings. *Am. J. Public Health* 82, 799–803. doi: 10.2105/AJPH.82.6.799

Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z.-W., Gao, X.-B., et al. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 51, 801–810. doi: 10.1016/j.neuron.2006.08.023

Imaki, T., Nahan, J. L., Rivier, C., Sawchenko, P. E., and Vale, W. (1991). Differential regulation of corticotropin-releasing factor messenger-rna in rat-brain regions by glucocorticoids and stress. *J. Neurosci.* 11, 585–599.

Izzo, E., Sanna, P. P., and Koob, G. F. (2005). Impairment of dopaminergic system function after chronic treatment with corticotropin-releasing factor. *Pharmacol.*

Biochem. Behav. 81, 701–708. doi: 10.1016/j.pbb.2005.04.017

Jerlhag, E., Egecioglu, E., Dickson, S. L., and Engel, J. A. (2010). Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference. *Psychopharmacology* 211, 415–422. doi: 10.1007/s00213-010-1907-7

Jerlhag, E., Egecioglu, E., Dickson, S. L., Douhan, A., Svensson, L., and Engel, J. A. (2007). Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addict. Biol.* 12, 6–16. doi: 10.1111/j.1369-1600.2006.00041.x

Koob, G. F., and Heinrichs, S. C. (1999). A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. *Brain Res.* 848, 141–152. doi: 10.1016/S0006-8993(99)01991-5

Kosten, T. R., Rounsaville, B. J., and Kleber, H. D. (1983). Relationship of depression to psychosocial stressors in heroin addicts. *J. Nerv. Ment. Dis.* 171, 97–104.

Kosten, T. R., Rounsaville, B. J., and Kleber, H. D. (1986). A 2.5-year follow-up of depression, life crises, and treatment effects on abstinence among opioid addicts. *Arch. Gen. Psychiatry* 43, 733–738. doi: 10.1001/archpsyc.1986.01800080019003

Krahn, D. D., Gosnell, B. A., Grace, M., and Levine, A. S. (1986). CRF antagonist partially reverses CRF- and stress-induced effects on feeding. *Brain Res. Bull.* 17, 285–289. doi: 10.1016/0361-9230(86)90233-9

Krahn, D., Kurth, C., Demitrack, M., and Drewnowski, A. (1992). The relationship of dieting severity and bulimic behaviors to alcohol and other drug use in young women. *J. Subst. Abuse*. 4, 341–353. doi: 10.1016/0899-3289(92)90041-U

Lu, L., Shepard, J. D., Scott Hall, F., and Shaham, Y. (2003). Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. *Neurosci. Biobehav. Rev*. 27, 457–491. doi: 10.1016/S0149-7634(03)00073-3

Maric, T., Sedki, F., Ronfard, B., Chafetz, D., and Shalev, U. (2011). A limited role for ghrelin in heroin self-administration and food deprivation-induced reinstatement of heroin seeking in rats. *Addict. Biol.* doi: 10.1111/j.1369-1600.2011.00396.x

Marinelli, M., Le Moal, M., and Piazza, P. V. (1996). Acute pharmacological blockade of corticosterone secretion reverses food restriction-induced sensitization of the locomotor response to cocaine. *Brain Res*. 724, 251–255. doi: 10.1016/0006-8993(96)00309-5

Matheny, K. B., and Weatherman, K. E. (1998). Predictors of smoking cessation and maintenance. *J. Clin. Psychol.* 54, 223–235. doi: 10.1002/(SICI)1097-4679(199802)54:2<223::AID-JCLP12>3.0.CO;2-L

Mällo, T., Berggård, C., Eller, M., Damberg, M., Oreland, L., and Harro, J. (2004). Effect of long-term blockade of CRF(1) receptors on exploratory behaviour, monoamines and transcription factor AP-2. *Pharmacol. Biochem. Behav.* 77, 855–865. doi: 10.1016/j.pbb.2004.02.016

Moffett, M. C., and Goeders, N. E. (2006). CP-154,526, a CRF type-1 receptor antagonist, attenuates the cue-and methamphetamine-induced reinstatement of extinguished methamphetamine-seeking behavior in rats. *Psychopharmacology* 190, 171–180. doi: 10.1007/s00213-006-0625-7

Piazza, P. V., and Le Moal, M. (1998). The role of stress in drug self-administration. *Trends Pharmacol Sci*. 19, 67–74. doi: 10.1016/S0165-6147(97)01115-2

Piazza, P. V., and Le Moal, M. L. (1996). Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annu. Rev. Pharmacol. Toxicol.* 36, 359–378. doi: 10.1146/annurev.pa.36.040196.002043

Shaham, Y., Funk, D., Erb, S., Brown, T. J., Walker, C. D., and Stewart, J. (1997). Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. *J. Neurosci.* 17, 2605–2614. doi: 10.1016/j.physbeh.2011.08.020

Shalev, U., Finnie, P. S., Quinn, T., Tobin, S., and Wahi, P. (2006). A role for corticotropin-releasing factor, but not corticosterone, in acute food-deprivation-induced reinstatement of heroin seeking in rats. *Psychopharmacology* 187, 376–384. doi: 10.1007/s00213-006-0427-y

Shalev, U., Highfield, D., Yap, J., and Shaham, Y. (2000). Stress and relapse to drug seeking in rats: studies on the generality of the effect. *Psychopharmacology* 150, 337–346. doi: 10.1007/s002130000441

Shalev, U., Marinelli, M., Baumann, M. H., Piazza, P.-V., and Shaham, Y. (2003). The role of corticosterone in food deprivation-induced reinstatement of cocaine seeking in the rat. *Psychopharmacology* 168, 170–176. doi: 10.1007/s00213-002-1200-5

Shalev, U., Yap, J., and Shaham, Y. (2001). Leptin attenuates acute food deprivation-induced relapse to heroin seeking. *J. Neurosci.* 21, RC129.

Simms, J. A., Haass-Koffler, C. L., Bito-Onon, J., Li, R., and Bartlett, S. E. (2012). Mifepristone in the central nucleus of the amygdala reduces yohimbine stress-induced reinstatement of ethanol-seeking. *Neuropsychopharmacology* 37, 906–918. doi: 10.1038/npp.2011.268

Sinha, R. (2001). How does stress increase risk of drug abuse and relapse? *Psychopharmacology* 158, 343–359. doi: 10.1007/s002130100917

Sinha, R. (2008). Chronic Stress, Drug Use, and Vulnerability to Addiction. *Ann. N.Y. Acad. Sci.* 1141, 105–130. doi: 10.1196/annals.1441.030

Sinha, R., and O'Malley, S. S. (1999). Craving for alcohol: findings from the clinic and the laboratory. *Alcohol Alcohol.* 34, 223–230. doi: 10.1093/alcalc/34.2.223

Sinha, R., Garcia, M., Paliwal, P., Kreek, M. J., and Rounsaville, B. J. (2006). Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. *Arc. Gen. Psychiatry* 63:324. doi: 10.1001/archpsyc.63.3.324

Stuber, G. D., Evans, S. B., Higgins, M. S., Pu, Y., and Figlewicz, D. P. (2002). Food restriction modulates amphetamine-conditioned place preference and nucleus accumbens dopamine release in the rat. *Synapse* 46, 83–90. doi: 10.1002/syn.10120

Tessari, M., Catalano, A., Pellitteri, M., Di Francesco, C., Marini, F., Gerrard, P. A., et al. (2007). Correlation between serum ghrelin levels and cocaine-seeking behaviour triggered by cocaine-associated conditioned stimuli in rats. *Addict. Biol.* 12, 22–29. doi: 10.1111/j.1369-1600.2007.00052.x

Tomiyama, A. J., Mann, T., Vinas, D., Hunger, J. M., DeJager, J., and Taylor, S. E. (2010). Low Calorie Dieting Increases Cortisol. *Psychosom. Med*. 72, 357–364. doi: 10.1097/PSY.0b013e3181d9523c

Tsigos, C., and Chrousos, G. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J. Psychosom. Res*. 53, 865–871. doi: 10.1016/S0022-3999(02)00429-4

Zacny, J. P., and de Wit, H. (1991). Effects of food deprivation on subjective effects and self-administration of marijuana in humans. *Psychol. Rep.* 68, 1263–1274.

Zacny, J. P., and de Wit, H. (1992). The effects of a restricted feeding regimen on cigarette smoking in humans. *Addict. Behav*. 17, 149–157. doi: 10.1016/0306-4603(92)90019-R

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Stress and eating: a dual role for bombesin-like peptides

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The current obesity "epidemic" in the developed world is a major health concern; over half of adult Canadians are now classified as overweight or obese. Although the reasons for high obesity rates remain unknown, an important factor appears to be the role stressors play in overconsumption of food and weight gain. In this context, increased stressor exposure and/or perceived stress may influence eating behavior and food choices. Stress-induced anorexia is often noted in rats exposed to chronic stress (e.g., repeated restraint) and access to standard Chow diet; associated reduced consumption and weight loss. However, if a similar stressor exposure takes place in the presence of palatable, calorie dense food, rats often consume an increase proportion of palatable food relative to Chow, leading to weight gain and obesity. In humans, a similar desire to eat palatable or "comfort" foods has been noted under stressful situations; it is thought that this response may potentially be attributable to stress-buffering properties and/or through activation of reward pathways. The complex interplay between stress-induced anorexia and stress-induced obesity is discussed in terms of the overlapping circuitry and neurochemicals that mediate feeding, stress and reward pathways. In particular, this paper draws attention to the bombesin family of peptides (BBs) initially shown to regulate food intake and subsequently shown to mediate stress response as well. Evidence is presented to support the hypothesis that BBs may be involved in stress-induced anorexia under certain conditions, but that the same peptides could also be involved in stress-induced obesity. This hypothesis is based on the unique distribution of BBs in key cortico-limbic brain regions involved in food regulation, reward, incentive salience and motivationally driven behavior.

Keywords: gastrin-releasing peptide, neuromedin B, stress, reward, obesity, anorexia

INTRODUCTION

The worldwide prevalence of obesity has doubled since 1980 and we have entered what is being called a "tsunami of obesity." According to the 2008 Statistics Canada report, 61% of adult Canadians were overweight or obese, contributing to and/or exacerbating outcomes of various health conditions including cardiovascular disease, type II diabetes, sleep apnea, as well as many psycho-social disorders (Stein and Colditz, 2004).

While the causes of the obesity epidemic are complex, stress has been identified as an important factor. Increased rates of obesity have been accompanied by a concomitant rise in perceived stress in North America. In humans, greater reported stress is associated with greater desire to eat, including binge eating (Warne, 2009). Further, high levels of perceived stress correlate with weight gain and obesity, as women who self-identify as high-stress responders to a laboratory stressor, have significantly greater BMI and sagittal diameters, than low-stress responders (Tomiyama et al., 2011). Similarly, students who self-identify as stress-eaters have higher levels of stress hormones like cortisol, during stressful periods, such that the elevated cortisol may be associated with their increased desire to eat (Epel et al., 2004).

While increased indices of stress in society are a tempting explanation for the obesity epidemic, it may be deceptively simple, as stress appears to affect feeding in a bidirectional manner. In humans, stress causes increased food intake in one subset of the population and conversely causes decreased food intake in another; why some people lose weight and other gain weight is not yet well understood (Stone and Brownell, 1994; Epel et al., 2004). Stress-induced anorexia is also commonly seen in animal research, where rats fed a standard chow diet lose weight or decrease food intake in response to chronic stress (e.g., repeated restraint or variable stressors) (Martí et al., 1994; Harris et al., 1998; Pecoraro et al., 2004). Indeed stress severity can alter Chow intake, such that the greater the severity of the stressor, the greater the suppression of Chow intake (Torres and Nowson, 2007; Maniam and Morris, 2012). However, there is also evidence of rodents that alternatively increase food consumption or gain weight in response to chronic stress, in particular, repeated social defeat (Foster et al., 2006; Tamashiro et al., 2007a,b). It bears noting that the stressor paradigms used by researchers, vary widely in terms of duration, intensity and nature (i.e., systemic, neurogenic, psychosocial etc.), making it exceedingly challenging to categorize the varied feeding responses.

Beyond the nature of the stressor itself, the type of food available appears to vary greatly, potentially contributing to the discrepant findings ranging from stress-induced anorexia to stress-induced obesity. In rats, while Chow consumption often decreases following repeated stress, consumption of tasty, calorie dense "palatable" food (typically high fat/high sugar content) remains unaffected (Ortolani et al., 2011) and the proportion of palatable food eaten relative to standard Chow can increase (Pecoraro et al., 2004). Additionally, while stress often induces weight loss in rats fed standard lab Chow, this weight loss can be reversed if given access to a palatable food diet (Harris et al., 1998; Pecoraro et al., 2004; Ortolani et al., 2011). Further, our lab has shown that palatable food consumption exacerbated the effects of a stressed rat's ability to handle a glucose load challenge and led to increased accumulation of visceral fat (MacKay et al., 2011), indicating that the combination of stressor exposure and access to palatable food may predispose individuals to developing metabolic syndrome and/or obesity later in life.

The interactions between feeding regulation and stress must be complex to produce such varied phenotypes; indeed, feeding regulation under non-stress conditions involves many interacting signals, and the mechanism(s) becomes increasingly complex when stress is introduced (Torres and Nowson, 2007; Maniam and Morris, 2012). It is not surprising, then, that many of the regulatory systems and circuitry that govern feeding are sensitive to stress. Several neural signals, neuropeptides in particular, serve dual roles as regulators of both feeding and stress response, and are thus well-positioned to mediate stress-induced changes in feeding behavior. These peptides include (but are not limited to) corticotropin-releasing factor (CRF), leptin, ghrelin, orexin, neuropeptide Y, melanocortin and cholecystokinin (Crawley and Corwin, 1994; Dallman et al., 1995; Hanson and Dallman, 1995; Merali et al., 1998; Koob and Heinrichs, 1999; Ahima and Flier, 2000; Vergoni and Bertolini, 2000; Dhillo et al., 2002; Ueta et al., 2003; Spinazzi et al., 2006; Stevanović et al., 2007; Kirsz and Zieba, 2011; Barson et al., 2013).

Another family of peptides similarly implicated in both feeding and stress is the bombesin-like peptides (which will henceforth be referred to as BBs). Bombesin, a 14 amino acid peptide first isolated from the skin of the frog *Bombina bombina* (Erspamer et al., 1970), originally generated intense interest because of its potent biological actions in mammals (Panula, 1986). Two mammalian bombesin homologs were subsequently discovered including gastrin-releasing peptide (GRP) and neuromedin B (NMB) (McDonald et al., 1979; Minamino et al., 1983, 1988). Appropriate receptors have also been identified (Minamino et al., 1988; Spindel et al., 1990; Battey and Wada, 1991; Jensen et al., 2008): whereas GRP has a greater affinity for BB_2 receptors, NMB preferentially activates the BB_1 receptor subtype (Spindel et al., 1990; Battey and Wada, 1991; Jensen et al., 2008), and the BB_3 receptor is a structurally related orphan receptor whose endogenous ligand remains unidentified (Weber et al., 1998).

BBs have long been recognized for their satiety properties as they are able to shorten meal size and duration of all mammals tested [for reviews, see (Merali et al., 1999; Yamada et al., 2002)]. Exogenous BB administration also activates the hypothalamic-pituitary-adrenal (HPA) axis and endogenous BBs are released during stressor exposure suggesting a role in mediation and/or modulation of the stress response (Merali et al., 2002). These facts, which will be expanded upon below, provide the framework for our first contention; that BBs play a role in stress-induced anorexia. However, beyond this more obvious role, we also contend that when stressor exposure is combined with a palatable food diet, the satiety effects of BB are superseded by extra-hypothalamic (cortico-limbic) BBs that *promote* obesity. This contention is based on the following which will be outlined in detail below: BBs (1) are released in response to not only aversive events (stressor exposure), but appetitive (food reward) events as well (Merali et al., 1998); and (2) are specifically localized in key brain regions involved in both stress and reward circuits, where they influence motivationally driven behavior (Merali et al., 2004, 2011, 2013; Mountney et al., 2008).

PALATABLE FOOD IMPACTS FEEDING RESPONSE TO STRESS

Palatable food may be distinguished from regular Chow because it is capable of activating neural reward circuitry. The powerful rewarding properties of food have been paralleled to those of drugs of abuse, and thus overeating has been compared to addiction (Dagher, 2009; Avena and Gold, 2011). Removal of a palatable diet can induce withdrawal-like behaviors (Cottone et al., 2009) and can cause rodents to endure aversive stimuli in order to regain access to palatable food (Pickering et al., 2009). Interestingly, stress is implicated in the reinstatement of not only substance abuse among abstinent drug users but also of failure among dieters (Adam and Epel, 2007). Stress and food reward both activate a broad array of neurocircuits involving several brain regions, including limbic [amygdala, nucleus accumbens (NAcc)] and cortical areas [anterior cingulate cortex (ACC)] (Lutter and Nestler, 2009; Dallman, 2010); circuits that often overlap.

It is noteworthy that the ability of palatable food to activate reward circuitry is associated with another phenomenon, whereby access to palatable food can mitigate or dampen the effects of stressors (Pecoraro et al., 2004; Dallman et al., 2005; Ulrich-Lai et al., 2010). Access to so-called "comfort food" appears to diminish the activation of the HPA axis in response to stress. This is reflected by attenuated release of adrenocorticotropic hormone (ACTH) and corticosterone following acute (restraint) stress (Kinzig et al., 2008; Foster et al., 2009; Christiansen et al., 2011a) and chronic stress (Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Maniam and Morris, 2010b). In addition, consumption of palatable food has also been linked to improved emotional states, as reflected by reduced anxiety- and depressive-type behaviors (Maniam and Morris, 2010a,b; Ulrich-Lai et al., 2010). Consistent with these reports, our lab recently showed that resting and stressor-induced levels of corticosterone were attenuated in rats with access to the palatable (or comfort) foods, compared to controls that only had access to Rat Chow (or "mundane" food). In addition, episodic stressor exposure during the juvenile period is also associated with profound long-term anxiety and this effect is attenuated by access and consumption of comfort food (MacKay et al., 2011). The ability of comfort food to dampen the effects of stress appears to be linked to its hedonic value, as oral consumption of sucrose or non-caloric sweetener

also provides stress-buffering effects, while intra-gastric gavage of sucrose does not (Ulrich-Lai et al., 2010).

The increasing levels of perceived societal stress accompanied by stress-dampening properties of comfort food may, in part, contribute to the obesity epidemic. It has been suggested that the stress relief provides negative reinforcement for the consumption of palatable food (Parylak et al., 2011). The learned association between comfort food and stress relief may result in the habitual consumption of comfort food in response to stress (Dallman, 2010), particularly given that stress promotes habitual behavior at the expense of goal-directed behaviors (Schwabe and Wolf, 2009).

FEEDING AND STRESS: OVERLAPPING NEURAL CIRCUITRY

The decision to eat or not to eat is regulated by two parallel and interacting systems, namely the homeostatic and the non-homeostatic systems (Kelley et al., 2005; Lutter and Nestler, 2009). The homeostatic system includes classic hypothalamic and brainstem pathways that govern energy balance in response to nutrient availability (Suzuki et al., 2010). The hypothalamus is a key region where many feeding circuits converge, (Benarroch, 2010; Maniam and Morris, 2012; Sinha and Jastreboff, 2013); this region is also known to participate in the mediation of the stress response. Stress activates the HPA axis, causing cascading release of CRF from the hypothalamus, ACTH from the anterior pituitary, and finally glucocorticoids (GCs) from the adrenals. It is noteworthy that these essential stress signals also have effects on feeding; in fact, central administration of CRF inhibits feeding (Arase et al., 1988), while central administration of GCs promotes feeding (Dallman et al., 2007). Within the hypothalamus, the arcuate nucleus contains two populations of neurons; one that stimulates feeding and one that inhibits feeding (Benarroch, 2010; Suzuki et al., 2010). These neurons project to the lateral hypothalamus, which communicates with reward circuitry (to be further discussed later), and the paraventricular nucleus of the hypothalamus (PVN) where CRF-producing neurons initiate the HPA axis cascade of stress response (Suzuki et al., 2010; Pandit et al., 2011). The hypothalamic nuclei are also responsive to several feeding signals, including insulin, leptin, and GCs (Dallman et al., 2006; Maniam and Morris, 2012; Sinha and Jastreboff, 2013). It is worthy of note that BBs, (particularly GRP) and their receptors as well as BB_3 receptor mRNA are highly localized in the hypothalamus at key feeding sites including the PVN and arcuate nucleus (Battey and Wada, 1991; Ladenheim et al., 1992; Zhang et al., 2013).

The homeostatic pathways are then embedded in a much larger neural circuitry referred to as the non-homeostatic, or cortico-limbic, system (Kelley et al., 2005; Lutter and Nestler, 2009); this is supported anatomically as many hypothalamic nuclei receive inputs from several relevant cortico-limbic regions (Benarroch, 2010; Berthoud, 2011; Stanley et al., 2011). The non-homeostatic system coordinates metabolic needs with external factors including external challenges, habits, and pleasurable feelings, and enables consumption of palatable foods well beyond the point when energy demands have been met (Kampe et al., 2009; Zheng et al., 2009; La Fleur et al., 2010). The limbic circuitry is known for its involvement in emotion (Davidson and Irwin, 1999), but cortico-limbic circuitry also mediates the rewarding aspects of food, including "liking," which is the pleasure associated with actual food consumption, and "wanting," which is the motivation to obtain food (Berridge, 1996).

As stipulated earlier, within the cortico-limbic circuitry are sites involved in feeding, stress and reward. The NAcc appears to be a critical region in feeding, especially of palatable food (Kelley et al., 2005; Alsiö et al., 2010; Miner et al., 2010). Indeed, food reward is capable of eliciting dopamine (DA) release from the NAcc in the same way as do addictive drugs such as cocaine and amphetamine (Hernandez and Hoebel, 1988; Pandit et al., 2011). Parenthetically, stress also elicits DA release from the NAcc (Abercrombie et al., 1989; Deutch and Cameron, 1992; Kalivas and Duffy, 1995), and stress-induced DA release from the NAcc is absent in rats that cannot produce GCs (Rougé-Pont et al., 1998). Importantly, extremely high densities of both BB_1 and BB_2 receptors are localized at the NAcc (Ladenheim et al., 1992). In contrast, only low to moderate levels of BB_3 receptor mRNA are expressed at this site (Zhang et al., 2013).

The amygdala is activated by both pleasant and aversive tastes (O'Doherty et al., 2001b) and contains two nuclei of interest, namely the basolateral amygdala (BLA) and the central amygdala (CeA). Both nuclei are implicated in stress (Davis and Whalen, 2001) as well as reward circuitry (Ahn and Phillips, 2002; Carelli et al., 2003), particularly in the conditioning of reward cues (Mahler and Berridge, 2009; Jones et al., 2010), including food-related cues (Petrovich et al., 2009; Petrovich, 2011). Within the amygdala, a moderate density of BB_1, BB_2, and BB_3 receptor mRNA are expressed at the CeA (Ladenheim et al., 1992), whereas BB_2 receptor mRNA is highly expressed in the lateral amygdala (part of the BLA complex) (Shumyatsky et al., 2002).

The ACC, which is innervated by the hypothalamic arcuate nucleus via the lateral hypothalamus (Kampe et al., 2009), is involved in emotion (Shackman et al., 2011) as well as higher order processes such as decision-making (Rosenbloom et al., 2012), self-awareness (Allman et al., 2010), attention (Weible, 2013), and reward (O'Doherty et al., 2001a; Berthoud, 2011). Imaging and electrophysiological studies further support involvement of the ACC in food reward as it is responsive to the sensory or hedonic properties as well as the palatability of food (O'Doherty et al., 2001b; Verhagen et al., 2003; Rolls, 2005). We recently showed that activation of GRP receptors in the ACC elicits GRP, but not CRF, release at the BLA, suggesting a functional pathway between these two regions utilizing BBs (Merali et al., 2013). It is of interest to note that there is a population of specialized neurons within the ACC of humans and primates, that selectively express NMB and GRP (Allman et al., 2010); the so called von economo neurons (VENs) are involved in consciously motivated behavior. While they are not as clearly delineated in the brains of lower mammals, NMB and GRP mRNA, are expressed in a restricted population of neurons in the ACC of rodents (Allman et al., 2011) thought to be homologous to VENs in humans and represent an intriguing target for investigation with respect to ingestion-related processes.

FEEDING AND STRESS: OVERLAPPING NEURAL MODULATORS

Beyond the overlapping circuitry between stress and feeding, there are also overlapping neurochemical signaling systems. Indeed, it is increasingly being recognized that many of the peptides involved in the regulation of food intake also seem to influence the stress response. Such peptides thus are well positioned to play a role in stress-induced changes in feeding behavior, including stress-induced anorexia or stress-induced obesity. For example, cholecystokinin, a satiety peptide involved in meal termination (Moran, 2006) activates the HPA axis (Antonijevic et al., 2000; Karlsson et al., 2005) and is also a powerful panicogenic agent (Zwanzger et al., 2012). Conversely, the orexigenic peptide neuropeptide Y, suppresses HPA activity and has anxiolytic properties (Antonijevic et al., 2000; Karlsson et al., 2005). In addition, both orexin and ghrelin which promote food intake or leptin which suppresses food intake all stimulate the HPA axis (Ahima and Flier, 2000; Asakawa et al., 2001; Spinazzi et al., 2006; Barson et al., 2013; Uchida et al., 2013). Likewise, BBs appear to have a dual function in feeding and stress responses, which will be further discussed below.

BBs IN FEEDING AND STRESS: POTENTIAL ROLE IN STRESS-INDUCED ANOREXIA

BBs influence a wide range of biological processes including thermoregulation, itch sensation, smooth muscle contraction, cell growth, endocrine response as well as numerous behavioral effects (Schjoldager et al., 1991; Itoh et al., 1995; Shumyatsky et al., 2002; Mountney et al., 2008; Merali et al., 2011, 1999; Su and Ko, 2011; Saito et al., 2013). However, this family of peptides, which is distributed throughout the gastrointestinal tract and brain, are widely recognized for their ability to influence digestion and food intake. As the name of one mammalian form, GRP, implies, BBs dose dependently stimulate gastrin and gastric acid secretion when administered peripherally (Knigge et al., 1984; Hildebrand et al., 2001), however, when injected into the brain, BB and GRP are potent inhibitors of gastric acid secretion (Martinez and Taché, 2000). On a behavioral level, both systemic and central administration of BBs suppress food intake and evoke behavioral and physiological responses akin to spontaneous satiety (Kulkosky et al., 1982; Smith and Gibbs, 1984; Merali et al., 1999). Bombesin is the most potent at suppressing food intake (due to activation of both BB_1 and BB_2 receptors), followed by GRP and then NMB (Sayegh, 2013). The satiety effects of exogenously administered BBs will not be outlined in further detail as they have been well described in several review papers (Gibbs and Smith, 1988; Merali et al., 1999; Yamada et al., 2002; Majumdar and Weber, 2011; Sayegh, 2013). Additional evidence for a role of BBs in the regulation of food intake comes from studies showing changes in peptide levels or mRNA expression in different metabolic states. For example, our lab has shown changes in tissue levels of immunoreactive (ir)-BBs at specific gut and brain regions in response to food ingestion and deprivation (Merali and Kateb, 1993; Plamondon and Merali, 1997). During a spontaneous meal ingestion, levels of ir-BBs increased significantly at hypothalamic structures including the PVN, arcuate nucleus and dorsomedial nucleus (Plamondon and Merali, 1997). Moreover, interstitial levels of BBs (assessed using push-pull perfusion) at the PVN were higher before meal ingestion and after the meal, as compared to those noted during food ingestion (Plamondon and Merali, 1994). More recently it was shown that food deprivation decreased GRP mRNA expression at the PVN, while a melanocortin agonist increased GRP mRNA at this site (Ladenheim et al., 2009).

The use of knockout strategy has further revealed that a lack of BB_3 receptors results in hyperphagia, leptin and insulin resistance, glucose metabolism dysregulation and the development of late onset obesity (Ohki-Hamazaki et al., 1997). Moreover, treatment with a novel synthetic BB_3 agonist results in weight loss and increased metabolic rate in mice and dogs (Guan et al., 2011), supporting evaluation of the BB_3 receptor as a potential therapeutic target for obesity (Zhang et al., 2013). It is also noteworthy that mice lacking BB_2 receptors eat more food during a meal than wild type mice and gain more weight over the long term, consistent with a role for this receptor subtype in satiety (Ohki-Hamazaki et al., 1997; Ladenheim et al., 2002). In contrast, mice lacking the BB_1 receptor showed no alterations in food intake or body weight gain (Ohki-Hamazaki et al., 1999), however, human genetic studies support a strong association between polymorphisms on the NMB gene and increased adiposity and obesity (Bouchard et al., 2004; Spálová et al., 2008; Pigeyre et al., 2010). Interestingly, in adolescence, the association between the polymorphism on the NMB gene and obesity was exacerbated in families of lower socio-economic status (Pigeyre et al., 2010). High fat diets, low physical activity and exposure to chronic stress are more prevalent in families of low socio-economic status (James et al., 1997; Baum et al., 1999).

BBs are also implicated in the mediation of the stress response. BBs are located in all major nodes of the HPA axis including the PVN, the anterior pituitary and the adrenal gland in addition to other stress responsive regions (Merali et al., 2002). Central administration of BBs activates both the HPA axis and the sympathetic branch of the autonomic nervous system as reflected by increased release of ACTH, corticosterone, norepinephrine and epinephrine; these effects are blocked by pretreatment with competitive and specific BB receptor antagonists (Brown et al., 1979, 1988; Gunion et al., 1989; Carver-Moore et al., 1991; Olsen et al., 1992; Malendowicz and Nussdorfer, 1995; Okuma et al., 1996; Au et al., 1997; Garrido et al., 1998, 1999; Malendowicz, 1998).

Considerable evidence suggests that BBs exert some of these effects via activation of CRF neurons. For example, pretreatment with a CRF receptor antagonist can block the endocrine, sympathetic and behavioral effects of central GRP administration (Garrido et al., 1998, 2002; Kent et al., 2001b). Moreover, we reported that central BB administration stimulates the release of CRF from the median eminence (the primary source of CRF release during HPA activation) translating into an increased availability of this peptide downstream at the anterior pituitary (Kent et al., 2001a). Interestingly, there is also recent evidence of co-localization of BB_3 and CRF receptors within the hypothalamus, including at the PVN and dorsomedial nucleus, yet the functional significance of this overlapping circuitry remains to be determined (Zhang et al., 2013).

Moreover, the BB systems are stress responsive as site-specific alterations in the endogenous levels of BBs and BB receptor densities are observed in response to acute stressor (restraint) exposure, including increased BBs at the hypothalamus and increased BB receptors at the PVN (Kent et al., 1998). Finally, we observed that acute restraint elicits the release of both CRF and BBs at the CeA (Merali et al., 1998), whereas chronic restraint exposure is associated with elevated interstitial levels of GRP at the anterior pituitary (Merali et al., 2009).

Taken together, these results suggest that under normal, non-stressful conditions, BBs have satiety effects. Given that BBs are released in response to stressor exposure (both acute and chronic), this peptidergic system is likely involved in stress-induced anorexia. Logically, then, weight gain associated with some models of stress (i.e., chronic psychosocial stress) may be linked to a decreased ability to respond to BBs' satiety effects, which is supported by the finding that obese women are less sensitive to BB-induced satiety than lean women (Lieverse et al., 1998), and this is also consistent with the obesity seen in BB receptor knockout mice. We could speculate that reduced sensitivity to BB may be the result of stressor-induced alterations in BB signaling leading to down-regulation of BB receptors at feeding relevant sites, attributable to prolonged release and exposure to BBs. Indeed, we have observed enhanced interstitial level of BBs at the anterior pituitary (located downstream of the hypothalamus) in response to chronic stressor (14 once daily restraint sessions) exposure (Merali et al., 2009), which could provide a mechanism for a stress-induced down-regulation of BB receptors. In support of this contention, we recently observed reduced mRNA expression of BB_2 receptors at the PVN following chronic corticosterone exposure (unpublished finding; see **Figure 1**). Moreover, sustained BB exposure (via chronic infusion) resulted in a down-regulation of BB receptors at the PVN and a tolerance development to the feeding suppressant effects of BB (Plamondon et al., 1998).

BBs IN INCENTIVE SALIENCE AND REWARD PROCESSES: POTENTIAL ROLE IN STRESS-INDUCED OBESITY

It was originally thought that the classic feeding regulators acted predominantly on homeostatic systems to control energy balance; however, increasing evidence suggests that food intake is a much more complex process, involving a much broader array of functions. In keeping with the contention of dual roles in feeding and stress, several years ago we reported that both appetitive (palatable food; graham crackers) and aversive (restraint stress) stimuli provoked *in vivo* release of BBs and CRF at the CeA, with a parallel rise in circulating corticosterone levels (Merali et al., 1998) (see **Figure 2**). To explain similar neurochemical responses to both aversive and appetitive stimuli, we suggested that rather than evoking fear and anxiety, these so-called "satiety/stress peptides" may serve to draw attention to biologically significant events (or cues) such as those associated with food availability as well as those posing physical threat. This would be akin to dopaminergic responses that might act in a similar capacity (Richardson and Gratton, 1996; Wickelgren, 1997). Indeed, dopaminergic neurons within the prefrontal cortex and NAcc, once thought to be exclusively involved in reward, were subsequently found to be responsive to stressors or stimuli with a negative valence (Horvitz, 2000). These observations led to the suggestions that dopaminergic signals contribute to specific cognitive functions and/or arousal (Richardson and Gratton, 1996; Horvitz, 2000). With time, this idea of stressor-induced increased incentive salience became a cornerstone to Dallman's "comfort food theory of obesity"(Dallman et al., 2005; Dallman, 2010). Her work showed that chronic stressor exposure elicits high levels of GCs and increases synthesis of CRF at stress/reward-responsive cortico-limbic sites like the CeA, which in turn enhances both the drive to consume as well as the salience of palatable foods (Foster et al., 2009). Once consumed, comfort foods themselves activate reward centers to subsequently reduce HPA activity.

Like BBs, the CRF family of peptides potently suppress food intake, and have been implicated in stressor-induced decreases in food intake (Dunn and Berridge, 1990; Koob and Heinrichs, 1999). However, beyond satiety effects, CRF has also

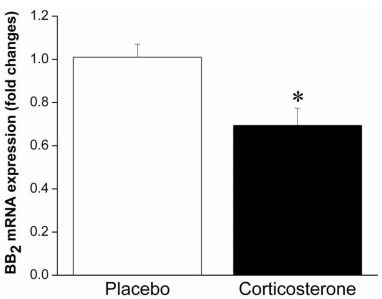

FIGURE 1 | Mean ± SEM (fold change) of mRNA expression of BB_2 at the PVN in placebo or corticosterone pellet (100 mg, 21 day slow release) implanted rats. Chronic corticosterone exposure resulted in a significant reduction in BB_2 mRNA expression at the PVN. *Significantly different from placebo at $p < 0.05$.

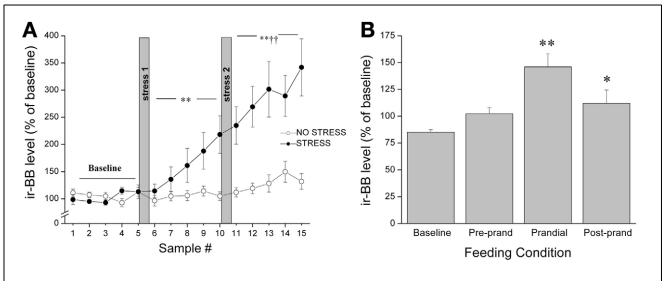

FIGURE 2 | Release of BBs at the CeA as measured by *in vivo* microdialysis in response to (A) restraint stress exposure (20 min hand restraint on two separate occasions called stress 1 and stress 2) or (B) presentation and ingestion of a palatable snack where microdialysis samples were collected continually and pooled every 30 min for 5 h. The quantity of food ingested during the 30 min bin was noted and the 30 min period before meal initiation was considered the preprandial period, and the 30 min sample preceding this was considered the baseline. Both stressor exposure and ingestion of a palatable snack elicited a significant increase in the release of BBs (and CRF) at the CeA accompanied by a parallel increase in plasma corticosterone levels. *,**Significantly different from baseline at $p < 0.05$ and $p < 0.01$, respectively. ††Significantly different from stress 1 at $p < 0.01$.

been implicated in reward pathways and incentive salience. For instance, increase in the release of CRF or its mRNA expression is provoked by natural rewards and incentive cues, at relevant cortico-limbic sites including the ACC and CeA (Merali et al., 2004; Foster et al., 2009). Moreover, CRF at the NAcc amplifies positive motivation for cued rewards by magnifying incentive salience (Peciña et al., 2006) and injection of CRF at this site elicits conditioned place preference for the chamber paired with CRF; the conditioning is dependent on CRF-induced DA release in this region (Lemos et al., 2012). Finally, in humans, low dose CRF administration increased palatable food consumption in a cortisol dependent manner (George et al., 2010).

Despite the provocative data, BBs involvement in incentive salience and reward has yet to be fully investigated. Given the similarities between BBs and CRF, and the fact that many of BBs stress effects appear to be mediated by CRF (as described above), we expect that BBs may similarly affect reward processes. As mentioned earlier, like CRF, BBs are released at the CeA in response to both stressor exposure and ingestion of a palatable snack (Merali et al., 1998). Notably, rats exposed to high chronic (14 days) doses of corticosterone (via systemic pump implants) show exaggerated stressor-provoked GRP (and CRF) release at the CeA and medial prefrontal cortex (which encompasses the ACC) (Merali et al., 2008) (see **Figure 3**). These findings are consistent with the notion of a sensitized BB release at cortico-limbic structures under stressful conditions (characterized by high GC levels).

In further support of the contended role of BBs' in motivation/reward, these peptides appear to interact with DA at key reward structures. DA is the neurotransmitter most closely associated with reward processes (Wise, 2004, 2006; Covey and Howard, 2011; Volkow et al., 2011), and has been implicated as one of the mediators of food reward processes (Wise et al., 1978; Bassareo and Di Chiara, 1999; Volkow et al., 2011). Our lab has shown robust meal-related fluctuations in levels of BBs at the NAcc (Plamondon and Merali, 1997). Microinjection of BBs at the NAcc elicits a marked increase in locomotor activity that is blocked by pretreatment with a D1 receptor antagonist, implicating BBs' capacity to modulate dopaminergic activity within this structure (Schulz et al., 1984; Johnston and Merali, 1988). Blockade of D1 and/or D2 receptors also attenuates the central BB-elicited increase in locomotor activity and grooming (Piggins and Merali, 1989; Merali and Piggins, 1990). Additionally, BBs increased DA synthesis in the dorsal striatum, olfactory tubercles, and hypothalamus (Widerlöv et al., 1984) and increased the activity of tuberoinfundibular and tuberohypophysial DA neurons (Manzanares et al., 1991).

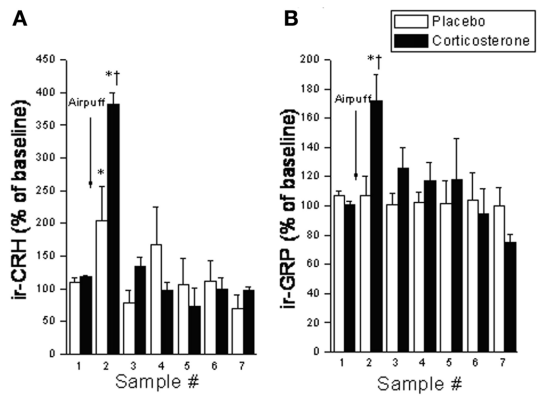

FIGURE 3 | Interstitial levels of (A) immunoreactive (ir) -CRF or (B) ir-GRP (expressed as a percentage of baseline values) at the CeA under basal conditions and following exposure to an airpuff stressor (5 airpuffs; 5 s puff/min) in placebo or corticosterone pellet (100 mg, 21 day slow release) implanted rats. Chronic corticosterone exposure potentiated the stressor-elicited release of CRF and GRP. *Significantly different from respective baseline sample at $p < 0.05$. †Significantly different from (sample-matched) placebo control condition at $p < 0.05$.

It is also noteworthy that BBs interact with the inhibitory neurotransmitter GABA. The GABAergic system is critical for the regulation of both reward (Wirtshafter and Stratford, 2010; Welberg, 2012), and stress (Herman et al., 2004), and is thought to be tied to motivational aspects of feeding (Truong et al., 2002; Takagi et al., 2003). Injection of $GABA_A$ or $GABA_B$ agonists at the NAcc shell produces profound hyperphagia in satiated rats (Stratford and Kelley, 1997; Basso and Kelley, 1999; Baldo et al., 2005). Moreover, chronic stress increased expression of the GABA-producing enzyme glutamic acid decarboxylase (GAD65) at the anterior hypothalamus but decreased GAD65 expression at the dorsal hypothalamus, effects reversed by palatable diet (sucrose) consumption (Christiansen et al., 2011b). GABA is also a key signal in reward pathways, as both a $GABA_A$ agonist and amphetamine injected at the NAcc shell increase the breaking point of lever pressing for food reward (Wirtshafter and Stratford, 2010), suggesting the rodents will "work harder" to attain reward. GRP infusion increases GABA efflux at the ventral hippocampus, an effect blocked by a BB_2 receptor antagonist (Andrews et al., 2000). Moreover, GABAergic interneurons at the lateral amygdala abundantly express BB_2 receptors (Shumyatsky et al., 2002), and application of GRP stimulates these interneurons to enhance inhibition of principal neurons (Cao et al., 2010). GRP application also facilitates GABA release at the ACC and amygdala (Cao et al., 2010). While the relationship between BBs and GABA at the amygdala is thought to modulate learned fear (Shumyatsky et al., 2002), their interactions in reward pathways, potentially at the NAcc, have yet to be fully elucidated and present an intriguing avenue for further investigation.

Taken together, BBs act at reward sites, potentially through modulation of DA and/or GABA functioning. As such, the necessary "hardware" is available to support a role for BBs in reward processes. Additionally, our lab now has preliminary evidence indicating that microinjection of BBs at the NAcc elicits DA release at this site. Moreover, like for CRF, injection of BBs at the NAcc is capable of eliciting conditioned place preference (manuscript in preparation). Thus, we hypothesize that, like CRF, BBs may act to increase the incentive salience associated with food reward. It has been suggested that the combination of GCs and CRF may act to associate the feeling of stress with the relief of stress by palatable food (Dallman, 2010). Similarly, BBs' ability to increase incentive salience, through GCs, and/or through interactions with DA or GABA, could strengthen a learned association between stress and palatable food, to enhance the rewarding

properties of palatable food and to promote palatable food consumption.

In the realm of addiction research, which may also apply to excessive palatable food intake, an alternate, more common view for CRF, is that rather than increasing incentive salience or reward, it actually decreases reward (increases the threshold for reward). Indeed, behavioral consequences of stressor exposure and CRF release are typically characterized by increased anxiety and anhedonia (Cottone et al., 2009; Koob, 2013). Therefore, increased intake of drugs of abuse or palatable food during stress may provide a means to counteract the negative aversive (allostatic) state (Cottone et al., 2009; George et al., 2012; Koob, 2013). Likewise it may be argued that under stress, BBs increase the "hedonic threshold" resulting in the need for increased palatable food consumption to achieve reward. While this remains a possibility, the ability of BBs (and CRF) to increase conditioned place preference when injected into the NAcc cannot be explained within this framework. Also inconsistent with this theory, is the ability of BBs to improve emotional states. For example, central administration of GRP (injected i.c.v or localized at the CeA, BLA or ACC), attenuated the fear potentiated startle (FPS) response as well as the expression of learned fear (as seen by reduced levels of freezing) in response to contextual cues (i.e., in the context in which animals had previously been exposed to shock), and to a tone that had previously been paired with a shock (Mountney et al., 2006, 2008; Merali et al., 2011). Moreover, mice lacking BB_2 receptors exhibit depressive-like behaviors (Monje et al., 2011).

Overall, therefore, based on evidence presented, we maintain the hypothesis that activation of BBs within cortico-limbic circuitry may, under certain circumstances, increase incentive salience/reward which may ultimately lead to weight gain/obesity. In suggesting this hypothesis, it is recognized that at first blush, it appears incompatible with the observed link between BB receptor knockout models (BB_2 and BB_3) and eventual weight gain/obesity. However, it should be emphasized that BB receptor knockout strategy impacts all receptors and related circuitry. In the case of CRF, research has shown that whereas the impact of chronic stressor exposure (or chronic GC exposure) predictably down-regulates hypothalamic CRF (particularly at the PVN), it "paradoxically" up-regulates or sensitizes the CRF system at cortico-limbic sites such as the CeA (Swanson and Simmons, 1989; Makino et al., 1994, 1999; Cook, 2002). Likewise it is possible that the food/stress elicited changes in specific circuits endowed with BB receptors may respond differentially; an effect not functionally captured through knockout strategy.

CONCLUSIONS

In sum, we propose a dual function for BBs in stress and feeding. Most obvious is a role for BBs in stress-induced anorexia. Exogenous administration of BBs potently suppress food intake and BBs are released centrally in response to stressor exposure (both acute and chronic). Weight gain associated with some models of chronic stress could be linked to an inability to respond to satiety signals, including those of BBs. As previously alluded, there is evidence of BB receptor down-regulation (at feeding relevant brain sites) following prolonged corticosterone exposure or chronic BB administration (Plamondon et al., 1998) which could be a mechanism for reduced sensitivity to the satiety effects of BBs (disinhibition).

While increased BB signaling within feeding relevant homeostatic circuitry may contribute to stress-induce anorexia or conversely, an impairment of BB signaling within this same circuitry may promote stress-induced obesity (under some circumstances), we further propose that *increased* BB signaling within cortico-limbic circuitry may also contribute to stress-induced obesity. It is when stressor exposure is combined with a palatable food diet, that we believe this second scenario becomes relevant. BBs are uniquely distributed within key cortico-limbic brain regions linked to reward; most notably at the Nacc, ACC, and amygdala. Moreover, we now have direct evidence that BBs, at the NAcc, induce conditioned place preference which strongly supports their involvement in reward-mediated processes. It is our contention that release of BBs at these cortico-limbic structures may serve to increase incentive salience and/or reward associated with palatable food; indeed future studies directly linking BB-induced reward/incentive salience with increased palatable food consumption need to be carried out to fully validate this hypothesis. Through their interaction with GCs, DA and/or GABA, BBs may enhance the rewarding/stress buffering properties of palatable food and/or strengthen a learned association between stress and palatable food, which may in turn further promote palatable food consumption ultimately leading to obesity.

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REFERENCES

Abercrombie, E. D., Keefe, K. A., DiFrischia, D. S., and Zigmond, M. J. (1989). Differential effect of stress on *in vivo* dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *J. Neurochem.* 52, 1655–1658. doi: 10.1111/j.1471-4159.1989.tb09224.x

Adam, T. C., and Epel, E. S. (2007). Stress, eating and the reward system. *Physiol. Behav.* 91, 449–458. doi: 10.1016/j.physbeh.2007.04.011

Ahima, R. S., and Flier, J. S. (2000). Leptin. *Annu. Rev. Physiol.* 62, 413–437. doi: 10.1146/annurev.physiol.62.1.413

Ahn, S., and Phillips, A. G. (2002). Modulation by central and basolateral amygdalar nuclei of dopaminergic correlates of feeding to satiety in the rat nucleus accumbens and medial prefrontal cortex. *J. Neurosci.* 22, 10958–10965.

Allman, J. M., Tetreault, N. A., Hakeem, A. Y., Manaye, K. F., Semendeferi, K., Erwin, J. M., et al. (2010). The von Economo neurons in frontoinsular and anterior cingulate cortex in great apes and humans. *Brain Struct. Funct.* 214, 495–517. doi: 10.1007/s00429-010-0254-0

Allman, J. M., Tetreault, N. A., Hakeem, A. Y., and Park, S. (2011). The von Economo neurons in apes and humans. *Am. J. Hum. Biol.* 23, 5–21. doi: 10.1002/ajhb.21136

Alsiö, J., Olszewski, P. K., Norbäck, A. H., Gunnarsson, Z. E. A., Levine, A. S., Pickering, C., et al. (2010). Dopamine D1 receptor gene expression decreases in the nucleus accumbens upon long-term exposure to palatable food and differs depending on diet-induced obesity phenotype in rats. *Neuroscience* 171, 779–787. doi: 10.1016/j.neuroscience.2010.09.046

Andrews, N., Davis, B., Gonzalez, M. I., Oles, R., Singh, L., and McKnight, A. T. (2000). Effect of gastrin-releasing peptide on rat hippocampal extracellular GABA levels and seizures in the audiogenic seizure-prone DBA/2 mouse. *Brain Res.* 859, 386–389. doi: 10.1016/S0006-8993(00)02010-2

Antonijevic, I. A., Murck, H., Bohlhalter, S., Frieboes, R. M., Holsboer, F., and Steiger, A. (2000).

Neuropeptide Y promotes sleep and inhibits ACTH and cortisol release in young men. *Neuropharmacology* 39, 1474–1481. doi: 10.1016/S0028-3908(00)00057-5

Arase, K., York, D. A., Shimizu, H., Shargill, N., and Bray, G. A. (1988). Effects of corticotropin-releasing factor on food intake and brown adipose tissue thermogenesis in rats. *Am. J. Physiol.* 255, E255–E259.

Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Fujimiya, M., et al. (2001). A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. *Neuroendocrinology* 74, 143–147. doi: 10.1159/000054680

Au, C. L., Canny, B. J., Farnworth, P. G., and Giraud, A. S. (1997). Bombesin regulation of adrenocorticotropin release from ovine anterior pituitary cells. *Peptides* 18, 995–1000. doi: 10.1016/S0196-9781(97)00049-1

Avena, N. M., and Gold, M. S. (2011). Food and addiction–sugars, fats and hedonic overeating. *Addiction* 106, 1214–1215. discussion: 1219–1220. doi: 10.1111/j.1360-0443.2011.03373.x

Baldo, B. A., Alsene, K. M., Negron, A., and Kelley, A. E. (2005). Hyperphagia induced by GABAA receptor-mediated inhibition of the nucleus accumbens shell: dependence on intact neural output from the central amygdaloid region. *Behav. Neurosci.* 119, 1195–1206. doi: 10.1037/0735-7044.119.5.1195

Barson, J. R., Morganstern, I., and Leibowitz, S. F. (2013). Complementary roles of orexin and melanin-concentrating hormone in feeding behavior. *Int. J. Endocrinol.* 2013, 983964. doi: 10.1155/2013/983964

Bassareo, V., and Di Chiara, G. (1999). Differential responsiveness of dopamine transmission to food-stimuli in nucleus accumbens shell/core compartments. *Neuroscience* 89, 637–641. doi: 10.1016/S0306-4522(98)00583-1

Basso, A. M., and Kelley, A. E. (1999). Feeding induced by GABA(A) receptor stimulation within the nucleus accumbens shell: regional mapping and characterization of macronutrient and taste preference. *Behav. Neurosci.* 113, 324–336. doi: 10.1037/0735-7044.113.2.324

Battey, J., and Wada, E. (1991). Two distinct receptor subtypes for mammalian bombesin-like peptides. *Trends Neurosci.* 14, 524–528. doi: 10.1016/0166-2236(91)90005-F

Baum, A., Garofalo, J. P., and Yali, A. M. (1999). Socioeconomic status and chronic stress. Does stress account for SES effects on health? *Ann. N.Y. Acad. Sci.* 896, 131–144. doi: 10.1111/j.1749-6632.1999.tb08111.x

Benarroch, E. E. (2010). Neural control of feeding behavior: overview and clinical correlations. *Neurology* 74, 1643–1650. doi: 10.1212/WNL.0b013e3181df0a3f

Berridge, K. C. (1996). Food reward: brain substrates of wanting and liking. *Neurosci. Biobehav. Rev.* 20, 1–25. doi: 10.1016/0149-7634(95)00033-B

Berthoud, H.-R. (2011). Metabolic and hedonic drives in the neural control of appetite: who is the boss. *Curr. Opin. Neurobiol.* 21, 888–896. doi: 10.1016/j.conb.2011.09.004

Bouchard, L., Drapeau, V., Provencher, V., Lemieux, S., Chagnon, Y., Rice, T., et al. (2004). Neuromedin beta: a strong candidate gene linking eating behaviors and susceptibility to obesity. *Am. J. Clin. Nutr.* 80, 1478–1486.

Brown, M., Tache, Y., and Fisher, D. (1979). Central nervous system action of bombesin: mechanism to induce hyperglycemia. *Endocrinology* 105, 660–665. doi: 10.1210/endo-105-3-660

Brown, M. R., Carver, K., and Fisher, L. A. (1988). Bombesin: central nervous system actions to affect the autonomic nervous system. *Ann. N.Y. Acad. Sci.* 547, 174–182. doi: 10.1111/j.1749-6632.1988.tb23885.x

Cao, X., Mercaldo, V., Li, P., Wu, L.-J., and Zhuo, M. (2010). Facilitation of the inhibitory transmission by gastrin-releasing peptide in the anterior cingulate cortex. *Mol. Pain* 6, 52. doi: 10.1186/1744-8069-6-52

Carelli, R. M., Williams, J. G., and Hollander, J. A. (2003). Basolateral amygdala neurons encode cocaine self-administration and cocaine-associated cues. *J. Neurosci.* 23, 8204–8211.

Carver-Moore, K., Gray, T. S., and Brown, M. R. (1991). Central nervous system site of action of bombesin to elevate plasma concentrations of catecholamines. *Brain Res.* 541, 225–231. doi: 10.1016/0006-8993(91)91022-S

Christiansen, A. M., Dekloet, A. D., Ulrich-Lai, Y. M., and Herman, J. P. (2011a). "Snacking" causes long term attenuation of HPA axis stress responses and enhancement of brain FosB/deltaFosB expression in rats. *Physiol. Behav.* 103, 111–116. doi: 10.1016/j.physbeh.2011.01.015

Christiansen, A. M., Herman, J. P., and Ulrich-Lai, Y. M. (2011b). Regulatory interactions of stress and reward on rat forebrain opioidergic and GABAergic circuitry. *Stress* 14, 205–215. doi: 10.3109/10253890.2010.531331

Cook, C. J. (2002). Glucocorticoid feedback increases the sensitivity of the limbic system to stress. *Physiol. Behav.* 75, 455–464. doi: 10.1016/S0031-9384(02)00650-9

Cottone, P., Sabino, V., Roberto, M., Bajo, M., Pockros, L., Frihauf, J. B., et al. (2009). CRF system recruitment mediates dark side of compulsive eating. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20016–20020. doi: 10.1073/pnas.0908789106

Covey, D. P., and Howard, C. D. (2011). Dopaminergic signaling in cost-benefit analyses: a matter of time, effort, or uncertainty. *J. Neurosci.* 31, 1561–1562. doi: 10.1523/JNEUROSCI.5841-10.2011

Crawley, J. N., and Corwin, R. L. (1994). Biological actions of cholecystokinin. *Peptides* 15, 731–755. doi: 10.1016/0196-9781(94)90104-X

Dagher, A. (2009). The neurobiology of appetite: hunger as addiction. *Int. J. Obes.* 33(Suppl. 2), S30–S33. doi: 10.1038/ijo.2009.69

Dallman, M. F. (2010). Stress-induced obesity and the emotional nervous system. *Trends Endocrinol. Metab.* 21, 159–165. doi: 10.1016/j.tem.2009.10.004

Dallman, M. F., Akana, S. F., Strack, A. M., Hanson, E. S., and Sebastian, R. J. (1995). The neural network that regulates energy balance is responsive to glucocorticoids and insulin and also regulates HPA axis responsivity at a site proximal to CRF neurons. *Ann. N.Y. Acad. Sci.* 771, 730–742. doi: 10.1111/j.1749-6632.1995.tb44724.x

Dallman, M. F., Pecoraro, N. C., and la Fleur, S. E. (2005). Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain. Behav. Immun.* 19, 275–280. doi: 10.1016/j.bbi.2004.11.004

Dallman, M. F., Pecoraro, N. C., La Fleur, S. E., Warne, J. P., Ginsberg, A. B., Akana, et al. (2006). Glucocorticoids, chronic stress, and obesity. *Prog. Brain Res.* 153, 75–105. doi: 10.1016/S0079-6123(06)53004-3

Dallman, M. F., Warne, J. P., Foster, M. T., and Pecoraro, N. C. (2007). Glucocorticoids and insulin both modulate caloric intake through actions on the brain. *J. Physiol.* 583, 431–436. doi: 10.1113/jphysiol.2007.136051

Davidson, R. J., and Irwin, W. (1999). The functional neuroanatomy of emotion and affective style. *Trends Cogn. Sci.* 3, 11–21. doi: 10.1016/S1364-6613(98)01265-0

Davis, M., and Whalen, P. J. (2001). The amygdala: vigilance and emotion. *Mol. Psychiatry* 6, 13–34. doi: 10.1038/sj.mp.4000812

Deutch, A. Y., and Cameron, D. S. (1992). Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. *Neuroscience* 46, 49–56. doi: 10.1016/0306-4522(92)90007-O

Dhillo, W. S., Small, C. J., Seal, L. J., Kim, M.-S., Stanley, S. A., Murphy, K. G., et al. (2002). The hypothalamic melanocortin system stimulates the hypothalamo-pituitary-adrenal axis *in vitro* and *in vivo* in male rats. *Neuroendocrinology* 75, 209–216. doi: 10.1159/000054712

Dunn, A. J., and Berridge, C. W. (1990). Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses. *Brain Res. Brain Res. Rev.* 15, 71–100. doi: 10.1016/0165-0173(90)90012-D

Epel, E., Jimenez, S., Brownell, K., Stroud, L., Stoney, C., and Niaura, R. (2004). Are stress eaters at risk for the metabolic syndrome. *Ann. N.Y. Acad. Sci.* 1032, 208–210. doi: 10.1196/annals.1314.022

Erspamer, V., Erpamer, G. F., and Inselvini, M. (1970). Some pharmacological actions of alytesin and bombesin. *J. Pharm. Pharmacol.* 22, 875–876. doi: 10.1111/j.2042-7158.1970.tb08465.x

Foster, M. T., Solomon, M. B., Huhman, K. L., and Bartness, T. J. (2006). Social defeat increases food intake, body mass, and adiposity in Syrian hamsters. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290, R1284–R1293. doi: 10.1152/ajpregu.00437.2005

Foster, M. T., Warne, J. P., Ginsberg, A. B., Horneman, H. F., Pecoraro, N. C., Akana, S. F., et al. (2009). Palatable foods, stress, and energy stores sculpt corticotropin-releasing factor, adrenocorticotropin, and corticosterone concentrations after restraint. *Endocrinology* 150, 2325–2333. doi: 10.1210/en.2008-1426

Garrido, M. M., Fuentes, J. A., and Manzanares, J. (2002). Gastrin-releasing peptide mediated regulation of 5-HT neuronal activity in the hypothalamic paraventricular nucleus under basal and restraint stress conditions. *Life Sci.* 70, 2953–2966. doi: 10.1016/S0024-3205(02)01558-8

Garrido, M. M., Manzanares, J., and Fuentes, J. A. (1999).

Hypothalamus, anterior pituitary and adrenal gland involvement in the activation of adrenocorticotropin and corticosterone secretion by gastrin-releasing peptide. *Brain Res.* 828, 20–26. doi: 10.1016/S0006-8993(99)01318-9

Garrido, M. M., Martin, S., Ambrosio, E., Fuentes, J. A., and Manzanares, J. (1998). Role of corticotropin-releasing hormone in gastrin-releasing peptide-mediated regulation of corticotropin and corticosterone secretion in male rats. *Neuroendocrinology* 68, 116–122. doi: 10.1159/000054357

George, O., Le Moal, M., and Koob, G. F. (2012). Allostasis and addiction: role of the dopamine and corticotropin-releasing factor systems. *Physiol. Behav.* 106, 58–64. doi: 10.1016/j.physbeh.2011.11.004

George, S. A., Khan, S., Briggs, H., and Abelson, J. L. (2010). CRH-stimulated cortisol release and food intake in healthy, non-obese adults. *Psychoneuroendocrinology* 35, 607–612. doi: 10.1016/j.psyneuen.2009.09.017

Gibbs, J., and Smith, G. P. (1988). The actions of bombesin-like peptides on food intake. *Ann. N.Y. Acad. Sci.* 547, 210–216. doi: 10.1111/j.1749-6632.1988.tb23889.x

Guan, X.-M., Metzger, J. M., Yang, L., Raustad, K. A., Wang, S.-P., Spann, S. K., et al. (2011). Antiobesity effect of MK-5046, a novel bombesin receptor subtype-3 agonist. *J. Pharmacol. Exp. Ther.* 336, 356–364. doi: 10.1124/jpet.110.174763

Gunion, M. W., Taché, Y., Rosenthal, M. J., Miller, S., Butler, B., and Zib, B. (1989). Bombesin microinfusion into the rat hypothalamic paraventricular nucleus increases blood glucose, free fatty acids and corticosterone. *Brain Res.* 478, 47–58. doi: 10.1016/0006-8993(89)91476-5

Hanson, E. S., and Dallman, M. F. (1995). Neuropeptide, Y (NPY) may integrate responses of hypothalamic feeding systems and the hypothalamo-pituitary-adrenal axis. *J. Neuroendocrinol.* 7, 273–279. doi: 10.1111/j.1365-2826.1995.tb00757.x

Harris, R. B., Zhou, J., Youngblood, B. D., Rybkin, I. I., Smagin, G. N., and Ryan, D. H. (1998). Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets. *Am. J. Physiol.* 275, R1928–R1938.

Herman, J. P., Mueller, N. K., and Figueiredo, H. (2004). Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. *Ann. N.Y. Acad. Sci.* 1018, 35–45. doi: 10.1196/annals.1296.004

Hernandez, L., and Hoebel, B. G. (1988). Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. *Life Sci.* 42, 1705–1712. doi: 10.1016/0024-3205(88)90036-7

Hildebrand, P., Lehmann, F. S., Ketterer, S., Christ, A. D., Stingelin, T., Beltinger, J., et al. (2001). Regulation of gastric function by endogenous gastrin releasing peptide in humans: studies with a specific gastrin releasing peptide receptor antagonist. *Gut* 49, 23–28. doi: 10.1136/gut.49.1.23

Horvitz, J. C. (2000). Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. *Neuroscience* 96, 651–656. doi: 10.1016/S0306-4522(00)00019-1

Itoh, S., Takashima, A., Itoh, T., and Morimoto, T. (1995). Effects of neuromedins and related peptides on the body temperature of rats. *Jpn. J. Physiol.* 45, 37–45. doi: 10.2170/jjphysiol.45.37

James, W. P., Nelson, M., Ralph, A., and Leather, S. (1997). Socioeconomic determinants of health. The contribution of nutrition to inequalities in health. *BMJ* 314, 1545–1549. doi: 10.1136/bmj.314.7093.1545

Jensen, R. T., Battey, J. F., Spindel, E. R., and Benya, R. V. (2008). International Union of Pharmacology. LXVIII. Mammalian bombesin receptors: nomenclature, distribution, pharmacology, signaling, and functions in normal and disease states. *Pharmacol. Rev.* 60, 1–42. doi: 10.1124/pr.107.07108

Johnston, S. A., and Merali, Z. (1988). Specific neuroanatomical and neurochemical correlates of locomotor and grooming effects of bombesin. *Peptides* 9(Suppl. 1), 245–256. doi: 10.1016/0196-9781(88)90250-1

Jones, J. L., Day, J. J., Wheeler, R. A., and Carelli, R. M. (2010). The basolateral amygdala differentially regulates conditioned neural responses within the nucleus accumbens core and shell. *Neuroscience* 169, 1186–1198. doi: 10.1016/j.neuroscience.2010.05.073

Kalivas, P. W., and Duffy, P. (1995). Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. *Brain Res.* 675, 325–328. doi: 10.1016/0006-8993(95)00013-G

Kampe, J., Tschöp, M. H., Hollis, J. H., and Oldfield, B. J. (2009). An anatomic basis for the communication of hypothalamic, cortical and mesolimbic circuitry in the regulation of energy balance. *Eur. J. Neurosci.* 30, 415–430. doi: 10.1111/j.1460-9568.2009.06818.x

Karlsson, R.-M., Holmes, A., Heilig, M., and Crawley, J. N. (2005). Anxiolytic-like actions of centrally-administered neuropeptide Y, but not galanin, in C57BL/6J mice. *Pharmacol. Biochem. Behav.* 80, 427–436. doi: 10.1016/j.pbb.2004.12.009

Kelley, A. E., Baldo, B. A., Pratt, W. E., and Will, M. J. (2005). Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol. Behav.* 86, 773–795. doi: 10.1016/j.physbeh.2005.08.066

Kent, P., Anisman, H., and Merali, Z. (1998). Are bombesin-like peptides involved in the mediation of stress response. *Life Sci.* 62, 103–114. doi: 10.1016/S0024-3205(97)01057-6

Kent, P., Anisman, H., and Merali, Z. (2001a). Central bombesin activates the hypothalamic-pituitary-adrenal axis. Effects on regional levels and release of corticotropin-releasing hormone and arginine-vasopressin. *Neuroendocrinology* 73, 203–214. doi: 10.1159/000054637

Kent, P., Bédard, T., Khan, S., Anisman, H., and Merali, Z. (2001b). Bombesin-induced HPA and sympathetic activation requires CRH receptors. *Peptides* 22, 57–65. doi: 10.1016/S0196-9781(00)00355-7

Kinzig, K. P., Hargrave, S. L., and Honors, M. A. (2008). Binge-type eating attenuates corticosterone and hypophagic responses to restraint stress. *Physiol. Behav.* 95, 108–113. doi: 10.1016/j.physbeh.2008.04.026

Kirsz, K., and Zieba, D. A. (2011). Ghrelin-mediated appetite regulation in the central nervous system. *Peptides* 32, 2256–2264. doi: 10.1016/j.peptides.2011.04.010

Knigge, U., Holst, J. J., Knuhtsen, S., Petersen, B., Krarup, T., Holst-Pedersen, J., et al. (1984). Gastrin-releasing peptide: pharmacokinetics and effects on gastro-entero-pancreatic hormones and gastric secretion in normal men. *J. Clin. Endocrinol. Metab.* 59, 310–315. doi: 10.1210/jcem-59-2-310

Koob, G. F. (2013). Addiction is a reward deficit and stress surfeit disorder. *Front. Psychiatry* 4:72. doi: 10.3389/fpsyt.2013.00072

Koob, G. F., and Heinrichs, S. C. (1999). A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. *Brain Res.* 848, 141–152. doi: 10.1016/S0006-8993(99)01991-5

Kulkosky, P. J., Gibbs, J., and Smith, G. P. (1982). Behavioral effects of bombesin administration in rats. *Physiol. Behav.* 28, 505–512. doi: 10.1016/0031-9384(82)90147-0

La Fleur, S. E., van Rozen, A. J., Luijendijk, M. C. M., Groeneweg, F., and Adan, R. A. H. (2010). A free-choice high-fat high-sugar diet induces changes in arcuate neuropeptide expression that support hyperphagia. *Int. J. Obes.* 34, 537–546. doi: 10.1038/ijo.2009.257

Ladenheim, E. E., Behles, R. R., Bi, S., and Moran, T. H. (2009). Gastrin-releasing peptide messenger ribonucleic acid expression in the hypothalamic paraventricular nucleus is altered by melanocortin receptor stimulation and food deprivation. *Endocrinology* 150, 672–678. doi: 10.1210/en.2008-0559

Ladenheim, E. E., Hampton, L. L., Whitney, A. C., White, W. O., Battey, J. F., and Moran, T. H. (2002). Disruptions in feeding and body weight control in gastrin-releasing peptide receptor deficient mice. *J. Endocrinol.* 174, 273–281. doi: 10.1677/joe.0.1740273

Ladenheim, E. E., Jensen, R. T., Mantey, S. A., and Moran, T. H. (1992). Distinct distributions of two bombesin receptor subtypes in the rat central nervous system. *Brain Res.* 593, 168–178. doi: 10.1016/0006-8993(92)91305-X

Lemos, J. C., Wanat, M. J., Smith, J. S., Reyes, B. A. S., Hollon, N. G., Van Bockstaele, E. J., et al. (2012). Severe stress switches CRF action in the nucleus accumbens from appetitive to aversive. *Nature* 490, 402–406. doi: 10.1038/nature11436

Lieverse, R. J., Masclee, A. A., Jansen, J. B., Lam, W. F., and Lamers, C. B. (1998). Obese women are less sensitive for the satiety effects of bombesin than lean women. *Eur. J. Clin. Nutr.* 52, 207–212. doi: 10.1038/sj.ejcn.1600541

Lutter, M., and Nestler, E. J. (2009). Homeostatic and hedonic signals interact in the regulation of food intake. *J. Nutr.* 139, 629–632. doi: 10.3945/jn.108.097618

MacKay, J. C., Patterson, Z. R., James, J., Kent, P., Abizaid, A., and Merali, Z. (2011). The impact of juvenile stress on palatable feeding and behavioural markers of depression and anxiety in juvenility. International Behavioral Neuroscience Society Abstract.

Mahler, S. V., and Berridge, K. C. (2009). Which cue to "want?" Central amygdala opioid activation enhances and focuses incentive salience on a prepotent reward cue. *J. Neurosci.* 29, 6500–6513. doi: 10.1523/JNEUROSCI.3875-08.2009

Majumdar, I. D., and Weber, H. C. (2011). Biology of mammalian bombesin-like peptides and their receptors. *Curr. Opin. Endocrinol. Diabetes Obes.* 18, 68–74. doi: 10.1097/MED.0b013e328340ff93

Makino, S., Gold, P. W., and Schulkin, J. (1994). Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus. *Brain Res.* 640, 105–112. doi: 10.1016/0006-8993(94)91862-7

Makino, S., Shibasaki, T., Yamauchi, N., Nishioka, T., Mimoto, T., Wakabayashi, I., et al. (1999). Psychological stress increased corticotropin-releasing hormone mRNA and content in the central nucleus of the amygdala but not in the hypothalamic paraventricular nucleus in the rat. *Brain Res.* 850, 136–143. doi: 10.1016/S0006-8993(99)02114-9

Malendowicz, L. K. (1998). Role of neuromedins in the regulation of adrenocortical function. *Horm. Metab. Res.* 30, 374–383. doi: 10.1055/s-2007-978901

Malendowicz, L. K., and Nussdorfer, G. G. (1995). Investigations on the acute effects of neuropeptides on the pituitary-adrenocortical function in normal and cold-stressed rats. I. Bombesin and neuromedin B. *Exp. Toxicol. Pathol.* 47, 31–34. doi: 10.1016/S0940-2993(11)80279-4

Maniam, J., and Morris, M. J. (2010a). Palatable cafeteria diet ameliorates anxiety and depression-like symptoms following an adverse early environment. *Psychoneuroendocrinology* 35, 717–728. doi: 10.1016/j.psyneuen.2009.10.013

Maniam, J., and Morris, M. J. (2010b). Voluntary exercise and palatable high-fat diet both improve behavioural profile and stress responses in male rats exposed to early life stress: role of hippocampus. *Psychoneuroendocrinology* 35, 1553–1564. doi: 10.1016/j.psyneuen.2010.05.012

Maniam, J., and Morris, M. J. (2012). The link between stress and feeding behaviour. *Neuropharmacology* 63, 97–110. doi: 10.1016/j.neuropharm.2012.04.017

Manzanares, J., Toney, T. W., Lookingland, K. J., and Moore, K. E. (1991). Activation of tuberoinfundibular and tuberohypophysial dopamine neurons following intracerebroventricular administration of bombesin. *Brain Res.* 565, 142–147. doi: 10.1016/0006-8993(91)91745-M

Martí, O., Martí, J., and Armario, A. (1994). Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. *Physiol. Behav.* 55, 747–753. doi: 10.1016/0031-9384(94)90055-8

Martinez, V., and Taché, Y. (2000). Bombesin and the brain-gut axis. *Peptides* 21, 1617–1625. doi: 10.1016/S0196-9781(00)00293-X

McDonald, T. J., Jörnvall, H., Nilsson, G., Vagne, M., Ghatei, M., Bloom, S. R., et al. (1979). Characterization of a gastrin releasing peptide from porcine non-antral gastric tissue. *Biochem. Biophys. Res. Commun.* 90, 227–233. doi: 10.1016/0006-291X(79)91614-0

Merali, Z., Anisman, H., James, J. S., Kent, P., and Schulkin, J. (2008). Effects of corticosterone on corticotrophin-releasing hormone and gastrin-releasing peptide release in response to an aversive stimulus in two regions of the forebrain (central nucleus of the amygdala and prefrontal cortex). *Eur. J. Neurosci.* 28, 165–172. doi: 10.1111/j.1460-9568.2008.06281.x

Merali, Z., Hayley, S., Kent, P., McIntosh, J., Bédard, T., and Anisman, H. (2009). Impact of repeated stressor exposure on the release of corticotropin-releasing hormone, arginine-vasopressin and bombesin-like peptides at the anterior pituitary. *Behav. Brain Res.* 198, 105–112. doi: 10.1016/j.bbr.2008.10.025

Merali, Z., and Kateb, C. C. (1993). Rapid alterations of hypothalamic and hippocampal bombesin-like peptide levels with feeding status. *Am. J. Physiol.* 265, R420–R425.

Merali, Z., Kent, P., and Anisman, H. (2002). Role of bombesin-related peptides in the mediation or integration of the stress response. *Cell. Mol. Life Sci.* 59, 272–287. doi: 10.1007/s00018-002-8422-x

Merali, Z., McIntosh, J., and Anisman, H. (1999). Role of bombesin-related peptides in the control of food intake. *Neuropeptides* 33, 376–386. doi: 10.1054/npep.1999.0054

Merali, Z., McIntosh, J., and Anisman, H. (2004). Anticipatory cues differentially provoke *in vivo* peptidergic and monoaminergic release at the medial prefrontal cortex. *Neuropsychopharmacology* 29, 1409–1418. doi: 10.1038/sj.npp.1300441

Merali, Z., McIntosh, J., Kent, P., Michaud, D., and Anisman, H. (1998). Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. *J. Neurosci.* 18, 4758–4766.

Merali, Z., Mountney, C., Kent, P., and Anisman, H. (2011). Effects of intracerebral ventricular administration of gastrin-releasing peptide and its receptor antagonist RC-3095 on learned fear responses in the rat. *Behav. Brain Res.* 216, 519–524. doi: 10.1016/j.bbr.2010.08.027

Merali, Z., Mountney, C., Kent, P., and Anisman, H. (2013). Activation of gastrin-releasing peptide receptors at the infralimbic cortex elicits gastrin-releasing peptide release at the basolateral amygdala: implications for conditioned fear. *Neuroscience.* 243, 97–103. doi: 10.1016/j.neuroscience.2013.03.056

Merali, Z., and Piggins, H. (1990). Effects of dopamine D1 and D2 receptor agonists and antagonists on bombesin-induced behaviors. *Eur. J. Pharmacol.* 191, 281–293. doi: 10.1016/0014-2999(90)94160-Y

Minamino, N., Kangawa, K., and Matsuo, H. (1983). Neuromedin B: a novel bombesin-like peptide identified in porcine spinal cord. *Biochem. Biophys. Res. Commun.* 114, 541–548. doi: 10.1016/0006-291X(83)90814-8

Minamino, N., Kangawa, K., and Matsuo, H. (1988). Neuromedin B and neuromedin Ca. Two mammalian bombesin-like peptides identified in porcine spinal cord and brain. *Ann. N.Y. Acad. Sci.* 547, 373–390. doi: 10.1111/j.1749-6632.1988.tb23904.x

Miner, P., Borkuhova, Y., Shimonova, L., Khaimov, A., and Bodnar, R. J. (2010). GABA-A and GABA-B receptors mediate feeding elicited by the GABA-B agonist baclofen in the ventral tegmental area and nucleus accumbens shell in rats: reciprocal and regional interactions. *Brain Res.* 1355, 86–96. doi: 10.1016/j.brainres.2010.07.109

Monje, F. J., Kim, E.-J., Cabatic, M., Lubec, G., Herkner, K. R., and Pollak, D. D. (2011). A role for glucocorticoid-signaling in depression-like behavior of gastrin-releasing peptide receptor knock-out mice. *Ann. Med.* 43, 389–402. doi: 10.3109/07853890.2010.538716

Moran, T. H. (2006). Gut peptide signaling in the controls of food intake. *Obesity (Silver Spring)* 14(Suppl. 5), 250S–253S. doi: 10.1038/oby.2006.318

Mountney, C., Anisman, H., and Merali, Z. (2008). Effects of gastrin-releasing peptide agonist and antagonist administered to the basolateral nucleus of the amygdala on conditioned fear in the rat. *Psychopharmacology (Berl.)* 200, 51–58. doi: 10.1007/s00213-008-1118-7

Mountney, C., Sillberg, V., Kent, P., Anisman, H., and Merali, Z. (2006). The role of gastrin-releasing peptide on conditioned fear: differential cortical and amygdaloid responses in the rat. *Psychopharmacology (Berl.)* 189, 287–296. doi: 10.1007/s00213-006-0585-y

O'Doherty, J., Kringelbach, M. L., Rolls, E. T., Hornak, J., and Andrews, C. (2001a). Abstract reward and punishment representations in the human orbitofrontal cortex. *Nat. Neurosci.* 4, 95–102. doi: 10.1038/82959

O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R., and McGlone, F. (2001b). Representation of pleasant and aversive taste in the human brain. *J. Neurophysiol.* 85, 1315–1321.

Ohki-Hamazaki, H., Sakai, Y., Kamata, K., Ogura, H., Okuyama, S., Watase, K., et al. (1999). Functional properties of two bombesin-like peptide receptors revealed by the analysis of mice lacking neuromedin B receptor. *J. Neurosci.* 19, 948–954.

Ohki-Hamazaki, H., Watase, K., Yamamoto, K., Ogura, H., Yamano, M., Yamada, K., et al. (1997). Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. *Nature* 390, 165–169. doi: 10.1038/36568

Okuma, Y., Yokotani, K., and Osumi, Y. (1996). Brain prostaglandins mediate the bombesin-induced increase in plasma levels of catecholamines. *Life Sci.* 59, 1217–1225. doi: 10.1016/0024-3205(96)00445-6

Olsen, L., Knigge, U., and Warberg, J. (1992). Gastrin-releasing peptide stimulation of corticotropin secretion in male rats. *Endocrinology* 130, 2710–2716. doi: 10.1210/en.130.5.2710

Ortolani, D., Oyama, L. M., Ferrari, E. M., Melo, L. L., and Spadari-Bratfisch, R. C. (2011). Effects of comfort food on food intake, anxiety-like behavior and the stress response in rats. *Physiol. Behav.* 103, 487–492. doi: 10.1016/j.physbeh.2011.03.028

Pandit, R., de Jong, J. W., Vanderschuren, L. J. M. J., and Adan, R. A. H. (2011). Neurobiology of overeating and obesity: the role of melanocortins and beyond. *Eur. J. Pharmacol.* 660, 28–42. doi: 10.1016/j.ejphar.2011.01.034

Panula, P. (1986). Histochemistry and function of bombesin-like peptides. *Med. Biol.* 64, 177–192.

Parylak, S. L., Koob, G. F., and Zorrilla, E. P. (2011). The dark side of food addiction. *Physiol. Behav.* 104, 149–156. doi: 10.1016/j.physbeh.2011.04.063

Peciña, S., Schulkin, J., and Berridge, K. C. (2006). Nucleus accumbens corticotropin-releasing factor increases cue-triggered motivation for sucrose reward: paradoxical positive incentive effects in stress. *BMC Biol.* 4:8. doi: 10.1186/1741-7007-4-8

Pecoraro, N., Reyes, F., Gomez, F., Bhargava, A., and Dallman, M. F. (2004). Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. *Endocrinology* 145, 3754–3762. doi: 10.1210/en.2004-0305

Petrovich, G. D. (2011). Learning and the motivation to eat: forebrain circuitry. *Physiol. Behav.* 104, 582–589. doi: 10.1016/j.physbeh.2011.04.059

Petrovich, G. D., Ross, C. A., Mody, P., Holland, P. C., and Gallagher, M. (2009). Central, but not basolateral, amygdala is critical for control of feeding by aversive learned cues. *J. Neurosci.* 29, 15205–15212. doi: 10.1523/JNEUROSCI.3656-09.2009

Pickering, C., Alsiö, J., Hulting, A.-L., and Schiöth, H. B. (2009). Withdrawal from free-choice high-fat high-sugar diet induces craving only in obesity-prone animals. *Psychopharmacology (Berl.)* 204, 431–443. doi: 10.1007/s00213-009-1474-y

Pigeyre, M., Bokor, S., Romon, M., Gottrand, F., Gilbert, C. C., Valtueña, J., et al. (2010). Influence of maternal educational level on the association between the rs3809508 neuromedin B gene polymorphism and the risk of obesity in the HELENA study. *Int. J. Obes.* 34, 478–486. doi: 10.1038/ijo.2009.260

Piggins, H., and Merali, Z. (1989). The effects of concurrent D-1 and D-2 dopamine receptor blockade with SCH 23390 and eticlopride, on bombesin-induced behaviours. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 13, 583–594. doi: 10.1016/0278-5846(89)90150-4

Plamondon, H., Lambert, C., and Merali, Z. (1998). Sustained bombesin exposure results in receptor down-regulation and tolerance to the chronic but not acute effects of bombesin on ingestion. *Brain Res.* 782, 202–211. doi: 10.1016/S0006-8993(97)01279-1

Plamondon, H., and Merali, Z. (1994). Push-pull perfusion reveals meal-dependent changes in the release of bombesin-like peptides in the rat paraventricular nucleus. *Brain Res.* 668, 54–61. doi: 10.1016/0006-8993(94)90510-X

Plamondon, H., and Merali, Z. (1997). Regulation of ingestion by CRF and bombesin-like peptides: distinct meal-related peptide level changes. *Am. J. Physiol.* 272, R268–R274.

Richardson, N. R., and Gratton, A. (1996). Behavior-relevant changes in nucleus accumbens dopamine transmission elicited by food reinforcement: an electrochemical study in rat. *J. Neurosci.* 16, 8160–8169.

Rolls, E. T. (2005). Taste and related systems in primates including humans. *Chem. Senses* 30(Suppl. 1), i76–i77. doi: 10.1093/chemse/bjh121

Rosenbloom, M. H., Schmahmann, J. D., and Price, B. H. (2012). The functional neuroanatomy of decision-making. *J. Neuropsychiatry Clin. Neurosci.* 24, 266–277. doi: 10.1176/appi.neuropsych.11060139

Rougé-Pont, F., Deroche, V., Le Moal, M., and Piazza, P. V. (1998). Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. *Eur. J. Neurosci.* 10, 3903–3907. doi: 10.1046/j.1460-9568.1998.00438.x

Saito, H., Nakamachi, T., Inoue, K., Ikeda, R., Kitamura, K., Minamino, N., et al. (2013). Autocrine effects of neuromedin B stimulate the proliferation of rat primary osteoblasts. *J. Endocrinol.* 217, 141–150. doi: 10.1530/JOE-12-0488

Sayegh, A. I. (2013). The role of bombesin and bombesin-related peptides in the short-term control of food intake. *Prog. Mol. Biol. Transl. Sci.* 114, 343–370. doi: 10.1016/B978-0-12-386933-3.00010-8

Schjoldager, B., Poulsen, S. S., Schmidt, P., Coy, D. H., and Holst, J. J. (1991). Gastrin-releasing peptide is a transmitter mediating porcine gallbladder contraction. *Am. J. Physiol.* 260, G577–G585.

Schulz, D. W., Kalivas, P. W., Nemeroff, C. B., and Prange, A. J. Jr. (1984). Bombesin-induced locomotor hyperactivity: evaluation of the involvement of the mesolimbic dopamine system. *Brain Res.* 304, 377–382. doi: 10.1016/0006-8993(84)90343-3

Schwabe, L., and Wolf, O. T. (2009). Stress prompts habit behavior in humans. *J. Neurosci.* 29, 7191–7198. doi: 10.1523/JNEUROSCI.0979-09.2009

Shackman, A. J., Salomons, T. V., Slagter, H. A., Fox, A. S., Winter, J. J., and Davidson, R. J. (2011). The integration of negative affect, pain and cognitive control in the cingulate cortex. *Nat. Rev. Neurosci.* 12, 154–167. doi: 10.1038/nrn2994

Shumyatsky, G. P., Tsvetkov, E., Malleret, G., Vronskaya, S., Hatton, M., Hampton, L., et al. (2002). Identification of a signaling network in lateral nucleus of amygdala important for inhibiting memory specifically related to learned fear. *Cell* 111, 905–918. doi: 10.1016/S0092-8674(02)01116-9

Sinha, R., and Jastreboff, A. M. (2013). Stress as a common risk factor for obesity and addiction. *Biol. Psychiatry* 73, 827–835. doi: 10.1016/j.biopsych.2013.01.032

Smith, G. P., and Gibbs, J. (1984). Gut peptides and postprandial satiety. *Fed. Proc.* 43, 2889–2892.

Spálová, J., Zamrazilová, H., Vcelák, J., Vanková, M., Lukásová, P., Hill, M., et al. (2008). Neuromedin beta: P73T polymorphism in overweight and obese subjects. *Physiol. Res.* 57(Suppl. 1), S39–S48.

Spinazzi, R., Andreis, P. G., Rossi, G. P., and Nussdorfer, G. G. (2006). Orexins in the regulation of the hypothalamic-pituitary-adrenal axis. *Pharmacol. Rev.* 58, 46–57. doi: 10.1124/pr.58.1.4

Spindel, E. R., Gibson, B. W., Reeve, J. R. Jr. and Kelly, M. (1990). Cloning of cDNAs encoding amphibian bombesin: evidence for the relationship between bombesin and gastrin-releasing peptide. *Proc. Natl. Acad. Sci. U.S.A.* 87, 9813–9817. doi: 10.1073/pnas.87.24.9813

Stanley, B. G., Urstadt, K. R., Charles, J. R., and Kee, T. (2011). Glutamate and GABA in lateral hypothalamic mechanisms controlling food intake. *Physiol. Behav.* 104, 40–46. doi: 10.1016/j.physbeh.2011.04.046

Stein, C. J., and Colditz, G. A. (2004). The Epidemic of obesity. *J. Clin. Endocrinol. Metab.* 89, 2522–2525. doi: 10.1210/jc.2004-0288

Stevanović, D., Milosević, V., Starcević, V. P., and Severs, W. B. (2007). The effect of centrally administered ghrelin on pituitary ACTH cells and circulating ACTH and corticosterone in rats. *Life Sci.* 80, 867–872. doi: 10.1016/j.lfs.2006.11.018

Stone, A. A., and Brownell, K. D. (1994). The stress-eating paradox: multiple daily measurements in adult males and females. *Psychol. Heal.* 9, 425–436. doi: 10.1080/08870449408407469

Stratford, T. R., and Kelley, A. E. (1997). GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. *J. Neurosci.* 17, 4434–4440.

Su, P.-Y., and Ko, M.-C. (2011). The role of central gastrin-releasing peptide and neuromedin b receptors in the modulation of scratching behavior in rats. *J. Pharmacol. Exp. Ther.* 337, 822–829. doi: 10.1124/jpet.111.178970

Suzuki, K., Simpson, K. A., Minnion, J. S., Shillito, J. C., and Bloom, S. R. (2010). The role of gut hormones and the hypothalamus in appetite regulation. *Endocr. J.* 57, 359–372. doi: 10.1507/endocrj.K10E-077

Swanson, L. W., and Simmons, D. M. (1989). Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. *J. Comp. Neurol.* 285, 413–435. doi: 10.1002/cne.902850402

Takagi, T., Bungo, T., Tachibana, T., Saito, E.-S., Saito, S., Yamasaki, I., et al. (2003). Intracerebroventricular administration of GABA-A and GABA-B receptor antagonists attenuate feeding and sleeping-like behavior induced by L-pipecolic acid in neonatal chicks. *J. Neurosci. Res.* 73, 270–275. doi: 10.1002/jnr.10656

Tamashiro, K. L. K., Hegeman, M. A., Nguyen, M. M. N., Melhorn, S. J., Ma, L. Y., Woods, S. C., et al. (2007a). Dynamic body weight and body composition changes in response to subordination stress. *Physiol. Behav.* 91, 440–448. doi: 10.1016/j.physbeh.2007.04.004

Tamashiro, K. L. K., Nguyen, M. M. N., Ostrander, M. M., Gardner, S. R., Ma, L. Y., Woods, S. C., et al. (2007b). Social stress and recovery: implications for body weight and body composition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293, R1864–R1874. doi: 10.1152/ajpregu.00371.2007

Tomiyama, A. J., Dallman, M. F., and Epel, E. S. (2011). Comfort food is comforting to those most stressed: evidence of the chronic stress response network in high stress

women. *Psychoneuroendocrinology* 36, 1513–1519. doi: 10.1016/j.psyneuen.2011.04.005

Torres, S. J., and Nowson, C. A. (2007). Relationship between stress, eating behavior, and obesity. *Nutrition* 23, 887–894. doi: 10.1016/j.nut.2007.08.008

Truong, B. G., Magrum, L. J., and Gietzen, D. W. (2002). GABA(A) and GABA(B) receptors in the anterior piriform cortex modulate feeding in rats. *Brain Res.* 924, 1–9. doi: 10.1016/S0006-8993(01)03213-9

Uchida, A., Zigman, J. M., and Perelló, M. (2013). Ghrelin and eating behavior: evidence and insights from genetically-modified mouse models. *Front. Neurosci.* 7:121. doi: 10.3389/fnins.2013.00121

Ueta, Y., Ozaki, Y., Saito, J., and Onaka, T. (2003). Involvement of novel feeding-related peptides in neuroendocrine response to stress. *Exp. Biol. Med. (Maywood)* 228, 1168–1174.

Ulrich-Lai, Y. M., Christiansen, A. M., Ostrander, M. M., Jones, A. A., Jones, K. R., Choi, D. C., et al. (2010). Pleasurable behaviors reduce stress via brain reward pathways. *Proc. Natl. Acad. Sci. U.S.A.* 107, 20529–20534. doi: 10.1073/pnas.1007740107

Ulrich-Lai, Y. M., Ostrander, M. M., Thomas, I. M., Packard, B. A., Furay, A. R., Dolgas, C. M., et al. (2007). Daily limited access to sweetened drink attenuates hypothalamic-pituitary-adrenocortical axis stress responses. *Endocrinology* 148, 1823–1834. doi: 10.1210/en.2006-1241

Vergoni, A. V., and Bertolini, A. (2000). Role of melanocortins in the central control of feeding. *Eur. J. Pharmacol.* 405, 25–32. doi: 10.1016/S0014-2999(00)00538-0

Verhagen, J. V., Rolls, E. T., and Kadohisa, M. (2003). Neurons in the primate orbitofrontal cortex respond to fat texture independently of viscosity. *J. Neurophysiol.* 90, 1514–1525. doi: 10.1152/jn.00320.2003

Volkow, N. D., Wang, G.-J., and Baler, R. D. (2011). Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn. Sci.* 15, 37–46. doi: 10.1016/j.tics.2010.11.001

Warne, J. P. (2009). Shaping the stress response: interplay of palatable food choices, glucocorticoids, insulin and abdominal obesity. *Mol. Cell. Endocrinol.* 300, 137–146. doi: 10.1016/j.mce.2008.09.036

Weber, H. C., Hampton, L. L., Jensen, R. T., and Battey, J. F. (1998). Structure and chromosomal localization of the mouse bombesin receptor subtype 3 gene. *Gene* 211, 125–131. doi: 10.1016/S0378-1119(98)00050-X

Weible, A. P. (2013). Remembering to attend: the anterior cingulate cortex and remote memory. *Behav. Brain Res.* 245, 63–75. doi: 10.1016/j.bbr.2013.02.010

Welberg, L. (2012). Reward: high expectations for GABA. *Nat. Rev. Neurosci.* 13, 150–151. doi: 10.1038/nrn3194

Wickelgren, I. (1997). Getting the brain's attention. *Science* 278, 35–37. doi: 10.1126/science.278.5335.35

Widerlöv, E., Mueller, R. A., Frye, G. D., and Breese, G. R. (1984). Bombesin increases dopamine function in rat brain areas. *Peptides* 5, 523–528. doi: 10.1016/0196-9781(84)90081-0

Wirtshafter, D., and Stratford, T. R. (2010). Evidence for motivational effects elicited by activation of GABA-A or dopamine receptors in the nucleus accumbens shell. *Pharmacol. Biochem. Behav.* 96, 342–346. doi: 10.1016/j.pbb.2010.06.004

Wise, R. A. (2004). Dopamine, learning and motivation. *Nat. Rev. Neurosci.* 5, 483–494. doi: 10.1038/nrn1406

Wise, R. A. (2006). Role of brain dopamine in food reward and reinforcement. *Philos. Trans. R. Soc. B Biol. Sci.* 361, 1149–1158. doi: 10.1098/rstb.2006.1854

Wise, R. A., Spindler, J., deWit, H., and Gerberg, G. J. (1978). Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. *Science* 201, 262–264. doi: 10.1126/science.566469

Yamada, K., Wada, E., Santo-Yamada, Y., and Wada, K. (2002). Bombesin and its family of peptides: prospects for the treatment of obesity. *Eur. J. Pharmacol.* 440, 281–290. doi: 10.1016/S0014-2999(02)01435-8

Zhang, L., Parks, G. S., Wang, Z., Wang, L., Lew, M., and Civelli, O. (2013). Anatomical characterization of bombesin receptor subtype-3 mRNA expression in the rodent central nervous system. *J. Comp. Neurol.* 521, 1020–1039. doi: 10.1002/cne.23216

Zheng, H., Lenard, N. R., Shin, A. C., and Berthoud, H.-R. (2009). Appetite control and energy balance regulation in the modern world: reward-driven brain overrides repletion signals. *Int. J. Obes.* 33(Suppl. 2), S8–S13. doi: 10.1038/ijo.2009.65

Zwanzger, P., Domschke, K., and Bradwejn, J. (2012). Neuronal network of panic disorder: the role of the neuropeptide cholecystokinin. *Depress. Anxiety* 29, 762–774. doi: 10.1002/da.21919

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The central GLP-1: implications for food and drug reward

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Glucagon-like-peptide-1 (GLP-1) and its long acting analogs comprise a novel class of type 2 diabetes (T2D) treatment. What makes them unique among other T2D drugs is their concurrent ability to reduce food intake, a great benefit considering the frequent comorbidity of T2D and obesity. The precise neural site of action underlying this beneficial effect is vigorously researched. In accordance with the classical model of food intake control GLP-1 action on feeding has been primarily ascribed to receptor populations in the hypothalamus and the hindbrain. In contrast to this common view, relevant GLP-1 receptor populations are distributed more widely, with a prominent mesolimbic complement emerging. The physiological relevance of the mesolimbic GLP-1 is suggested by the demonstration that similar anorexic effects can be obtained by independent stimulation of the mesolimbic and hypothalamic GLP-1 receptors (GLP-1R). Results reviewed here support the idea that mesolimbic GLP-1R are sufficient to reduce hunger-driven feeding, the hedonic value of food and food-motivation. In parallel, emerging evidence suggests that the range of action of GLP-1 on reward behavior is not limited to food-derived reward but extends to cocaine, amphetamine, and alcohol reward. The new discoveries concerning GLP-1 action on the mesolimbic reward system significantly extend the potential therapeutic range of this drug target.

Keywords: food reward, ethanol reward, GLP-1, gut peptides, ventral tegmental area, dopamine, exendin 9–39, liraglutide

INTRODUCTION

The alarming rates of obesity in the western world clearly indicate that we have not yet adapted to the dietary challenges engendered by the environment of readily available cheap calories. Inability to limit excessive food intake is likely a key process contributing to uncontrolled weight gain. It is clear that food, especially palatable, calorie-dense, obesogenic food, is rewarding. The high hedonic value and motivational incentive of food are the main culprits for overeating or eating beyond the immediate metabolic need; here referred to as food reward behavior. Thus, in order to develop effective anti-obesity treatments it is of high interest to discover the mechanisms that can limit the hedonically-driven eating and food reward behavior. One potentially promising therapeutic is glucagon-like-peptide 1 (GLP-1). Endogenous GLP-1 is produced in the intestinal L-cells and the hindbrain (Han et al., 1986; Jin et al., 1988; Larsen et al., 1997a; Reimann et al., 2008). GLP-1 receptors (GLP-1R) can also be stimulated exogenously via long lasting GLP-1 analogs (Hayes et al., 2010; Graham et al., 2012; Parkes et al., 2013). Several GLP-1 analogs, among them exendin 4 (EX4; Byetta) or liraglutide (Victoza), are approved for clinical use in type 2 diabetes (T2D) patients to improve glycemic control (Wang et al., 1997; Greig et al., 1999; Agerso et al., 2002; Drucker et al., 2010). Much has been learned about the anatomical, neurochemical, and functional suppressive effects of GLP-1 or its analogs on food intake; GLP-1's ability to suppress food reward behavior is a new concept.

The goal of this review is to extend the understanding of the neural circuitry mediating the intake inhibitory effects of GLP-1 beyond the hypothalamus and the hindbrain and into the mesolimbic areas. Also discussed here will be the behavioral consequences of the aforementioned expansion of the range of GLP-1 impact, into the mesolimbic system. Namely the new behavioral and physiological effects of GLP-1, such as the regulation of food and drug reward.

GLP-1 ANOREXIA BEYOND THE DIRECT HYPOTHALAMUS/HINDBRAIN ACTION

Central or peripheral GLP-1 injection reduces food intake in rodents and man (Turton et al., 1996; Larsen et al., 1997b; Naslund et al., 1999; Langhans, 2000; Hayes et al., 2008; Astrup et al., 2009, 2012). To date the literature has primarily focused on hypothalamic and brainstem nuclei as the key central nervous system (CNS) targets for anorexic and to some extent glucoregulatory effects of GLP-1 (Shughrue et al., 1996; McMahon and Wellman, 1998; Schick et al., 2003; Hayes et al., 2008, 2009; Sandoval et al., 2008). This topic has already been discussed by a number of excellent reviews (Holst, 2004; Hayes et al., 2010; Trapp and Hisadome, 2011). This hypothalamus/hindbrain focused model of GLP-1 action, however, does not easily accommodate the recent findings showing that GLP-1R stimulation reduces food reward behavior (Dickson et al., 2012). This observation was important as it supports the presumption that the range of impact of GLP-1 on food intake extends beyond homeostatic (or metabolic, need based) food intake. It brings attention to potential activity of GLP-1 in areas classically associated with reward behavior such as the ventral tegmental areas (VTA) and the nucleus accumbens (NAc). The VTA and its dopaminergic projections to the NAc orchestrate goal-directed motivated

behavior to obtain natural reinforcements like food or sex (Wise and Bozarth, 1984; Wise, 2004a,b, 2006). It is also increasingly clear that addictive drugs (Koob, 1992) can hijack the reward system, a system originally evolved to motivate the drive for natural rewards. While the reward control brain centers are neuroanatomically separated they are not entirely disconnected from the classic homeostatic centers, and bidirectional communication between both regions takes place under physiological conditions. Many of the classic hormones regulating feeding have a direct impact on the mesolimbic VTA/NAc neurons (Abizaid et al., 2006; Fulton et al., 2006; Hommel et al., 2006; Palmiter, 2007; Abizaid, 2009; Vucetic and Reyes, 2010; Skibicka and Dickson, 2011; Skibicka et al., 2011; DiLeone et al., 2012). GLP-1 is the newest member of this group.

MESOLIMBIC REWARD SYSTEM GLP-1 RECEPTOR EXPRESSION

Already in 1999 the first neuroanatomical indication for a potential role of GLP-1 in reward emerged. GLP-1R mRNA and GLP-1 immunoreactivity was detected in the VTA and NAc, as well as other reward associated areas including lateral hypothalamus, lateral habenula, hippocampus, and substantia nigra (Merchenthaler et al., 1999). Identification of the peptide and receptors, however, does not by itself substantiate a functional relevance, but evidence for a physiological role of these receptor populations emerged 13 years later and will be discussed here.

AFFERENT AND EFFERENT INNERVATION OF THE HINDBRAIN GLP-1-PRODUCING NEURONS

Unlike many of its gut or fat hormone counterparts (ghrelin or leptin) for which there is little evidence of central production, GLP-1 can be made in the brain. At the level of the CNS GLP-1 is produced in the hindbrain, primarily in the nucleus of the solitary tract (NTS) (Han et al., 1986; Jin et al., 1988; Larsen et al., 1997a; Reimann et al., 2008). This is a very strategic location for the GLP-1 neurons because of the impressive range of energy balance relevant inputs received by the NTS (**Figure 1**).

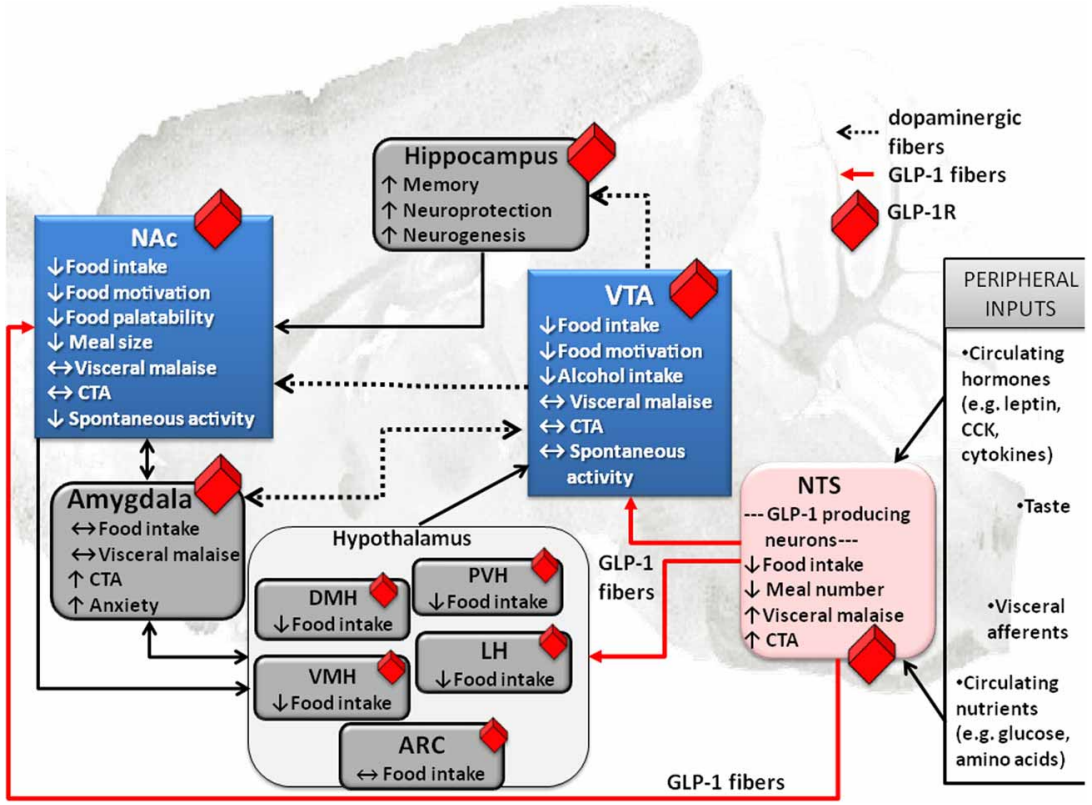

FIGURE 1 | Effect of GLP-1 on food intake and associated behaviors is neuroanatomicaly distributed. Local application of GLP-1 or GLP-1 analogs (e.g., EX4) into the VTA or the NAc alters food motivation/reward. Moreover, many other GLP-1R expressing CNS sites that directly respond to GLP-1 have clear connections to the mesolimbic dopamine circuitry, key in food reward behaviors (indicated in blue). This neuroanatomical distribution of GLP-1R potentially allows for a multi-center, wide-spread impact of GLP-1on food reward behavior, at several levels of the CNS. Furthermore, it appears that GLP-1 influences feeding in different brain regions by partly overlapping and partly distinct mechanisms. Several GLP-1 terminal sites have been confirmed (in red). Notably two of them are mesolimbic; VTA and NAc. Presumably, however, most GLP-1R expressing sites would receive their GLP-1 supply from the only source of GLP-1 in the brain, the hindbrain NTS GLP-1-producing neurons. This neuroanatomical architecture places the GLP-1 system as a central sensor of an array of key circulating factors and neural inputs from the viscera and tongue that is immediately able to integrate and relay the information to the mesolimbic centers. Prefrontal cortex, PFC; nucleus tractus solitarius, NTS; ventral tegmental area, VTA; paraventricular nucleus of the hypothalamus, PVH; lateral hypothalamus, LH; arcuate nucleus of the hypothalamus, ARC; ventromedial nucleus of the hypothalamus, VMH; dorsomedial nucleus of the hypothalamus, DMH; conditioned taste aversion, CTA.

NTS receives vagal input from the gastrointestinal tract and gustatory input from the tongue (Grill and Kaplan, 2002; Grill and Hayes, 2009, 2012). Furthermore, the NTS located GLP-1 neurons extend their dendrites into the circumventricular area postrema, an area with fenestrated capillaries that can sample blood borne factors (Llewellyn-Smith et al., 2011). Also projections of these hindbrain GLP-1 neurons are widely distributed, giving neuroanatomical support for the diverse physiological and behavioral effects of the endogenous GLP-1. Key mesolimbic reward areas, like the VTA and the NAc, are innervated by the GLP-1 producing neurons, potentially allowing hindbrain GLP-1 to modulate modulate reward behavior directly. Ascending fibers from the caudal NTS, identified with anterograde tracing, terminate in the VTA and the NAc (Rinaman, 2010). In fact, nearly one third of all the NTS GLP-1-producing neurons send ascending fibers to the VTA and the NAc (core and shell regions) (Dossat et al., 2011; Alhadeff et al., 2012). Thus, GLP-1 neurons are placed in a very influential position, enabling them to sample the hormonal milieu, visceral sensory and gustatory input, integrate and carry this information directly to the mesolimbic system without any intermediate stops.

GLP-1R-DRIVEN ACTIVATION OF THE MESOLIMBIC NEURONS

On the basis of immediate early gene expression analysis it seems that GLP-1 or its analogs can increase neuronal activity in the mesolimbic system. Local application of GLP-1 to the core of the NAc increases the number of neurons expressing the immediate early gene, c-fos, in this region (Dossat et al., 2011). Similar activation can even be obtained with a peripheral GLP-1 analog, EX4, injection (Labouesse et al., 2012). Given that EX4 is administered peripherally as a part of the anti-diabetic treatment regimen, data linking peripheral GLP-1 analog injections to altered neuronal activity in reward areas may be relevant in the clinical and therapeutic setting.

MESOLIMBIC GLP-1R DRIVEN FOOD-ORIENTED BEHAVIORS

Even though the research on the physiological role of the mesolimbic GLP-1 activation is in its early stages the first studies addressing this question provide compelling evidence for an important role of the GLP-1 system in the mesolimbic circuitry and resulting reduction in food intake. Based on the earlier discussed receptor expression, innervation and their crucial role in behavioral control two mesolimbic nuclei, the VTA and the NAc, became the first targets of studies exploring behavioral consequences of the mesolimbic GLP-1R activation. The VTA is a key nucleus that modulates reward behavior and that harbors the cell bodies of dopamine neurons (Wise and Bozarth, 1984; Koob, 1992). Selective and local VTA microinjection of EX4 consistently yields reduction in food intake and body weight. These intake suppressive effects are not macronutrient specific since the intake of both palatable (high-fat or high-sugar) food and normal chow (Alhadeff et al., 2012; Dickson et al., 2012) is reduced by GLP-1R activation. The finding that exogenous stimulation of GLP-1R results in suppression of food intake irrespective of its macronutrient content is perhaps consistent with previous data that indicate that all macronutrients (carbohydrates, fat, proteins) can induce the release of GLP-1 from the intestinal L-cells (Reimann, 2010; Diakogiannaki et al., 2012). Intake of chow, however, is only reduced if the rats are overnight fasted or, in *ad libitum* fed rats, if the chow is available as the only source of calories (Dickson et al., 2012). In contrast, if a choice between chow and high-fat diet is given to satiated rats EX4 appears to selectively reduce the high-fat intake but surprisingly increase the chow intake (Alhadeff et al., 2012). These findings can lead us to conclude that VTA GLP-1R activation might result in a lack of preference for high-energy/fat food. Similar results were reported for the selective stimulation of the GLP-1R in the NAc (Dossat et al., 2011; Alhadeff et al., 2012; Dickson et al., 2012). NAc is a terminal site for the dopaminergic projections originating in the VTA; it can also directly communicate with the lateral hypothalamus, an important interface between the homeostatic and reward circuits. It can be divided into two subregions, the shell and the core; contributions of these two subregions to the control of motivated reward behavior might differ (Di Chiara, 2002). GLP-1 projections are neuroanatomically positioned to influence both subregions, since GLP-1R and innervating fibers can be found in both the shell and the core. Like the VTA GLP-1R, those in the NAc contribute to the intake suppressive responses by GLP-1. Localized delivery of EX4 to the shell or core region reduces high-fat or sucrose intake and body weight. However, in NAc higher doses of EX4 are required to reduce chow intake in both fasted and *ad libitum* fed rats compared with those effective in the VTA, indicating perhaps that NAc is somewhat less sensitive to the intake reducing effects of EX4 (Alhadeff et al., 2012; Dickson et al., 2012). The intake suppressive effect is not unique to the GLP-1 analogs as the native GLP-1 peptide can similarly reduce chow intake when locally delivered to the NAc core (but not shell) (Dossat et al., 2011). Noteworthy, the reported reductions in food consumption appear to be associated with a reduction in body weight (Alhadeff et al., 2012; Dickson et al., 2012). This is an important observation as it may imply that targeting the mesolimbic reward system with GLP-1 agonists may be a viable weight loss strategy.

Together, these data support a role for mesolimbic GLP-1Rs in the regulation of food intake, irrespective of the macronutrient composition of the food. These results are important from a clinical perspective as a reduction in food intake accompanied by weight loss is a desirable outcome in an obese patient. They do not, however, address the question of the physiological role of the endogenously released GLP-1 in these mesolimbic areas. This question has been pursued through focal microinjections of a GLP-1R antagonist into the mesolimbic nuclei. Utilizing this methodology, several reports provide convincing evidence that endogenous GLP-1 released in the VTA and the NAc is in fact necessary for food intake control and blockade of its signal can lead to increased food intake (Dossat et al., 2011; Alhadeff et al., 2012; Dossat et al., 2013). Further support for this idea is provided by Dossat et al. (2013) who report that tonic release of GLP-1 in the NAc core is necessary to reduce the palatability of a sucrose solution and also participates in limiting the size of the sucrose meal. Supportively, both the size of a sucrose meal in rats and also the rate of licking during the first meal, an effect often indicative of a perceived increase in palatability, were

increased by local microinjection of a GLP-1R antagonist in the NAc core (Dossat et al., 2013). Interestingly, the recently reported NAc manipulation did not alter non-caloric sweet saccharine consumption (Dossat et al., 2013), suggesting that GLP-1 released in the NAc likely interacts with calories but not taste of the sugary solution. It is worth noting that bypassing the taste signaling by infusing glucose directly into the stomach is sufficient for evoking the rewarding properties of glucose and the associated dopamine release in the NAc (Ren et al., 2010). Thus, it is plausible that the hindbrain GLP-1 neurons are a part of the ascending pathway activated by intragastric glucose, with a role to limit reward responses perhaps.

In the previous paragraphs we have reviewed evidence for the clear suppression of food intake by mesolimbic GLP-1 activation. What is left unresolved is the mechanism(s) responsible for this reduction. Previously discussed data hint at a potential reduction in palatability evoked from the core of the NAc, understood as less pleasure obtained from food and hence leading to less food consumption. The mesolimbic system is, however, key to regulating the incentive salience or motivation (Berridge, 1996). Thus, in the next section the available data on a potential role of the GLP-1 system in motivation will be discussed.

Reviewing the literature it can be somewhat difficult to obtain a consensus on a definition of the now frequently used term, "food reward." As mentioned earlier, for the purposes of this review this term will refer to eating beyond the immediate caloric need, eating for the hedonic value of food, or heightened incentive salience or motivation to eat. Thus, reducing the motivation to eat is one way to reduce food reward. Food motivation can be measured in animal models (and recently also in human subjects) with an operant procedure for food in which earning each food pellet requires more work than the one before, reflecting craving and wanting for food; this test is called progressive ratio operant procedure (Hodos, 1961; Miras et al., 2012). It is a plausible mechanism via which GLP-1 may lead to intake suppression since activation of GLP-1 via EX4 injection was shown to decrease the motivation to obtain food (Dickson et al., 2012), in a progressive ratio operant conditioning. This effect was rather striking as the central (ventricular) EX4 injection produced an impressive 80% reduction in rewards earned. The food reward-reducing effect of EX4 is further confirmed in another test of food reward, the conditioned place preference test, in which rats conditioned to spend most of their time in an environment previously paired to chocolate pellets lost that conditioned preference when injected with EX4 (Dickson et al., 2012). Of course, these results obtained with peripheral or central infusions of the agonist, while of some clinical relevance (peripheral route), do not address the issue of the neural substrate underlying these reward effects. This issue has been pursued through combining localized mesolimbic delivery of EX4 with the sucrose-motivated progressive ratio test. The reported results are consistent with the hypothesis that the key mesolimbic areas, the VTA and the NAc, represent the neural substrate driving the food reward suppressing effect of GLP-1R stimulation. Selective intra-VTA or intra-NAc EX4 application reduces the incentive value for sucrose (Dickson et al., 2012). Again, the VTA was more sensitive to GLP-1R stimulation. This VTA GLP-1R driven reduction in sucrose reward behavior was shown in both overnight food restricted and *ad libitum* fed rats indicating that this response is robust (Dickson et al., 2012). When sucrose-driven operant behavior is performed in food-restricted rats, the motivation to work for sugar is generally reliable and high for all rats. This is in contrast to the *ad libitum* condition in which some rats still choose to expend a significant amount of work for their sugar reward (high-responders); others, however, expend only a fraction of the work they are willing to do in a restricted state (low-responders) (Dickson et al., 2012). This interesting observation was used to show that these innate differences in *ad libitum* fed rats may interact with GLP-1R-driven reward responses. This is reflected by data showing that EX4 is mostly effective in high-responders, leaving the responses of low-responders intact.

The classic mesolimbic reward areas are clearly important in driving the effect of GLP-1 on food reward; GLP-1R, however, are detected in several other nuclei that contribute to the different aspects of reward control. Substantia nigra is one such interesting candidate area. The crucial role of the substantia nigra dopaminergic neurons in operant conditioning for food emerged from data showing that selective restoration of dopamine production in substantia nigra or its primary terminal target, the dorsal striatum, is sufficient to restore the motivation to work for food in mice that lack dopamine otherwise (Sotak et al., 2005; Robinson et al., 2006, 2007; Palmiter, 2008). GLP-1R has been detected in the substantia nigra (Merchenthaler et al., 1999), its role in food reward behavior is unexplored, but is certainly worth considering in future studies. Interestingly, while we do not know the role of GLP-1 in this circuit on food reward several studies have explored the therapeutic potential of nigrostriatal GLP-1 action in animal models of Parkinson's disease (Harkavyi et al., 2008; Kim et al., 2009; Abuirmeileh et al., 2012; Holscher, 2012) with great success culminating now with clinical trials.

COMPETING AND CONFOUNDING BEHAVIORS

Certain limitations should always be considered when a suppression of ingestive or motivated behavior is obtained. In the case of GLP-1 two most prominent limitations to drawing specific conclusions about the role of the peptide in regulation of reward are (1) nausea or visceral illness and (2) the non-specific motor disturbance.

Nausea/aversion

The concern with nausea resulting from GLP-1 should not come as a surprise as this is in fact the most common side effect reported in patients receiving GLP-1 analog treatment (Calara et al., 2005). This clinical observation is closely mimicked in preclinical rodent studies, in which signs of visceral illness, nausea or conditioned taste aversion after EX4 or GLP-1 treatment have been clearly demonstrated (Thiele et al., 1997, 1998; Rinaman, 1999a,b; Seeley et al., 2000; Kinzig et al., 2002; Kanoski et al., 2012). It seems, however, that while stimulation of some GLP-1R expressing neuronal populations is responsible for reducing food intake in association with nausea or aversion stimulation of

others can lead to food intake reduction via mechanisms independent of nausea (McMahon and Wellman, 1998; Alhadeff et al., 2012; Dickson et al., 2012; Kanoski et al., 2012). It is, of course, warranted to consider this possibility carefully as the mesolimbic system underlies the establishment of aversion responses. Three reports to date suggest that VTA and NAc GLP-1Rs induced suppression of food intake and reward is not associated with visceral illness (Dossat et al., 2011; Alhadeff et al., 2012; Dickson et al., 2012). The fact that a similar conclusion, the lack of association between nausea and GLP-1 in reward areas, was obtained across different laboratories, diverse methods of visceral illness assessment and different GLP-1R agonists makes this a much more compelling argument. Relevant studies were conducted in rodents in which conditioned taste aversion and kaolin consumption were used to evaluate potential nausea or malaise associated with EX4 or GLP-1. Kaolin consumption is a form of PICA response (consumption of non-nutritive substances) that is indicative of visceral malaise (Takeda et al., 1993; De Jonghe et al., 2009). The PICA response has been used extensively to study visceral malaise in rats, a species that cannot vomit. VTA and NAc shell directed EX4 application, at doses that clearly reduce food intake and reward behavior, does not induce consumption of kaolin. Similarly intra-NAc core GLP-1 administration at a dose that reduced chow intake did not elicit a conditioned taste aversion to saccharine, indicating that the rats did not find the accumbal GLP-1R stimulation aversive (Dossat et al., 2011). The collective value of these findings is not only in offering support to the hypothesis that mesolimbic GLP-1 has a specific role in reward behavior but also in emphasizing the neuroanatomical separation of the reward and visceral illness mechanisms of GLP-1. Currently this information is of little benefit to the patients receiving GLP-1 based therapy that is applied peripherally and provides drug access presumably to all brain GLP-1R populations simultaneously. However, the fact that the potentially clinically beneficial food reward suppressing effects of GLP-1R stimulation can be disassociated from the clinically undesirable nausea offers promise for a future therapy selectively targeting the mesolimbic reward system GLP-1R populations that could be free of this most common adverse effect of GLP-1 analog therapy.

Motor disturbances/hypoactivity

Another concern with therapies targeting the mesolimbic system is that the behavioral outcomes obtained are not specific to ingestive behavior and mediated via a general increase or decrease in physical activity. This becomes a valid concern for GLP-1 as some studies indicate that peripheral GLP-1R agonist application can result in a reduction in spontaneous motor activity (Mack et al., 2006; Erreger et al., 2012). Other reports, however, fail to find this GLP-1/EX4 associated hypoactivity in otherwise normal rats or mice (Talsania et al., 2005; Hayes et al., 2008). Local, intra-VTA GLP-1R activation appears to align with the latter reports and does not result in any changes in non-goal oriented motor activity (Dickson et al., 2012). This is in contrast to the NAc GLP-1R stimulation which led to a brief (10 minute long) reduction in activity, during the 1 h long testing period. Considering how short-lived the hypoactivity period was, it seems unlikely that it is a major contributing factor to reward-associated behavioral effects of EX4 that lasted for a period of several hours. However, this possibility could not be entirely eliminated based on the available data. Nevertheless, the results obtained with VTA GLP-1R population suggest that the suppression of food reward and intake can be fully disassociated from any changes in general motor activity. It is worth mentioning that in the literature evaluating the rewarding effects of psychostimulants a change in physical activity is not considered problematic, as many psychostimulants like cocaine or amphetamine are associated with hyperactivity, and this behavior seems to be intimately linked to increases in dopamine (Imperato and Di Chiara, 1986; Wise and Bozarth, 1987). Thus, a reduction of hyperactivity is often translated to mean a reduction in NAc dopamine release and used as a proxy measure for a reduction of the rewarding/addictive properties of a given psychostimulant. If this line of thinking is applied to the mesolimbic GLP-1R activation, it is plausible that while NAc GLP-1R activation is associated with a suppression of the dopamine signal, the VTA GLP-1R reduce reward behavior via another mechanism. This is, however, rather speculative and requires future investigation that should involve a direct measurement of the dopamine release after intra-VTA or intra-NAc GLP-1 application during a food reward task *in vivo*.

GLP-1 IMPACT ON ALCOHOL INTAKE AND REWARD

Sugars are clearly rewarding; when sugars undergo alcoholic fermentation alcohol is one of the resulting products. While alcohol is reinforcing in itself (Henningfield and Meisch, 1975), it is the only addictive substance that also provides a source of calories. A number of studies highlight the importance of the gut-brain signaling in the regulation of alcohol intake (Crespi, 1998; Leggio, 2010). Thus, it is not surprising that the initial discovery of the impact of GLP-1 on food reward opened up the question whether GLP-1R stimulation could be beneficial for curbing alcohol intake. The link between alcohol and GLP-1 starts already in the gut, as alcohol intake can result in an elevated level of gut-produced GLP-1 in rats (Davis et al., 2012b) suggesting a possible relationship between alcohol intake and GLP-1. Direct evidence for this relationship followed this initial discovery and included findings showing that peripheral injections of GLP-1 or the GLP-1 analog, EX4, reduce alcohol consumption in rodents (Davis et al., 2012b; Shirazi et al., 2013). Interestingly, in both studies this ability to reduce alcohol consumption seems to be dependent on the baseline consumption levels. The consumption of alcohol is significantly reduced, but only in genetically-determined alcohol-preferring rats or those selected from an outbred population for high-alcohol consumption. In contrast, low-alcohol consuming rats show little change in their alcohol drinking after GLP-1R stimulation. The suppressing effect of GLP-1 on alcohol intake is linked to suppressed alcohol reward, an idea now tested in both rats and mice with two different tests of alcohol reward behavior, adding strength to the conclusion. Mice treated with GLP-1 do not show a conditioned place preference to alcohol (Shirazi et al., 2013) and alcohol-preferring rats injected with EX4 reduce their operant responding for an alcohol solution (Davis et al., 2012a). Peripheral injection of EX4 also

appears to reduce alcohol-induced accumbal dopamine release, findings consistent with the idea that a peripheral EX4 treatment can have an impact on the mesolimbic dopamine system (Egecioglu et al., 2013). Together these data indicate that the stimulation of GLP-1R is sufficient to reduce alcohol consumption; however, they do not speak to the ability of the endogenous GLP-1 to regulate alcohol intake. To this end, we recently reported that blockade of GLP-1R via a peripheral injection of a GLP-1R antagonist in outbred Wistar rats results in increased alcohol consumption (Shirazi et al., 2013). Thus, endogenous GLP-1 makes a significant contribution to the normal regulation of alcohol intake and when that endogenous signal is taken away the rats drink more alcohol. In contrast, the same GLP-1R blockade was not sufficient to elevate alcohol drinking in alcohol-preferring rats, perhaps reflecting a differential alcohol intake regulation in this strain (Davis et al., 2012b). Importantly, the effect of GLP-1 on alcohol intake may be driven locally by mesolimbic VTA GLP-1R activation, since acute intra-VTA injection of GLP-1 or EX4 reduces overnight alcohol consumption by nearly 30%, (Shirazi et al., 2013). This newly emerging alcohol-suppressing effect of GLP-1 is certainly of clinical interest. Since GLP-1 analogs are already approved for clinical use in T2D and deemed clinically safe, it is surely an attractive possibility to consider their use for alcohol disorders. It is important to note, however, that the data summarized here include only preclinical studies; thus, future studies are necessary to determine whether this newly discovered effect can be generalized to a clinical population. Furthermore, all discussed reports evaluate only acute effects of the treatment, and evaluation of long-term effects of the GLP-1R stimulation on the alcohol intake has not yet been reported. Nevertheless, collectively these findings provide further support for a pleiotropic impact of GLP-1 on the mesolimbic circuitry and reward behavior.

GLP-1 IMPACT ON PSYCHOSTIMULANT AND NICOTINE REWARD

Considering that the mesolimbic reward system is a key target not only for food and alcohol, but also for psychostimulants and nicotine (Koob, 1992), it is perhaps not surprising that recent work has explored the potential of GLP-1 analogs to alter psychostimulant reward. It must be noted that the effects of a substance to reduce food reward cannot be simply extrapolated to suggest a psychostimulant reward-reducing effect. Melanocortins, for example, are one of the most potent anorexic neuropeptides (Cone, 2005), they reduce food reward behavior (Davis et al., 2011) but may increase mesolimbic dopamine release and psychostimulant reward (Lindblom et al., 2001; Hsu et al., 2005). Thus, a careful and direct evaluation of the role of GLP-1 on the psychostimulant reward was necessary and proved fruitful, since an impact of GLP-1 on both cocaine and amphetamine behavioral response was revealed (Erreger et al., 2012; Graham et al., 2012). Peripheral delivery of EX4 attenuated cocaine reward behavior in mice in a cocaine-induced conditioned place preference test. Perhaps surprisingly, this attenuation of the conditioned preference response was not associated with a reduction in cocaine-induced hyperactivity, even though the doses used were well into the range of those previously shown to induce hypoactivity and 10 to 100-fold higher than those needed for an anorexic response. Since it is often suggested that the cocaine-induced hyperactivity might be a correlate of dopamine release in the NAc, the lack of an effect of EX4 on cocaine hyperactivity might indicate that the reduction in reward value of cocaine occurs via a mechanism outside of or downstream of the dopamine release. In contrast, a peripheral injection of EX4 in rats reduced both basal locomotor activity and amphetamine-induced hyperactivity, which may be indicative of a reduced dopamine signal. If the results of these two studies were to be integrated it could be concluded that the impact of GLP-1R activation on psychostimulant-induced hyperactivity, and by extension dopamine release, may be species (mice vs. rats) or drug (cocaine vs. amphetamine) dependent. Interestingly, peripheral injection of liraglutide was shown to reduce behavior induced by apomorphine (a non-selective dopamine agonist) (Dixit et al., 2013). The effect of liraglutide was strikingly comparable to that achieved by a well-established antipsychotic medication, haloperidol (Dixit et al., 2013). While these are very promising results, it is clear that future studies are needed to determine the mechanisms (intracellular signals and downstream neurotransmitters) behind the impact of EX4 on the specific psychostimulant reward. Furthermore, nothing is known about the contribution of the endogenous GLP-1 to the psychostimulant reward; it would certainly be worthwhile to explore whether changes/dysfunction in the endogenous GLP-1 system are a contributing factor to psychostimulant reward and addiction. This essential contribution of endogenously produced GLP-1 to drug reward is, however, suggested by Tuesta et al. (2012). They show that nicotine reward, tested via a conditioned place preference in mice lacking the GLP-1R, was significantly enhanced (Tuesta et al., 2012). Moreover, the GLP-1R knockout mice self-administered more nicotine than their wildtype counterparts (Tuesta et al., 2012); which could suggest an enhanced motivational salience of nicotine in these mice. These compelling data suggest that the endogenous GLP-1 is necessary for curbing the reward experience from drugs.

CONTRIBUTION OF GLP-1 TO CHANGES IN REWARD BEHAVIOR AFTER BARIATRIC SURGERY

Bariatric surgery results in an impressive weight loss, reduced food intake and a lack of compensatory energy expenditure reduction (le Roux and Bloom, 2005; Sjostrom et al., 2007; Buchwald and Oien, 2009; Buchwald, 2010; Carlsson et al., 2012). Some studies also report reduced food reward post-surgery (Shin and Berthoud, 2011; Miras et al., 2012) but this reduction is not detected by others (Mathes et al., 2012). The effect of bariatric surgery on the GLP-1 system is also well-established (Cummings, 2009). The peripheral GLP-1 system is more responsive to both meals and alcohol after bariatric surgery (Davis et al., 2012b). Some reports also suggest that this surgery can lead to reduced alcohol intake and reward in both humans and rodents (Davis et al., 2012b). Thus, it is likely that an elevated circulating GLP-1 contributes to the reduced food and alcohol reward after bariatric surgery. In fact, pharmacological blockade of GLP-1R that is ineffective in sham rats, restores the chow intake of Roux-en-Y gastric (RYGB) operated rats (Abegg et al., 2013). This essential

contribution of GLP-1 was not, however, found in a mouse model of vertical sleeve gastrectomy (Wilson-Pérez et al., 2013). Further studies evaluating the effect of longer-term or central GLP-1R stimulation are still needed to entirely eliminate the requirement of GLP-1 signaling for the food/alcohol suppressing effect of bariatric surgery. It is also noteworthy that one recent report indicates an elevated ethanol intake and reward in diet-induced obese rats after bariatric surgery (Hajnal et al., 2012) that might be associated with higher levels of another gut peptide, ghrelin. This is consistent with reports indicating that a small but significant subpopulation of baratric surgery patients drinks more alcohol after the surgery. The involvement of GLP-1 was not suggested by either of these studies. These surprisingly conflicting results in preclinical bariatric surgery models might be associated with the different rat strains used: lean outbred rats, lean alcohol preferring rats vs. diet-induced obese rats, an idea in need of further experimental support. The question: which preclinical bariatric surgery model is the most relevant for the human condition remains to be answered.

FUTURE PROSPECTS

The data reviewed here paint a clear picture of the potent and robust food and drug reward suppressing action of the GLP-1 system. The GLP-1-reward field is, however, very young and much work remains to be done. The results reviewed here need to be confirmed in a clinical population. There is good reason to expect that similar food/drug reward suppressing effects would be obtained in human subjects since most beneficial effects of GLP-1 reported to date are easily translated from preclinical to clinical studies. Nonetheless, direct evidence is still missing. Furthermore, almost all available evidence reflects the acute effects of GLP-1 manipulation on reward; thus long-term studies are still needed to confirm that the reward-suppressing action can be maintained over time and with repeated GLP-1 analog administration.

The food-reward suppressing effect of GLP-1R stimulation needs to be confirmed in the target preclinical and clinical population—obese subjects. Chronic high-fat or high-sugar food intake is associated with changes in the mesolimbic reward system and reward behavior (Li et al., 2009; Vucetic et al., 2011; Sharma et al., 2013). There seems to be a lack of consensus on the exact impact of this chronic overeating on the reward circuitry. Both enhanced and reduced food reward behavior have been suggested, either as a contributor or a result of obesity (Berthoud et al., 2011). In an attempt to reconcile the disparate findings it has been suggested that the activity of food responsive reward circuitry is high before development of obesity and becomes suppressed with time due to excessive consumption of the rewarding food. Thus, it is clear that an effect of a drug on reward in non-obese animals cannot be simply extrapolated to those that are obese and it will be crucial to determine whether the food reward suppressive effects of GLP-1 and analogs is relevant and beneficial in an obese model. Moreover, while GLP-1 analog therapy is clearly effective in regulating blood glucose of obese patients, the anorexic effects of the acute peripheral injections of GLP-1 or EX4 may be attenuated in rats fed a high-fat diet (Williams et al., 2011). Yet evidence also exists suggesting that the anorexic response to a GLP-1 analog, liraglutide, is in fact enhanced and lasts much longer in high-fat fed rats (Mul et al., 2013). Which of these findings is relevant for food reward regulation by GLP-1 remains to be determined.

Studies exploring the CNS effects of GLP-1 could also greatly benefit from a neuroanatomical dissection of the hindbrain GLP-1-producing neuronal population and its projection targets. Peripheral or ventricular exogenous drug application can likely allow for stimulation of most GLP-1R-expressing cell populations simultaneously. It would be of great interest to determine whether it is possible to stimulate, also in a clinical setting, specific GLP-1 populations. One way this could be achieved would be if different hindbrain GLP-1-producing neurons have divergent projection targets and a different set of inputs. For example, if one population activated by factor X projects specifically to the amygdala but another activated by factor Y to the VTA, it would be possible to achieve more precise and selective VTA GLP-1R activation by simply applying factor Y. Elegant studies of this type have been done for example by Leshan and colleagues to show that only a subpopulation of VTA dopamine neurons projecting specifically to the amygdala but not the NAc is activated by leptin (Leshan et al., 2010). The idea of diverse subpopulations for GLP-1 producing neurons is, at this point, purely speculative and remains to be investigated. Furthermore, understanding the functionality of the GLP-1R may help in development of more specific GLP-1 analogs (Patterson et al., 2013). The neurochemical mediators downstream from the mesolimbic GLP-1R are also largely unexplored. Thus, both the intracellular signals engaged by the GLP-1R expressing cells and the downstream neuropeptides and neurotransmitters released await a detailed analysis.

CONCLUDING REMARKS

Behavioral feeding responses are evolutionarily conserved and necessary for survival. The neurocircuitry underlying feeding control is complex and requires the coordinated involvement of many likely redundant and parallel brain circuits. Thus, it is likely that the most efficient way to achieve a reduction in food intake would have to involve a simultaneous impact on a number of CNS regions controlling different aspects of feeding regulation from the cognitive, decision-making, memory, and rewarding aspects to the feeling of satiety and hunger. The evidence reviewed here supports the idea that GLP-1 action on the CNS is distributed with many brain nuclei being engaged simultaneously via central GLP-1 to regulate metabolism, and reward-seeking behaviors.

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REFERENCES

Abegg, K., Schiesser, M., Lutz, T. A., and Bueter, M. (2013). Acute peripheral GLP-1 receptor agonism or antagonism does not alter energy expenditure in rats after Roux-en-Y gastric bypass. *Physiol. Behav*. doi: 10.1016/j.physbeh.2013.03.027. [Epub ahead of print].

Abizaid, A. (2009). Ghrelin and dopamine: new insights on the peripheral regulation of appetite. *J. Neuroendocrinol.* 21, 787–793. doi: 10.1111/j.1365-2826.2009.01896.x

Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., et al. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J. Clin. Invest.* 116, 3229–3239. doi: 10.1172/JCI29867

Abuirmeileh, A., Harkavyi, A., Rampersaud, N., Lever, R., Tadross, J. A., Bloom, S. R., et al. (2012). Exendin-4 treatment enhances L-DOPA evoked release of striatal dopamine and decreases dyskinetic movements in the 6-hydoxydopamine lesioned rat. *J. Pharm. Pharmacol.* 64, 637–643. doi: 10.1111/j.2042-7158.2011.01394.x

Agerso, H., Jensen, L. B., Elbrond, B., Rolan, P., and Zdravkovic, M. (2002). The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men. *Diabetologia* 45, 195–202. doi: 10.1007/s00125-001-0719-z

Alhadeff, A. L., Rupprecht, L. E., and Hayes, M. R. (2012). GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake. *Endocrinology* 153, 647–658. doi: 10.1210/en.2011-1443

Astrup, A., Carraro, R., Finer, N., Harper, A., Kunesova, M., Lean, M. E., et al. (2012). Safety, tolerability and sustained weight loss over 2 years with the once-daily human GLP-1 analog, liraglutide. *Int. J. Obes. (Lond.)* 36, 843–854. doi: 10.1038/ijo.2011.158

Astrup, A., Rossner, S., Van Gaal, L., Rissanen, A., Niskanen, L., Al Hakim, M., et al. (2009). Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. *Lancet* 374, 1606–1616. doi: 10.1016/S0140-6736(09)61375-1

Berridge, K. C. (1996). Food reward: brain substrates of wanting and liking. *Neurosci. Biobehav. Rev.* 20, 1–25. doi: 10.1016/0149-7634(95)00033-B

Berthoud, H. R., Lenard, N. R., and Shin, A. C. (2011). Food reward, hyperphagia, and obesity. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300, R1266–R1277. doi: 10.1152/ajpregu.00028.2011

Buchwald, H. (2010). Metabolic surgery: a brief history and perspective. *Surg. Obes. Relat. Dis.* 6, 221–222. doi: 10.1016/j.soard.2009.09.001

Buchwald, H., and Oien, D. M. (2009). Metabolic/bariatric surgery Worldwide 2008. *Obes. Surg.* 19, 1605–1611. doi: 10.1007/s11695-009-0014-5

Calara, F., Taylor, K., Han, J., Zabala, E., Carr, E. M., Wintle, M., et al. (2005). A randomized, open-label, crossover study examining the effect of injection site on bioavailability of exenatide (synthetic exendin-4). *Clin. Ther.* 27, 210–215. doi: 10.1016/j.clinthera.2005.02.008

Carlsson, L. M., Peltonen, M., Ahlin, S., Anveden, A., Bouchard, C., Carlsson, B., et al. (2012). Bariatric surgery and prevention of type 2 diabetes in Swedish obese subjects. *N. Engl. J. Med.* 367, 695–704. doi: 10.1056/NEJMoa1112082

Cone, R. D. (2005). Anatomy and regulation of the central melanocortin system. *Nat. Neurosci.* 8, 571–578. doi: 10.1038/nn1455

Crespi, F. (1998). The role of cholecystokinin (CCK), CCK-A or CCK-B receptor antagonists in the spontaneous preference for drugs of abuse (alcohol or cocaine) in naive rats. *Methods Find. Exp. Clin. Pharmacol.* 20, 679–697. doi: 10.1358/mf.1998.20.8.487502

Cummings, D. E. (2009). Endocrine mechanisms mediating remission of diabetes after gastric bypass surgery. *Int. J. Obes. (Lond.)* 33(Suppl. 1), S33–S40. doi: 10.1038/ijo.2009.15

Davis, J. F., Choi, D. L., Shurdak, J. D., Krause, E. G., Fitzgerald, M. F., Lipton, J. W., et al. (2011). Central melanocortins modulate mesocorticolimbic activity and food seeking behavior in the rat. *Physiol. Behav.* 102, 491–495. doi: 10.1016/j.physbeh.2010.12.017

Davis, J. F., Hakala-Finch, A., and Benoit, S. C. (2012a). "GLP-1 signaling decreases operant responding for ethanol in ethanol preferring rats," in *Society for Neuroscience,* (New Orleans, LA: Society for Neuroscience 2012 Book 5), 8.

Davis, J. F., Schurdak, J. D., Magrisso, I. J., Mul, J. D., Grayson, B. E., Pfluger, P. T., et al. (2012b). Gastric bypass surgery attenuates ethanol consumption in ethanol-preferring rats. *Biol. Psychiatry* 72, 354–360. doi: 10.1016/j.biopsych.2012.01.035

De Jonghe, B. C., Lawler, M. P., Horn, C. C., and Tordoff, M. G. (2009). Pica as an adaptive response: kaolin consumption helps rats recover from chemotherapy-induced illness. *Physiol. Behav.* 97, 87–90. doi: 10.1016/j.physbeh.2009.02.009

Diakogiannaki, E., Gribble, F. M., and Reimann, F. (2012). Nutrient detection by incretin hormone secreting cells. *Physiol. Behav.* 106, 387–393. doi: 10.1016/j.physbeh.2011.12.001

Di Chiara, G. (2002). Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behav. Brain Res.* 137, 75–114. doi: 10.1016/S0166-4328(02)00286-3

Dickson, S. L., Shirazi, R. H., Hansson, C., Bergquist, F., Nissbrandt, H., and Skibicka, K. P. (2012). The glucagon-like peptide 1 (GLP-1) analogue, exendin-4, decreases the rewarding value of food: a new role for mesolimbic GLP-1 receptors. *J. Neurosci.* 32, 4812–4820. doi: 10.1523/JNEUROSCI.6326-11.2012

DiLeone, R. J., Taylor, J. R., and Picciotto, M. R. (2012). The drive to eat: comparisons and distinctions between mechanisms of food reward and drug addiction. *Nat. Neurosci.* 15, 1330–1335. doi: 10.1038/nn.3202

Dixit, T. S., Sharma, A. N., Lucot, J. B., and Elased, K. M. (2013). Antipsychotic-like effect of GLP-1 agonist liraglutide but not DPP-IV inhibitor sitagliptin in mouse model for psychosis. *Physiol. Behav.* 114–115, 38–41. doi: 10.1016/j.physbeh.2013.03.008

Dossat, A. M., Diaz, R., Gallo, L., Panagos, A., Kay, K., and Williams, D. L. (2013). Nucleus accumbens GLP-1 receptors influence meal size and palatability. *Am. J. Physiol. Endocrinol. Metab.* 304, E1314–E1320. doi: 10.1152/ajpendo.00137.2013

Dossat, A. M., Lilly, N., Kay, K., and Williams, D. L. (2011). Glucagon-like peptide 1 receptors in nucleus accumbens affect food intake. *J. Neurosci.* 31, 14453–14457. doi: 10.1523/JNEUROSCI.3262-11.2011

Drucker, D. J., Dritselis, A., and Kirkpatrick, P. (2010). Liraglutide. *Nat. Rev. Drug Discov.* 9, 267–268. doi: 10.1038/nrd3148

Egecioglu, E., Steensland, P., Fredriksson, I., Feltmann, K., Engel, J. A., and Jerlhag, E. (2013). The glucagon-like peptide 1 analogue Exendin-4 attenuates alcohol mediated behaviors in rodents. *Psychoneuroendocrinology* 38, 1259–1270. doi: 10.1016/j.psyneuen.2012.11.009

Erreger, K., Davis, A. R., Poe, A. M., Greig, N. H., Stanwood, G. D., and Galli, A. (2012). Exendin-4 decreases amphetamine-induced locomotor activity. *Physiol. Behav.* 106, 574–578. doi: 10.1016/j.physbeh.2012.03.014

Fulton, S., Pissios, P., Manchon, R. P., Stiles, L., Frank, L., Pothos, E. N., et al. (2006). Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* 51, 811–822. doi: 10.1016/j.neuron.2006.09.006

Graham, D. L., Erreger, K., Galli, A., and Stanwood, G. D. (2012). GLP-1 analog attenuates cocaine reward. *Mol. Psychiatry* 106, 574–578. doi: 10.1038/mp.2012.141

Greig, N. H., Holloway, H. W., De Ore, K. A., Jani, D., Wang, Y., Zhou, J., et al. (1999). Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations. *Diabetologia* 42, 45–50. doi: 10.1007/s001250051111

Grill, H. J., and Hayes, M. R. (2009). The nucleus tractus solitarius: a portal for visceral afferent signal processing, energy status assessment and integration of their combined effects on food intake. *Int. J. Obes. (Lond.)* 33(Suppl. 1), S11–S15. doi: 10.1038/ijo.2009.10

Grill, H. J., and Hayes, M. R. (2012). Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance. *Cell Metab.* 16, 296–309. doi: 10.1016/j.cmet.2012.06.015

Grill, H. J., and Kaplan, J. M. (2002). The neuroanatomical axis for control of energy balance. *Front. Neuroendocrinol.* 23, 2–40. doi: 10.1006/frne.2001.0224

Hajnal, A., Zharikov, A., Polston, J. E., Fields, M. R., Tomasko, J., Rogers, A. M., et al. (2012). Alcohol reward is increased after Roux-en-Y gastric bypass in dietary obese rats with differential effects following ghrelin antagonism. *PLoS ONE* 7:e49121. doi: 10.1371/journal.pone.0049121

Han, V. K., Hynes, M. A., Jin, C., Towle, A. C., Lauder, J. M., and Lund, P. K. (1986). Cellular localization of proglucagon/glucagon-like peptide I messenger RNAs in rat brain. *J. Neurosci. Res.* 16, 97–107. doi: 10.1002/jnr.490160110

Harkavyi, A., Abuirmeileh, A., Lever, R., Kingsbury, A. E., Biggs, C. S., and

Whitton, P. S. (2008). Glucagon-like peptide 1 receptor stimulation reverses key deficits in distinct rodent models of Parkinson's disease. *J. Neuroinflammation* 5, 19. doi: 10.1186/1742-2094-5-19

Hayes, M. R., Bradley, L., and Grill, H. J. (2009). Endogenous hindbrain glucagon-like peptide-1 receptor activation contributes to the control of food intake by mediating gastric satiation signaling. *Endocrinology* 150, 2654–2659. doi: 10.1210/en.2008-1479

Hayes, M. R., De Jonghe, B. C., and Kanoski, S. E. (2010). Role of the glucagon-like-peptide-1 receptor in the control of energy balance. *Physiol. Behav.* 100, 503–510. doi: 10.1016/j.physbeh.2010.02.029

Hayes, M. R., Skibicka, K. P., and Grill, H. J. (2008). Caudal brainstem processing is sufficient for behavioral, sympathetic, and parasympathetic responses driven by peripheral and hindbrain glucagon-like-peptide-1 receptor stimulation. *Endocrinology* 149, 4059–4068. doi: 10.1210/en.2007-1743

Henningfield, J. E., and Meisch, R. A. (1975). Ethanol-reinforced responding and intake as a function of volume per reinforcement. *Pharmacol. Biochem. Behav.* 3, 437–441. doi: 10.1016/0091-3057(75)90053-2

Hodos, W. (1961). Progressive ratio as a measure of reward strength. *Science* 134, 943–944. doi: 10.1126/science.134.3483.943

Holscher, C. (2012). Potential role of glucagon-like peptide-1 (GLP-1) in neuroprotection. *CNS Drugs* 26, 871–882. doi: 10.2165/11635890-000000000-00000

Holst, J. J. (2004). On the physiology of GIP and GLP-1. *Horm. Metab. Res.* 36, 747–754. doi: 10.1055/s-2004-826158

Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z. W., Gao, X. B., et al. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 51, 801–810. doi: 10.1016/j.neuron.2006.08.023

Hsu, R., Taylor, J. R., Newton, S. S., Alvaro, J. D., Haile, C., Han, G., et al. (2005). Blockade of melanocortin transmission inhibits cocaine reward. *Eur. J. Neurosci.* 21, 2233–2242. doi: 10.1111/j.1460-9568.2005.04038.x

Imperato, A., and Di Chiara, G. (1986). Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J. Pharmacol. Exp. Ther.* 239, 219–228.

Jin, S. L., Han, V. K., Simmons, J. G., Towle, A. C., Lauder, J. M., and Lund, P. K. (1988). Distribution of glucagonlike peptide I (GLP-I), glucagon, and glicentin in the rat brain: an immunocytochemical study. *J. Comp. Neurol.* 271, 519–532. doi: 10.1002/cne.902710405

Kanoski, S. E., Rupprecht, L. E., Fortin, S. M., De Jonghe, B. C., and Hayes, M. R. (2012). The role of nausea in food intake and body weight suppression by peripheral GLP-1 receptor agonists, exendin-4 and liraglutide. *Neuropharmacology* 62, 1916–1927. doi: 10.1016/j.neuropharm.2011.12.022

Kim, S., Moon, M., and Park, S. (2009). Exendin-4 protects dopaminergic neurons by inhibition of microglial activation and matrix metalloproteinase-3 expression in an animal model of Parkinson's disease. *J. Endocrinol.* 202, 431–439. doi: 10.1677/JOE-09-0132

Kinzig, K. P., D'Alessio, D. A., and Seeley, R. J. (2002). The diverse roles of specific GLP-1 receptors in the control of food intake and the response to visceral illness. *J. Neurosci.* 22, 10470–10476.

Koob, G. F. (1992). Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol. Sci.* 13, 177–184. doi: 10.1016/0165-6147(92)90060-J

Labouesse, M. A., Stadlbauer, U., Weber, E., Arnold, M., Langhans, W., and Pacheco-Lopez, G. (2012). Vagal afferents mediate early satiation and prevent flavour avoidance learning in response to intraperitoneally infused exendin-4. *J. Neuroendocrinol.* 24, 1505–1516. doi: 10.1111/j.1365-2826.2012.02364.x

Langhans, W. (2000). Anorexia of infection: current prospects. *Nutrition* 16, 996–1005. doi: 10.1016/S0899-9007(00)00421-4

Larsen, P. J., Tang-Christensen, M., Holst, J. J., and Orskov, C. (1997a). Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience* 77, 257–270. doi: 10.1016/S0306-4522(96)00434-4

Larsen, P. J., Tang-Christensen, M., and Jessop, D. S. (1997b). Central administration of glucagon-like peptide-1 activates hypothalamic neuroendocrine neurons in the rat. *Endocrinology* 138, 4445–4455. doi: 10.1210/en.138.10.4445

Leggio, L. (2010). Role of the ghrelin system in alcoholism: acting on the growth hormone secretagogue receptor to treat alcohol-related diseases. *Drug News Perspect.* 23, 157–166. doi: 10.1358/dnp.2010.23.3.1429490

le Roux, C. W., and Bloom, S. R. (2005). Why do patients lose weight after Roux-en-Y gastric bypass. *J. Clin. Endocrinol. Metab.* 90, 591–592. doi: 10.1210/jc.2004-2211

Leshan, R. L., Opland, D. M., Louis, G. W., Leinninger, G. M., Patterson, C. M., Rhodes, C. J., et al. (2010). Ventral tegmental area leptin receptor neurons specifically project to and regulate cocaine- and amphetamine-regulated transcript neurons of the extended central amygdala. *J. Neurosci.* 30, 5713–5723. doi: 10.1523/JNEUROSCI.1001-10.2010

Li, Y., South, T., Han, M., Chen, J., Wang, R., and Huang, X. F. (2009). High-fat diet decreases tyrosine hydroxylase mRNA expression irrespective of obesity susceptibility in mice. *Brain Res.* 1268, 181–189. doi: 10.1016/j.brainres.2009.02.075

Lindblom, J., Opmane, B., Mutulis, F., Mutule, I., Petrovska, R., Klusa, V., et al. (2001). The MC4 receptor mediates alpha-MSH induced release of nucleus accumbens dopamine. *Neuroreport* 12, 2155–2158. doi: 10.1097/00001756-200107200-00022

Llewellyn-Smith, I. J., Reimann, F., Gribble, F. M., and Trapp, S. (2011). Preproglucagon neurons project widely to autonomic control areas in the mouse brain. *Neuroscience* 180, 111–121. doi: 10.1016/j.neuroscience.2011.02.023

Mack, C. M., Moore, C. X., Jodka, C. M., Bhavsar, S., Wilson, J. K., Hoyt, J. A., et al. (2006). Antiobesity action of peripheral exenatide (exendin-4) in rodents: effects on food intake, body weight, metabolic status and side-effect measures. *Int. J. Obes. (Lond.)* 30, 1332–1340. doi: 10.1038/sj.ijo.0803284

Mathes, C. M., Bueter, M., Smith, K. R., Lutz, T. A., le Roux, C. W., and Spector, A. C. (2012). Roux-en-Y gastric bypass in rats increases sucrose taste-related motivated behavior independent of pharmacological GLP-1-receptor modulation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302, R751–R767. doi: 10.1152/ajpregu.00214.2011

McMahon, L. R., and Wellman, P. J. (1998). PVN infusion of GLP-1-(7-36) amide suppresses feeding but does not induce aversion or alter locomotion in rats. *Am. J. Physiol.* 274, R23–R29.

Merchenthaler, I., Lane, M., and Shughrue, P. (1999). Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J. Comp. Neurol.* 403, 261–280. doi: 10.1002/(SICI)1096-9861(19990111)403:2<261::AID-CNE8>3.0.CO;2-5

Miras, A. D., Jackson, R. N., Jackson, S. N., Goldstone, A. P., Olbers, T., Hackenberg, T., et al. (2012). Gastric bypass surgery for obesity decreases the reward value of a sweet-fat stimulus as assessed in a progressive ratio task. *Am. J. Clin. Nutr.* 96, 467–473. doi: 10.3945/ajcn.112.036921

Mul, J. D., Begg, D. P., Barrera, J. G., Li, B., Matter, E. K., D'Alessio, D. A., et al. (2013). High-fat diet changes the temporal profile of GLP-1 receptor-mediated hypophagia in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 305, R68–R77. doi: 10.1152/ajpregu.00588.2012

Naslund, E., Barkeling, B., King, N., Gutniak, M., Blundell, J. E., Holst, J. J., et al. (1999). Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int. J. Obes. Relat. Metab. Disord.* 23, 304–311. doi: 10.1038/sj.ijo.0800818

Palmiter, R. D. (2007). Is dopamine a physiologically relevant mediator of feeding behavior. *Trends Neurosci.* 30, 375–381. doi: 10.1016/j.tins.2007.06.004

Palmiter, R. D. (2008). Dopamine signaling in the dorsal striatum is essential for motivated behaviors: lessons from dopamine-deficient mice. *Ann. N.Y. Acad. Sci.* 1129, 35–46. doi: 10.1196/annals.1417.003

Parkes, D. G., Mace, K. F., and Trautmann, M. E. (2013). Discovery and development of exenatide: the first antidiabetic agent to leverage the multiple benefits of the incretin hormone, GLP-1. *Expert Opin. Drug Discov.* 8, 219–244. doi: 10.1517/17460441.2013.741580

Patterson, J. T., Li, P., Day, J. W., Gelfanov, V. M., and DiMarchi, R. D. (2013). A hydrophobic site on the GLP-1 receptor extracellular domain orients the peptide ligand for signal transduction. *Mol. Metab.* 2, 86–91. doi: 10.1016/j.molmet.2013.01.003

Reimann, F. (2010). Molecular mechanisms underlying nutrient detection by incretin-secreting cells. *Int. Dairy J.* 20, 236–242. doi: 10.1016/j.idairyj.2009.11.014

Reimann, F., Habib, A. M., Tolhurst, G., Parker, H. E., Rogers, G. J.,

and Gribble, F. M. (2008). Glucose sensing in L cells: a primary cell study. *Cell Metab.* 8, 532–539. doi: 10.1016/j.cmet.2008.11.002

Ren, X., Ferreira, J. G., Zhou, L., Shammah-Lagnado, S. J., Yeckel, C. W., and de Araujo, I. E. (2010). Nutrient selection in the absence of taste receptor signaling. *J. Neurosci.* 30, 8012–8023. doi: 10.1523/JNEUROSCI.5749-09.2010

Rinaman, L. (1999a). A functional role for central glucagon-like peptide-1 receptors in lithium chloride-induced anorexia. *Am. J. Physiol.* 277, R1537–R1540.

Rinaman, L. (1999b). Interoceptive stress activates glucagon-like peptide-1 neurons that project to the hypothalamus. *Am. J. Physiol.* 277, R582–R590.

Rinaman, L. (2010). Ascending projections from the caudal visceral nucleus of the solitary tract to brain regions involved in food intake and energy expenditure. *Brain Res.* 1350, 18–34. doi: 10.1016/j.brainres.2010.03.059

Robinson, S., Rainwater, A. J., Hnasko, T. S., and Palmiter, R. D. (2007). Viral restoration of dopamine signaling to the dorsal striatum restores instrumental conditioning to dopamine-deficient mice. *Psychopharmacology (Berl.)* 191, 567–578. doi: 10.1007/s00213-006-0579-9

Robinson, S., Sotak, B. N., During, M. J., and Palmiter, R. D. (2006). Local dopamine production in the dorsal striatum restores goal-directed behavior in dopamine-deficient mice. *Behav. Neurosci.* 120, 196–200. doi: 10.1037/0735-7044.120.1.000

Sandoval, D. A., Bagnol, D., Woods, S. C., D'Alessio, D. A., and Seeley, R. J. (2008). Arcuate glucagon-like peptide 1 receptors regulate glucose homeostasis but not food intake. *Diabetes* 57, 2046–2054. doi: 10.2337/db07-1824

Schick, R. R., Zimmermann, J. P., vorm Walde, T., and Schusdziarra, V. (2003). Peptides that regulate food intake: glucagon-like peptide 1-(7-36) amide acts at lateral and medial hypothalamic sites to suppress feeding in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284, R1427–R1435.

Seeley, R. J., Blake, K., Rushing, P. A., Benoit, S., Eng, J., Woods, S. C., et al. (2000). The role of CNS glucagon-like peptide-1 amide receptors in mediating the visceral illness effects of lithium chloride. *J. Neurosci.* 20, 1616–1621.

Sharma, S., Fernandes, M. F., and Fulton, S. (2013). Adaptations in brain reward circuitry underlie palatable food cravings and anxiety induced by high-fat diet withdrawal. *Int. J. Obes. (Lond.)*. 37, 1183–1191.

Shin, A. C., and Berthoud, H. R. (2011). Food reward functions as affected by obesity and bariatric surgery. *Int. J. Obes. (Lond.)* 35(Suppl. 3), S40–S44. doi: 10.1038/ijo.2011.147

Shirazi, R. H., Dickson, S. L., and Skibicka, K. P. (2013). Gut peptide GLP-1 and its analogue, exendin-4, decrease alcohol intake and reward. *PLoS ONE* 8:e61965. doi: 10.1371/journal.pone.0061965

Shughrue, P. J., Lane, M. V., and Merchenthaler, I. (1996). Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. *Endocrinology* 137, 5159–5162. doi: 10.1210/en.137.11.5159

Sjostrom, L., Narbro, K., Sjostrom, C. D., Karason, K., Larsson, B., Wedel, H., et al. (2007). Effects of bariatric surgery on mortality in Swedish obese subjects. *N. Engl. J. Med.* 357, 741–752. doi: 10.1056/NEJMoa066254

Skibicka, K. P., and Dickson, S. L. (2011). Ghrelin and food reward: the story of potential underlying substrates. *Peptides* 32, 2265–2273. doi: 10.1016/j.peptides.2011.05.016

Skibicka, K. P., Hansson, C., Alvarez-Crespo, M., Friberg, P. A., and Dickson, S. L. (2011). Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* 180, 129–137. doi: 10.1016/j.neuroscience.2011.02.016

Sotak, B. N., Hnasko, T. S., Robinson, S., Kremer, E. J., and Palmiter, R. D. (2005). Dysregulation of dopamine signaling in the dorsal striatum inhibits feeding. *Brain Res.* 1061, 88–96. doi: 10.1016/j.brainres.2005.08.053

Takeda, N., Hasegawa, S., Morita, M., and Matsunaga, T. (1993). Pica in rats is analogous to emesis: an animal model in emesis research. *Pharmacol. Biochem. Behav.* 45, 817–821. doi: 10.1016/0091-3057(93)90126-E

Talsania, T., Anini, Y., Siu, S., Drucker, D. J., and Brubaker, P. L. (2005). Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. *Endocrinology* 146, 3748–3756. doi: 10.1210/en.2005-0473

Thiele, T. E., Seeley, R. J., D'Alessio, D., Eng, J., Bernstein, I. L., Woods, S. C., et al. (1998). Central infusion of glucagon-like peptide-1-(7-36) amide (GLP-1) receptor antagonist attenuates lithium chloride-induced c-Fos induction in rat brainstem. *Brain Res.* 801, 164–170. doi: 10.1016/S0006-8993(98)00584-8

Thiele, T. E., Van Dijk, G., Campfield, L. A., Smith, F. J., Burn, P., Woods, S. C., et al. (1997). Central infusion of GLP-1, but not leptin, produces conditioned taste aversions in rats. *Am. J. Physiol.* 272, R726–R730.

Trapp, S., and Hisadome, K. (2011). Glucagon-like peptide 1 and the brain: central actions-central sources. *Auton. Neurosci.* 161, 14–19. doi: 10.1016/j.autneu.2010.09.008

Tuesta, L. M., Fowler, C. D., Lu, Q., and Kenny, P. J. (2012). "Role of glucagon-like peptide-1 (GLP-1) receptors in nicotine reinforcement," in *Society for Neuroscience, Vol. Program No. 455.08/Q4 2012 Neuroscience Meeting Planner*. New Orleans, LA: Society for Neuroscience.

Turton, M. D., O'Shea, D., Gunn, I., Beak, S. A., Edwards, C. M., Meeran, K., et al. (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379, 69–72. doi.org/10.1038/379069a0

Vucetic, Z., Kimmel, J., and Reyes, T. M. (2011). Chronic high-fat diet drives postnatal epigenetic regulation of μ-opioid receptor in the brain. *Neuropsychopharmacology* 36, 1199–1206. doi: 10.1038/npp.2011.4

Vucetic, Z., and Reyes, T. M. (2010). Central dopaminergic circuitry controlling food intake and reward: implications for the regulation of obesity. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 2, 577–593. doi: 10.1002/wsbm.77

Wang, Y., Perfetti, R., Greig, N. H., Holloway, H. W., DeOre, K. A., Montrose-Rafizadeh, C., et al. (1997). Glucagon-like peptide-1 can reverse the age-related decline in glucose tolerance in rats. *J. Clin. Invest.* 99, 2883–2889. doi: 10.1172/JCI119482

Williams, D. L., Hyvarinen, N., Lilly, N., Kay, K., Dossat, A., Parise, E., et al. (2011). Maintenance on a high-fat diet impairs the anorexic response to glucagon-like-peptide-1 receptor activation. *Physiol. Behav.* 103, 557–564. doi: 10.1016/j.physbeh.2011.04.005

Wilson-Pérez, H. E., Chambers, A. P., Ryan, K. K., Li, B., Sandoval, D. A., Stoffers, D., et al. (2013). Vertical sleeve gastrectomy is effective in two genetic mouse models of glucagon-like Peptide 1 receptor deficiency. *Diabetes* 62, 2380–2385. doi: 10.2337/db12-1498

Wise, R. A. (2004a). Dopamine and food reward: back to the elements. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 286, R13. doi: 10.1152/ajpregu.00590.2003

Wise, R. A. (2004b). Dopamine, learning and motivation. *Nat. Rev. Neurosci.* 5, 483–494. doi: 10.1038/nrn1406

Wise, R. A. (2006). Role of brain dopamine in food reward and reinforcement. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 361, 1149–1158. doi: 10.1098/rstb.2006.1854

Wise, R. A., and Bozarth, M. A. (1984). Brain reward circuitry: four circuit elements "wired" in apparent series. *Brain Res. Bull.* 12, 203–208. doi: 10.1016/0361-9230(84)90190-4

Wise, R. A., and Bozarth, M. A. (1987). A psychomotor stimulant theory of addiction. *Psychological. Rev.* 94, 469–492. doi: 10.1037/0033-295X.94.4.469

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A paradoxical association of an oxytocin receptor gene polymorphism: early-life adversity and vulnerability to depression

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Several prosocial behaviors may be influenced by the hormone oxytocin. In line with this perspective, the oxytocin receptor (OXTR) gene single nucleotide polymorphism (SNP), rs53576, has been associated with a broad range of social behaviors. In this regard, the G allele of the OXTR SNP has been accompanied by beneficial attributes such as increased empathy, optimism, and trust. In the current study among university students ($N = 288$), it was shown that early-life maltreatment was associated with depressive symptoms, and that the OXTR genotype moderated this relationship, such that under high levels of childhood maltreatment, only individuals with GG/GA genotype demonstrated increased depressive symptomatology compared to those with the AA genotype. In addition, the role of distrust in mediating the relation between childhood maltreatment and depression seemed to be more important among G allele carriers compared to individuals with the AA genotype. Thus, a breach in trust (i.e., in the case of early-life abuse or neglect) may have a more deleterious effect among G carriers, who have been characterized as more prosocial and attuned to social cues. The data suggested that G carriers of the OXTR might favor social sensitivity and thus might have been more vulnerable to the effects of early-life adversity.

Keywords: depression, distrust, early-life, maltreatment, oxytocin, SNP, stress

INTRODUCTION

Oxytocin, a neuropeptide produced in the hypothalamus, is involved in a broad range of physiological and behavioral processes. The role of oxytocin in pair-bonding and reproductive behaviors has been well-established (Carter, 1998; Gimpl and Fahrenholz, 2001), and this peptidergic system has become the focus of understanding the biological processes underlying prosocial behaviors (Donaldson and Young, 2008). In this regard, intranasal oxytocin has been shown to increase trusting behavior (Kosfeld et al., 2005), positive communication (Ditzen et al., 2009), and in-group favoritism (De Dreu et al., 2011). Furthermore, intranasal oxytocin has been associated with reduced social stress reactivity (Heinrichs et al., 2003), and attenuated amygdala activity in response to emotional stimuli (Domes et al., 2007).

In addition to intranasal manipulations of oxytocin, studies examining human social behaviors have also recently focused on the oxytocin receptor gene (OXTR) located on chromosome 3p25 (Inoue et al., 1994). A single nucleotide polymorphism (SNP), rs53576, involving a guanine (G) to adenine (A) substitution located in the third intron of the OXTR has emerged as an important candidate in the understanding of human social behaviors. Individuals homozygous for the G allele compared to those with the GA or AA genotypes exhibit greater empathy and an increased ability to detect emotion from pictures of human faces in which only the eyes were shown (Rodrigues et al., 2009), greater maternal sensitivity (Bakermans-Kranenburg and van Ijzendoorn, 2008) and higher self-esteem and optimism (Saphire-Bernstein et al., 2011). However, optimism was not associated with the OXTR rs53576 in a large cohort of Caucasian women (Cornelis et al., 2012). It was also revealed that in a sample of Caucasian males, those with the GG genotype displayed higher trust-related behaviors in comparison to A allele carriers (Krueger et al., 2012). Individuals with one or two copies of the G allele also showed lower cortisol levels compared to individuals with the AA genotype assessed in the Trier Social Stress Test after receiving social support (Chen et al., 2011). Furthermore, the GG/GA genotypes seek more emotional social support in Caucasian but not Asian participants (Kim et al., 2010) and have higher positive affect (Lucht et al., 2009) compared to AA genotypes.

Together, these findings indicate that having one or two copies of the G allele is associated with several positive features. However, the possibility exists that the seemingly positive effects of being a G carrier might be absent under conditions of adversity, especially those that involve negative early-life experiences. For instance, in an African American sample GG carriers exposed to severe childhood maltreatment displayed greater disorganized attachments styles and increased risk for emotional dysregulation compared to GA/AA carriers (Bradley et al., 2011). As well, among a Caucasian sample of depressed individuals, the GG genotype was associated with higher levels of adult separation anxiety compared to A carriers (Costa et al., 2009). It seems that in the face of

adversity, the more prosocial and socially attuned individuals may be more sensitive and therefore more likely to be affected by adverse experiences such as abuse.

It might be expected that although the G allele has been associated with greater trust, a violation of that trust, as in the case of early-life abuse and neglect could be more upsetting for those individuals. Accordingly, in the present study we examined the OXTR SNP in relation to childhood maltreatment and mental health outcomes in a culturally diverse sample. It was predicted that individuals with one or two copies of the G allele who self-reported early-life maltreatment would display elevated depression scores compared to those with two copies of the A allele. Furthermore, the betrayal associated with childhood maltreatment may culminate into a general distrust for others (Doyle, 2001) which may have important ramifications related to depression (Lynch and Cicchetti, 1998). Thus, we predicted that levels of distrust may be a mediator through which maltreatment might lead to depressive symptoms. Further, given that OXTR has been implicated in trust-related behaviors (Krueger et al., 2012), we hypothesized that feelings of distrust following maltreatment would be more detrimental for those with a G allele.

METHODS

PARTICIPANTS

Participants included 288 Carleton University first and second year students (213 females and 75 males), with a mean age of 19.99 ($SD = 3.17$) who were recruited through the university's online computerized recruitment system. Self-reported ethnicity included White (58.0%, $n = 167$), Black (11.8%, $n = 34$), Asian (8%, $n = 23$), Arab (5.7%, $n = 17$), South Asian (5.6%, $n = 16$), South East Asian (2.1%, $n = 6$), Latin American (1.4%, $n = 4$), Aboriginal (1.4%, $n = 4$), and other (e.g., mixed ethnicity, 5.9%, $n = 17$).

PROCEDURE

Once signed informed consent was obtained, participants responded to a series of demographic questions as well as measures of current depressive symptoms, childhood maltreatment, and distrust and cynicism. Saliva samples for DNA genotyping were taken following the questionnaires. Upon completion of the study, which took up to 1 h to complete, participants were debriefed and compensated with course credit. All procedures in the current study were approved by the Carleton University Ethics Committee for Psychological Research.

GENOTYPING

Samples for genotyping were collected using Oragene OG-500 collection kits (DNA Genotek, Inc., Ottawa, ON, Canada). Genomic DNA was extracted from the sample collection kit according to the manufacturer's instructions and diluted to approximately equal concentration (20 ng/μL). Genotyping was conducted using quantitative polymerase chain reaction (qPCR). Amplification reactions were performed in a total volume of 15 μl, containing ∼1 μL (20 ng) of genomic template, 0.6 μL of each primer (concentration 10 μM), 1.2 μL of dNTP, 1.5 μL 10X Buffer, 1.5 μL of $MgCl_2$, 0.3 μL of Salmon Sperm DNA, 0.15 μL of Taq polymerase, 0.015 of SYBR green, and 8.135 μL of water. All q-PCR plates were run in duplicate. Following this, all qPCR products were electrophoresed on 2% agarose gel and then visualized to verify qPCR results. The Bio-Rad Iq5 Primer sequences used for qPCR were as follows: OXTR F1 forward: TCCCTGTTTCTGTGGGACTGAGGAC, OXTR F2 forward: TCCCTGTTTCTGTGGGACTGAGGAT, OXTR reverse: ACCCAAGAGGCTGGTTTGGGGTT.

The allele distribution of the OXTR polymorphism was 118 GG individuals (22 male, 96 female) and 119 GA (38 male, 81 female) and 43 AA individuals (14 male, 29 female). The genotype distributions met Hardy-Weinberg Equilibrium expectations, $\chi^2_{(1)} = 1.99$, $p = 0.16$. Based on earlier studies, there was reason to collapse across the GG and GA genotypes, but there was also precedent for collapsing across the GA and AA carriers. In the present study the depressive scores associated with the A allele were somewhat lower than among G carriers (AA compared to G carriers), albeit not significantly so, and thus the AA recessive allele was considered in comparison to the pooled G carriers. As will be seen, this approach fit the data better than the alternative procedure, which did not distinguish between these conditions on any of the variables measured in the present investigation. This same procedure was used in studies showing that individuals with the GG/GA genotypes seek more emotional social support (Kim et al., 2010), have higher positive affect (Lucht et al., 2009), and showed lower cortisol responses to stress after social support compared to individuals with two copies of the A allele (Chen et al., 2011).

Eight individuals were excluded from analyses including genotype because we were unable to determine a genotype from the samples provided. No significant differences were found between the genotype groups based on sex, $\chi^2_{(1)} = 0.98$, $p = 0.32$, and past or current mental disorders, $\chi^2_{(1)} = 0.004$, $p = 0.95$. A trend was apparent for differences between genotypes based on self-reported ethnicity, $\chi^2_{(8)} = 14.13$, $p = 0.08$. This is not surprising, as the distribution of the OXTR polymorphism differs between ethnicities. More specifically, among Asian ethnic groups the AA genotype is most prevalent, whereas it is least prevalent among Caucasians (Kim et al., 2011; Saphire-Bernstein et al., 2011). Similarly, in the current study, of the 21 Asian participants whose samples could be genotyped, eight of these were of the AA genotype, which is a much higher frequency than that found among Caucasians. Thus, as the Asian OXTR genotype distributions differed from that of Caucasian individuals, all of the main analyses were performed both with and without Asian participants. In both instances the observed results were very similar.

MEASURES

Depressive symptoms

The 21-item Beck Depression inventory (BDI) (Beck et al., 1961) was used to assess depressive symptoms. For each item participants responded to one of four options which ranged from low to high depression symptomatology. Total scores were calculated by summing across all items ($\alpha = 0.91$).

Childhood maltreatment

The 31-item Childhood Maltreatment Questionnaire (short form) (Demare, 1996) assessed levels of maltreatment

comprising psychological ($\alpha = 0.95$), physical ($\alpha = 0.90$), and sexual abuse ($\alpha = 0.89$) as well as neglect ($\alpha = 0.85$). Each item can be rated from 1 (never) to 5 (very often) indicating the frequency of experiences.

Distrust and Cynicism

The 8-item Distrust and Cynicism Scale (derived from the Cook–Medley Hostility Scale) has been used as a reliable and valid measure of cynical distrust (Greenglass and Julkunen, 1989, 1991). Each item can be rated from 0 (completely disagree) to 3 (completely agree). Total scores were calculated by taking the mean across all items ($\alpha = 0.82$). Items such as; "*It is safer to trust nobody*" can be found in this scale.

STATISTICAL ANALYSES

The statistical analyses were performed using SPSS for Windows 18.0 (SPSS Science, Chicago, IL, USA). Statistical significance was determined at $p < 0.05$ (two-tailed). Analyses assessing differences on depression scores, childhood maltreatment, and distrust were assessed using independent samples *t*-tests. Correlational analysis was performed using Pearson product moment correlations. Moderations were analyzed using hierarchical linear regressions, and the significant moderations were followed up using a web utility for simple slopes (Preacher et al., 2006). Mediation analyses were conducted using Sobel's test for estimating indirect effects (Preacher and Hayes, 2004). Moderated mediation analyses were conducted using bootstrapping procedures and confidence intervals based on 5000 resamples (Preacher et al., 2007). In all regression analyses standardized scores were used.

RESULTS

The depression scores among individuals with the GG and AG genotype were very similar to one another ($M = 9.66$; $SE = 0.75$ and $M = 9.15$; $SE = 0.74$, respectively) and although somewhat elevated compared to that of the AA genotype ($M = 7.58$; $SE = 1.23$), these groups did not significantly differ from one another, $t_{(1,\ 278)} = 1.36$, $p = 0.18$. Childhood maltreatment scores also did not differ based on genotype for total maltreatment scores, $t_{(1,\ 51.3)} = -0.77$, $p = 0.45$, or on any subscales of maltreatment, including physical abuse, $t_{(1,\ 51.3)} = -0.70$, $p = 0.49$, psychological abuse, $t_{(1,\ 278)} = -0.67$, $p = 0.50$, sexual abuse, $t_{(1,\ 42.4)} = -0.93$, $p = 0.36$, and neglect, $t_{(1,\ 50.1)} = -0.96$, $p = 0.34$. There were too few participants who reported sexual abuse ($N = 2$) to provide meaningful results. As seen in **Table 1**, levels of maltreatment experienced in the current study were appreciable, with the most common form of abuse being of a psychological nature, whereas neglect and physical abuse were less common. In the present study ~10% of participants reported experiencing physical abuse or neglect at least sometimes and up to very often. Additionally, up to 30% reported experiences of psychological abuse to the same degree. Furthermore, there were no differences on levels of distrust, $t_{(1,278)} = -0.27$, $p = 0.79$, between genotype groups. For the Student *t*-test, the *p*-value for equal variances not assumed was reported when Levene's test was significant ($p < 0.05$).

Females were found to have higher depressive scores than males, $t_{(1,\ 163.6)} = 2.60$, $p = 0.01$, and they also reported higher levels of childhood maltreatment, $t_{(1,\ 225.4)} = 2.49$, $p = 0.01$. However, there were no gender differences on distrust levels $t_{(1,\ 286)} = -0.15$, $p = 0.89$, nor was the Gender × Gene interaction significant in relation to depressive symptoms, childhood maltreatment, or distrust. Furthermore, in an effort to control for the potential influence of population stratification, analyses assessing Ethnicity × Gene interactions on depressive symptoms, distrust, and childhood maltreatment were not found to be significant. In this regard, these analyses included all nine ethnicities as one of the factors, as well as a further analysis in which these ethnicities were collapsed into 5 ethnic conditions.

Table 1 | Percentage of childhood maltreatment experienced.

Childhood maltreatment	Never/ Rarely (%)	Sometimes (%)	Often/Very often (%)
Psychological	70.7	15.0	14.3
Neglect	90.2	4.9	4.9
Physical	89.2	7.1	3.7

The relation between childhood maltreatment and depressive symptoms revealed that total scores on childhood maltreatment were related to severity of depressive symptoms $r = 0.44$, $p < 0.001$. Likewise, psychological abuse, $r = 0.46$, $p < 0.001$, physical abuse, $r = 0.30$, $p < 0.001$, and neglect, $r = 0.34$, $p < 0.001$, were positively related to depression scores. The possible moderating effect of genotype was explored regarding the relationship between total childhood maltreatment scores, psychological abuse, physical abuse, and neglect with symptoms of depression.

To examine the possible interaction between total childhood maltreatment scores and depressive symptoms with OXTR genotype, a hierarchical linear regression was conducted. Genotype and total maltreatment scores were entered on the first step, and the Genotype × maltreatment interaction term was entered on the second step. The moderating role of OXTR genotype on the relation between childhood maltreatment and depression was significant, $\Delta R^2 = 0.02$, $b = -2.47$, $t = -2.42$, $p = 0.02$. Follow up simple slope analyses (Preacher et al., 2006), as shown in **Figure 1**, revealed that levels of depressive symptoms did not differ between genotypes at low levels of childhood maltreatment. However, at high levels of childhood maltreatment, depressive symptoms were significantly increased among those with one or two copies of the G allele ($p < 0.001$), an effect not seen among individuals with the AA genotype ($p = 0.07$). To further examine whether differences existed between individuals with one or more copies of the G allele, orthogonal contrasts were carried out in a hierarchical linear regression. As expected, individuals with the GG and GA genotypes displayed a similar relation between childhood maltreatment and depressive symptoms, and thus did not moderate this relationship. This moderation analysis was also conducted with ethnicity as a covariate, in an effort to control for population stratification, and the analysis remained significant.

We examined the potential interactive effects between the different forms of maltreatment (i.e., psychological abuse, physical abuse, and neglect) and genotype in predicting depression. A multiple hierarchical linear regression was conducted between genotype and each of psychological abuse, physical abuse, and

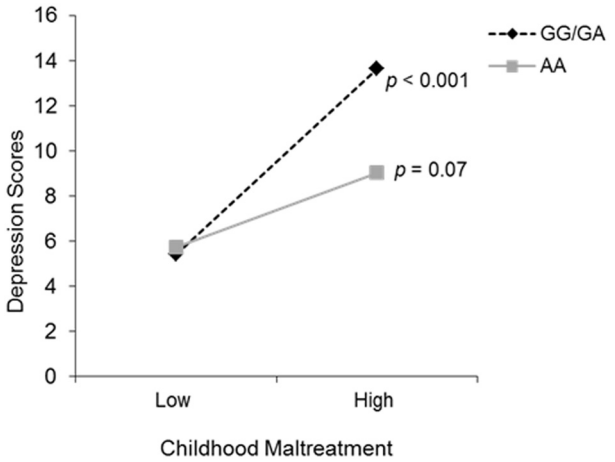

FIGURE 1 | The relation between childhood maltreatment and depression scores as a function of the OXTR rs53576 genotype (GG/GA vs. AA). The simple slopes analyses revealed that genotype groups did not differ at lower levels of childhood maltreatment. However, depressive symptoms increased significantly when higher levels of childhood maltreatment were experienced, but only among those individuals with the GG/GA genotype.

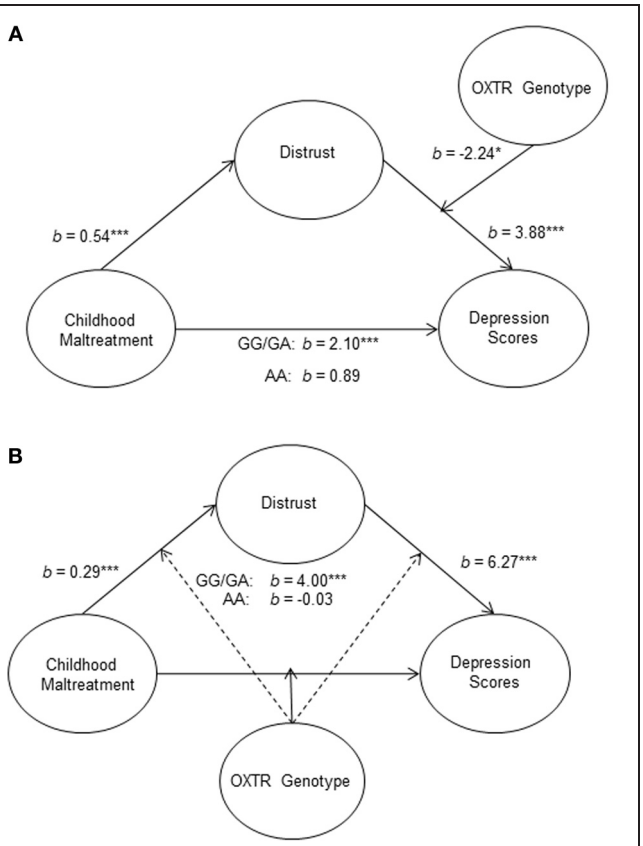

FIGURE 2 | Schematic representations of the moderated mediation models. The relation between childhood maltreatment and depression scores through distrust was moderated by OXTR rs53576 genotype (GG/GA vs. AA). This mediation model was found only to be significant among G carriers **(A)**. An alternative model was tested, in which the relationship between childhood maltreatment and depression scores through distrust was moderated at all three pathways. This time, only the direct path was moderated and again the effect was only observed among individuals with the GG/GA genotype **(B)**. $^{*}p < 0.05$, and $^{***}p < 0.001$.

neglect. Genotype and maltreatment subscales were entered on the first step, and the Genotype × Maltreatment subscale interaction terms were entered on the second step. Neither psychological abuse, physical abuse, nor neglect interacted with genotype to predict depression scores, $\Delta R^2 = 0.02$, $\Delta F_{(3,\ 272)} = 2.07$, $p = 0.11$. Thus, it seems that the relation between specific forms of maltreatment and depressive symptoms were not uniquely moderated by genotype, whereas the interaction was evident when maltreatment as a whole was considered.

It was of interest to explore potential pathways through which childhood maltreatment predicted depressive symptoms. In this regard, level of distrust was examined as a possible mediator through which childhood maltreatment predicts depression scores. As expected, distrust was positively related to depression scores, $r = 0.53$, $p < 0.001$. A mediation analyses was conducted, using bootstrapping techniques based on 5000 resamples to determine 95% confidence limits (Preacher and Hayes, 2004). The mediated effect of distrust in the relation between childhood maltreatment and severity of depressive symptoms was significant (95% CI {1.19, 2.63}), although the relation between maltreatment and depressive symptoms remained significantly evident, $b = 1.88$, $p < 0.001$. An alternative model was tested in which depressive symptoms mediated the relation between childhood maltreatment and distrust, and this model was also significant, (95% CI {0.11, 0.24}).

We further examined whether this mediation relationship was moderated by OXTR genotype. Moderated mediation analyses conducted using bootstrapping procedures and confidence intervals based on 5000 resamples (Preacher et al., 2007) showed that, as expected, the OXTR genotype moderated the mediating role of distrust in the relation between childhood maltreatment and depression scores (**Figure 2A**). Specifically, the relation between maltreatment and depression scores was mediated by distrust, but this mediated effect was only significant for G carriers. This moderated mediation analysis was also conducted with ethnicity as a covariate and remained significant.

An alternative moderation model, in which the OXTR genotype moderated the relation between childhood maltreatment and distrust was found not to be significant. However, when a final alternative model wherein the moderating effects of genotype on all three pathways were considered simultaneously (**Figure 2B**), only the moderation of the direct path between childhood maltreatment and depression scores accounted for unique variance. Thus, although distrust among those individuals with a G allele appeared to be more strongly associated with depressive symptoms than among those with AA genotype, when reports of childhood maltreatment were taken into consideration, their sensitivity to early-life maltreatment preclude effects attributable to distrust in the evolution of depressive symptoms.

DISCUSSION

In the current investigation, there was no direct associated found between the OXTR genotypes and depression scores. This is in contrast to the previous finding that A-allele carriers displayed greater depressive symptomatology (Saphire-Bernstein et al., 2011), although these variations may be due to the fact that different measures were used to assess depressive symptoms. Furthermore in the current investigation GG and GA genotypes were collapsed together, whereas Saphire-Bernstein et al. collapsed OXTR A carriers together. Importantly, in the current study, the OXTR genotype interacted with experiences of childhood maltreatment to predict depressive symptoms. Specifically, individuals with one or two copies of the G allele reported greater severity of depressive symptoms when they reported high levels of maltreatment compared to those individuals with the AA genotype. Thus, having experienced a negative early-life environment, the more sensitive G allele carriers appeared to be at greater risk of exhibiting depressive symptoms. These findings are consistent with the report that African American individuals with the GG genotype were at increased risk for emotional dysregulation provided that they experienced three or more types of childhood maltreatment (Bradley et al., 2011). Those with the GG or GA genotype in the present study also displayed higher depressive symptoms provided that they had experienced high levels childhood maltreatment. However, it is uncertain from the available data whether this relation was apparent with multiple different forms of maltreatment. This said, in the current investigation, the OXTR SNP interacted with total childhood maltreatment scores in predicting depressive symptoms and not with the individual subscales of maltreatment (i.e., psychological abuse, physical abuse, and neglect). In effect, the different forms of maltreatment do not account for unique variance, and it is all forms of maltreatment that was sufficient to increase depression symptoms among G carriers.

These findings suggest that individuals carrying one or two copies of the G allele may be more affected by previous experiences, regardless of whether it is positive or negative. Considering the research implicating the beneficial traits associated with having the G allele of the OXTR SNP, the current findings might seem counterintuitive. However, our results are in line with the view that the same genetic factors that make individuals relatively sensitive to a negative environment also influence sensitivity to a positive environment. In this respect, it has been suggested that "for better or for worse" certain genotypes are considered to promote greater plasticity and susceptible to the environment, (Belsky and Pluess, 2009; Belsky et al., 2009). Essentially, individuals with the GG or GA genotype of the OXTR SNP may thrive in a positive environment, (e.g., one that is high in social support) but, this same allele may encourage susceptibility in a negative environment. In fact, similar findings were reported with regard to the BDNF polymorphism (Val66Met) and vulnerability to childhood maltreatment. Specifically, the Val/Val genotype (which at first blush would seem to be associated with diminished vulnerability to stress-related pathology), were more negatively affected by experiences of early-life adversity compared to individuals with the Val/Met and Met/Met genotypes (Caldwell et al., 2013).

Evidently when examining the OXTR genotypes and their associations, environmental influences matter, and this extends to research focusing on other domains of the oxytocin system. In this regard, the effects of intranasal oxytocin administration on social behaviors are often moderated by contextual factors, thus leading to weak and/or inconsistent findings (Bartz et al., 2011). Furthermore, it was suggested that endogenous oxytocin levels across individuals are highly variable, and could be an indicator of sensitivity to social cues. Essentially, although high plasma oxytocin levels might be related to increased sensitivity and in turn elevated pro-social behaviors in general, the increased sensitivity among these individuals might result in elevated distress under conditions where their social needs are not met (Taylor, 2006; Bartz et al., 2011). From this perspective, oxytocin may confer a disposition toward increased sensitivity to social cues that can be either beneficial or detrimental depending on the environmental context (Bartz et al., 2011).

Beyond contextual factors, personal characteristics such as having a distrusting outlook on the world may be important when explaining the relationship between early-life adversity and depressive symptoms among specific OXTR genotypes. It has been reported that individuals with GG genotypes display greater trust in an investor-trustee money transfer game (Krueger et al., 2012). However, it has also been reported that no association existed between the OXTR SNP and human trust behaviors (Apicella et al., 2010). Thus, it was suggested that the OXTR SNP may be associated with trust-specific behaviors, but not lend itself to a general increase in trustworthy behaviors (Krueger et al., 2012). As the scale in the current investigation measured general feelings of distrust, this might account for why the OXTR genotypes did not differ in levels of distrust. Nevertheless, distrust was found to mediate the pathway between childhood maltreatment and depressive symptoms. Although, the directionality cannot be confirmed as alternative models were also significant. Furthermore, this mediation only occurred among the G carriers, and not among individuals with the AA genotype. Thus, distrust may have greater ramifications on measures of well-being among those individuals with one or more G alleles. Given that the G allele has been associated with greater levels of trust as well as greater sensitivity, it is possible that a breach of this trust could be particularly damaging for those individuals. However, when the mediation model was tested with OXTR genotype moderating all three pathways, (as seen in **Figure 2B**) only the direct path was significant. This suggests that, although genotype influences the extent of the relation between distrust and depressive symptoms, it seems that the differential sensitivity to negative early-life experiences is sufficiently overwhelming that when taken into consideration, the role of distrust is obfuscated. It should also be noted that when examining emotional reactions to betrayals in trust and the OXTR rs53576, no association between these variables was reported (Tabak et al., 2013). However, given the different methodologies used and especially considering the current findings were in the context of childhood maltreatment, the different outcomes are not particularly surprising.

There are several limitations associated with the current findings. Early-life maltreatment was determined based on retrospective self-reports. The use of self-reports, although common,

might be biased by the individuals' current affective state, and indeed individuals might be unaware of events that occurred years earlier. The present findings are limited in the scope of the early-life adverse events that were considered, and generalizations beyond this population, in which maltreatment was moderate, would be inappropriate. Additionally, in the current investigation, G carriers were collapsed and compared to individuals with the AA genotype. This method provided the best fit to our data and has been used in previous studies, although there have also been reports in which A carriers were combined (GA/AA). However, additional analyses revealed that individuals with the GG versus GA genotypes did not differ from one another. Importantly, the functionality of the OXTR rs53576 remains unknown. Although it has been suggested that intron 3, in which this particular OXTR SNP is located, may contribute to transcription suppression (Mizumoto et al., 1997), it is also possible that the observed associations are largely due to linkage disequilibrium associations with other functional OXTR polymorphisms (Lin et al., 2007). Finally, the current study included a heterogeneous cultural sample. Ideally, a much larger sample size would allow for analyses to be done separately for each ethnic group as there may be important Gene × Environment differences across ethnicities. A larger sample size might also have permitted analyses to determine whether one or another form of abuse, interacting with genotype, was more closely aligned with depression. Thus, the inability to detect an interactive effect between the OXTR SNP and specific forms of maltreatment could be due to a lack of power stemming from the relatively small number of participants. A power analysis indicated that with the N used in the current study only a small-medium effect size could be detected.

Summarizing, the results of the present study indicated that the OXTR SNP interacts with early-life adversity in the form of maltreatment to predict depressive symptoms. Specifically, depressive symptoms were most prominent among individuals with the GG/GA genotype who experienced abuse and neglect. Being correlational, the data do not allow for causal connections to be made. Nonetheless, the present findings are consistent with the view that the OXTR genotype might favor social sensitivity so that early experiences might affect later mood states. Although the G allele has typically been characterized as being associated with beneficial attributes, it seems as if those with the G allele might be most sensitive to environmental influences, whereas individuals with the AA genotype (who are typically viewed as less socially attune or prosocial), were least affected by early-life adversity. One can imagine a breach in trust might be less detrimental to an individual who is less sensitive to their social environment, as seems to be true for the individuals with two copies of the A allele. These data provide support to the likelihood that the G allele in the OXTR SNP rs53576 is not always advantageous, and in some instances the AA genotype does not necessarily suggest an unfortunate fate.

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REFERENCES

Apicella, C. L., Cesarini, D., Johannesson, M., Dawes, C. T., Lichtenstein, P., Wallace, B., et al. (2010). No association between oxytocin receptor (OXTR) gene polymorphisms and experimentally elicited social preferences. *PLoS ONE* 5:e111153. doi: 10.1371/journal.pone.0011153

Bakermans-Kranenburg, M. J., and van Ijzendoorn, M. H. (2008). Oxytocin receptor (OXTR) and serotonin transporter (5-HTT) genes associated with observed parenting. *Soc. Cogn. Affect. Neurosci.* 3, 128–134. doi: 10.1093/scan/nsn004

Bartz, J. A., Zaki, J., Bolger, N., and Ochsner, K. N. (2011). Social effects of oxytocin in humans: context and person matter. *Trends Cogn. Sci.* 15, 301–309.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., and Erbaugh, J. (1961). An inventory for measuring depression. *Arch. Gen. Psychiatry* 4, 561–571. doi: 10.1001/archpsyc.1961.01710120031004

Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., and Williams, R. (2009). Vulnerability genes or plasticity genes? *Mol. Psychiatry* 14, 746–754. doi: 10.1038/mp.2009.44

Belsky, J., and Pluess, M. (2009). Beyond diathesis stress: differential susceptibility to environmental influences. *Psychol. Bull.* 135, 885–908. doi: 10.1037/a0017376

Bradley, B., Westen, D., Mercer, K. B., Binder, E. B., Jovanovic, T., Crain, D., et al. (2011). Association between childhood maltreatment and adult emotional dysregulation in a low-income, urban, African American sample: moderation by oxytocin receptor gene. *Dev. Psychopathol.* 23, 439–452. doi: 10.1017/S0954579411000162

Caldwell, W., McInnis, O. A., McQuaid, R. J., Liu, G., Stead, J. D., Anisman, H., et al. (2013). The role of the Val66Met polymorphism of the brain derived neurotrophic factor gene in coping strategies relevant to depressive symptoms. *PLoS ONE* 8:e65547. doi: 10.1371/journal.pone.0065547

Carter, C. S. (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology* 23, 779–818. doi: 10.1016/S0306-4530(98)00055-9

Chen, F. S., Kumsta, R., von Dawans, B., Monakhov, M., Ebstein, R. P., and Heinrichs, M. (2011). Common oxytocin receptor gene (OXTR) polymorphism and social support interact to reduce stress in humans. *Proc. Natl. Acad. Sci. U.S.A.* 108, 19937–19942.

Cornelis, M. C., Glymour, M. M., Chang, S.-C., Tchetgen, E. J. T., Liang, L., Koenen, K. C., et al. (2012). Oxytocin receptor (OXTR) is not associated with optimism in the Nurses' Health Study. *Mol. Psychiatry* 17, 1157–1159.

Costa, B., Pini, S., Gabelloni, P., Abelli, M., Lari, L., Cardini, A., et al. (2009). Oxytocin receptor polymorphism and adult attachment style in patients with depression. *Psychoneuroendocrinology* 34, 1506–1514. doi: 10.1016/j.psyneuen.2009.05.006

De Dreu, C. K. W., Greer, L. L., Van Kleef, G. A., Shalvi, S., and Handgraaf, M. J. J. (2011). Oxytocin promotes human ethnocentrism. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1262–1266. doi: 10.1073/pnas.1015316108

Demare, D. (1996). "The childhood maltreatment questionnaire: examining long-term correlates of childhood maltreatment," in *Paper presented at APA* (Toronto, ON).

Ditzen, B., Schaer, M., Gabriel, B., Bodenmann, G., Ehlert, U., and Heinrichs, M. (2009). Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. *Biol. Psychiatry* 65, 728–731. doi: 10.1016/j.biopsych.2008.10.011

Domes, G., Heinrichs, M., Gläscher, J., Büchel, C., Braus, D. F., and Herpertz, S. C. (2007). Oxytocin attenuates amygdala response to emotional faces regardless of valence. *Biol. Psychiatry* 62, 1187–1190. doi: 10.1016/j.biopsych.2007.03.025

Donaldson, Z. R., and Young, L. J. (2008). Oxytocin, vasopressin, and the neurogenetics of sociality. *Science* 322, 900–904. doi: 10.1126/science.1158668

Doyle, C. (2001). Surviving and coping with emotional abuse in childhood. *Clin. Child Psychol. Psychiatry* 6, 387–402. doi: 10.1177/1359104501006003008

Gimpl, G., and Fahrenholz, F. (2001). The oxytocin receptor system: structure, function, and regulation. *Physiol. Rev.* 81, 629–683.

Greenglass, E. R., and Julkunen, J. (1989). Construct validity and sex

differences in Cook-Medley hostility. *Pers. Individ. Dif.* 10, 209–218. doi: 10.1016/0191-8869(89)90206-7

Greenglass, E. R., and Julkunen, J. (1991). Cook-Medley hostility, anger, and the Type A behavior pattern in Finland. *Psychol. Rep.* 68, 1059–1066.

Heinrichs, M., Baumgartner, T., Kirschbaum, C., and Ehlert, U. (2003). Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol. Psychiatry* 54, 1389–1398. doi: 10.1016/S0006-3223(03)00465-7

Inoue, T., Kimura, T., Azuma, C., Inazawa, J., Takemura, M., Kikuchi, T., et al. (1994). Structural organization of the human oxytocin receptor gene. *J. Biol. Chem.* 269, 32451–32456.

Kim, H. S., Sherman, D. K., Sasaki, J. Y., Xu, J., Chu, T. Q., Ryu, C., et al. (2010). Culture, distress and oxytocin receptor polymorphism (OXTR) interact to influence emotional support seeking. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15717–15721. doi: 10.1073/pnas.1010830107

Kim, H. S., Sherman, D. K., Mojaverian, T., Sasaki, J. Y., Park, J., Suh, E. M., et al. (2011). Gene-culture interaction: oxytocin receptor polymorphism (OXTR) and emotion regulation. *Soc. Psychol. Pers. Sci.* 2, 665–672.

Kosfeld, M., Heinrichs, M., Zak, P. J., Fischbacher, U., and Fehr, E. (2005). Oxytocin increases trust in humans. *Nature* 435, 673–676. doi: 10.1038/nature03701

Krueger, F., Parasuraman, R., Iyengar, V., Thornburg, M., Weel, J., Lin, M., et al. (2012). Oxytocin receptor genetic variation promotes human trust behavior. *Front. Hum. Neurosci.* 6:4. doi: 10.3389/fnhum.2012.00004

Lin, P. I., Vance, J. M., Pericak-Vance, M. A., and Martin, E. R. (2007). No gene is an island: the flip-flop phenomenon. *Am. J. Hum. Genet.* 80, 531–538. doi: 10.1086/512133

Lucht, M. J., Barnow, S., Sonnenfeld, C., Rosenberger, A., Grabe, H. J., Schroeder, W., et al. (2009). Associations between the oxytocin receptor gene (OXTR) and affect, loneliness and intelligence in normal subject. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 860–866.

Lynch, M., and Cicchetti, D. (1998). An ecological-transactional analysis of children and contexts: the longitudinal interplay among child maltreatment, community violence and children's symptomatology. *Dev. Psychopathol.* 10, 235–257. doi: 10.1017/S095457949800159X

Mizumoto, Y., Kimura, T., and Iveil, R. (1997). A genomic element within the third intron of the human oxytocin receptor gene may be involved in transcriptional suppression. *Mol. Cell. Endocrinol.* 135, 129–138. doi: 10.1016/S0303-7207(97)00195-0

Preacher, K. J., Curran, P. J., and Bauer, D. J. (2006). Computational tools for probing interaction effects in multiple linear regression, multilevel modeling, and latent curve analysis. *J. Educ. Behav. Stat.* 31, 437–448. doi: 10.3102/10769986031004437

Preacher, K. J., and Hayes, A. F. (2004). SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behav. Res. Methods Instrum. Comput.* 36, 717–731.

Preacher, K. J., Rucker, D. D., and Hayes, A. F. (2007). Addressing moderated mediation hypotheses: theory, methods, and prescriptions. *Multivariate Behav. Res.* 42, 185–227. doi: 10.1080/00273170701341316

Rodrigues, S. M., Saslow, L. R., Garcia, N., John, O. P., and Keltner, D. (2009). Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21437–21441.

Saphire-Bernstein, S., Way, B. M., Kim, H. S., Sherman, D. K., and Taylor, S. E. (2011). Oxytocin receptor gene (OXTR) is related to psychological resources. *Proc. Natl. Acad. Sci. U.S.A.* 108, 15118–15122. doi: 10.1073/pnas.1113137108

Tabak, B. A., McCullough, M. E., Carver, C. S., Pedersen, E. J., and Cuccaro, M. L. (2013). Variation in oxytocin receptor gene (OXTR) polymorphisms is associated with emotional and behavioral reactions to betrayal. *Soc. Cogn. Affect. Neurosci.* doi: 10.1093/scan/nst042. [Epub ahead of print].

Taylor, S. E. (2006). Tend and befriend: biobehavioral bases of affiliation under stress. *Curr. Dir. Psychol. Sci.* 15, 273–277. doi: 10.1111/j.1467-8721.2006.00451.x

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GENERAL COMMENTARY
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Oxytocin and social salience: a call for gene-environment interaction research

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A commentary on

A paradoxical association of an oxytocin receptor gene polymorphism: early-life adversity and vulnerability to depression
by McQuaid, R. J., McInnis, O. A., Stead, J. D., Matheson, K., and Anisman, H. (2013). Front. Neurosci. 7:128. doi: 10.3389/fnins.2013.00128

The role of the neuropeptide oxytocin in social cognition and prosocial behavior continues to fascinate the scientific community (Bartz et al., 2011b). However, as our knowledge of oxytocin's influence on social processes increases, a more complex picture continues to emerge. Studies have begun to identify individual differences and environmental contexts that substantially alter the effects of oxytocin administration (Bartz et al., 2011b; Macdonald, 2012). In addition, interpersonal distress has been positively correlated with plasma oxytocin (e.g., Tabak et al., 2011), and evidence for potential negative consequences of oxytocin administration continues to accumulate (Miller, 2013)—calling into question the view that oxytocin represents a social panacea (Pfeiffer, 2013). Although questions remain about how to properly measure oxytocin (Szeto et al., 2011; McCullough et al., 2013), and whether intranasal administration of oxytocin crosses the blood-brain barrier (Churchland and Winkielman, 2012; but see, Neumann et al., 2013), a developing theme in human oxytocin research is that early environmental experiences and/or attachment styles appear to moderate the effects of oxytocin administration (Bartz et al., 2010, 2011a; van IJzendoorn et al., 2011; De Dreu, 2012a; Riem et al., 2013).

Translational research has demonstrated that early environmental experiences can alter the oxytocin system (Champagne et al., 2001; Bales and Perkeybile, 2012), which may have long-lasting effects that impact adult human functioning (e.g., Heim et al., 2009; Strathearn et al., 2009; Feldman et al., 2013). Based on findings such as these, an increasing number of studies have begun to investigate how variation on the gene encoding for the oxytocin receptor (OXTR) may relate to socially relevant normative and pathological phenotypes. To date, the most commonly studied marker on OXTR is rs53576, an adenine-to-guanine single nucleotide polymorphism (SNP) that lies in the third intronic region. Although most published studies have found associations with the rs53576 G allele and beneficial aspects of social cognition and behavior (e.g., Saphire-Bernstein et al., 2011; Krueger et al., 2012), several studies have found no relationship between rs53576 genotype and similar social phenotypes (e.g., Apicella et al., 2010; Cornelis et al., 2012; Tabak et al., 2013), or opposite relationships (i.e., where the A allele is protective; Costa et al., 2009). Although some of these discrepancies likely result from known issues in genetic association studies (e.g., Ioannidis et al., 2001; Lin et al., 2007), findings from studies of endogenous oxytocin (Strathearn et al., 2009), oxytocin administration (van IJzendoorn et al., 2011), and OXTR (McQuaid et al., 2013) suggest that one method through which studies might yield more consistent findings is to include measures of environmental experiences in order to examine gene-environment interactions.

In a study recently published in this journal, McQuaid et al. (2013) examined how rs53576 genotype moderated the relationship between childhood maltreatment and depression symptoms in a racially/ethnically diverse sample. The authors found an association between individuals who carried the G allele on rs53576 and increased depression symptomology compared to those with the AA genotype. Furthermore, the authors discovered a meditational role of distrust/cynicism, suggesting that depression symptoms resulted (or co-occurred) in part from developing a distrustful attitude toward others after having suffered childhood maltreatment. McQuaid et al.'s (2013) findings are in agreement with Bradley et al. (2011) who studied a larger sample ($n = 595$) of 18–90 year old African Americans with low-incomes: they found that individuals with the GG genotype on rs53576 who experienced severe childhood maltreatment were more likely to have disorganized attachment styles and higher levels of emotional dysregulation.

Thus, in contrast to the majority of published studies focusing on rs53576, Bradley et al. (2011) and McQuaid et al. (2013) found that it was the G allele that represented risk rather than protection when examined in the context of childhood maltreatment. Initially, these findings seem to contradict those from previous studies; however, these results are in agreement with the "social salience" hypothesis of oxytocin, which proposes that oxytocin increases the salience of both positive and negative social cues (Shamay-Tsoory et al., 2009). Following this logic, enhanced social salience may improve social cognition and increase prosocial behavior for individuals in positive environments, but have detrimental effects for others in negative environments (Bartz et al., 2011b; Bakermans-Kranenburg and van IJzendoorn, 2013), perhaps because these environments encourage

the view that others are untrustworthy (Bakermans-Kranenburg et al., 2012). This hypothesis has gained some traction in light of recent findings demonstrating that oxytocin does not promote (or reduces) prosocial tendencies toward anonymous others or out-group members (Declerck et al., 2010; De Dreu et al., 2010; Radke and de Bruijn, 2012). However, the ways in which oxytocin administration may differentially affect people depending on attachment anxiety and/or avoidance continue to be explored (Bartz, 2012; De Dreu, 2012b).

Findings from Bradley et al. (2011) and McQuaid et al. (2013) involving rs53576 are also in agreement with the differential susceptibility hypothesis (Belsky et al., 2009), which posits that certain genetic variants can represent risk or protection depending upon whether or not the environment is positive or negative (for a discussion of differential susceptibility as related to another OXTR SNP, rs2254298, see Brüne, 2012). Importantly, the function of rs53576 is unknown. In addition, results from McQuaid et al. (2013) were based on a cross-sectional design, retrospective self-reports, and a small heterogeneous sample, which increases the potential for false-positive findings (Moffitt et al., 2006). As a result, the authors appropriately note that replication with larger samples and more sophisticated methodology is needed. Nonetheless, results from McQuaid et al. (2013) are in agreement with studies demonstrating the plasticity of the oxytocin system in both animals and humans and suggest that it is in the best interest of researchers investigating genetic associations (as well as epigenetic mechanisms; Kumsta et al., 2013) in oxytocin system relevant genes to measure both positive and negative environmental experiences for the purpose of examining gene-environment interactions.

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REFERENCES

Apicella, C. L., Cesarini, D., Johannesson, M., Dawes, C. T., Lichtenstein, P., Wallace, B., et al. (2010). No association between oxytocin receptor (OXTR) gene polymorphisms and experimentally elicited social preferences. *PLoS ONE* 5:e11153. doi: 10.1371/journal.pone.0011153

Bakermans-Kranenburg, M. J., and van IJzendoorn, M. H. (2013). Sniffing around oxytocin: review and meta-analyses of trials in healthy and clinical groups with implications for pharmacotherapy. *Transl. Psychiatry* 3, e258. doi: 10.1038/tp.2013.34

Bakermans-Kranenburg, M. J., van Ijzendoorn, M. H., Riem, M. M., Tops, M., and Alink, L. R. (2012). Oxytocin decreases handgrip force in reaction to infant crying in females without harsh parenting experiences. *Soc. Cogn. Affect. Neurosci.* 7, 951–957. doi: 10.1093/scan/nsr067

Bales, K. L., and Perkeybile, A. M. (2012). Developmental experiences and the oxytocin receptor system. *Horm. Behav.* 61, 313–319. doi: 10.1016/j.yhbeh.2011.12.013

Bartz, J. A. (2012). Oxytocin, attachment, betrayal and self-interest: a commentary on "Oxytocin modulates the link between adult attachment and cooperation through reduced betrayal aversion" by Carsten, K. W. De Dreu, Psychoneuroendocrinology, *Psychoneuroendocrinology* 37, 1106–1108. doi: 10.1016/j.psyneuen.2011.10.003

Bartz, J. A., Simeon, D., Hamilton, H., Kim, S., Crystal, S., Braun, A., et al. (2011a). Oxytocin can hinder trust and cooperation in borderline personality disorder. *Soc. Cogn. Affect. Neurosci.* 6, 556–563. doi: 10.1093/scan/nsq085

Bartz, J. A., Zaki, J., Bolger, N., and Ochsner, K. N. (2011b). Social effects of oxytocin in humans: context and person matter. *Trends Cogn. Sci.* 15, 301–309. doi: 10.1016/j.tics.2011.05.002

Bartz, J. A., Zaki, J., Ochsner, K. N., Bolger, N., Kolevzon, A., Ludwig, N., et al. (2010). Effects of oxytocin on recollections of maternal care and closeness. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21371–21375. doi: 10.1073/pnas.1012669107

Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., and Williams, R. (2009). Vulnerability genes or plasticity genes. *Mol. Psychiatry* 14, 746–754. doi: 10.1038/mp.2009.44

Bradley, B., Westen, D., Mercer, K. B., Binder, E. B., Jovanovic, T., Crain, D., et al. (2011). Association between childhood maltreatment and adult emotional dysregulation in a low-income, urban, African American sample: moderation by oxytocin receptor gene. *Dev. Psychopathol.* 23, 439–452. doi: 10.1017/S0954579411000162

Brüne, M. (2012). Does the oxytocin receptor (OXTR) polymorphism (rs2254298) confer vulnerability for psychopathology or differential susceptibility. Insights from evolution. *BMC Medicine* 10:38. doi: 10.1186/1741-7015-10-38

Champagne, F., Diorio, J., Sharma, S., and Meaney, M. J. (2001). Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12736–12741. doi: 10.1073/pnas.221224598

Churchland, P. S., and Winkielman, P. (2012). Modulating social behavior with oxytocin: how does it work. What does it mean? *Horm. Behav.* 61, 392–399. doi: 10.1016/j.yhbeh.2011.12.003

Cornelis, M. C., Glymour, M. M., Chang, S. C., Tchetgen, E. J., Liang, L., Koenen, K. C., et al. (2012). Oxytocin receptor (OXTR) is not associated with optimism in the Nurses' Health Study. *Mol. Psychiatry* 17, 1157–1159. doi: 10.1038/mp.2011.178

Costa, B., Pini, S., Gabelloni, P., Abelli, M., Lari, L., Cardini, A., et al. (2009). Oxytocin receptor polymorphisms and adult attachment style in patients with depression. *Psychoneuroendocrinology* 34, 1506–1514. doi:10.1016/j.psyneuen.2009.05.006

Declerck, C. H., Boone, C., and Kiyonari, T. (2010). Oxytocin and cooperation under conditions of uncertainty: the modulating role of incentives and social information. *Horm. Behav.* 57, 368–374. doi: 10.1016/j.yhbeh.2010.01.006

De Dreu, C. K. (2012a). Oxytocin modulates the link between adult attachment and cooperation through reduced betrayal aversion. *Psychoneuroendocrinology* 37, 871–880. doi: 10.1016/j.psyneuen.2011.10.003

De Dreu, C. K. (2012b). Oxytocin, attachment, and self-regarding preferences in humans: rejoinder to Bartz. *Psychoneuroendocrinology* 37, 1108–1110. doi: 10.1016/j.psyneuen.2012.03.004

De Dreu, C. K., Greer, L. L., Handgraaf, M. J., Shalvi, S., Van Kleef, G. A., Baas, M., et al. (2010). The neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans. *Science* 328, 1408–1411. doi: 10.1126/science.1189047

Feldman, R., Gordon, I., Influs, M., Gutbir, T., and Ebstein, R. P. (2013). Parental oxytocin and early caregiving jointly shape children's oxytocin response and social reciprocity. *Neuropsychopharmacology* 38, 1154–1162. doi: 10.1038/npp.2013.22

Heim, C., Young, L. J., Newport, D. J., Mletzko, T., Miller, A. H., and Nemeroff, C. B. (2009). Lower CSF oxytocin concentrations in women with a history of childhood abuse. *Mol. Psychiatry* 14, 954–958. doi: 10.1038/mp.2008.112

Ioannidis, J. P., Ntzani, E. E., Trikalinos, T. A., and Contopoulos-Ioannidis, D. G. (2001). Replication validity of genetic association studies. *Nat. Genet.* 29, 306–309. doi: 10.1038/ng749

Krueger, F., Parasuraman, R., Iyengar, V., Thornburg, M., Weel, J., Lin, M., et al. (2012). Oxytocin receptor genetic variation promotes human trust behavior. *Front. Hum. Neurosci.* 6:4. doi: 10.3389/fnhum.2012.00004

Kumsta, R., Hummel, E., Chen, F. S., and Heinrichs, M. (2013). Epigenetic regulation of the oxytocin receptor gene: implications for behavioral neuroscience. *Front. Neurosci.* 7:83. doi: 10.3389/fnins.2013.00083

Lin, P. I., Vance, J. M., Pericak-Vance, M. A., and Martin, E. R. (2007). No gene is an island: the flip-flop phenomenon. *Am. J. Hum. Genet.* 80, 531–538. doi: 10.1086/512133

Macdonald, K. S. (2012). Sex, receptors, and attachment: a review of individual factors influencing response to oxytocin. *Front. Neurosci.* 6:194. doi: 10.3389/fnins.2012.00194

McCullough, M. E., Churchland, P. S., and Mendez, A. J. (2013). Problems with measuring peripheral

oxytocin: can the data on oxytocin and human behavior be trusted? *Neurosci. Biobehav. Rev.* 37, 1485–1492. doi: 10.1016/j.neubiorev.2013.04.018

McQuaid, R. J., McInnis, O. A., Stead, J. D., Matheson, K., and Anisman, H. (2013). A paradoxical association of an oxytocin receptor gene polymorphism: early-life adversity and vulnerability to depression. *Front. Neurosci.* 7:128. doi: 10.3389/fnins.2013.00128

Miller, G. (2013). The promise and perils of oxytocin. *Science* 339, 267–269. doi: 10.1126/science.339.6117.267

Moffitt, T. E., Caspi, A., and Rutter, M. (2006). Measured gene-environment interactions in psychopathology: concepts, research strategies, and implications for research, intervention, and public understanding of genetics. *Perspect. Psychol. Sci.* 1, 5–27. doi: 10.1111/j.1745-6916.2006.00002.x

Neumann, I. D., Maloumby, R., Beiderbeck, D. I., Lukas, M., and Landgraf, R. (2013). Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. *Psychoneuroendocrinology* 38, 1985–1993. doi: 10.1016/j.psyneuen.2013.03.003

Pfeiffer, U. J. (2013). Oxytocin-not always a moral molecule. *Front. Hum. Neurosci.* 6:193. doi: 10.3389/fnhum.2013.00010

Radke, S., and de Bruijn, E. R. (2012). The other side of the coin: oxytocin decreases the adherence to fairness norms. *Front. Hum. Neurosci.* 6:193. doi: 10.3389/fnhum.2012.00193

Riem, M. E. M., van IJzendoorn, M. H., Tops, M., Boksem, M. A. S., Rombouts, S. A. R. B., and Bakermans-Kranenburg, M. J. (2013). Oxytocin effects on complex brain networks are moderated by experiences of maternal love withdrawal. *Eur. Neuropsychopharmacol.* 10, 1288–1295. doi: 10.1016/j.euroneuro.2013.01.011

Saphire-Bernstein, S., Way, B. M., Kim, H. S., Sherman, D. K., and Taylor, S. E. (2011). Oxytocin receptor gene (OXTR) is related to psychological resources. *Proc. Natl. Acad. Sci. U.S.A.* 108, 15118–15122. doi: 10.1073/pnas.1113137108

Shamay-Tsoory, S. G., Fischer, M., Dvash, J., Hariri, A. R., Perach-Bloom, N., and Levkovitz, Y. (2009). Intranasal administration of oxytocin increases envy and schadenfreude (gloating). *Biol. Psychiatry* 66, 864–870. doi: 10.1016/j.biopsych.2009.06.009

Strathearn, L., Fonagy, P., Amico, J., and Montague, P. R. (2009). Adult attachment predicts maternal brain and oxytocin response to infant cues. *Neuropsychopharmacology* 34, 2655–2666. doi: 10.1038/npp.2009.103

Szeto, A., McCabe, P. M., Nation, D. A., Tabak, B. A., Rossetti, M. A., McCullough, M. E., et al. (2011). Evaluation of enzyme immunoassay and radioimmunoassay methods for the measurement of plasma oxytocin. *Psychosom. Med.* 73, 393–400. doi: 10.1097/PSY.0b013e31821df0c2

Tabak, B. A., McCullough, M. E., Carver, C. S., Pedersen, E. J., and Cuccaro, M. L. (2013). Variation in oxytocin receptor gene (OXTR) polymorphisms is associated with emotional and behavioral reactions to betrayal. *Soc. Cogn. Affect. Neurosci.* doi: 10.1093/scan/nst042. [Epub ahead of print].

Tabak, B. A., McCullough, M. E., Szeto, A., Mendez, A. J., and McCabe, P. M. (2011). Oxytocin indexes relational distress following interpersonal harms in women. *Psychoneuroendocrinology* 36, 115–122. doi: 10.1016/j.psyneuen.2010.07.004

van IJzendoorn, M. H., Huffmeijer, R., Alink, L. R., Bakermans-Kranenburg, M. J., and Tops, M. (2011). The impact of oxytocin administration on charitable donating is moderated by experiences of parental love-withdrawal. *Front. Psychol.* 2:258. doi: 10.3389/fpsyg.2011.00258

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Metabolic disturbances connecting obesity and depression

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Obesity markedly increases the odds of developing depression. Depressed mood not only impairs motivation, quality of life and overall functioning but also increases the risks of obesity complications. Abdominal obesity is a better predictor of depression and anxiety risk than overall adipose mass. A growing amount of research suggests that metabolic abnormalities stemming from central obesity that lead to metabolic disease may also be responsible for the increased incidence of depression in obesity. As reviewed here, a higher mass of dysfunctional adipose tissue is associated with several metabolic disturbances that are either directly or indirectly implicated in the control of emotions and mood. To better comprehend the development of depression in obesity, this review pulls together select findings addressing the link between adiposity, diet and negative emotional states and discusses the evidence that alterations in glucocorticoids, adipose-derived hormones, insulin and inflammatory signaling that are characteristic of central obesity may be involved.

Keywords: mood disorders, abdominal obesity, saturated fatty acids, leptin, insulin, adiponectin, resistin, inflammation

INTRODUCTION

Consistent with its broad impact on physiology and health, obesity is increasingly linked to impairments in central nervous system (CNS) function. Mood disorders are now well recognized as significant risks of obesity and related metabolic illnesses. Obese individuals have about a 55% increased odds of developing depression (Luppino et al., 2010) whereas diabetes is estimated to double the incidence of depression (Anderson et al., 2001). Depression is among the primary causes of disability and its relentless impact can contribute substantially to the burden of metabolic disorders. Beyond diminishing quality of life and functioning, depressed mood presents additional threats to obese individuals by counteracting adherence to treatment and lifestyle changes and increasing the risk of complications. Abdominal adiposity and poor diet quality have been implicated in the development of depressed mood during obesity (Roberts et al., 2003; Dong et al., 2004; Simon et al., 2006; Zhao et al., 2011; Hamer et al., 2012). There is also a bidirectional association between obesity and depression such that depressed individuals are more likely to gain excessive weight due to poor food choices and reduced physical activity (Luppino et al., 2010; Pan et al., 2012). Recent work is beginning to illuminate the biological mechanisms that promote depressed mood in obesity. This review focuses specifically on the impact of adiposity, diet and associated metabolic signals in the development of depression and negative emotional states. First, select findings on the influence of palatable foods, obesity, visceral fat accumulation and dietary fats on emotional states and depression are presented. With an aim to shed light on potential mechanisms, we then review the link between depressive symptomology and some adipose-related metabolic signals, namely glucocorticoids (GCs), leptin, adiponectin, resistin, insulin and inflammatory signals. There are excellent and highly pertinent reviews on the neurobiology of depression (Nemeroff and Vale, 2005; Krishnan and Nestler, 2008; Willner et al., 2012; Sun et al., 2013) and so this subject will not be covered here, rather the potential role of metabolic signals are discussed with regards to what is known about their central actions to affect anxiety, depressive-like behavior and mood.

MOOD, FOOD, AND ADIPOSITY

Emotions such as joy, frustration and fear can profoundly affect appetite and food choice. In turn, eating palatable foods can modify emotional states by producing feelings of comfort, gratification or disgust which can strongly influence our approach or avoidance of such foods in the future. Positive emotional reactions (i.e., rewarding and hedonic effects) to food are considered to play a major role in overeating and the development of obesity (Fulton, 2010). External or psychological stressors can have divergent effects on feeding behavior such that some individuals increase food intake in response to a stressful experience while others eat less (Oliver and Wardle, 1999; Gibson, 2006; Dallman, 2010). Similarly, conditions of chronic stress can lead to diminished appetite and weight loss in some individuals whereas it can have an opposite effect in others by stimulating consumption of palatable and rewarding foods that blunt stress responses (Adam and Epel, 2007). For example, a recent study found that women reporting higher chronic stress with low cortisol reactivity to an acute social stress test consumed more calories from chocolate cake and less vegetables than those experiencing low chronic stress (Tryon et al., 2013). Likewise, a similar positive correlation between social stress and choice of palatable, energy rich foods over fruits and vegetables is observed in children (Cartwright et al., 2003). Stress-induced preference for palatable food has been documented in several human (Stone and Brownell, 1994; Epel et al., 2004) and animal studies (Dallman et al., 2003, 2005; Cottone et al., 2009) while other work demonstrates the effects

of such foods to dampen signs of stress and anxiety following exposure to a stressor or conditions of chronic unpredictable stress (Pecoraro et al., 2004; la Fleur et al., 2005; Maniam and Morris, 2010; Ulrich-Lai et al., 2010; Finger et al., 2011, 2012). There is a strong connection between the consumption of high-fat and high-sugar foods and positive emotions, and despite the divergence in eating behavior, an overall increase in tasty, energy-rich foods is reported, independent of stress-induced hyperphagia or hypophagia (Gibson, 2006; Dallman, 2010).

Mood states such as anxiety and depression can also affect food choice and energy metabolism. Individuals experiencing depressed mood show preference for and consumption of palatable "comfort foods" and self-report use of these foods as means to alleviate negative feelings (Macht, 2008). While short-term consumption of palatable foods can provide relief from negative emotions and mood states, chronic consumption of calorically-rich foods and subsequent increases in adiposity may promote vulnerability to depression and anxiety (Novick et al., 2005; Simon et al., 2006; Kloiber et al., 2007; Sharma and Fulton, 2013). In a recent study, we found that mice consuming a saturated high-fat diet (HFD) for 12 weeks show depressive-like features characterized by greater immobility in the forced swim task and reduced exploratory behavior in elevated plus maze and open field tests (Sharma and Fulton, 2013). HFD-induced behavioral changes were accompanied by elevated basal hypothalamic-pituitary-adrenal (HPA) activity and reactivity in response to stress. Separate findings suggest that negative emotional states and increased stress sensitivity caused by prolonged high-fat feeding do not rely on severe diet-induced obesity (DIO): six weeks of saturated HFD, leading to a more moderate gain in weight (∼11% relative to low-fat diet controls) and without influencing basal corticosterone levels, produced sucrose anhedonia (reduced ability to experience pleasure/reward), anxiety-like behavior and heightened stress-induced HPA activation (Sharma et al., 2012). Furthermore, when high-fat fed mice were returned to a normal chow diet craving for sucrose and high-fat food and behavioral and biochemical signs of anxiety were potentiated (Sharma et al., 2012). Several groups have shown that removal of a high-fat or high-sugar diet following prolonged intermittent or regular intake can increase behavioral and physiological signs of depression and anxiety (Avena et al., 2008a; Teegarden et al., 2008; Cottone et al., 2009; Iemolo et al., 2012; Sharma et al., 2012) or withdrawal (Avena et al., 2008b; Pickering et al., 2009; Sharma et al., 2012). Results of a new study suggest endocannabinoid signaling in the central nucleus of the amygdala is increased as a means to counteract increased anxiety elicited by palatable food withdrawal (Blasio et al., 2013). Thus, not only can chronic high-fat feeding promote negative emotional states but it may also enhance sensitivity to stress, including that triggered by dieting, to perpetuate a vicious cycle of overeating, weight gain and depressed mood.

Evidence suggests that diets high in saturated fat and relatively low in polyunsaturated and monounsaturated fatty acids contribute to the pathogenesis of both mood and metabolic disorders during obesity. The consumption of foods rich in saturated and/or trans-fat, like the Western diet, is associated with an increased incidence of depression whereas diets containing mostly unsaturated fats, such as the Mediterranean diet, appear to reduce the odds of depression (Sanchez-Villegas and Martinez-Gonzalez, 2013). Other reports indicate that inadequate dietary polyunsaturated fatty acids (PUFA) are associated with a higher incidence of depression (Peet et al., 1998) and that increasing omega-3 PUFA by greater consumption of fish can either decrease depressive symptoms in humans (Lin and Su, 2007; Sanchez-Villegas et al., 2007; Oddy et al., 2011; Park et al., 2012a) and rodents (Moranis et al., 2012; Park et al., 2012b) or have no effect on mood (Ruusunen et al., 2011). A considerable amount of data shows that diets rich in saturated fatty acids are associated with increases in overall adiposity and bias fat accumulation in abdominal stores. As compared to individuals on a Mediterranean diet, those consuming a diet high in saturated fat have increased weight gain, a greater volume of visceral adipose tissue, larger waist circumference and more cardiovascular disease mortality (Schulze et al., 2006; Molenaar et al., 2009; Romaguera et al., 2009, 2010; Mozaffarian et al., 2011; Estruch et al., 2013; Nazare et al., 2013). The accumulation of adipose tissue in abdominal stores is more important than the total amount of body fat in predicting the risk of several complications of obesity including insulin resistance and the metabolic syndrome (cluster of metabolic abnormalities and cardiovascular risk factors with impaired insulin sensitivity) (Despres and Lemieux, 2006; Tchernof and Despres, 2013) whereas the Mediterranean diet has been shown to be largely protective against these metabolic risks (Riccardi and Rivellese, 2000). The higher mass of dysfunctional adipose tissue in obesity may not only cause or exacerbate metabolic abnormalities that give rise to metabolic disease but also neurobiological impairments that give rise to mood disorders. Indeed, central adiposity is a better predictor of depression and anxiety risk than body weight or body mass index (BMI) (Weber-Hamann et al., 2002; van Reedt Dortland et al., 2013a). These findings suggest that excessive saturated fat intake and metabolic alterations that produce or arise from high abdominal fat mass may serve as common causal elements for both metabolic and mood disorders.

Apart from stimulating visceral fat accumulation, saturated fat intake increases circulating concentrations of fatty acids like palmitate which can enter the brain to have deleterious effects on neural functions. Indeed, palmitate has been shown to impair leptin and insulin receptor signal transduction in the hypothalamus to thereby reduce their catabolic actions and promote weight gain (Benoit et al., 2009; Kleinridders et al., 2009). As described below, leptin and insulin are implicated in enhanced mood and thus it is conceivable that reduced responses to these hormones elicited by saturated high fat feeding could contribute to negative emotional states. Supporting the possibility that high CNS palmitate levels modulate neural mechanisms controlling emotions, a recent study demonstrates that serum concentrations of palmitate correlated positively with depression in humans (Tsuboi et al., 2013). In addition, we recently found that male adult Wistar rats consuming a palm oil HFD for 8 weeks and with higher serum levels of palmitate show greater anxiety-like behavior in an open field test relative to rats consuming an isocaloric olive oil HFD (Hryhorczuk et al., unpublished). Interestingly, these effects were not accompanied by changes in body weight, glycemia and plasma leptin and insulin levels suggesting that the anxiogenic

phenotype could be related to the effects of saturated fats to stimulate HPA disturbances and/or inflammation. As elaborated in the next section, visceral fat deposition and dyslipidemia are associated with several endocrine and metabolic changes that have been implicated in the CNS control of emotional states and mood.

METABOLIC SIGNALS AND EMOTIONAL STATES

A meta-analysis of longitudinal data figures obesity (BMI $\geq$ 30) to increase the overall risk of onset of depression by 55% in Americans while overweight (BMI 25–29.9) to heighten the incidence of depression by 27% (Luppino et al., 2010). Despite the comorbidity of obesity and depression, it is important to note that obese individuals classified as "metabolically healthy," that is without associated cardiometabolic risk factors (high blood pressure, reduced high-density lipoprotein cholesterol and increased triglycerides, glycated haemoglobin and C-reactive protein), do not appear to have a heightened risk for depression (Hamer et al., 2012). Accordingly, an increasing amount of data points toward unhealthy diet, visceral adiposity and associated metabolic changes as culprits in obesity-induced depression (Roberts et al., 2003; Dong et al., 2004; Simon et al., 2006). As discussed here and summarized in **Figure 1**, several endocrine and metabolic abnormalities have been linked to depressed mood or depressive-like behavior including hypercortisolemia, insulin and leptin resistance and metabolic inflammatory signals. These signals are positively associated with central fat accumulation and thus are important candidate molecules tying obesity to depression.

GLUCOCORTICOIDS

HPA axis dysfunction and elevated GCs are implicated in the pathophysiology of both obesity and depression. GCs exert pleiotropic effects on metabolic, endocrine, immune and behavioral functions. Under normal physiological conditions of acute stress, the HPA axis is activated to release GCs which have an adaptive value to restore energy balance by increasing insulin, motivation for palatable food (Piazza and Le Moal, 1997; Dallman et al., 2006; Dallman, 2010) and mobilizing stored energy and directing it to central stores (Mann and Thakore, 1999). The critical actions of GCs on adipose tissue deposition were demonstrated by early studies showing that adrenalectomy prevents obesity in Zucker rats and this is reversed by replacement of corticosterone (Freedman et al., 1985; Castonguay et al., 1986). Fat mass is associated with increased cortisol rise at the time of waking and high cortisol reactivity under stressful situations (Therrien et al., 2007, 2008; Mujica-Parodi et al., 2009). But while cortisol production and turnover are increased in obesity, overall systemic cortisol levels have been shown to be normal or low in obesity (Hautanen et al., 1997). Rather, dysregulation of HPA axis activity is more a function of body fat distribution rather than total fat mass as abnormal diurnal variation in circulating cortisol levels is positively associated with the waist-to-hip ratio (Lasikiewicz et al., 2008). Over-activation of the HPA axis in subjects with abdominal obesity is suggested by elevated response to corticotrophin releasing hormone (CRH) stimulation, and increased basal and stimulated response to stress (Pasquali, 2012). Excess systemic cortisol has been shown to result in a 2- to 5-fold increase in visceral adipose tissue (Brown et al., 2004), and as such individuals under chronic stress are likely to have more visceral fat (Adam and Epel, 2007; Kyrou and Tsigos, 2009).

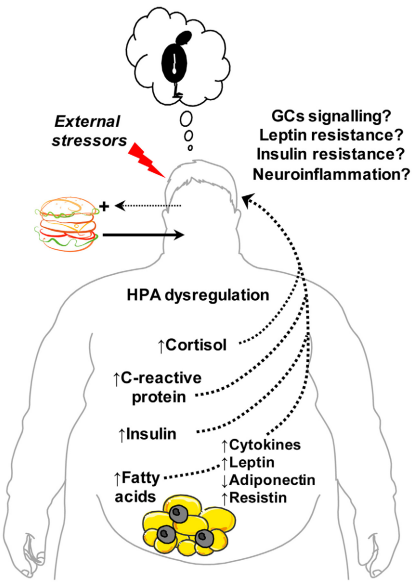

FIGURE 1 | Metabolic signals and disturbances linking obesity and abdominal adiposity with depression. Central obesity and related metabolic changes correlate positively with depression. Excessive intake of foods high in saturated fat promotes weight gain, visceral fat accumulation and increased risk of depressed mood. Endocrine changes associated with abdominal obesity include hypothalamic-pituitary-adrenal (HPA) dysregulation and altered plasma levels of cortisol, leptin, adiponectin, resistin and insulin—hormones implicated in the central control of emotion and mood. Obesity-induced impairments in brain glucocorticoids (GCs), leptin and insulin receptor signaling are posited to link hypercortisolemia and leptin and insulin resistance to depression. Central fat accumulation also stimulates the release of inflammatory cytokines (e.g., tumour necrosis factor-α, interleukin-1β) and signals (C-reactive protein) that can promote neuroinflammatory responses and depressive behavior. Increased entry of saturated free fatty acids into the brain may also propagate neuroinflammation, leptin/insulin resistance and, consequently, depression. In parallel, increased vulnerability to external stressors, negative emotional states and adverse cognitive style (e.g., poor self-image) associated with overweight and obesity can potentiate consumption of energy-dense "comfort" foods to fuel a vicious cycle of central obesity, metabolic dysfunction and depression. This figure was produced using *Servier Medical Art*.

Studies have found small elevations in serum cortisol concentrations associated with depression (Parker et al., 2003; Raison and Miller, 2003; Stetler and Miller, 2011). Further, individuals with Cushing's syndrome (characterized by cortisol hypersecretion and abdominal obesity) exhibit behavioral and neurobiological features of depression (Sonino et al., 1998). GCs not only act peripherally to maintain energy homeostasis but also feedback to the CNS to modulate HPA activity and the emotional and behavioral effects of stress (Herman et al., 2003). GCs are known to target GC receptors [including mineralocorticoid (MC) receptors] in midbrain and limbic circuits that regulate reward and emotional processes (Arnett et al., 2011; Solomon et al., 2012; Wang et al., 2013). Repeated administration of corticosterone to rodents is reported to produce depressive-like behavior

(Kalynchuk et al., 2004; Gregus et al., 2005) whereas GC receptor overexpression in the forebrain increases depressive-like behavior in the forced swim test and anxiety-like behavior in the elevated plus maze task (Wei et al., 2004). In contrast, loss of forebrain GC receptors has also been shown to increase depressive-like behavior in the forced swim and tail suspension tasks (Boyle et al., 2005) and increase stress-induced locomotor activation (Boyle et al., 2006). Finally, suggesting a particular role for MC receptor signaling in anxiety, MC receptor overexpression in the forebrain (Rozeboom et al., 2007) or specifically in basolateral amygdala (Mitra et al., 2009) reduces anxiety-like behavior. These contradictory results may be reconciled by findings that increasing forebrain GR receptor signaling enhances the magnitude of both positive and negative emotional responses depending upon the test employed suggesting that GC are important for "emotional lability" (Wei et al., 2004). On the whole, however, many findings support a pro-depressive effect of GC surplus, especially in the context of their strong influence to inhibit brain development and growth. Excess GCs promote hippocampal atrophy and decrease neurogenesis in the hippocampus—actions that have been well-implicated in the effect of GC on depressive behavior (McEwen, 1997; Bremner, 2006; Goebel et al., 2010; Schoenfeld and Gould, 2012).

It is important to note that hypercortisolism is often associated with melancholic depression, a subtype of clinical depression that is accompanied by anhedonia, hypophagia and weight loss; while atypical depression, the most common form of depression, is characterized by reduced HPA activity, improved mood in response to positive events and increased appetite, carbohydrate craving and frequently weight gain (Juruena and Cleare, 2007). Despite these categorizations, there is evidence of an association between hypercortisolemic depression and abdominal fat accumulation (Weber-Hamann et al., 2002). Hypercortisolemia in depressed individuals is accompanied by decreased GC-mediated negative feedback and increased release of CRH from the paraventricular nucleus (PVN) (Holsboer, 2000). Along these lines, we found that the increased anxiety-like behavior of the rats subjected to the saturated palm oil HFD was accompanied by elevated basal corticosterone levels and reduced GC-mediated negative feedback (dexamethasone suppression test) relative to rats consuming the calorically-matched olive oil HFD (Hryhorczuk et al., unpublished). As no differences in weight, leptin, insulin and blood glucose were detected, we hypothesize that increased corticosterone and/or reduced GC receptor signaling in limbic areas may in part mediate the effects of a saturated HFD to elicit negative emotional states. It is interesting to speculate that GC surplus in obesity may be associated with central impairments in the intracellular metabolism of GCs by 11β-hydroxysteroid dehydrogenases (11β-HSDs), enzymes that are well-implicated in visceral fat accumulation and affiliated peripheral metabolic abnormalities such as insulin resistance (Tchernof and Despres, 2013). 11βHSDs are widely expressed in the brain, including the hippocampus and amygdala, and are linked to changes in emotional and cognitive functions (Wyrwoll et al., 2011). It is clear that HPA abnormalities serve as common elements in the pathophysiology of both obesity and depression, but their direct contribution to emotional disturbances elicited by DIO have not been established. Notably, the data collectively suggest that diets rich in saturated fat and visceral fat accumulation, but not body mass *per se*, are important mediators of HPA disturbances and depressive symptomology associated with obesity.

ADIPOSE-DERIVED HORMONES

Leptin

As an endocrine organ, adipose tissue secretes numerous peptide hormones that target the brain and other tissues to regulate metabolism and behavior. Leptin is an adipokine that circulates in proportion to the size of the fat mass (Maffei et al., 1995). Apart from its numerous actions on physiological processes such as appetite, energy expenditure and neuroendocrine function, leptin is linked to human depression and has been shown to have antidepressant and anxiolytic effects in rodents (Asakawa et al., 2003; Liu et al., 2010b; Yamada et al., 2011). Epidemiological findings on leptin levels and depression are conflicting. Several studies find that individuals with major depressive disorder (MDD) have lower plasma leptin levels compared to healthy controls with similar BMI (Kraus et al., 2001; Atmaca et al., 2002, 2008; Westling et al., 2004; Jow et al., 2006). However, other reports show that plasma leptin levels are increased in women with depressive disorder (Rubin et al., 2002; Esel et al., 2005; Zeman et al., 2009) and that leptin levels are either increased (Kraus et al., 2002; Esel et al., 2005; Schilling et al., 2013) or not changed (Kraus et al., 2002; Schilling et al., 2013) by antidepressant treatment. In depressed individuals suffering from loss of appetite plasma leptin concentrations were reported not to differ from those of healthy controls (Deuschle et al., 1996) whereas another study found higher serum leptin only in atypical depressive patients in which increased appetite is often observed (Gecici et al., 2005). In older men, the combination of elevated visceral fat and high leptin levels was associated with depression onset (Milaneschi et al., 2012). Rodent studies, on the other hand, present more conclusive findings. Lack of leptin (obese *ob/ob* mice) or its receptor (obese *db/db* mice) is associated with increased behavioral despair in the forced swim task (Collin et al., 2000; Sharma et al., 2010; Yamada et al., 2011). Repeated leptin treatment has also been shown to reduce anxiety in *ob/ob* mice (Asakawa et al., 2003). Systemic or central leptin administration in normal mice has antidepressant effects as measured by the tail suspension test, forced swim task and social interaction test and has anxiolytic effects in the elevated plus maze (Liu et al., 2010a; Yamada et al., 2011). In sum, a greater part of the human research ties low leptin levels to depression but there is discordancy amongst these investigations which may be a function of the subtype of depression, the age and sex of participants and perhaps, as elaborated below, the onset of leptin insensitivity generated by high leptin levels.

One of the ways in which leptin may affect emotions is via its influence on HPA activity. A negative correlation between basal concentrations of leptin and mobilization of cortisol is reported in humans (Komorowski et al., 2000). Leptin deficient *ob/ob* mice have elevated corticosterone which is reduced by leptin replacement (Garthwaite et al., 1980; Arvaniti et al., 2001). Systemic leptin administration lowers corticosterone levels and prevents the induction of CRH synthesis in the PVN and the activation of CRH neurons observed in food-deprived

ob/ob mice (Huang et al., 1998). In turn, chronic unpredictable mild stress in rats which leads to depressive-like behaviors activates the HPA axis and decreases serum leptin levels (Ge et al., 2013). Beyond modulating HPA activity, leptin also targets the long-form of its receptor (LepRb) in midbrain and forebrain loci to affect emotional processes. Leptin has antidepressant effects when administered into the hippocampus, an area mediating cognitive impairments of depression whereas genetic deletion of hippocampal LepRb results in a depression-like phenotype (Asakawa et al., 2003; Lu et al., 2006; Finger et al., 2010; Liu et al., 2010a; Guo et al., 2013). Loss of LepRb specifically in glutamatergic neurons of the forebrain (mainly hippocampus and prefrontal cortex) was recently reported to elicit depressive-like behavior without affecting anxiety (Guo et al., 2012). Leptin also functionally activates dopamine neurons in the ventral tegmental area (VTA) of the midbrain where it reduces dopamine neuronal firing while increasing dopamine availability (Fulton et al., 2006; Hommel et al., 2006). Selective deletion of LepRb from midbrain dopamine neurons has been shown to increase anxiety-like behavior, but not depressive-like behavior, in several behavioral tests via increased D1 receptor signaling in the central nucleus of the amygdala (Liu et al., 2011). Finally, recent work shows that leptin levels are positively associated with stress-induced dopamine release (Burghardt et al., 2012). Collectively, the data demonstrates that the antidepressant actions of leptin are mediated by LepRb signaling in limbic and prefrontal nuclei whereas leptin action in dopamine neurons of the ventral midbrain that innervate the central nucleus of the amygdala underlie the anxiolytic actions of leptin.

Leptin levels are associated with risk of depression onset in men with a significant amount of visceral fat (Milaneschi et al., 2012) and correlate positively with depressive symptoms in patients with type 2 diabetes (Labad et al., 2012). In conditions of obesity, and particularly central obesity that favors insulin resistance and type 2 diabetes, leptin sensitivity is diminished owing to lower CNS leptin entry (decreased CSF: plasma leptin ratio) and defective LepRb signaling (Myers et al., 2012). This phenomenon known as leptin resistance is characteristic of obesity and may explain how the risk of mood disorders is elevated in obese states associated with high circulating leptin levels. Compatible with this notion, mice rendered obese by a HFD show reduced sensitivity to the antidepressant actions of leptin and to the effects of leptin to increase hippocampal brain-derived neurotrophic factor (BDNF) concentrations (protective against depression) relative to low-fat diet controls (Yamada et al., 2011). Further, leptin insensitivity may exacerbate HPA dysregulation in obesity (Collura et al., 2009) and thereby enhance the mass of dysfunctional central adipose stores in a cortisol-dependent manner. Features of leptin resistance have been reported in the midbrain VTA where mesolimbic DA neurons reside (Matheny et al., 2011), although it is not yet known whether or not reduced leptin sensitivity in this region has anxiogenic effects as one would predict. Leptin resistance would appear to affect multiple neural and endocrine pathways that affect emotions and mood including hippocampal and mesolimbic dopamine pathways and HPA activity, and thus represents another strong candidate mechanism underlying depression in obesity.

Adiponectin

Adiponectin is another adipose-derived hormone that is well implicated in energy homeostasis and insulin resistance (Turer and Scherer, 2012) and that has more recently been tied to depression. While plasma adiponectin is found at high concentrations in healthy subjects it often correlates negatively with obesity, waist circumference and visceral fat in humans (Arita et al., 1999; Cnop et al., 2003; Ryo et al., 2004; Hanley et al., 2007; Cohen et al., 2011; Mente et al., 2013) and rodents (Maeda et al., 2001; Milan et al., 2002; Delporte et al., 2004; Ye et al., 2007). However, metabolically healthy obese subjects have plasma levels of adiponectin similar to lean controls (Aguilar-Salinas et al., 2008; Morrison et al., 2011; Doumatey et al., 2012) suggesting that adiponectin levels changes are secondary to metabolic disturbances found in obesity. In humans, plasma adiponectin levels are reported to negatively correlate with depressive measures in patients with MDD and in women with depressive disorder (Zeman et al., 2009). Alternatively, other studies report a positive relationship between adiponectin levels and depressive and anxiety symptoms (Wilhelm et al., 2013) and men with subsyndromal depression (Jeong et al., 2012); or no change in patients with MDD (Jeong et al., 2012). Antidepressant therapy in individuals with MDD does not appear to affect adiponectin and resistin levels (Lehto et al., 2010). Recent work shows that chronic social defeat in mice decreases the level of plasma adiponectin and that recapitulating low levels of adiponectin by using haploinsufficient mice (Adipo±) increases the incidence of stress-induced depressive-like behaviors due to impaired HPA axis negative feedback (Liu et al., 2012). Moreover, it was found that, like leptin, central administration of adiponectin has antidepressant effects as assessed in the forced swim test and tail suspension test (Liu et al., 2012). In summary, while animal studies suggest an antidepressant action of adiponectin, the link between plasma adiponectin concentrations and depression in humans is less clear which may be connected to the type of depressive disorder and the effects of sex and antidepressant treatment.

Resistin

Adipocyte-derived resistin is linked to insulin resistance in rodent models, however, human resistin is released from macrophages and its association with cardiometabolic disease is less defined (Schwartz and Lazar, 2011). Plasma resistin levels in relation to obesity are not as clear as those for adiponectin. Early studies found that circulating resistin levels are elevated in genetic and DIO in mice (Steppan et al., 2001) and obese humans (Degawa-Yamauchi et al., 2003; Owecki et al., 2011; Sadashiv et al., 2012) whereas other studies found that resistin is downregulated in human obesity (Way et al., 2001; Ye et al., 2013) and rodent obesity (Milan et al., 2002; Maebuchi et al., 2003). As for the relationship between resistin and depression in humans, resistin levels were found to be positively correlated with atypical but not typical depression (Lehto et al., 2010). In addition, human resistin levels were positively linked with salivary cortisol in depressed patients (Weber-Hamann et al., 2007) which is in line with a study reporting higher plasma resistin levels in women suffering from Cushing's syndrome (Krsek et al., 2004). Conversely, resistin levels are lower in patients receiving antidepressant treatment who

have remitted from depression (Weber-Hamann et al., 2007). As it currently stands, there are no documented reports exploring the impact of resistin treatment or its loss of function in animal studies of anxiety and depression. The epidemiological data would predict resistin treatment to have pro-depressant actions, a potential finding that could be related to resistin's status as an inflammatory marker.

INSULIN

Depression is at least twice as common among those with diabetes and is associated with a more unfavorable prognosis (Anderson et al., 2001). Depression often appears at the prediabetes stage which is marked by insulin resistance (Kan et al., 2013). Individuals with insulin resistance that are overweight or obese (Platt et al., 2013), with metabolic syndrome (Koponen et al., 2008; Akbaraly et al., 2009; Almeida et al., 2009; Pulkki-Raback et al., 2009) or with abdominal obesity and metabolic syndrome (Hamer et al., 2012) are more likely to develop depression. However, some literature points toward little or no association between metabolic syndrome/insulin resistance and depression (Adriaanse et al., 2006; Platt et al., 2013; Shen and Bergquist-Beringer, 2013). A recent systematic review and meta-analysis found a small but significant cross-sectional association between depression and insulin resistance (Kan et al., 2013). Another recent study found that treating patients with MDD and abdominal obesity or metabolic syndrome with the insulin-sensitizing drug pioglitazone for 12 weeks alone or in combination with an antidepressant therapy reduced signs of depression and anxiety (Kemp et al., 2012). Attenuation of depressive symptoms correlated positively with the reduction in insulin resistance. Similarly, rosiglitazone administered to normal chow-fed mice and rats was reported to decrease immobility time in tail suspension and forced swim tests suggestive of an antidepressant action (Eissa Ahmed et al., 2009). Collectively, the data suggest that insulin resistance may be causal to depressed mood during obesity while increasing sensitivity to insulin has antidepressant actions.

Several studies have sought to determine the role of CNS insulin in the control of emotional states. Third ventricle injection of an insulin receptor antisense RNA in rats was reported to stimulate depressive-like behavior by increasing immobility in forced swim test and decreasing open arm entries in elevated plus maze (Grillo et al., 2011). Intranasal insulin treatment provides a means to deliver insulin to the brain by bypassing the blood–brain barrier. Daily intranasal insulin treatment for 8 weeks in healthy men was shown to ameliorate self-reported mood (Benedict et al., 2004). Other clinical studies show that intranasal insulin reduces cortisol levels and thus may have antidepressant actions by lessening visceral obesity (Chapman et al., 2013). Intranasal insulin delivery in mice for 7 days was shown to have anxiolytic properties in the light/dark box test, but insulin did not have any effect on anxiety behavior in the marble-burying test and elevated plus maze. In prediabetic (hyperglycaemic and hyperinsulinemic) mice fed for 55 weeks with a 30% kcal fat diet, the anxiolytic effects of intranasal insulin are somewhat diminished (Marks et al., 2009): intranasal insulin failed to reverse the anxiogenic effects of DIO in the light/dark box test while it attenuated anxiety in the elevated plus maze and the marble-burying test. These data suggest that DIO may provoke increased anxiety through impairments in central insulin signaling. In support of this possibility, chronic intake of HFD elicits impairments in hypothalamic insulin receptor signaling (De Souza et al., 2005) and similar resistance to insulin is documented in other brain areas (Kim and Feldman, 2012). By and large, available evidence suggests that insulin has mood-enhancing effects or point to a positive correlation between insulin resistance and depression. However, it will be important to determine if intranasal insulin has antidepressant effects in depressed individuals and, if so, whether this action is maintained in obesity. In addition, more research is required to identify the brain sites mediating the effects of insulin on mood. One likely site of action is the VTA as recent evidence demonstrates that insulin inhibits excitatory glutamatergic inputs onto dopamine neurons (Labouebe et al., 2013). In view of leptin's actions to diminish behavioral despair and anxiety via hippocampal and ventral midbrain nuclei, respectively, regions where insulin receptors are also expressed, it is interesting to speculate that these sites could be important for an influence of insulin on emotional states.

INFLAMMATORY SIGNALS

Obesity is characterized by a state of prolonged low-grade inflammation and several lines of evidence implicate immune-mediated tissue inflammation as an important element linking obesity to insulin resistance. Likewise, numerous findings implicate immune activation in the pathogenesis of depression (Dunn et al., 2005; Dantzer et al., 2008). Elevated levels of inflammatory signals have been associated with the severity of depression (Levine et al., 1999; Dunn et al., 2005). In addition, cancer patients receiving cytokine treatment are more likely to experience depressive symptoms (Musselman et al., 2001). Cytokines are important mediators of innate and adaptive immunity. Both peripherally- and centrally-derived cytokines can stimulate CNS cytokine receptors and several findings point to cytokines as strong modulators of mood. Administration of lipopolysaccharide generates the release of interleukin 1β (IL1β) and tumour-necrosis factor-alpha (TNFα) in the brain to produce sickness behaviors characterized by social withdrawal, decreased exploratory behavior and depressive-like symptoms (Dunn et al., 2005). Direct administration of IL1β and TNFα (but not IL6) to the brain produces depressive symptomology in rodents (Goshen et al., 2008) whereas central administration of an IL1β receptor antagonist during chronic stress (Koo and Duman, 2008) or targeted deletion of the gene encoding TNFα produces antidepressant like effects (Simen et al., 2006). Similarly, preclinical studies show that blocking pro-inflammatory cytokine signaling can produce an antidepressant effect. An important transcriptional target of IL1β and other cytokines is the inhibitor of nuclear factor kappa B kinase (IκK)/nuclear factor kappa B (NFκB) pathway. Activation of IκK/NFκB specifically in the nucleus accumbens (NAc), a region viewed to mediate hedonic deficits in depression, using viral-mediated gene transfer was shown to increase anxiety- and depressive-like behavior using the openfield and forced

swim tests, respectively (Koo et al., 2010). In contrast, inhibition of IκK/NFκB in the NAc decreased depressive-like behavior (Christoffel et al., 2012). Together, these findings suggest that elevated levels of pro-inflammatory cytokines in the brain and NAc IκK/NFκB signaling increases anxiety and depressive symptomology.

Obesity provides a means by which inflammatory responses in the brain can be generated. Consistent with this view, a recent study demonstrates that several gene markers of neuroinflammation are upregulated in the hypothalamus of rodents during high-fat feeding including IL6, TNFα, IKKβ and NFκB (Thaler et al., 2012). This metabolic inflammatory response in the hypothalamus appeared rapidly after the onset of high-fat feeding, waned and then escalated in magnitude and diversity during chronic high-fat feeding. Increased infiltration, activation and proliferation of microglia and astrocytes, called reactive gliosis, was also noted in rodents following 4 weeks of high-fat feeding along with similar postmortem signs of gliosis and neuronal injury in the hypothalami of obese individuals (Thaler et al., 2012). At a functional level, elevated inflammatory conditions in the hypothalamus have been shown to contribute to leptin resistance (Kleinridders et al., 2009), insulin resistance (De Souza et al., 2005) and to neuroendocrine impairments, obesity and glucose intolerance in a manner that depends on IKKβ/NFκB signaling (Zhang et al., 2008).

A recent, large-scale epidemiological study evaluated the impact of HPA activity, autonomic nervous system, inflammation and lifestyle factors on adverse associations of anxiety and depression severity with high and low-density lipoprotein cholesterol, triglycerides, BMI and waist circumference. Interestingly, the inflammatory marker C-reactive protein (CRP) had the most consistent impact explaining 14–53% of the associations of anxiety and depression severity with abdominal obesity and dyslipidemia (van Reedt Dortland et al., 2013b). In a separate study, men with low-grade inflammation, high CRP levels and depressive symptoms were more likely to develop abdominal obesity during the 11-year follow-up (Valtonen et al., 2012). In addition, individuals with diabetes and MDD were found to have significantly higher CRP levels than non-diabetic patients with depression (Alvarez et al., 2013). A new epidemiological study showed that obesity is associated with greater depressive symptoms and that CRP concentrations were independently associated with depression scores in analyses fully adjusted for sociodemographic and background variables (Daly, 2013). Finally, another recent and large epidemiological study of men and women aged 20–100 years observed that increasing CRP levels were associated with increasing risk for psychological distress and depression (Wium-Andersen et al., 2013). Interestingly, increased CRP levels have been show to exacerbate injury-induced gliosis in the brain (Hsuchou et al., 2012b). It is intriguing to speculate whether or not potentiation of injury-induced gliosis by CRP is due in part to its actions to increase blood-brain barrier permeability to noxious stimuli (Hsuchou et al., 2012a). Decreasing the permeability of the BBB to free fatty acids, cytokines and/or immune cells are avenues through which abdominal obesity and dyslipidemia can encourage neuroinflammation and subsequent mood impairments.

SUMMARY AND PERSPECTIVES

Obesity is largely driven by excessive energy intake which is a product of both the amount and caloric density of food consumed. Agricultural, economic and cultural factors contribute to an abundance of energy-rich and palatable foods in many parts of the Western world and favor the selection of products containing saturated oils and refined sugars. The psychological dynamics of the consumer weigh heavily at the food selection level. Emotional reactions to food can largely affect our future intake and can propel overeating. What is becoming increasingly evident is that, in turn, the nutrients we consume can over time influence our mood and behavior either via direct CNS actions or by their impact on energy metabolism, endocrine function and immunity. Findings from several fronts tie the development of depressed mood in obese individuals to overeating, central adipose mass and associated metabolic disruptions (**Figure 1**). Recent research findings propose that the overconsumption of saturated fats is a potential factor underlying obesity-induced depression. In view of the effect saturated fats to bias central fat deposition and the association between saturated fat intake and cardiometabolic (Micha and Mozaffarian, 2009, 2010) and neurological diseases (Takechi et al., 2010; Kanoski and Davidson, 2011), the link between saturated fats and depression is not surprising. Thus, to further our understanding of the development of depression during obesity we can build upon a wealth of knowledge about the metabolic disturbances associated with central obesity, how these disturbances affect signaling mechanisms and, while less vast, our expanding understanding of how these signals modulate neural functions. Identifying the root causes of depression during obesity will greatly benefit from much more neurobiological and behavioral research of the mood-regulating CNS sites and mechanisms through which metabolic, inflammatory and nutrient signals act.

Endocrine abnormalities are common in visceral obesity. As discussed, various findings suggest that alterations in cortisol, leptin, adiponectin and resistin levels are associated with depression and/or that these hormones have CNS actions to affect mood, anxiety and depressive-like behavior. Another hormone well-implicated in overeating an obesity development is the gut peptide ghrelin, and several studies suggest that ghrelin plays a key role at the interface of homeostatic control and neural circuits involved in reward and stress (*for review see Abizaid article in this issue*). We did not review a role for ghrelin, however, because there is currently no evidence specifically linking this hormone to depression. On the other hand, neuropeptide systems that intricately interact with metabolic hormones have been linked to the pathophysiology of mood disorders (for review see Mathe et al., 2007). For example, apart from its orexigenic action, neuropeptide Y (NPY) was shown to reduce signs of anxiety when injected centrally (Heilig et al., 1989; Heilig, 1995). In line with these earlier studies, recent work demonstrates that NPY mRNA levels were decreased in hippocampal homogenates of a rat genetic model of depression (Melas et al., 2013). NPY is also reduced in the CSF of patients with depressive disorder (Heilig et al., 2004) and this is reversed upon antidepressant therapy (Nikisch et al., 2005). Yet other central signaling molecules with strong ties to both energy metabolism and emotions are the endocannabinoids.

A role for endocannabinoid signaling via the CB1 receptor in anxiety and depressive symptoms is well established (Mangieri and Piomelli, 2007; Gorzalka and Hill, 2011). A recent study demonstrates the importance of endocannabinoid signaling in the amygdala in the anxiogenic effects of palatable food withdrawal (Mangieri and Piomelli, 2007; Gorzalka and Hill, 2011; McLaughlin and Gobbi, 2012; Mechoulam and Parker, 2013) and thus add to the growing evidence that endocannabinoids may be another link between obesity and mood disorders.

Endocrine dysfunction in central obesity typically coincides with a mild inflammatory state. Central adipose stores are a source of inflammatory cytokines secreted by local monocytes and macrophages, and the release of another inflammatory molecule, CRP, is upregulated during obesity and inflammation. Several findings demonstrate the pro-depressive effects of inflammatory signals whereas others studies are uncovering the deleterious impact of hypothalamic inflammation brought on by DIO. At present, the mechanisms that initiate and propagate neuroinflammation during high-fat feeding are not fully known. Moreover, it is not clear if limbic regions controlling emotions and mood also succumb to this "metabolic inflammation" during high-fat feeding. It also remains to be established if immune cells that are activated during obesity can infiltrate the brain as they do in other chronic inflammatory conditions and thereby contribute to the neuroinflammatory response. Apart from the involvement of humoral factors, one should also consider the action of metabolic signals on vagal efferents to the CNS. Cytokine stimulation of vagal nerves can trigger neuroinflammatory responses (Capuron and Miller, 2011) and therefore may prove to be an important pathway to diet-induced neuroinflammation. Establishing the direct impact of these metabolic and inflammatory signals to obesity-induced depression will not prove easy as they are often interconnected and temporally overlap. To surmount such difficulties controlled animal studies employing conditional and time-resolved loss- or gain-of-function strategies will be very valuable for determining the distinct involvement of each of these factors in the development of depressive behavior during high-fat feeding and obesity.

REFERENCES

Adam, T. C., and Epel, E. S. (2007). Stress, eating and the reward system. *Physiol. Behav.* 91, 449–458. doi: 10.1016/j.physbeh.2007.04.011

Adriaanse, M. C., Dekker, J. M., Nijpels, G., Heine, R. J., Snoek, F. J., and Pouwer, F. (2006). Associations between depressive symptoms and insulin resistance: the hoorn study. *Diabetologia* 49, 2874–2877. doi: 10.1007/s00125-006-0500-4

Aguilar-Salinas, C. A., Garcia, E. G., Robles, L., Riano, D., Ruiz-Gomez, D. G., Garcia-Ulloa, A. C., et al. (2008). High adiponectin concentrations are associated with the metabolically healthy obese phenotype. *J. Clin. Endocrinol. Metab.* 93, 4075–4079. doi: 10.1210/jc.2007-2724

Akbaraly, T. N., Kivimaki, M., Brunner, E. J., Chandola, T., Marmot, M. G., Singh-Manoux, A., et al. (2009). Association between metabolic syndrome and depressive symptoms in middle-aged adults: results from the Whitehall II study. *Diabetes Care* 32, 499–504. doi: 10.2337/dc08-1358

Almeida, O. P., Calver, J., Jamrozik, K., Hankey, G. J., and Flicker, L. (2009). Obesity and metabolic syndrome increase the risk of incident depression in older men: the health in men study. *Am. J. Geriatr. Psychiatry* 17, 889–898. doi: 10.1097/JGP.0b013e3181b047e3

Alvarez, A., Faccioli, J., Guinzbourg, M., Castex, M. M., Bayon, C., Masson, W., et al. (2013). Endocrine and inflammatory profiles in type 2 diabetic patients with and without major depressive disorder. *BMC Res. Notes* 6:61. doi: 10.1186/1756-0500-6-61

Anderson, R. J., Freedland, K. E., Clouse, R. E., and Lustman, P. J. (2001). The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care* 24, 1069–1078. doi: 10.2337/diacare.24.6.1069

Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J., et al. (1999). Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem. Biophys. Res. Commun.* 257, 79–83. doi: 10.1006/bbrc.1999.0255

Arnett, M. G., Kolber, B. J., Boyle, M. P., and Muglia, L. J. (2011). Behavioral insights from mouse models of forebrain–and amygdala-specific glucocorticoid receptor genetic disruption. *Mol. Cell. Endocrinol.* 336, 2–5. doi: 10.1016/j.mce.2010.11.011

Arvaniti, K., Huang, Q., and Richard, D. (2001). Effects of leptin and corticosterone on the expression of corticotropin-releasing hormone, agouti-related protein, and proopiomelanocortin in the brain of ob/ob mouse. *Neuroendocrinology* 73, 227–236. doi: 10.1159/000054639

Asakawa, A., Inui, A., Inui, T., Katsuura, G., Fujino, M. A., and Kasuga, M. (2003). Leptin treatment ameliorates anxiety in ob/ob obese mice. *J. Diabetes Complications* 17, 105–107. doi: 10.1016/S1056-8727(02)00185-X

Atmaca, M., Kuloglu, M., Tezcan, E., and Ustundag, B. (2008). Serum leptin and cholesterol values in violent and non-violent suicide attempters. *Psychiatry Res.* 158, 87–91. doi: 10.1016/j.psychres.2003.05.002

Atmaca, M., Kuloglu, M., Tezcan, E., Ustundag, B., Gecici, O., and Firidin, B. (2002). Serum leptin and cholesterol values in suicide attempters. *Neuropsychobiology* 45, 124–127. doi: 10.1159/000054950

Avena, N. M., Bocarsly, M. E., Rada, P., Kim, A., and Hoebel, B. G. (2008a). After daily bingeing on a sucrose solution, food deprivation induces anxiety and accumbens dopamine/acetylcholine imbalance. *Physiol. Behav.* 94, 309–315. doi: 10.1016/j.physbeh.2008.01.008

Avena, N. M., Rada, P., and Hoebel, B. G. (2008b). Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci. Biobehav. Rev.* 32, 20–39. doi: 10.1016/j.neubiorev.2007.04.019

Benedict, C., Hallschmid, M., Hatke, A., Schultes, B., Fehm, H. L., Born, J., et al. (2004). Intranasal insulin improves memory in humans. *Psychoneuroendocrinology* 29, 1326–1334. doi: 10.1016/j.psyneuen.2004.04.003

Benoit, S. C., Kemp, C. J., Elias, C. F., Abplanalp, W., Herman, J. P., Migrenne, S., et al. (2009). Palmitic acid mediates hypothalamic insulin resistance by altering PKC-theta subcellular localization in rodents. *J. Clin. Invest.* 119, 2577–2589. doi: 10.1172/JCI36714

Blasio, A., Iemolo, A., Sabino, V., Petrosino, S., Steardo, L., Rice, K. C., et al. (2013). Rimonabant precipitates anxiety in rats withdrawn from palatable food: role of the central amygdala. *Neuropsychopharmacology*. doi: 10.1038/npp.2013.153. [Epub ahead of print].

Boyle, M. P., Brewer, J. A., Funatsu, M., Wozniak, D. F., Tsien, J. Z., Izumi, Y., et al. (2005). Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. *Proc. Natl. Acad. Sci. U.S.A* 102, 473–478. doi: 10.1073/pnas.0406458102

Boyle, M. P., Kolber, B. J., Vogt, S. K., Wozniak, D. F., and Muglia, L. J. (2006). Forebrain glucocorticoid receptors modulate anxiety-associated locomotor activation and adrenal responsiveness. *J. Neurosci.* 26, 1971–1978. doi: 10.1523/JNEUROSCI.2173-05.2006

Bremner, J. D. (2006). Stress and brain atrophy. *CNS Neurol. Disord. Drug Targets* 5, 503–512. doi: 10.2174/187152706778559309

Brown, E. S., Varghese, F. P., and McEwen, B. S. (2004). Association of depression with medical illness: does cortisol play a role. *Biol. Psychiatry* 55, 1–9. doi: 10.1016/S0006-3223(03)00473-6

Burghardt, P. R., Love, T. M., Stohler, C. S., Hodgkinson, C., Shen, P. H., Enoch, M. A., et al. (2012). Leptin regulates dopamine responses to sustained stress in humans. *J. Neurosci.* 32, 15369–15376. doi: 10.1523/JNEUROSCI.2521-12.2012

Capuron, L., and Miller, A. H. (2011). Immune system to brain signaling: neuropsychopharmacological implications.

Pharmacol. Ther. 130, 226–238. doi: 10.1016/j.pharmthera.2011.01.014

Cartwright, M., Wardle, J., Steggles, N., Simon, A. E., Croker, H., and Jarvis, M. J. (2003). Stress and dietary practices in adolescents. *Health Psychol.* 22, 362–369. doi: 10.1037/0278-6133.22.4.362

Castonguay, T. W., Dallman, M. F., and Stern, J. S. (1986). Some metabolic and behavioral effects of adrenalectomy on obese Zucker rats. *Am. J. Physiol.* 251, R923–R933.

Chapman, C. D., Frey, W. H. 2nd., Craft, S., Danielyan, L., Hallschmid, M., Schioth, H. B., et al. (2013). Intranasal treatment of central nervous system dysfunction in humans. *Pharm. Res*. 30, 2475–2484. doi: 10.1007/s11095-012-0915-1

Christoffel, D. J., Golden, S. A., Heshmati, M., Graham, A., Birnbaum, S., Neve, R. L., et al. (2012). Effects of inhibitor of [kappa]B kinase activity in the nucleus accumbens on emotional behavior. *Neuropsychopharmacology* 37, 2615–2623. doi: 10.1038/npp.2012.121

Cnop, M., Havel, P. J., Utzschneider, K. M., Carr, D. B., Sinha, M. K., Boyko, E. J., et al. (2003). Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* 46, 459–469. doi: 10.1007/s00125-003-1074-z

Cohen, S. S., Gammon, M. D., Signorello, L. B., North, K. E., Lange, E. M., Fowke, J. H., et al. (2011). Serum adiponectin in relation to body mass index and other correlates in black and white women. *Ann. Epidemiol.* 21, 86–94. doi: 10.1016/j.annepidem.2010.10.011

Collin, M., Hakansson-Ovesjo, M. L., Misane, I., Ogren, S. O., and Meister, B. (2000). Decreased 5-HT transporter mRNA in neurons of the dorsal raphe nucleus and behavioral depression in the obese leptin-deficient ob/ob mouse. *Brain Res. Mol. Brain Res.* 81, 51–61. doi: 10.1016/S0169-328X(00)00167-4

Collura, L. A., Hoffman, J. B., and Wilson, M. E. (2009). Administration of human leptin differentially affects parameters of cortisol secretion in socially housed female rhesus monkeys. *Endocrine* 36, 530–537. doi: 10.1007/s12020-009-9250-7

Cottone, P., Sabino, V., Roberto, M., Bajo, M., Pockros, L., Frihauf, J. B., et al. (2009). CRF system recruitment mediates dark side of compulsive eating. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20016–20020. doi: 10.1073/pnas.0908789106

Dallman, M. F. (2010). Stress-induced obesity and the emotional nervous system. *Trends Endocrinol. Metab.* 21, 159–165. doi: 10.1016/j.tem.2009.10.004

Dallman, M. F., Pecoraro, N., Akana, S. F., la Fleur, S. E., Gomez, F., Houshyar, H., et al. (2003). Chronic stress and obesity: a new view of "comfort food." *Proc. Natl. Acad. Sci. U.S.A.* 100, 11696–11701. doi: 10.1073/pnas.1934666100

Dallman, M. F., Pecoraro, N. C., and la Fleur, S. E. (2005). Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain Behav. Immun.* 19, 275–280. doi: 10.1016/j.bbi.2004.11.004

Dallman, M. F., Pecoraro, N. C., la Fleur, S. E., Warne, J. P., Ginsberg, A. B., Akana, S. F., et al. (2006). Glucocorticoids, chronic stress, and obesity. *Prog. Brain Res.* 153, 75–105. doi: 10.1016/S0079-6123(06)53004-3

Daly, M. (2013). The relationship of C-reactive protein to obesity-related depressive symptoms: a longitudinal study. *Obesity (Silver Spring.)* 21, 248–250. doi: 10.1002/oby.20051

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., and Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56. doi: 10.1038/nrn2297

Degawa-Yamauchi, M., Bovenkerk, J. E., Juliar, B. E., Watson, W., Kerr, K., Jones, R., et al. (2003). Serum resistin (FIZZ3) protein is increased in obese humans. *J. Clin. Endocrinol. Metab.* 88, 5452–5455. doi: 10.1210/jc.2002-021808

Delporte, M. L., El Mkadem, S. A., Quisquater, M., and Brichard, S. M. (2004). Leptin treatment markedly increased plasma adiponectin but barely decreased plasma resistin of ob/ob mice. *Am. J. Physiol. Endocrinol. Metab.* 287, E446–E453. doi: 10.1152/ajpendo.00488.2003

De Souza, C. T., Araujo, E. P., Bordin, S., Ashimine, R., Zollner, R. L., Boschero, A. C., et al. (2005). Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology* 146, 4192–4199. doi: 10.1210/en.2004-1520

Despres, J. P., and Lemieux, I. (2006). Abdominal obesity and metabolic syndrome. *Nature* 444, 881–887. doi: 10.1038/nature05488

Deuschle, M., Blum, W. F., Englaro, P., Schweiger, U., Weber, B., Pflaum, C. D., et al. (1996). Plasma leptin in depressed patients and healthy controls. *Horm. Metab. Res.* 28, 714–717. doi: 10.1055/s-2007-979885

Dong, C., Sanchez, L. E., and Price, R. A. (2004). Relationship of obesity to depression: a family-based study. *Int. J. Obes. Relat. Metab. Disord.* 28, 790–795. doi: 10.1038/sj.ijo.0802626

Doumatey, A. P., Bentley, A. R., Zhou, J., Huang, H., Adeyemo, A., and Rotimi, C. N. (2012). Paradoxical hyperadiponectinemia is associated with the Metabolically Healthy Obese (MHO) phenotype in african americans. *J. Endocrinol. Metab.* 2, 51–65. doi: 10.4021/jem95W

Dunn, A. J., Swiergiel, A. H., and Beaurepaire, R. D. (2005). Cytokines as mediators of depression: What can we learn from animal studies. *Neurosci. Biobehav. Rev.* 29, 891–909. doi: 10.1016/j.neubiorev.2005.03.023

Eissa Ahmed, A. A., Al-Rasheed, N. M., and Al-Rasheed, N. M. (2009). Antidepressant-like effects of rosiglitazone, a PPARgamma agonist, in the rat forced swim and mouse tail suspension tests. *Behav. Pharmacol.* 20, 635–642. doi: 10.1097/FBP.0b013e328331b9bf

Epel, E., Jimenez, S., Brownell, K., Stroud, L., Stoney, C., and Niaura, R. (2004). Are stress eaters at risk for the metabolic syndrome. *Ann. N.Y. Acad. Sci.* 1032, 208–210. doi: 10.1196/annals.1314.022

Esel, E., Ozsoy, S., Tutus, A., Sofuoglu, S., Kartalci, S., Bayram, F., et al. (2005). Effects of antidepressant treatment and of gender on serum leptin levels in patients with major depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 29, 565–570. doi: 10.1016/j.pnpbp.2005.01.009

Estruch, R., Ros, E., Salas-Salvado, J., Covas, M. I., Corella, D., Aros, F., et al. (2013). Primary prevention of cardiovascular disease with a Mediterranean diet. *N. Engl. J. Med.* 368, 1279–1290. doi: 10.1056/NEJMoa1200303

Finger, B. C., Dinan, T. G., and Cryan, J. F. (2010). Leptin-deficient mice retain normal appetitive spatial learning yet exhibit marked increases in anxiety-related behaviours. *Psychopharmacology (Berl.)* 210, 559–568. doi: 10.1007/s00213-010-1858-z

Finger, B. C., Dinan, T. G., and Cryan, J. F. (2011). High-fat diet selectively protects against the effects of chronic social stress in the mouse. *Neuroscience* 192, 351–360. doi: 10.1016/j.neuroscience.2011.06.072

Finger, B. C., Dinan, T. G., and Cryan, J. F. (2012). The temporal impact of chronic intermittent psychosocial stress on high-fat diet-induced alterations in body weight. *Psychoneuroendocrinology* 37, 729–741. doi: 10.1016/j.psyneuen.2011.06.015

Freedman, M. R., Castonguay, T. W., and Stern, J. S. (1985). Effect of adrenalectomy and corticosterone replacement on meal patterns of Zucker rats. *Am. J. Physiol.* 249, R584–R594.

Fulton, S. (2010). Appetite and reward. *Front. Neuroendocrinol.* 31:85–103. doi: 10.1016/j.yfrne.2009.10.003

Fulton, S., Pissios, P., Manchon, R. P., Stiles, L., Frank, L., Pothos, E. N., et al. (2006). Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* 51, 811–822. doi: 10.1016/j.neuron.2006.09.006

Garthwaite, T. L., Martinson, D. R., Tseng, L. F., Hagen, T. C., and Menahan, L. A. (1980). A longitudinal hormonal profile of the genetically obese mouse. *Endocrinology* 107, 671–676. doi: 10.1210/endo-107-3-671

Ge, J. F., Qi, C. C., and Zhou, J. N. (2013). Imbalance of leptin pathway and hypothalamus synaptic plasticity markers are associated with stress-induced depression in rats. *Behav. Brain Res.* 249C, 38–43. doi: 10.1016/j.bbr.2013.04.020

Gecici, O., Kuloglu, M., Atmaca, M., Tezcan, A. E., Tunckol, H., Emul, H. M., et al. (2005). High serum leptin levels in depressive disorders with atypical features. *Psychiatry Clin. Neurosci.* 59, 736–738. doi: 10.1111/j.1440-1819.2005.01445.x

Gibson, E. L. (2006). Emotional influences on food choice: sensory, physiological and psychological pathways. *Physiol. Behav.* 89, 53–61. doi: 10.1016/j.physbeh.2006.01.024

Goebel, M., Fleming, S. M., Million, M., Stengel, A., Tache, Y., and Wang, L. (2010). Mice overexpressing corticotropin-releasing factor show brain atrophy and motor dysfunctions. *Neurosci. Lett.*

473, 11–15. doi: 10.1016/j.neulet.2010.01.068

Gorzalka, B. B., and Hill, M. N. (2011). Putative role of endocannabinoid signaling in the etiology of depression and actions of antidepressants. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 1575–1585. doi: 10.1016/j.pnpbp.2010.11.021

Goshen, I., Kreisel, T., Ben-Menachem-Zidon, O., Licht, T., Weidenfeld, J., Ben-Hur, T., et al. (2008). Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Mol. psychiatry* 13, 717–728. doi: 10.1038/sj.mp.4002055

Gregus, A., Wintink, A. J., Davis, A. C., and Kalynchuk, L. E. (2005). Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. *Behav. Brain. Res.* 156, 105–114. doi: 10.1016/j.bbr.2004.05.013

Grillo, C. A., Piroli, G. G., Kaigler, K. F., Wilson, S. P., Wilson, M. A., and Reagan, L. P. (2011). Downregulation of hypothalamic insulin receptor expression elicits depressive-like behaviors in rats. *Behav. Brain Res.* 222, 230–235. doi: 10.1016/j.bbr.2011.03.052

Guo, M., Huang, T. Y., Garza, J. C., Chua, S. C., and Lu, X. Y. (2013). Selective deletion of leptin receptors in adult hippocampus induces depression-related behaviours. *Int. J. Neuropsychopharmacol.* 16, 857–867. doi: 10.1017/S1461145712000703

Guo, M., Lu, Y., Garza, J. C., Li, Y., Chua, S. C., Zhang, W., et al. (2012). Forebrain glutamatergic neurons mediate leptin action on depression-like behaviors and synaptic depression. *Transl. Psychiatry* 2, e83. doi: 10.1038/tp.2012.9

Hamer, M., Batty, G. D., and Kivimaki, M. (2012). Risk of future depression in people who are obese but metabolically healthy: the English longitudinal study of ageing. *Mol. Psychiatry* 17, 940–945. doi: 10.1038/mp.2012.30

Hanley, A. J., Bowden, D., Wagenknecht, L. E., Balasubramanyam, A., Langfeld, C., Saad, M. F., et al. (2007). Associations of adiponectin with body fat distribution and insulin sensitivity in nondiabetic Hispanics and African-Americans. *J. Clin. Endocrinol. Metab.* 92, 2665–2671. doi: 10.1210/jc.2006-2614

Hautanen, A., Raikkonen, K., and Adlercreutz, H. (1997). Associations between pituitary-adrenocortical function and abdominal obesity, hyperinsulinaemia and dyslipidaemia in normotensive males. *J. Intern. Med.* 241, 451–461. doi: 10.1111/j.1365-2796.1997.tb00002.x

Heilig, M. (1995). Antisense inhibition of neuropeptide Y (NPY)-Y1 receptor expression blocks the anxiolytic-like action of NPY in amygdala and paradoxically increases feeding. *Regul. Pept.* 59, 201–205. doi: 10.1016/0167-0115(95)00103-I

Heilig, M., Soderpalm, B., Engel, J. A., and Widerlov, E. (1989). Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. *Psychopharmacology (Berl.)* 98, 524–529. doi: 10.1007/BF00441953

Heilig, M., Zachrisson, O., Thorsell, A., Ehnvall, A., Mottagui-Tabar, S., Sjogren, M., et al. (2004). Decreased cerebrospinal fluid neuropeptide Y (NPY) in patients with treatment refractory unipolar major depression: preliminary evidence for association with preproNPY gene polymorphism. *J. Psychiatr. Res.* 38, 113–121. doi: 10.1016/S0022-3956(03)00101-8

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., et al. (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front. Neuroendocrinol.* 24:151–180. doi: 10.1016/j.yfrne.2003.07.001

Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23, 477–501. doi: 10.1016/S0893-133X(00)00159-7

Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z.-W., Gao, X.-B., et al. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 51, 801–810. doi: 10.1016/j.neuron.2006.08.023

Hsuchou, H., Kastin, A. J., Mishra, P. K., and Pan, W. (2012a). C-reactive protein increases BBB permeability: implications for obesity and neuroinflammation. *Cell. Physiol. Biochem.* 30, 1109–1119. doi: 10.1159/000343302

Hsuchou, H., Kastin, A. J., and Pan, W. (2012b). Blood-borne metabolic factors in obesity exacerbate injury-induced gliosis. *J. Mol. Neurosci.* 47, 267–277. doi: 10.1007/s12031-012-9734-4

Huang, Q., Rivest, R., and Richard, D. (1998). Effects of leptin on corticotropin-releasing factor (CRF) synthesis and CRF neuron activation in the paraventricular hypothalamic nucleus of obese (ob/ob) mice. *Endocrinology* 139, 1524–1532. doi: 10.1210/en.139.4.1524

Iemolo, A., Valenza, M., Tozier, L., Knapp, C. M., Kornetsky, C., Steardo, L., et al. (2012). Withdrawal from chronic, intermittent access to a highly palatable food induces depressive-like behavior in compulsive eating rats. *Behav. Pharmacol.* 23, 593–602. doi: 10.1097/FBP.0b013e328357697f

Jeong, H. G., Min, B. J., Lim, S., Kim, T. H., Lee, J. J., Park, J. H., et al. (2012). Plasma adiponectin elevation in elderly individuals with subsyndromal depression. *Psychoneuroendocrinology* 37, 948–955. doi: 10.1016/j.psyneuen.2011.11.002

Jow, G. M., Yang, T. T., and Chen, C. L. (2006). Leptin and cholesterol levels are low in major depressive disorder, but high in schizophrenia. *J. Affect. Disord.* 90, 21–27. doi: 10.1016/j.jad.2005.09.015

Juruena, M. F., and Cleare, A. J. (2007). Overlap between atypical depression, seasonal affective disorder and chronic fatigue syndrome. *Rev. Bras. Psiquiatr.* 29(Suppl. 1), S19–S26. doi: 10.1590/S1516-44462007000500005

Kalynchuk, L. E., Gregus, A., Boudreau, D., and Perrot-Sinal, T. S. (2004). Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. *Behav. Neurosci.* 118, 1365–1377. doi: 10.1037/0735-7044.118.6.1365

Kan, C., Silva, N., Golden, S. H., Rajala, U., Timonen, M., Stahl, D., et al. (2013). A systematic review and meta-analysis of the association between depression and insulin resistance. *Diabetes Care* 36, 480–489. doi: 10.2337/dc12-1442

Kanoski, S. E., and Davidson, T. L. (2011). Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. *Physiol. Behav.* 103, 59–68. doi: 10.1016/j.physbeh.2010.12.003

Kemp, D. E., Ismail-Beigi, F., Ganocy, S. J., Conroy, C., Gao, K., Obral, S., et al. (2012). Use of insulin sensitizers for the treatment of major depressive disorder: a pilot study of pioglitazone for major depression accompanied by abdominal obesity. *J. Affect. Disord.* 136, 1164–1173. doi: 10.1016/j.jad.2011.06.033

Kim, B., and Feldman, E. L. (2012). Insulin resistance in the nervous system. *Trends Endocrinol. Metab.* 23, 133–141. doi: 10.1016/j.tem.2011.12.004

Kleinridders, A., Schenten, D., Konner, A. C., Belgardt, B. F., Mauer, J., Okamura, T., et al. (2009). MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity. *Cell Metab.* 10, 249–259. doi: 10.1016/j.cmet.2009.08.013

Kloiber, S., Ising, M., Reppermund, S., Horstmann, S., Dose, T., Majer, M., et al. (2007). Overweight and obesity affect treatment response in major depression. *Biol. Psychiatry* 62, 321–326. doi: 10.1016/j.biopsych.2006.10.001

Komorowski, J., Jankiewicz-Wika, J., and Stepien, H. (2000). Effects of Gn-RH, TRH, and CRF administration on plasma leptin levels in lean and obese women. *Neuropeptides* 34, 89–97. doi: 10.1054/npep.2000.0799

Koo, J. W., and Duman, R. S. (2008). IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc. Natl. Acad. Sci. U.S.A.* 105, 751–756. doi: 10.1073/pnas.0708092105

Koo, J. W., Russo, S. J., Ferguson, D., Nestler, E. J., and Duman, R. S. (2010). Nuclear factor-kappaB is a critical mediator of stress-impaired neurogenesis and depressive behavior. *Proc. Natl. Acad. Sci. U.S.A.* 107, 2669–2674. doi: 10.1073/pnas.0910658107

Koponen, H., Jokelainen, J., Keinanen-Kiukaanniemi, S., Kumpusalo, E., and Vanhala, M. (2008). Metabolic syndrome predisposes to depressive symptoms: a population-based 7-year follow-up study. *J. Clin. Psychiatry* 69, 178–182. doi: 10.4088/JCP.v69n0202

Kraus, T., Haack, M., Schuld, A., Hinze-Selch, D., Koethe, D., and Pollmacher, T. (2002). Body weight, the tumor necrosis factor system, and leptin production during treatment with mirtazapine or venlafaxine. *Pharmacopsychiatry* 35, 220–225. doi: 10.1055/s-2002-36390

Kraus, T., Haack, M., Schuld, A., Hinze-Selch, D., and Pollmacher, T. (2001). Low leptin levels but normal body mass indices in patients with depression or schizophrenia.

Neuroendocrinology 73, 243–247. doi: 10.1159/000054641

Krishnan, V., and Nestler, E. J. (2008). The molecular neurobiology of depression. *Nature* 455, 894–902. doi: 10.1038/nature07455

Krsek, M., Silha, J. V., Jezkova, J., Hana, V., Marek, J., Weiss, V., et al. (2004). Adipokine levels in Cushing's syndrome; elevated resistin levels in female patients with Cushing's syndrome. *Clin. Endocrinol. (Oxf.)* 60, 350–357. doi: 10.1111/j.1365-2265.2003.01987.x

Kyrou, I., and Tsigos, C. (2009). Stress hormones: physiological stress and regulation of metabolism. *Curr. Opin. Pharmacol.* 9, 787–793. doi: 10.1016/j.coph.2009.08.007

Labad, J., Price, J. F., Strachan, M. W., Fowkes, F. G., Deary, I. J., Seckl, J. R., et al. (2012). Leptin levels and depressive symptoms in people with type 2 diabetes: the edinburgh type 2 diabetes study. *Psychosom. Med.* 74, 39–45. doi: 10.1097/PSY.0b013e31823ba8af

Labouebe, G., Liu, S., Dias, C., Zou, H., Wong, J. C., Karunakaran, S., et al. (2013). Insulin induces long-term depression of ventral tegmental area dopamine neurons via endocannabinoids. *Nat. Neurosci.* 16, 300–308. doi: 10.1038/nn.3321

la Fleur, S. E., Houshyar, H., Roy, M., and Dallman, M. F. (2005). Choice of lard, but not total lard calories, damps adrenocorticotropin responses to restraint. *Endocrinology* 146, 2193–2199. doi: 10.1210/en.2004-1603

Lasikiewicz, N., Hendrickx, H., Talbot, D., and Dye, L. (2008). Exploration of basal diurnal salivary cortisol profiles in middle-aged adults: associations with sleep quality and metabolic parameters. *Psychoneuroendocrinology* 33, 143–151. doi: 10.1016/j.psyneuen.2007.10.013

Lehto, S. M., Huotari, A., Niskanen, L., Tolmunen, T., Koivumaa-Honkanen, H., Honkalampi, K., et al. (2010). Serum adiponectin and resistin levels in major depressive disorder. *Acta Psychiatr. Scand.* 121, 209–215. doi: 10.1111/j.1600-0447.2009.01463.x

Levine, J., Barak, Y., Chengappa, K. N., Rapoport, A., Rebey, M., and Barak, V. (1999). Cerebrospinal cytokine levels in patients with acute depression. *Neuropsychobiology* 40, 171–176. doi: 10.1159/000026615

Lin, P. Y., and Su, K. P. (2007). A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. *J. Clin. Psychiatry* 68, 1056–1061. doi: 10.4088/JCP.v68n0712

Liu, J., Garza, J., Bronner, J., Kim, C., Zhang, W., and Lu, X.-Y. (2010a). Acute administration of leptin produces anxiolytic-like effects: a comparison with fluoxetine. *Psychopharmacology* 207, 535–545. doi: 10.1007/s00213-009-1684-3

Liu, J., Garza, J. C., Bronner, J., Kim, C. S., Zhang, W., and Lu, X. Y. (2010b). Acute administration of leptin produces anxiolytic-like effects: a comparison with fluoxetine. *Psychopharmacology (Berl.)* 207, 535–545. doi: 10.1007/s00213-009-1684-3

Liu, J., Guo, M., Zhang, D., Cheng, S. Y., Liu, M., Ding, J., et al. (2012). Adiponectin is critical in determining susceptibility to depressive behaviors and has antidepressant-like activity. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12248–12253. doi: 10.1073/pnas.1202835109

Liu, J., Perez, S. M., Zhang, W., Lodge, D. J., and Lu, X. Y. (2011). Selective deletion of the leptin receptor in dopamine neurons produces anxiogenic-like behavior and increases dopaminergic activity in amygdala. *Mol. Psychiatry* 16, 1024–1038. doi: 10.1038/mp.2011.36

Lu, X.-Y., Kim, C. S., Frazer, A., and Zhang, W. (2006). Leptin: a potential novel antidepressant. *Proc. Natl. Acad. Sci. U.S.A.* 103, 1593–1598. doi: 10.1073/pnas.0508901103

Luppino, F. S., de Wit, L. M., Bouvy, P. F., Stijnen, T., Cuijpers, P., Penninx, B. W., et al. (2010). Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch. Gen. Psychiatry* 67, 220–229. doi: 10.1001/archgenpsychiatry.2010.2

Macht, M. (2008). How emotions affect eating: a five-way model. *Appetite* 50, 1–11. doi: 10.1016/j.appet.2007.07.002

Maebuchi, M., Machidori, M., Urade, R., Ogawa, T., and Moriyama, T. (2003). Low resistin levels in adipose tissues and serum in high-fat fed mice and genetically obese mice: development of an ELISA system for quantification of resistin. *Arch. Biochem. Biophys.* 416, 164–170. doi: 10.1016/S0003-9861(03)00279-0

Maeda, N., Takahashi, M., Funahashi, T., Kihara, S., Nishizawa, H., Kishida, K., et al. (2001). PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 50, 2094–2099. doi: 10.2337/diabetes.50.9.2094

Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., et al. (1995). Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat. Med.* 1, 1155–1161. doi: 10.1038/nm1195-1155

Mangieri, R. A., and Piomelli, D. (2007). Enhancement of endocannabinoid signaling and the pharmacotherapy of depression. *Pharmacol. Res.* 56, 360–366. doi: 10.1016/j.phrs.2007.09.003

Maniam, J., and Morris, M. J. (2010). Palatable cafeteria diet ameliorates anxiety and depression-like symptoms following an adverse early environment. *Psychoneuroendocrinology* 35, 717–728. doi: 10.1016/j.psyneuen.2009.10.013

Mann, J. N., and Thakore, J. H. (1999). Melancholic depression and abdominal fat distribution: a mini-review. *Stress* 3, 1–15. doi: 10.3109/10253899909001108

Marks, D. R., Tucker, K., Cavallin, M. A., Mast, T. G., and Fadool, D. A. (2009). Awake intranasal insulin delivery modifies protein complexes and alters memory, anxiety, and olfactory behaviors. *J. Neurosci.* 29, 6734–6751. doi: 10.1523/JNEUROSCI.1350-09.2009

Mathe, A. A., Husum, H., El Khoury, A., Jimenez-Vasquez, P., Gruber, S. H., Wortwein, G., et al. (2007). Search for biological correlates of depression and mechanisms of action of antidepressant treatment modalities. Do neuropeptides play a role? *Physiol. Behav.* 92, 226–231. doi: 10.1016/j.physbeh.2007.05.016

Matheny, M., Shapiro, A., Tumer, N., and Scarpace, P. J. (2011). Region-specific diet-induced and leptin-induced cellular leptin resistance includes the ventral tegmental area in rats. *Neuropharmacology* 60, 480–487. doi: 10.1016/j.neuropharm.2010.11.002

McEwen, B. S. (1997). Possible mechanisms for atrophy of the human hippocampus. *Mol. Psychiatry* 2, 255–262. doi: 10.1038/sj.mp.4000254

McLaughlin, R. J., and Gobbi, G. (2012). Cannabinoids and emotionality: a neuroanatomical perspective. *Neuroscience* 204, 134–144. doi: 10.1016/j.neuroscience.2011.07.052

Mechoulam, R., and Parker, L. A. (2013). The endocannabinoid system and the brain. *Annu. Rev. Psychol.* 64, 21–47. doi: 10.1146/annurev-psych-113011-143739

Melas, P. A., Lennartsson, A., Vakifahmetoglu-Norberg, H., Wei, Y., Aberg, E., Werme, M., et al. (2013). Allele-specific programming of Npy and epigenetic effects of physical activity in a genetic model of depression. *Transl. Psychiatry* 3, e255. doi: 10.1038/tp.2013.31

Mente, A., Meyre, D., Lanktree, M. B., Heydarpour, M., Davis, A. D., Miller, R., et al. (2013). Causal relationship between adiponectin and metabolic traits: a mendelian randomization study in a multiethnic population. *PLoS ONE* 8:e66808. doi: 10.1371/journal.pone.0066808

Micha, R., and Mozaffarian, D. (2009). Trans fatty acids: effects on metabolic syndrome, heart disease and diabetes. *Nat. Rev. Endocrinol.* 5, 335–344. doi: 10.1038/nrendo.2009.79

Micha, R., and Mozaffarian, D. (2010). Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. *Lipids* 45, 893–905. doi: 10.1007/s11745-010-3393-4

Milan, G., Granzotto, M., Scarda, A., Calcagno, A., Pagano, C., Federspil, G., et al. (2002). Resistin and adiponectin expression in visceral fat of obese rats: effect of weight loss. *Obes. Res.* 10, 1095–1103. doi: 10.1038/oby.2002.149

Milaneschi, Y., Simonsick, E. M., Vogelzangs, N., Strotmeyer, E. S., Yaffe, K., Harris, T. B., et al. (2012). Leptin, abdominal obesity, and onset of depression in older men and women. *J. Clin. Psychiatry* 73, 1205–1211. doi: 10.4088/JCP.11m07552

Mitra, R., Ferguson, D., and Sapolsky, R. M. (2009). Mineralocorticoid receptor overexpression in basolateral amygdala reduces corticosterone secretion and anxiety. *Biol. Psychiatry* 66, 686–690. doi: 10.1016/j.biopsych.2009.04.016

Molenaar, E. A., Massaro, J. M., Jacques, P. F., Pou, K. M., Ellison, R. C., Hoffmann, U., et al. (2009). Association of lifestyle factors with abdominal subcutaneous and visceral adiposity: the Framingham Heart Study. *Diabetes Care* 32, 505–510. doi: 10.2337/dc08-1382

Moranis, A., Delpech, J. C., De Smedt-Peyrusse, V., Aubert, A., Guesnet, P., Lavialle, M., et al. (2012). Long term adequate n-3 polyunsaturated fatty acid diet protects from depressive-like behavior but not

from working memory disruption and brain cytokine expression in aged mice. *Brain Behav. Immun.* 26, 721–731. doi: 10.1016/j.bbi.2011.11.001

Morrison, J. A., Glueck, C. J., Daniels, S., Wang, P., and Stroop, D. (2011). Paradoxically high adiponectin in obese 16-year-old girls protects against appearance of the metabolic syndrome and its components seven years later. *J. Pediatr.* 158, 208–214 e201. doi: 10.1016/j.jpeds.2010.08.012

Mozaffarian, D., Hao, T., Rimm, E. B., Willett, W. C., and Hu, F. B. (2011). Changes in diet and lifestyle and long-term weight gain in women and men. *N. Engl. J. Med.* 364, 2392–2404. doi: 10.1056/NEJMoa1014296

Mujica-Parodi, L. R., Renelique, R., and Taylor, M. K. (2009). Higher body fat percentage is associated with increased cortisol reactivity and impaired cognitive resilience in response to acute emotional stress. *Int. J. Obes. (Lond.)* 33, 157–165. doi: 10.1038/ijo.2008.218

Musselman, D. L., Lawson, D. H., Gumnick, J. F., Manatunga, A. K., Penna, S., Goodkin, R. S., et al. (2001). Paroxetine for the prevention of depression induced by high-dose interferon alfa. *N. Engl. J. Med.* 344, 961–966. doi: 10.1056/NEJM200103293441303

Myers, M. G. Jr., Heymsfield, S. B., Haft, C., Kahn, B. B., Laughlin, M., Leibel, R. L., et al. (2012). Challenges and opportunities of defining clinical leptin resistance. *Cell Metab.* 15, 150–156. doi: 10.1016/j.cmet.2012.01.002

Nazare, J. A., Smith, J., Borel, A. L., Almeras, N., Tremblay, A., Bergeron, J., et al. (2013). Changes in both global diet quality and physical activity level synergistically reduce visceral adiposity in men with features of metabolic syndrome. *J. Nutr.* 143, 1074–1083. doi: 10.3945/jn.113.175273

Nemeroff, C. B., and Vale, W. W. (2005). The neurobiology of depression: inroads to treatment and new drug discovery. *J. Clin. Psychiatry* 66(Suppl. 7), 5–13.

Nikisch, G., Agren, H., Eap, C. B., Czernik, A., Baumann, P., and Mathe, A. A. (2005). Neuropeptide Y and corticotropin-releasing hormone in CSF mark response to antidepressive treatment with citalopram. *Int. J. Neuropsychopharmacol.* 8, 403–410. doi: 10.1017/S1461145705005158

Novick, J. S., Stewart, J. W., Wisniewski, S. R., Cook, I. A., Manev, R., Nierenberg, A. A., et al. (2005). Clinical and demographic features of atypical depression in outpatients with major depressive disorder: preliminary findings from STAR*D. *J. Clin. Psychiatry* 66, 1002–1011. doi: 10.4088/JCP.v66n0807

Oddy, W. H., Hickling, S., Smith, M. A., O'Sullivan, T. A., Robinson, M., de Klerk, N. H., et al. (2011). Dietary intake of omega-3 fatty acids and risk of depressive symptoms in adolescents. *Depress. Anxiety* 28, 582–588. doi: 10.1002/da.20822

Oliver, G., and Wardle, J. (1999). Perceived effects of stress on food choice. *Physiol. Behav.* 66, 511–515. doi: 10.1016/S0031-9384(98)00322-9

Owecki, M., Miczke, A., Nikisch, E., Pupek-Musialik, D., and Sowinski, J. (2011). Serum resistin concentrations are higher in human obesity but independent from insulin resistance. *Exp. Clin. Endocrinol. Diabetes* 119, 117–121. doi: 10.1055/s-0030-1263111

Pan, A., Sun, Q., Czernichow, S., Kivimaki, M., Okereke, O. I., Lucas, M., et al. (2012). Bidirectional association between depression and obesity in middle-aged and older women. *Int. J. Obes. (Lond.)* 36, 595–602. doi: 10.1038/ijo.2011.111

Park, Y., Kim, M., Baek, D., and Kim, S. H. (2012a). Erythrocyte n-3 polyunsaturated fatty acid and seafood intake decrease the risk of depression: case-control study in Korea. *Ann. Nutr. Metab.* 61, 25–31. doi: 10.1159/000339264

Park, Y., Moon, H. J., and Kim, S. H. (2012b). N-3 polyunsaturated fatty acid consumption produces neurobiological effects associated with prevention of depression in rats after the forced swimming test. *J. Nutr. Biochem.* 23, 924–928. doi: 10.1016/j.jnutbio.2011.04.018

Parker, K. J., Schatzberg, A. F., and Lyons, D. M. (2003). Neuroendocrine aspects of hypercortisolism in major depression. *Horm. Behav.* 43, 60–66. doi: 10.1016/S0018-506X(02)00016-8

Pasquali, R. (2012). The hypothalamic-pituitary-adrenal axis and sex hormones in chronic stress and obesity: pathophysiological and clinical aspects. *Ann. N.Y. Acad. Sci.* 1264, 20–35. doi: 10.1111/j.1749-6632.2012.06569.x

Pecoraro, N., Reyes, F., Gomez, F., Bhargava, A., and Dallman, M. F. (2004). Chronic stress promotes palatable feeding, which reduces signs of stress: feed-forward and feedback effects of chronic stress. *Endocrinology* 145, 3754–3762. doi: 10.1210/en.2004-0305

Peet, M., Murphy, B., Shay, J., and Horrobin, D. (1998). Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biol. Psychiatry* 43, 315–319. doi: 10.1016/S0006-3223(97)00206-0

Piazza, P. V., and Le Moal, M. (1997). Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. *Brain Res. Brain Res. Rev.* 25, 359–372. doi: 10.1016/S0165-0173(97)00025-8

Pickering, C., Alsio, J., Hulting, A. L., and Schioth, H. B. (2009). Withdrawal from free-choice high-fat high-sugar diet induces craving only in obesity-prone animals. *Psychopharmacology (Berl.)* 204, 431–443. doi: 10.1007/s00213-009-1474-y

Platt, A. M., Egan, A. M., Berquist, M. J., Dreyer, M. L., Babar, G., and Ugrasbul, F. (2013). Health-related quality of life, depression, and metabolic parameters in overweight insulin-resistant adolescents. *J. Pediatr. Health Care* 27, 120–126. doi: 10.1016/j.pedhc.2011.06.015

Pulkki-Raback, L., Elovainio, M., Kivimaki, M., Mattsson, N., Raitakari, O. T., Puttonen, S., et al. (2009). Depressive symptoms and the metabolic syndrome in childhood and adulthood: a prospective cohort study. *Health Psychol.* 28, 108–116. doi: 10.1037/a0012646

Raison, C. L., and Miller, A. H. (2003). When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *Am. J. Psychiatry* 160, 1554–1565. doi: 10.1176/appi.ajp.160.9.1554

Riccardi, G., and Rivellese, A. A. (2000). Dietary treatment of the metabolic syndrome–the optimal diet. *Br. J. Nutr.* 83(Suppl. 1), S143–S148. doi: 10.1017/S0007114500001082

Roberts, R. E., Deleger, S., Strawbridge, W. J., and Kaplan, G. A. (2003). Prospective association between obesity and depression: evidence from the Alameda County Study. *Int. J. Obes. Relat. Metab. Disord.* 27, 514–521. doi: 10.1038/sj.ijo.0802204

Romaguera, D., Norat, T., Mouw, T., May, A. M., Bamia, C., Slimani, N., et al. (2009). Adherence to the Mediterranean diet is associated with lower abdominal adiposity in European men and women. *J. Nutr.* 139, 1728–1737. doi: 10.3945/jn.109.108902

Romaguera, D., Norat, T., Vergnaud, A. C., Mouw, T., May, A. M., Agudo, A., et al. (2010). Mediterranean dietary patterns and prospective weight change in participants of the EPIC-PANACEA project. *Am. J. Clin. Nutr.* 92, 912–921. doi: 10.3945/ajcn.2010.29482

Rozeboom, A. M., Akil, H., and Seasholtz, A. F. (2007). Mineralocorticoid receptor overexpression in forebrain decreases anxiety-like behavior and alters the stress response in mice. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4688–4693. doi: 10.1073/pnas.0606067104

Rubin, R. T., Rhodes, M. E., and Czambel, R. K. (2002). Sexual diergism of baseline plasma leptin and leptin suppression by arginine vasopressin in major depressives and matched controls. *Psychiatry Res.* 113, 255–268. doi: 10.1016/S0165-1781(02)00263-9

Ruusunen, A., Virtanen, J. K., Lehto, S. M., Tolmunen, T., Kauhanen, J., and Voutilainen, S. (2011). Serum polyunsaturated fatty acids are not associated with the risk of severe depression in middle-aged Finnish men: Kuopio Ischaemic Heart Disease Risk Factor (KIHD) study. *Eur. J. Nutr.* 50, 89–96. doi: 10.1007/s00394-010-0118-7

Ryo, M., Nakamura, T., Kihara, S., Kumada, M., Shibazaki, S., Takahashi, M., et al. (2004). Adiponectin as a biomarker of the metabolic syndrome. *Circ. J.* 68, 975–981. doi: 10.1253/circj.68.975

Sadashiv, Tiwari, S., Paul, B. N., Kumar, S., Chandra, A., Dhananjai, S., et al. (2012). Over expression of resistin in adipose tissue of the obese induces insulin resistance. *World J. Diabetes* 3, 135–141. doi: 10.4239/wjd.v3.i7.135

Sanchez-Villegas, A., Henriquez, P., Figueiras, A., Ortuno, F., Lahortiga, F., and Martinez-Gonzalez, M. A. (2007). Long chain omega-3 fatty acids intake, fish consumption and mental disorders in the SUN cohort study. *Eur. J. Nutr.* 46, 337–346. doi: 10.1007/s00394-007-0671-x

Sanchez-Villegas, A., and Martinez-Gonzalez, M. A. (2013). Diet, a new target to prevent depression. *BMC Med.* 11:3. doi: 10.1186/1741-7015-11-3

Schilling, C., Gilles, M., Blum, W. F., Daseking, E., Colla, M., Weber-Hamann, B., et al. (2013). Leptin plasma concentrations increase during antidepressant treatment with amitriptyline and mirtazapine, but not paroxetine and venlafaxine: leptin resistance mediated by

antihistaminergic activity. *J. Clin. Psychopharmacol.* 33, 99–103. doi: 10.1097/JCP.0b013e31827cb179

Schoenfeld, T. J., and Gould, E. (2012). Stress, stress hormones, and adult neurogenesis. *Exp. Neurol.* 233, 12–21. doi: 10.1016/j.expneurol.2011.01.008

Schulze, M. B., Fung, T. T., Manson, J. E., Willett, W. C., and Hu, F. B. (2006). Dietary patterns and changes in body weight in women. *Obesity (Silver Spring.)* 14, 1444–1453. doi: 10.1038/oby.2006.164

Schwartz, D. R., and Lazar, M. A. (2011). Human resistin: found in translation from mouse to man. *Trends Endocrinol. Metab.* 22, 259–265. doi:10.1016/j.tem.2011.03.005

Sharma, A. N., Elased, K. M., Garrett, T. L., and Lucot, J. B. (2010). Neurobehavioral deficits in db/db diabetic mice. *Physiol. Behav.* 101, 381–388. doi: 10.1016/j.physbeh.2010.07.002

Sharma, S., Fernandes, M. F., and Fulton, S. (2012). Adaptations in brain reward circuitry underlie palatable food cravings and anxiety induced by high-fat diet withdrawal. *Int. J. Obes. (Lond.)* 37, 1183–1189. doi: 10.1038/ijo.2012.197

Sharma, S., and Fulton, S. (2013). Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. *Int. J. Obes. (Lond.)* 37, 382–389. doi: 10.1038/ijo.2012.48

Shen, Q., and Bergquist-Beringer, S. (2013). The Relationship between major depression and insulin resistance: does it vary by gender or race/ethnicity among young adults aged 20-39 years. *J. Diabetes.* doi: 10.1111/1753-0407.12044. [Epub ahead of print].

Simen, B. B., Duman, C. H., Simen, A. A., and Duman, R. S. (2006). TNFalpha signaling in depression and anxiety: behavioral consequences of individual receptor targeting. *Biological. Psychiatry* 59, 775–785. doi: 10.1016/j.biopsych.2005.10.013

Simon, G. E., Von Korff, M., Saunders, K., Miglioretti, D. L., Crane, P. K., van Belle, G., et al. (2006). Association between obesity and psychiatric disorders in the US adult population. *Arch. Gen. Psychiatry* 63, 824–830. doi: 10.1001/archpsyc.63.7.824

Solomon, M. B., Furay, A. R., Jones, K., Packard, A. E., Packard, B. A., Wulsin, A. C., et al. (2012). Deletion of forebrain glucocorticoid receptors impairs neuroendocrine stress responses and induces depression-like behavior in males but not females. *Neuroscience* 203, 135–143. doi: 10.1016/j.neuroscience.2011.12.014

Sonino, N., Fava, G. A., Raffi, A. R., Boscaro, M., and Fallo, F. (1998). Clinical correlates of major depression in Cushing's disease. *Psychopathology* 31, 302–306. doi: 10.1159/000029054

Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., et al. (2001). The hormone resistin links obesity to diabetes. *Nature* 409, 307–312. doi: 10.1038/35053000

Stetler, C., and Miller, G. E. (2011). Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. *Psychosom. Med.* 73, 114–126. doi: 10.1097/PSY.0b013e31820ad12b

Stone, A. A., and Brownell, K. D. (1994). The stress-eating paradox: multiple daily measurements in adult males and females. *Psychol. Health* 9, 425–436. doi: 10.1080/08870449408407469

Sun, H., Kennedy, P. J., and Nestler, E. J. (2013). Epigenetics of the depressed brain: role of histone acetylation and methylation. *Neuropsychopharmacology* 38, 124–137. doi: 10.1038/npp.2012.73

Takechi, R., Galloway, S., Pallebage-Gamarallage, M. M., Lam, V., and Mamo, J. C. (2010). Dietary fats, cerebrovasculature integrity and Alzheimer's disease risk. *Prog. Lipid Res.* 49, 159–170. doi: 10.1016/j.plipres.2009.10.004

Tchernof, A., and Despres, J. P. (2013). Pathophysiology of human visceral obesity: an update. *Physiol. Rev.* 93, 359–404. doi: 10.1152/physrev.00033.2011

Teegarden, S. L., Nestler, E. J., and Bale, T. L. (2008). Delta FosB-mediated alterations in dopamine signaling are normalized by a palatable high-fat diet. *Biol. Psychiatry* 64, 941–950. doi: 10.1016/j.biopsych.2008.06.007

Thaler, J. P., Yi, C. X., Schur, E. A., Guyenet, S. J., Hwang, B. H., Dietrich, M. O., et al. (2012). Obesity is associated with hypothalamic injury in rodents and humans. *J. Clin. Invest.* 122, 153–162. doi: 10.1172/JCI59660

Therrien, F., Drapeau, V., Lalonde, J., Lupien, S. J., Beaulieu, S., Tremblay, A., et al. (2007). Awakening cortisol response in lean, obese, and reduced obese individuals: effect of gender and fat distribution. *Obesity (Silver Spring)* 15, 377–385. doi: 10.1038/oby.2007.509

Therrien, F., Drapeau, V., Lupien, S. J., Beaulieu, S., Dore, J., Tremblay, A., et al. (2008). Awakening cortisol response in relation to psychosocial profiles and eating behaviors. *Physiol. Behav.* 93, 282–288. doi: 10.1016/j.physbeh.2007.08.019

Tryon, M. S., Decant, R., and Laugero, K. D. (2013). Having your cake and eating it too: a habit of comfort food may link chronic social stress exposure and acute stress-induced cortisol hyporesponsiveness. *Physiol. Behav.* 114–115, 32–37. doi: 10.1016/j.physbeh.2013.02.018

Tsuboi, H., Watanabe, M., Kobayashi, F., Kimura, K., and Kinae, N. (2013). Associations of depressive symptoms with serum proportions of palmitic and arachidonic acids, and alpha-tocopherol effects among male population–a preliminary study. *Clin. Nutr.* 32, 289–293. doi: 10.1016/j.clnu.2012.07.011

Turer, A. T., and Scherer, P. E. (2012). Adiponectin: mechanistic insights and clinical implications. *Diabetologia* 55, 2319–2326. doi: 10.1007/s00125-012-2598-x

Ulrich-Lai, Y. M., Christiansen, A. M., Ostrander, M. M., Jones, A. A., Jones, K. R., Choi, D. C., et al. (2010). Pleasurable behaviors reduce stress via brain reward pathways. *Proc. Natl. Acad. Sci. U.S.A.* 107, 20529–20534. doi: 10.1073/pnas.1007740107

Valtonen, M. K., Laaksonen, D. E., Laukkanen, J. A., Tolmunen, T., Viinamäki, H., Lakka, H.-M., et al. (2012). Low-grade inflammation and depressive symptoms as predictors of abdominal obesity. *Scand. J. Public Health* 40, 674–680. doi: 10.1177/1403494812461730

van Reedt Dortland, A. K. B., Giltay, E. J., van Veen, T., Zitman, F. G., and Penninx, B. W. J. H. (2013a). Longitudinal relationship of depressive and anxiety symptoms with dyslipidemia and abdominal obesity. *Psychosom. Med.* 75, 83–89. doi: 10.1097/PSY.0b013e318274d30f

van Reedt Dortland, A. K. B., Vreeburg, S. A., Giltay, E. J., Licht, C. M. M., Vogelzangs, N., van Veen, T., et al. (2013b). The impact of stress systems and lifestyle on dyslipidemia and obesity in anxiety and depression. *Psychoneuroendocrinology* 38, 209–218. doi: 10.1016/j.psyneuen.2012.05.017

Wang, Q., Verweij, E. W., Krugers, H. J., Joels, M., Swaab, D. F., and Lucassen, P. J. (2013). Distribution of the glucocorticoid receptor in the human amygdala; changes in mood disorder patients. *Brain Struct. Funct.* doi: 10.1007/s00429-013-0589-4. [Epub ahead of print].

Way, J. M., Görgün, C. Z., Tong, Q., Uysal, K. T., Brown, K. K., Harrington, W. W., et al. (2001). Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor γ agonists. *J. Biol. Chem.* 276, 25651–25653. doi: 10.1074/jbc.C100189200

Weber-Hamann, B., Hentschel, F., Kniest, A., Deuschle, M., Colla, M., Lederbogen, F., et al. (2002). Hypercortisolemic depression is associated with increased intra-abdominal fat. *Psychosom. Med.* 64, 274–277.

Weber-Hamann, B., Kratzsch, J., Kopf, D., Lederbogen, F., Gilles, M., Heuser, I., et al. (2007). Resistin and adiponectin in major depression: the association with free cortisol and effects of antidepressant treatment. *J. Psychiatr. Res.* 41, 344–350. doi: 10.1016/j.jpsychires.2006.01.002

Wei, Q., Lu, X. Y., Liu, L., Schafer, G., Shieh, K. R., Burke, S., et al. (2004). Glucocorticoid receptor overexpression in forebrain: a mouse model of increased emotional lability. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11851–11856. doi: 10.1073/pnas.0402208101

Westling, S., Ahren, B., Traskman-Bendz, L., and Westrin, A. (2004). Low CSF leptin in female suicide attempters with major depression. *J. Affect. Disord.* 81, 41–48. doi: 10.1016/j.jad.2003.07.002

Wilhelm, C. J., Choi, D., Huckans, M., Manthe, L., and Loftis, J. M. (2013). Adipocytokine signaling is altered in Flinders sensitive line rats, and adiponectin correlates in humans with some symptoms of depression. *Pharmacol. Biochem. Behav.* 103, 643–651. doi: 10.1016/j.pbb.2012.11.001

Willner, P., Scheel-Kruger, J., and Belzung, C. (2012). The neurobiology of depression and antidepressant action. *Neurosci. Biobehav. Rev.* doi: 10.1016/j.neubiorev.2012.12.007. [Epub ahead of print].

Wium-Andersen, M., Ørsted, D., Nielsen, S., and Nordestgaard, B. (2013). Elevated c-reactive protein levels, psychological distress,

and depression in 73 131 individuals. *JAMA Psychiatry* 70, 176–184. doi: 10.1001/2013.jamapsychiatry.102

Wyrwoll, C. S., Holmes, M. C., and Seckl, J. R. (2011). 11beta-hydroxysteroid dehydrogenases and the brain: from zero to hero, a decade of progress. *Front. Neuroendocrinol.* 32, 265–286. doi: 10.1016/j.yfrne.2010.12.001

Yamada, N., Katsuura, G., Ochi, Y., Ebihara, K., Kusakabe, T., Hosoda, K., et al. (2011). Impaired CNS leptin action is implicated in depression associated with obesity. *Endocrinology* 152, 2634–2643. doi: 10.1210/en.2011-0004

Ye, H., Zhang, H. J., Xu, A., and Hoo, R. L. (2013). Resistin Production from Adipose Tissue Is Decreased in db/db Obese Mice, and Is Reversed by Rosiglitazone. *PLoS ONE* 8:e65543. doi: 10.1371/journal.pone.0065543

Ye, J., Gao, Z., Yin, J., and He, Q. (2007). Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. *Am. J. Physiol. Endocrinol. Metab.* 293, E1118–E1128. doi: 10.1152/ajpendo.00435.2007

Zeman, M., Jirak, R., Jachymova, M., Vecka, M., Tvrzicka, E., and Zak, A. (2009). Leptin, adiponectin, leptin to adiponectin ratio and insulin resistance in depressive women. *Neuro Endocrinol. Lett.* 30, 387–395.

Zhang, X., Zhang, G., Zhang, H., Karin, M., Bai, H., and Cai, D. (2008). Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. *Cell* 135, 61–73. doi: 10.1016/j.cell.2008.07.043

Zhao, G., Ford, E. S., Li, C., Tsai, J., Dhingra, S., and Balluz, L. S. (2011). Waist circumference, abdominal obesity, and depression among overweight and obese U.S. adults: National Health and Nutrition Examination Survey 2005–2006. *BMC Psychiatry* 11:130. doi: 10.1186/1471-244X-11-130

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