THE TWO-WAY LINK BETWEEN EATING BEHAVIOR AND BRAIN METABOLISM

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THE TWO-WAY LINK BETWEEN EATING BEHAVIOR AND BRAIN METABOLISM

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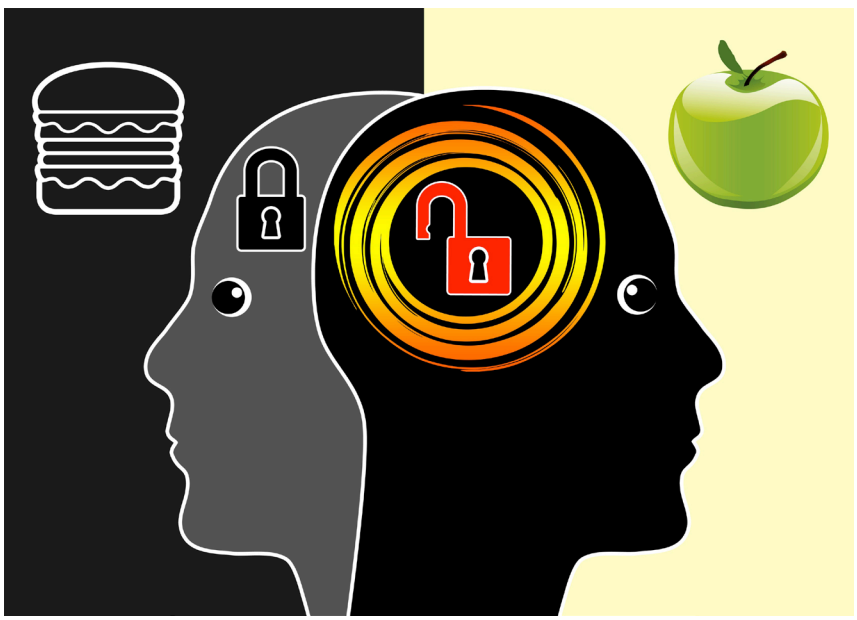

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This research topic collected and connected information concerning both the underlying metabolic mechanisms and consequences of eating behaviors. These two aspects are tremendously important for a better understanding of eating behavior abnormalities as well as for improving education on eating disorders and behaviors.

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Table of Contents

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EDITORIAL
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Appetite, reward, and obesity: the causes and consequences of eating behaviors

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Keywords: eating behavior, eating disorders, brain metabolism, obesity, reward, appetite, hedonic eating, energy expenditure

Eating behavior is constantly being shaped by a convergence of homeostatic demands and motivational parameters set to insure adequate behavioral patterns with a variety of metabolic consequences that send signals to brain structures to the brain structures responsible for the homeostatic parameters. Out of the scores of themes concerning eating behavior, the 18 papers comprising the current Research Topic, "*The two-way link between eating behavior and brain metabolism,*" happened to be distributed among the three large overlapping fields: reward, appetite, and obesity.

Reward

Understanding eating behavior is impossible without understanding the mechanisms of reward. Blum et al. (2014) looked at obesity as a result of over-consumption due to addictive processes and impaired reward functioning. They discuss the relevancy of the Reward Deficiency Syndrome: a genetic/epigenetic phenomenon resulting in a dysfunction of the dopaminergic system intertwined with the system of carbohydrate homeostasis. Natural palatable reward is powerfully mediated by sensory input stimulated by carbohydrates. The specifics of peripheral stimulation (oral, duodenal, or combined sensing of sweet) addressing to the brain areas involved with hedonic processes allowed an important conclusion regarding the role of visceral modulation in hedonic processes (Clouard et al., 2014). The rewarding nature of food mediated via dopaminergic system can be enhanced under the condition of excessive stress triggering a vicious circle and leading to maladaptive responses including overconsumption of palatable food and obesity (Sominsky and Spencer, 2014). The dopaminergic neurons are under influence of insulin and leptin—both potent regulators of eating behavior (Khanh et al., 2014), thus adding an important loop to the eating behavior control system.

The interplay between reward, gratification, and dopaminergic system can override hunger control. This was the main theme of Singh's review (Singh, 2014) summarizing the links between three domains: mood, food, and obesity. Godier and Park (2014) discussed the role of modulation of the glutaminergic and gamma-aminobutyric acid (GABA) pathways by dopaminergic system in functioning of the reward system and suggested a potential application of these mechanisms to treatment of eating disorders, compulsivity, and addictions. Dietrich et al. (2014) linked heightened or reduced reward sensitivity and concluded that this link plays one of the key roles in the development of obesity. They also showed that the link between body weight status and behavioral characteristics are gender-specific. Interestingly, Pendergast et al. (2014) demonstrated that the source of reward may be unrelated to eating behavior: an access to a running wheel in obese mice, being a source of not only exercise but also a reward, normalized circadian rhythms of eating behavior disturbed due to the high-fat, high-sugar obesogenic diet.

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Appetite

Watkins and Kim (2015) investigated the role of endocannabinoid system in macronutrient metabolism and discussed the possible appetite-stimulating role of polyunsaturated fatty acids, which are precursor ligands of cannabinoid receptors. The appetite-inhibiting effects of brain-derived neurotrophic factor midbrain dopaminergic system, hedonic eating, reward and addiction has been thoroughly reviewed by Takei et al. (2014). Basing on their original data, the authors suggested that these activities are mediated by Mammalian Target of Rapamycin, the role of which authors defined as "*a cellular crossroads for the regulation of food intake and metabolism by nutrients*" (p. 4).

The effect of the ketogenic diet on the appetite-inhibiting brain network recruiting Cholecystokinin, neuropeptide Y and Ghrelin as well as on an appetite-stimulating network involving adiponectin, GABA and adenosine monophosphate-activated protein kinase is pictured in details in the review of the EB-related effects of the ketogenic diet by Paoli et al. (2015). In a mini-review, McFadden et al. (2014) analyzed the rope of alpha-7 nicotinic acetylcholine receptor in eating behavior and its interaction with proopiomelanocortin, neuropeptide Y, GABA, serotonin, glutamate, melanin-concentrating hormone, and dopamine.

One of the feasible means of appetite control might be a generic meal replacement: in a sample of older, obese adults, it affected brain areas relevant to eating behavior where it lowered functional connectivity in insula, anterior cingulate cortex, superior temporal pole, amygdala, and hippocampus (Paolini et al., 2014). The study may have practical application to the obesity management.

Obesity

In agreement with the numerous data on the obesogenic diet, the combination of fat and sugars in snack foods triggered more profound overeating response compared with either fat or sugars or standard chow (Hoch et al., 2014). One of the most common consequences of obesity is hypertension. Smith et al. (2014) investigated the opposite link and found that an antihypertensive vasodilatory drug Losartan prevented obesity of rats fed on the obesogenic diet, perhaps via an increase in energy expenditure due to thermal dissipation through the skin.

Diet-induced obesity, human binge eating behavior and the mesolimbic pathway were analyzed by Perello et al. (2014). They discussed the integration of neuronal inputs from the hypothalamus with peripheral hormones and visceral sensory information in the arcuate hypothalamic neurons. Messina et al. (2014) reviewed the role of hypothalamic neurons, both excited and inhibited by glucose, and their interaction with Orexin-A in coordination of such processes as feeding, sleep-wakefulness, neuroendocrine function, vascular, and metabolic reactions. In the review discussing the obesity-depression relationship, Rossetti et al. (2014) described the coexisting pathways for energy homeostasis and mood balance and suggested that obesity might be considered a risk factor for depression but most likely it happens in the cases of either binge eating or metabolically precarious, abdominal adiposity.

Orexin-A can cause both hyperphagia and hypophagia; it can also modify energy expenditure through thermal dissipation (Messina et al., 2014). This is an important observation since the energy expenditure aspect is often overlooked in the obesity studies. Adding the possibility to voluntarily enhance energy expenditure eliminated the obesogenic diet's effect of high-fat, high-sucrose diet (Pendergast et al., 2014).

Conclusion

The Topic collected and connected information concerning both the underlying metabolic mechanisms and consequences of eating behaviors - the aspects tremendously important for a better understanding of normal and pathological eating behavior to better manage appetite and obesity as well as for improving professional and public education on eating and metabolic disorders.

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ORIGINAL RESEARCH ARTICLE
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Wheel-running activity modulates circadian organization and the daily rhythm of eating behavior

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Consumption of high-fat diet acutely alters the daily rhythm of eating behavior and circadian organization (the phase relationship between oscillators in central and peripheral tissues) in mice. Voluntary wheel-running activity counteracts the obesogenic effects of high-fat diet and also modulates circadian rhythms in mice. In this study, we sought to determine whether voluntary wheel-running activity could prevent the proximate effects of high-fat diet consumption on circadian organization and behavioral rhythms in mice. Mice were housed with locked or freely rotating running wheels and fed chow or high-fat diet for 1 week and rhythms of locomotor activity, eating behavior, and molecular timekeeping (PERIOD2::LUCIFERASE luminescence rhythms) in *ex vivo* tissues were measured. Wheel-running activity delayed the phase of the liver rhythm by 4 h in both chow- and high-fat diet-fed mice. The delayed liver phase was specific to wheel-running activity since an enriched environment without the running wheel did not alter the phase of the liver rhythm. In addition, wheel-running activity modulated the effect of high-fat diet consumption on the daily rhythm of eating behavior. While high-fat diet consumption caused eating events to be more evenly dispersed across the 24 h-day in both locked-wheel and wheel-running mice, the effect of high-fat diet was much less pronounced in wheel-running mice. Together these data demonstrate that wheel-running activity is a salient factor that modulates liver phase and eating behavior rhythms in both chow- and high-fat-diet fed mice. Wheel-running activity in mice is both a source of exercise and a self-motivating, rewarding behavior. Understanding the putative reward-related mechanisms whereby wheel-running activity alters circadian rhythms could have implications for human obesity since palatable food and exercise may modulate similar reward circuits.

Keywords: circadian, C57BL/6J, mice, voluntary exercise, eating behavior, metabolism, obesity, liver

INTRODUCTION

Circadian rhythms of physiology and behavior have endogenous ∼24-h periods that synchronize to environmental cycles of light/dark and food availability (Takahashi et al., 2001). In mammals, the master circadian clock, located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus, receives direct input from the retina about the environmental light-dark cycle and coordinates the timing of clocks in extra-SCN brain regions and peripheral tissues (Yamazaki et al., 2000; Abe et al., 2002; Yoo et al., 2004). Changes in environmental cycles of light/dark and timing of food intake, such as occurs with transmeridian air travel or rotating shift-work, induces internal desynchronization of circadian clocks so that physiological and behavioral rhythms are no longer optimally tuned to environmental conditions (Yamazaki et al., 2000).

Mice fed high-fat diet develop diet-induced obesity, which is characterized by increased body weight and fat mass (Surwit et al., 1988; Krawczewski Carhuatanta et al., 2011). Chronic high-fat diet consumption also alters daily rhythms of locomotor activity, body temperature, and food intake in mice (Kohsaka et al., 2007; Mendoza et al., 2008). We recently investigated the proximate effects of high-fat diet consumption on daily rhythms of behavior and on the phase relationship between tissue oscillators in mice (i.e., circadian organization). We measured the phases of the circadian gene fusion protein reporter rhythms [PERIOD2::LUCIFERASE (PER2::LUC)] in explanted tissues and found that most tissue clocks, including the SCN, arcuate nucleus of the hypothalamus, and white adipose tissue, are resistant to the acute effects of high-fat diet consumption (Pendergast et al., 2013). In contrast, the phase of the liver rhythm is markedly advanced after only 1 week of high-fat diet consumption. The expression of transcripts and metabolites in the liver are profoundly altered after only 3 days of high-fat diet consumption, demonstrating the acute metabolic consequences of high-fat diet on the mouse liver (Eckel-Mahan et al., 2013). In addition to the alteration of the liver rhythm, the daily rhythm of eating behavior is immediately altered by high-fat diet as eating events become uniformly distributed across the day and night.

Voluntary wheel-running activity decreases fat mass and increases energy expenditure in mice fed high-fat diets (Bell et al., 1995; Krawczewski Carhuatanta et al., 2011; Scarpace et al., 2012; Meek et al., 2013). Wheel-running activity also alters circadian rhythms in mice. For example, wheel-running activity shortens the period of the activity rhythm and accelerates re-entrainment to shifted light-dark cycles (Harrington et al., 2007; Leise et al., 2013). Wheel-running activity also alters the phase of the liver rhythm in chow-fed mice (Schroeder et al., 2012). In this study, we sought to determine whether voluntary wheel-running activity counteracts the proximate effects of high-fat diet consumption on circadian organization and behavioral rhythms in mice.

MATERIALS AND METHODS

ANIMALS

Male C57BL/6J heterozygous PER2::LUCIFERASE mice (Yoo et al., 2004) (N21–23; backcrossed to C57BL/6J mice from The Jackson Laboratory) were bred in the Vanderbilt University animal facility in a 12 h-light/12 h-dark cycle (12L:12D; light intensity ∼350 lux). Breeders were fed chow (13.5% kcal from fat, LabDiet 5L0D) and water *ad libitum*. Genotyping was performed by measuring light emission from a tail piece with a luminometer. Mice were euthanized by cervical dislocation followed by decapitation. The Vanderbilt University Institutional Animal Care and Use Committee approved all experiments (M/08/096).

EXPERIMENTAL PROTOCOL

At 21 days old, male heterozygous PER2::LUC mice were weaned and group housed (2–4 mice per cage). Chow and water were provided *ad libitum*. At 7 weeks old, mice were singly housed in cages (33 × 17 × 14 cm) and provided with chow *ad libitum*. The cages contained either locked wheels (wheels could not rotate) or freely rotating wheels and were housed in light-tight boxes in 12L:12D (light intensity 200–300 lux; temperature inside light-tight boxes: 25.5 ± 1.5°C). At 8 weeks old, the chow was replaced with either high-fat diet (45% kcal from fat; Research Diets D01060502) or with fresh chow. Food was changed within 3 h of lights off. One mouse was excluded from the analysis because it did not have any eating events for 12 h after high-fat diet was placed in the food hopper. To evaluate the effect of environmental enrichment without a running wheel, the experiment was performed as described above except that mice were single-housed in empty cages or in cages with 1 paper-based refuge hut and 2 sheets of nesting paper. At 9 weeks old, PER2::LUC expression was measured in *ex vivo* tissues. Body weight and food were measured at 7, 8, and 9 weeks old within 3 h before lights off. Total kcal consumed was calculated based on the grams of food consumed (chow: 3.02 kcal/g metabolizable energy; 45% HFD: 4.73 kcal/g).

LUMINESCENCE RECORDING

Cultures were prepared within 1.5 h before lights off as we have previously described (Yamazaki and Takahashi, 2005; Pendergast et al., 2013), "The gonadal white adipose tissue (surrounding the gonads; WAT), liver, lung, spleen, aorta, pituitary, SCN, arcuate complex (containing the arcuate nucleus of the hypothalamus and ependymal cell layer as described previously Guilding et al., 2009) were collected from the same mouse." We chose tissues that regulate metabolism (liver, WAT, arcuate), food intake (arcuate), cardiovascular function (aorta, lung), immune responses (spleen), hormone secretion (pituitary), and the master circadian pacemaker (SCN). As we previously described (Pendergast et al., 2013), "Bioluminescence was monitored in real-time (at 36.8 ± 0.02°C) with the LumiCycle (Actimetrics), and photon counts were integrated over 10-min intervals. LumiCycle software (Actimetrics) was used to subtract the 24-h moving average from the raw luminescence data and to smooth the data by 0.5-h adjacent averaging. To determine period and phase, the detrended and smoothed data were exported to ClockLab (Actimetrics). The period was determined by fitting a regression line to the acrophase of at least 3 days of the PER2::LUC rhythm. The period of the rhythm in liver explants spontaneously changes after 3–4 cycles in culture. The periods reported for liver (**Table 1**) are the first periods measured in culture. The phase was determined from the peak of PER2::LUC expression during the interval between 12 and 36 h in culture. LumiCycle software was used to determine the amplitude of the same cycle used to determine phase for each tissue using the sine fit curve-fitting method. Only one cycle was analyzed for amplitude because the period and damping rate of PER2::LUC expression in liver are not constant (Pendergast and Yamazaki, 2012). Samples with a goodness of fit less than 90% were excluded (the resulting number of samples analyzed for amplitude are reported in **Table 1**). The amplitudes of the rhythms from arcuate explants could not be analyzed because the goodness of fit was always less than 90% because the bioluminescence traces were noisy due to the low level of light emission from the tissue."

LOCOMOTOR ACTIVITY AND EATING BEHAVIOR

We measured general activity and eating behavior according to our previously reported method (Pendergast et al., 2013). General activity was monitored every minute with passive infrared sensors (sensors record a maximum of 1 count every 6 s; model 007.1, Visonic LTD) and the number of wheel revolutions was recorded every minute (Figure S1) using Clocklab (Actimetrics). One mouse was excluded from the analysis because it ran fewer than 10,000 revolutions/day on Day 7 of the protocol. As we previously described (Pendergast et al., 2013), "An infrared video camera (PYLE PLCM22IR Flush Mount Rear View Camera with 0.5 Lux Night Vision, Pyle Audio Inc, Brooklyn, NY, USA) was connected to a computer with the VideoSecu 4 Channel PCI DVR Card for CCTV Home Security Surveillance System C53 (VideoSecu, Carrollton, TX). DVR software by EYEONET (AMETA International Co. Ltd, Markham, Ontario, Canada) was used to record and analyze the images. To reduce the size of the video file, images were recorded only when the mouse approached the feeder. When the mouse entered a defined region around the feeder, images were captured 1 frame every second for 5 s. Observers (Julie S. Pendergast, Katrina L. Branecky) watched the videos and coded eating behavior in 1-min bins. Eating behavior was defined based on the following criteria: The mouse either: (1) took food from the feeder with its mouth or hands; or (2) moved food in the feeder with its mouth; or (3) ate food in its paws away from the feeder; and this eating behavior persisted for 3 or more seconds (to distinguish eating from other behaviors

Table 1 | Circadian parameters of bioluminescence rhythms in *ex vivo* tissues.

		Locked wheel		Free wheel		
	Tissue	**Chow: mean ± *SD* (*n*)**	**HFD: mean ± *SD* (*n*)**	**Chow: mean ± *SD* (*n*)**	**HFD: mean ± *SD* (*n*)**	***P****
Period (h)	SCN	24.34 ± 0.25 (7)	24.35 ± 0.11 (7)	24.29 ± 0.24 (6)	24.23 ± 0.19 (6)	NS
	Pituitary	23.37 ± 0.27 (6)	23.73 ± 0.59 (7)	23.47 ± 0.47 (6)	23.46 ± 0.50 (6)	NS
	Liver	21.59 ± 0.66 (5)	21.28 *h* ± 0.60 (5)	20.68 ± 1.03 (5)	20.24 ± 0.54 (6)	Wheel: $F_{(1,\ 17)} = 9.52$ $p < 0.01$
	Lung	23.98 ± 0.35 (6)	23.99 ± 0.45 (7)	24.00 ± 0.40 (6)	23.76 ± 0.18 (6)	NS
	Spleen	24.40 ± 0.44 (6)	24.34 ± 0.33 (7)	24.42 ± 0.83 (6)	24.15 ± 0.34 (6)	NS
	Aorta	24.54 ± 0.17 (4)	24.53 ± 0.33 (3)	24.03 ± 0.36 (6)	23.91 ± 0.49 (6)	Wheel: $F_{(1,\ 15)} = 9.66$ $p < 0.01$
	Arcuate complex	23.37 ± 0.88 (3)	23.17 ± 0.51 (5)	22.53 ± 0.36 (4)	22.35 ± 0.67 (4)	Wheel: $F_{(1,\ 12)} = 7.40$ $p = 0.01$
	Gonadal WAT	25.85 ± 0.54 (4)	25.59 ± 0.77 (6)	25.62 ± 0.51 (6)	25.74 ± 0.43 (6)	NS
Amplitude of bioluminescence (counts/s)	SCN	36.03 ± 14.35 (7)	27.61 ± 11.79 (7)	48.89 ± 12.90 (6)	52.17 ± 33.84 (6)	Wheel: $F_{(1,\ 22)} = 5.77$ $p = 0.03$
	Pituitary	37.61 ± 11.16 (7)	29.15 ± 11.23 (6)	54.58 ± 26.04 (6)	37.91 ± 10.24 (6)	NS
	Liver	9.43 ± 5.38 (6)	18.32 ± 13.82 (6)	8.97 ± 4.29 (5)	10.96 ± 10.62 (6)	NS
	Lung	26.71 ± 8.31 (7)	21.89 ± 8.68 (7)	34.47 ± 12.34 (6)	22.15 ± 8.69 (6)	Diet: $F_{(1,\ 22)} = 5.21$ $p = 0.03$
	Spleen	23.58 ± 9.98 (7)	21.57 ± 6.23 (7)	30.14 ± 12.92 (6)	27.24 ± 5.85 (6)	NS
	Aorta	25.61 ± 13.18 (3)	15.70 ± 2.59 (3)	38.32 ± 15.74 (5)	34.15 ± 19.65 (6)	NS
	Arcuate complex	ND	ND	ND	ND	ND
	Gonadal WAT	11.27 ± 6.3 (6)	15.81 ± 7.03 (3)	15.04 ± 6.33 (6)	9.13 ± 9.39 (6)	NS

**The data were compared by 2 × 2 factorial analysis. None of the tissues had a significant interaction between wheel and diet. Some tissues showed a significant effect of wheel or diet, as reported in the table. NS indicates that no significant differences were found.*

such as sniffing). When eating behavior occurred, that 1-min was coded as "1" (a 1-min bin could only have a maximum value of "1" regardless of whether eating persisted for 3 s or 1 min), while 1-min bins without eating behavior were coded as "0." General activity and wheel-running activity were plotted similarly to eating behavior, such that each 1-min bin was coded as either "1" when activity occurred or "0" when there was no activity" (Pendergast et al., 2013). For each mouse, eating, general activity, and wheel-running events during 1 day of chow (Day 7) and during 1 day of high-fat diet (Day 9) consumption were plotted in circular histogram plots in 2.5° bins (10-min) using Oriana 4.0 software (Kovach Computing Services, Wales, UK). Grand mean vectors were calculated for each group ($n = 5$ mice/group).

STATISTICAL ANALYSIS

A 2 × 2 factorial treatment arrangement followed by *post-hoc* Fisher's least significant difference (LSD) tests were used to analyze body weight gain, food intake, and the phases, periods, and amplitudes of tissue PER2::LUC rhythms using SPSS 13.0. Independent *t*-tests (two-tailed) were used to compare body weight, food intake, and the phases of liver PER2::LUC rhythms for the environmental enrichment experiment. Circular data were analyzed and plotted using Oriana 4.0. Rayleigh's Uniformity test was used to determine if the activity, wheel-running, and eating events of individual mice had a significant non-uniform direction (for individual mice). Hotelling's one sample test was used to test if there was a significant mean direction (for grand mean vectors). The mean angle (μ) ± circular standard deviation *(SD)* and vector length (r; degree of clustering) are reported. The grand mean vectors could not be compared by circular Two-Way ANOVA because they violated the assumptions of the test (the concentration, k, was not equivalent between groups and κ was not ≥ 2). All data are the mean ± *SD*. Significance was ascribed at $p < 0.05$.

RESULTS

EFFECTS OF HIGH-FAT DIET AND WHEEL-RUNNING ACTIVITY ON WEIGHT GAIN AND FOOD INTAKE

We first measured weight gain (**Figure 1A**) and food intake (**Figure 1B**) in male mice that were single-housed with either a locked wheel (the wheel was present but could not rotate) or a freely rotating wheel. Body weight did not differ between the groups at baseline when the mice were singly housed (7 weeks old) nor after 1 week of single-housing (8 weeks old; Table S1). All mice were fed chow for 1 week; mice with running wheels consumed significantly more chow (in kcal of metabolizable energy) than mice with locked wheels [$F_{(1,\ 32)} = 10.53$, $p < 0.01$, Table S1]. Mice were then fed either chow or HFD (45% kcal fat) for the subsequent week. Mice with locked wheels consuming HFD gained more weight than chow-fed mice [$F_{(1,\ 32)} = 4.39$, $p = 0.04$, *post-hoc* $p < 0.001$ **Figure 1A**]. In contrast, when mice were allowed to run on the wheels, weight gain did not differ between mice fed chow and HFD (**Figure 1A**). Food intake was

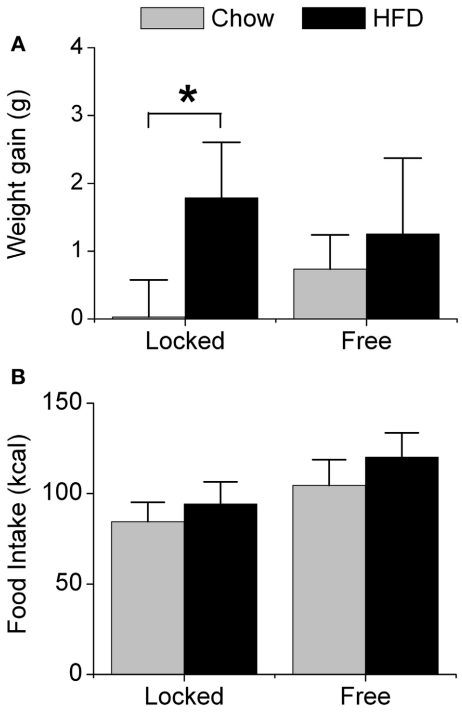

FIGURE 1 | Acute effect of wheel-running activity on body weight and food intake. Male heterozygous PER2::LUC mice were single-housed at 7 weeks old in cages with either locked or freely rotating running wheels and provided with chow *ad libitum* for 1 week. At 8 weeks old, mice were provided with fresh chow (gray) or high-fat diet (HFD; black) for 1 week. The mean (±*SD*) weight gain (**A**; grams) and food intake (**B**; kcal metabolizable energy) were determined during 1 week of chow or HFD feeding. There was a main effect of diet [$F_{(1,\ 32)} = 14.93$, $p < 0.001$] and a significant interaction between wheel and diet [$F_{(1,\ 32)} = 4.39$, $p = 0.04$] on body weight gain. Mice with locked wheels consuming HFD gained more weight than chow-fed mice (*$p < 0.001$). There were significant main effects of wheel [$F_{(1,\ 32)} = 27.15$, $p < 0.001$] and diet [$F_{(1,\ 32)} = 8.28$, $p < 0.01$] on food intake. Locked/chow $n = 7$; Locked/HFD $n = 12$; Free/chow $n = 6$; Free/HFD $n = 11$.

significantly increased by both wheel-running activity and HFD [**Figure 1B**; main effect of wheel: $F_{(1,\ 32)} = 27.15$, $p < 0.001$; main effect of diet: $F_{(1,\ 32)} = 8.28$, $p < 0.01$].

HIGH-FAT DIET AND WHEEL-RUNNING ACTIVITY INDEPENDENTLY ALTER CIRCADIAN ORGANIZATION

We next determined the effect of wheel-running activity on the circadian organization of mice fed chow or HFD by measuring the phases of the PER2::LUC bioluminescence rhythms in tissue explants (**Figure 2**; **Table 1**). We found that the phases of the aorta, arcuate complex, pituitary, white adipose tissue, and SCN were not altered by wheel-running activity or diet. The phase of the lung was delayed by wheel-running activity in both chow- and HFD-fed mice [main effect of wheel: $F_{(1,\ 22)} = 9.22$, $p = 0.006$], but was not altered by diet. The phase of the liver was delayed by wheel-running activity [main effect of wheel: $F_{(1,\ 20)} = 30.72$, $p < 0.001$] and advanced by high-fat diet [main effect of diet: $F_{(1,\ 20)} = 71.25$, $p < 0.001$], but there was no interaction between wheel activity and diet. Likewise, the phase of

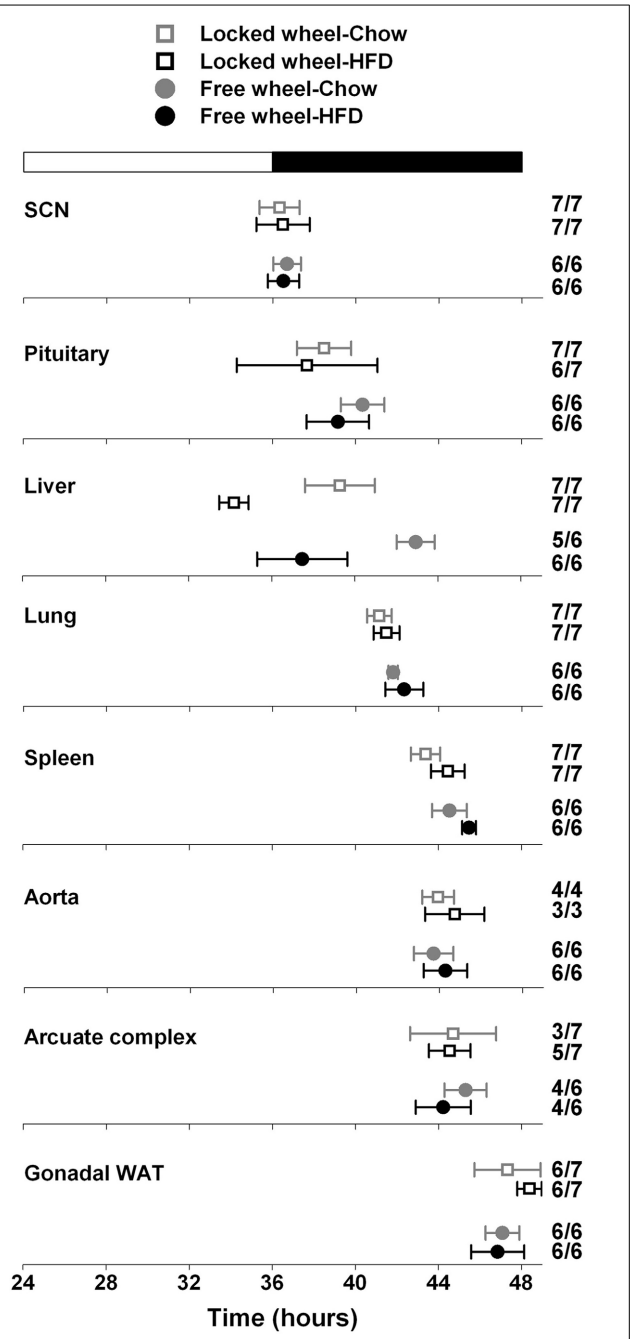

FIGURE 2 | Wheel-running activity affects the phase of the liver clock. Male heterozygous PER2::LUC mice were single-housed in cages with either locked (open squares) or freely rotating (filled circles) running wheels. Mice were fed chow (gray symbols) or HFD (black symbols) for 1 week. The mean (±*SD*) phases were determined from the peaks of PER2::LUC expression during the interval between 12 and 36 h in culture and were plotted relative to the time of last lights on where 24 h is lights on and 36 h is lights off (white and black bar at top). The sample size is shown (number of rhythmic tissues/number of tissues tested). Locked wheel data is taken from Pendergast et al. (2013). There were main effects of wheel on the phases of the lung [$F_{(1,\ 22)} = 9.22$, $p = 0.006$], liver [$F_{(1,\ 20)} = 30.72$, $p < 0.001$], and spleen [$F_{(1,\ 21)} = 15.29$, $p < 0.001$] and main effects of diet on the phases of the liver ($F = 71.25$, $p < 0.001$) and spleen ($F = 13.14$, $p = 0.002$). There was no effect of an interaction between wheel and diet on the phase of any tissues.

the spleen was delayed by wheel-running activity [main effect of wheel: $F_{(1,\ 21)} = 15.29$, $p < 0.001$] and advanced by HFD [main effect of diet: $F_{(1,\ 21)} = 13.14$, $p = 0.002$]. Together, these data demonstrate that circadian organization was altered by wheel-running activity or consumption of high-fat diet.

Compared to mice with locked wheels, the periods of the PER2::LUC rhythms in the liver, aorta, and arcuate complex were shortened by wheel-running activity, while the periods of the other tissues were not affected by activity or diet (**Table 1**). Wheel-running activity increased the amplitude of the PER2::LUC rhythm in the SCN in both chow- and HFD-fed mice [main effect of wheel: $F_{(1,\ 22)} = 5.77$, $p = 0.03$; **Table 1**]. The amplitude of the lung was greater in chow-fed compared to HFD-fed mice [main effect of diet: $F_{(1,\ 22)} = 5.21$, $p = 0.03$; **Table 1**]. The amplitudes of the other tissues were not altered by activity or diet (**Table 1**).

CIRCADIAN ORGANIZATION IS NOT ALTERED BY ENVIRONMENTAL ENRICHMENT WITHOUT A RUNNING WHEEL

A previous study demonstrated that mice in an enriched environment which included running wheels, toys, and mazes were protected from diet-induced obesity (Cao et al., 2011). Since we found that wheel-running activity delayed the phase of the liver (**Figure 2**), we next examined whether this effect was specific to wheel-running activity, or whether environmental enrichment without a running wheel could alter circadian organization. Mice were single-housed in either empty cages or in cages with environmental enrichment (1 refuge hut and 2 nesting papers) and fed HFD for 1 week (**Figure 3**). HFD consumption for 1 week caused body weight gain in mice housed in both the enriched environment (2.23 ± 0.51 g) and in empty cages (1.65 ± 0.19 g). There were no differences in body weight gain or food intake between mice housed in empty cages or in cages with environmental enrichment (Table S2). The phases of the PER2::LUC rhythms in liver, lung, and spleen were not altered by environmental enrichment (**Figure 3**). These data demonstrate that alteration of liver phase in mice housed with running wheels is specific to wheel-running activity and not attributed to an enriched environment.

EFFECTS OF DIET AND WHEEL-RUNNING ACTIVITY ON LOCOMOTOR ACTIVITY RHYTHMS

We next determined the effect of diet and wheel-running on daily rhythms of locomotor activity (**Figure 4**; Figures S3, S4). To visualize the distribution of activity over the 24-h day, we plotted locomotor activity in circular histograms. The phase and distribution of general activity (**Figures 4A,B,E,F**) and wheel-running activity (**Figures 4G,H**) were quantified for each mouse by determining the direction and length, respectively, of the vector of daily activity (Tables S3, S4). Consistent with our previous study, the pattern of general activity in mice with locked wheels, measured by passive infrared sensors, changed immediately upon addition of HFD (Figure S1A; **Figures 4A,B**). During chow consumption, mice had 5 to 6 bouts of consolidated activity during the light phase and these bouts dissipated during HFD consumption. A similar HFD-induced change in the pattern of general activity was observed in mice with freely rotating wheels (Figure S1C; **Figures 4E,F**). Compared to chow feeding, the mean

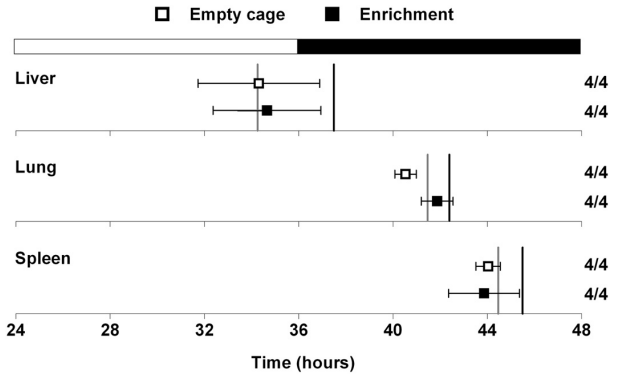

FIGURE 3 | Effect of environmental enrichment on tissue rhythms. Male heterozygous PER2::LUC mice were single-housed in either empty cages (open squares) or in cages with enrichment (1 cardboard hut and 2 pieces of shredding paper; filled squares). Mice were fed high-fat diet for 1 week. The mean (±*SD*) phases were determined from the peaks of PER2::LUC expression during the interval between 12 and 36 h in culture and were plotted relative to the time of last lights on where 24 h is lights on and 36 h is lights off (black and white bar at top). The sample size is shown (number of rhythmic tissues/number of tissues tested). The phases of the PER2::LUC rhythms in liver, lung, and spleen were not altered by environmental enrichment. For reference the gray vertical lines show the phases of the tissues from mice housed with locked wheels and the black vertical lines show the phases of the tissues from mice with freely rotating wheels (data shown in **Figure 2**).

phase of the general activity rhythm was delayed by HFD consumption in mice with either locked or freely rotating wheels (**Figure 4I**). Similarly, the phase of the wheel-running activity rhythm was delayed by HFD consumption compared to chow (**Figure 4J**).

WHEEL-RUNNING ACTIVITY MODULATES THE EFFECT OF HIGH-FAT DIET CONSUMPTION ON THE EATING BEHAVIOR RHYTHM

We previously found that high-fat diet altered the daily rhythm of eating behavior (Pendergast et al., 2013). To determine if wheel-running activity could modify the effect of HFD consumption on the daily rhythm of eating behavior, we continuously monitored eating events with an infrared camera and plotted eating events in circular histograms (**Figures 5**, S5). The phase and distribution of eating events were quantified for each mouse by determining the direction and length, respectively, of the vector of daily eating behavior (**Figures 5A–D**; Table S5). When mice were provided with chow, eating events were consolidated during the night (**Figures 5A,C**) and the direction and length of the mean vectors of eating behavior did not differ between mice with locked or freely rotating wheels (**Figure 5E**: black and green vectors; Table S5). Consistent with our previous results, the eating behavior rhythm was rapidly altered (within 1 day) in mice given HFD with locked wheels such that eating events became more evenly distributed across the day and night (**Figure 5B**), as indicated by a decrease in the length of the mean vector of eating behavior (**Figure 5E**: blue vector; Table S5). However, when mice had freely rotating wheels, the effect of HFD consumption on the distribution of eating events was less marked (**Figure 5D**). With running wheel activity, eating events occurred mostly during the

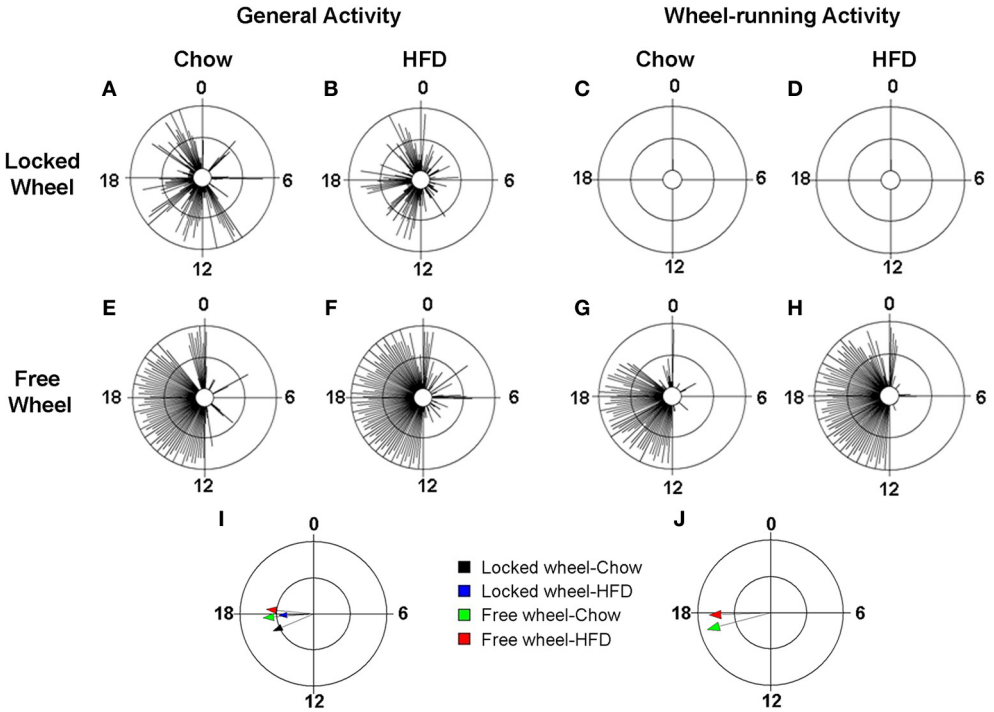

FIGURE 4 | Locomotor activity rhythms in chow and high-fat diet-fed mice. Male wild-type mice were single-housed in 12L: 12D with locked **(A–D)** or freely rotating **(E–H)** running wheels at 7 weeks old. Chow was provided *ad libitum* for 1 week (Days 1–7) and then chow was replaced with high-fat diet (HFD) for 1 week (Days 8–15). Representative circular histograms (**A–H**; plotted in 10-min bins; scale: inner circle, 0; middle circle, 5.5, outer circle, 11; units: activity counts per 10-min bin) show the distribution of general activity (**A,B,E,F**; measured with a passive infrared sensor) and wheel activity (**G,H**; no wheel revs in **C,D** because wheel was locked) for a mouse during chow (Day 7; left panels) and HFD (Day 9; right panels) consumption relative to the time of day (where 0 is lights on and 12 is lights off). Grand mean vectors of general activity (**I**; $n = 5$ mice/group) and wheel-running activity (**J**; $n = 5$ mice/group) during chow (black and green arrows) or HFD (blue and red arrows) feeding in mice with locked (black and blue arrows) or freely-rotating wheels are plotted on the circular histogram (plotted in 10-min bins; scale: inner circle, 0.35; outer circle, 0.7). The size and length of the arrow represents the uniformity of the distribution of activity where small, short arrows indicate that activity is more evenly distributed across the cycle.

night, with some events occurring during the day (**Figure 5D**). The length of the mean vector of HFD eating events for wheel-running mice (**Figure 5E**: red vector; Table S4) was intermediate to that of HFD eating in mice with locked wheels (**Figure 5E**: blue vector) and mice consuming chow (**Figure 5E**: black and blue vectors). The mean phase of the eating events was delayed in wheel-running mice (**Figure 5E**: green and red vectors; Table S4) compared to mice with locked wheels (**Figure 5E**: black and blue vectors).

DISCUSSION

Voluntary wheel-running activity prevents the obesogenic effects of high-fat diet in mice (Krawczewski Carhuatanta et al., 2011). We previously showed that after one week of HFD consumption, mice experienced a 10% increase in body mass, the phase of their liver clock was advanced by 5 h, and their eating behavior rhythm was less robust (Pendergast et al., 2013). Since wheel-running activity is effective in preventing diet-induced obesity and also has potent effects on circadian parameters of behavior, we postulated that it would modulate the effects of HFD consumption on tissue and behavior rhythms.

In our previous study, we identified the liver clock as a proximate target of high-fat diet consumption (Pendergast et al., 2013). After only 1 week of high-fat diet feeding in mice with locked wheels, the phase of the PER2::LUC rhythm in the liver was advanced by 5 h compared to chow-fed controls. In this study, we found that wheel-running activity delayed the phase of the liver PER2::LUC rhythm by 3 h compared to mice housed with locked wheels in both chow- and HFD-fed mice. Thus, the magnitude of the phase advance of the liver rhythm caused by HFD consumption was equivalent in wheel-running and locked-wheel mice. These data demonstrate that both wheel-running activity and HFD can alter the phase of the liver.

A complex enriched environment consisting of group-housed mice in cages with running wheels, mazes, and toys prevented weight gain in mice fed HFD for 4 weeks (Cao et al., 2011). In mice, consumption of HFD is akin to administration of drugs of abuse and environmental enrichment blunts the rewarding effects of drugs (Xu et al., 2007; El Rawas et al., 2009; Pandit et al., 2012). Thus it is possible that environmental enrichment decreases the reward associated with HFD, resulting in protection from diet-induced obesity. However, enriched environments typically contain a running wheel, so it is difficult to distinguish the effects of running wheel activity, which increases exercise and is itself a unique motivated behavior, from the other enhanced sensory and cognitive experiences of the enriched environment.

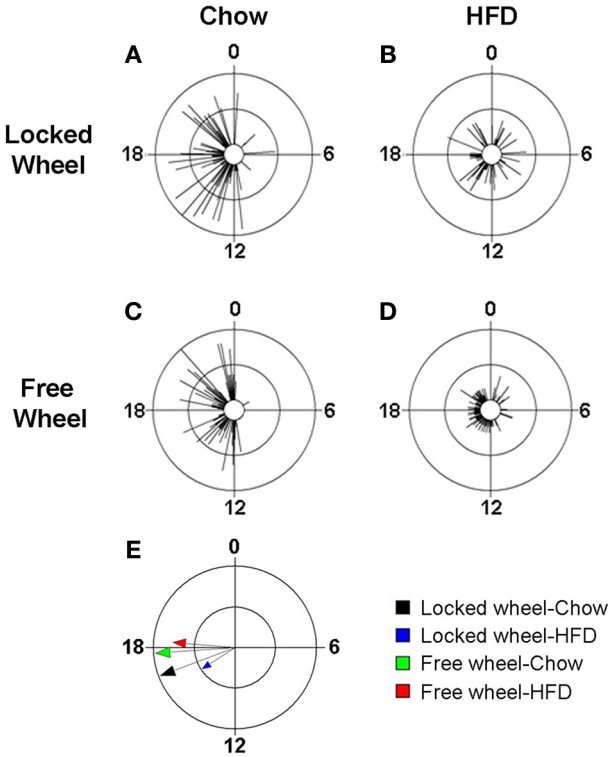

FIGURE 5 | Wheel-running activity alters the eating behavior rhythm in mice fed high-fat diet. Male wild-type mice were single-housed in 12L: 12D with a locked **(A,B)** or freely rotating **(C,D)** running wheels at 7 weeks old. Chow was provided *ad libitum* for 1 week (Days 1–7) and then chow was replaced with high-fat diet (HFD) for 1 week (Days 8–15). Representative circular histograms (**A–D**; plotted in 10-min bins; scale: inner circle, 0; middle circle, 5.5, outer circle, 11; units: eating events per 10-min bin) show the distribution of eating events for a mouse during chow (Day 7; left panels) and HFD (Day 9; right panels) consumption relative to the time of day (where 0 is lights on and 12 is lights off). Grand mean vectors of eating behavior (**E**; $n = 5$ mice/group) during chow (black and green arrows) or HFD (blue and red arrows) feeding in mice with locked (black and blue arrows) or freely-rotating wheels are plotted on the circular histogram (plotted in 10-min bins; scale: inner circle, 0.3; outer circle, 0.6). The size and length of the arrow represents the uniformity of the distribution of the eating events where small, short arrows indicate that eating events are more evenly distributed across the cycle.

We determined if the delay of the liver phase was specific to wheel-running activity or if an enriched environment without the running wheel could alter circadian organization. We found that circadian organization did not differ between HFD-fed mice in empty cages and those in cages with environmental enrichment without a running wheel. These findings suggest that the running wheel specifically alters circadian organization.

We found that the periods of the PER2::LUC rhythms in liver, aorta, and arcuate nucleus of the hypothalamus were shorter in wheel-running compared to locked-wheel mice. We do not know how wheel-running activity results in the shortening of the periods of tissue rhythms. However, it has been demonstrated that wheel-running activity alters physiological processes and gene expression in liver, aorta, and hypothalamus and it is possible that these changes could relate to alterations in the period of the circadian rhythm (Haskell-Luevano et al., 2009; Werner et al., 2009; Kim et al., 2010).

In this study, we examined the effect of HFD and wheel-running activity on three different behavioral outputs. We first assessed general activity measured by passive infrared sensors, which detected the spectrum of mouse behaviors, including grooming, eating, drinking, nesting, and exploring (climbing, sniffing, digging). We found that regardless of whether the mice had locked or freely rotating wheels, HFD consumption had similar effects on the pattern of general activity such that consolidated daytime bouts of activity dissipated during HFD. HFD consumption delayed the phase of general activity in both locked and wheel-running mice, but the distribution of activity (length of vector) was affected only in mice with locked wheels. General activity became more evenly distributed across the day and night during HFD consumption by mice with locked wheels.

We also measured wheel-running activity, which is postulated to be a motivated and/or rewarding behavior in mice (Sherwin, 1998). We found that the phase of the wheel-running rhythm was delayed by consumption of HFD compared to chow, but the number of wheel revolutions and the distribution of wheel activity (length of vector) were not altered by HFD.

We also specifically measured eating behavior by continuously recording mouse behavior at the feeder and scoring eating events. In contrast to general activity, we found that wheel-running activity modulated the effect of HFD consumption on the daily rhythm of eating behavior. While HFD consumption caused eating events to be more evenly dispersed across the 24 h-day in both locked-wheel and wheel-running mice, the effect of HFD was much less pronounced in wheel-running mice.

For both eating behavior and liver rhythms, wheel-running activity caused phase delays but the magnitudes of the delays in behavior and in the phases of liver explants were different (Figure S6). Thus, we hypothesize that HFD consumption and wheel-running activity act through distinct pathways to alter liver phase, but they converge to modulate eating behavior. These data suggest that the phase of the liver cannot be solely explained by the change in the eating behavior rhythm during *ad libitum* feeding. This is in contrast to previous studies where restricted meal timing entrained the phase of the liver (Stokkan et al., 2001, but see Hatori et al., 2012 for restricted feeding of high-fat diet). There are a couple of potential explanations for the apparent disconnect between the eating behavior rhythm and liver phase. First, we analyzed the eating behavior rhythm on the first full day of high-fat consumption while liver phase was analyzed after 1 week of HFD consumption. Second, future studies should examine whether the phases of food intake rhythms (as opposed to eating behavior) and liver rhythms are correlated in mice with locked and freely rotating wheels.

Wheel-running activity in mice is both a source of exercise and a self-motivating, rewarding behavior. It is unclear whether it is the exercise, the reward, or both properties of wheel-running activity that alter liver phase and the daily rhythm of eating behavior. Previous studies have demonstrated that wheel-running activity is salient in modulating circadian rhythms in rodents, while forced treadmill exercise may not be as effective, thus it

has been hypothesized that wheel-running activity may be a unique motivational state that impacts upon circadian rhythmicity (Mistlberger, 1991; Janik and Mrosovsky, 1993; Mistlberger et al., 1996 but see Marchant and Mistlberger, 1996; Wolff and Esser, 2012). Future experiments could examine the effect of forced treadmill running, which provides exercise without self-motivated reward, on circadian organization and eating behavior. Understanding the putative reward-related mechanisms whereby wheel-running activity alters circadian rhythms could have implications for human obesity since palatable food (like drugs of addiction) is rewarding and may promote dependence (Volkow et al., 2012).

AUTHOR CONTRIBUTIONS

Julie S. Pendergast designed and performed experiments, analyzed data, and wrote the manuscript. Katrina L. Branecky and Roya Huang performed experiments and analyzed data. Kevin D. Niswender contributed to the design of the experiments and participated in discussion and writing of the manuscript. Shin Yamazaki designed and performed experiments and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fpsyg.2014.00177/abstract

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Meal replacement: calming the hot-state brain network of appetite

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There is a growing awareness in the field of neuroscience that the self-regulation of eating behavior is driven by complex networks within the brain. These networks may be vulnerable to "hot states" which people can move into and out of dynamically throughout the course of a day as a function of changes in affect or visceral cues. The goal of the current study was to identify and determine differences in the Hot-state Brain Network of Appetite (HBN-A) that exists after a brief period of food restraint followed either by the consumption of a meal replacement (MR) or water. Fourteen overweight/obese adults came to our laboratory on two different occasions. Both times they consumed a controlled breakfast meal and then were restricted from eating for 2.5 h prior to an MRI scan. On one visit, they consumed a meal replacement (MR) liquid meal after this period of food restriction; on the other visit they consumed an equal amount of water. After these manipulations, the participants underwent a resting fMRI scan. Our first study aim employed an exploratory, data-driven approach to identify hubs relevant to the HBN-A. Using data from the water condition, five regions were found to be the hubs or nodes of the HBN-A: insula, anterior cingulated cortex, the superior temporal pole, the amygdala, and the hippocampus. We then demonstrated that the consumption of a liquid MR dampened interconnectivity between the nodes of the HBN-A as compared to water. Importantly and consistent with these network data, the consumption of a MR beverage also lowered state cravings and hunger.

Keywords: meal replacement, craving, eating behavior, obesity, brain networks, graph-theory

INTRODUCTION

There is a growing awareness in the field of neuroscience that the self-regulation of eating behavior is driven by complex networks within the brain that control "liking" and "wanting" of food. In extensive research, Berridge et al. (2010) have shown that "liking" represents the hedonic facet of this process whereas "wanting" refers to incentive salience motivation. The authors emphasize that "wanting" can be motivational even when hedonic "liking" does not arise. Although it is not possible to discern "liking" from "wanting" in most experimental paradigms, there is evidence that the desire to consume food is based upon activity in brain networks that vary as a function of individual differences (Rejeski et al., 2012), environmental stimuli (Stoeckel et al., 2009; Bullins et al., 2013; Kullmann et al., 2013), and homeostatic drive (Berthoud, 2012). In line with Loewenstein (2005), people can move in and out of "hot states" dynamically throughout the course of a day as a function of changes in affect or visceral cues; thus, the desire for food or the preoccupation with it is disproportionately higher in hot than cold states (see also Kavanaugh, 2005). The goal of the current study is to identify and determine differences in the hot-state brain network of appetite (HBN-A) that exists in a resting state after a brief period of food restraint followed either by the consumption of a meal replacement (MR) or water.

The study design we employed involved having participants (a) come to our laboratory on two occasions so that we could feed them a controlled morning meal, (b) insure that they did not eat for 2.5 h, (c) deliver on a randomized schedule either water or a MR, and (d) then have them participate in resting fMRI scans. Thus, we were interested in whether there was an identifiable HBN-A in a resting state following a brief period of food restraint and, whether MR calmed this network as compared to water. In identifying the HBN-A, we were guided by both existing research and an empirically-driven process. Specifically, a recent review by Stice and colleagues has summarized the integrative signaling of the brain reward system, including both the homeostatic and hedonic feeding systems (Lowe and Butryn, 2007; Stice et al., 2013). Much work has been done in this field by other investigators as well (Berridge et al., 2010; Kringelbach et al., 2012); however, the review by Stice et al. (2013) is one of the most comprehensive in identifying areas at all levels of the brain, from the subcortical to the neocortex, that are involved in eating behavior. Based upon this review and other published work (Bechara et al., 2000; Tracy et al., 2001; Kringelbach, 2004; Olson et al., 2007;

Stoeckel et al., 2008; Pessoa, 2010; Carnell et al., 2012; Kringelbach et al., 2012; Paolini et al., 2012; Rejeski et al., 2012), we anticipated that the HBN-A would consist of at least 4 primary regions: the insula, hippocampus, amygdala, and anterior cingulate cortex. Due to limitations of fMRI technology, we did not anticipate detecting small, deep brain structures such as the parabrachial nucleus or the ventral tegmental area identified by previous investigators (Kringelbach et al., 2012; Stice et al., 2013). Similarly, because our study involved resting state without active processing of food cues, we did not expect involvement of the orbitofrontal cortex (OPF) (Kringelbach et al., 2012).

Because we are unaware of existing fMRI research on this topic using graph-theory-based methodology, the first phase of our analysis was designed to qualitatively evaluate and empirically confirm the network hubs of relevance to the HBN-A. To ensure high sensitivity in this phase, we used a p value of 0.10 to identify network hubs. Once the structure of the HBN-A was established, we then examined whether the consumption of a MR altered connectivity in this network as compared to water. This second phase, which was the primary aim of the study, employed a per comparison error rate of $p = 0.05$. Based upon clinical research which has shown that MR products are effective in curbing appetite and in promoting weight loss (Rothacker et al., 2001; Heymsfield et al., 2003; Annunziato et al., 2009; Frestedt et al., 2012), we hypothesized that MR would decrease connectivity within the HBN-A when compared to water and that this effect would be evident for both direct and indirect connections within the HBN-A. We also evaluated state craving and hunger and expected both to be higher in the water than MR condition.

METHODS

PARTICIPANTS

A sample ($n = 14$) of older, overweight and obese (BMI $\geq$ 28 kg/m^2 but $\leq$ 40 kg/m^2) adults was recruited from Forsyth County, NC. All participants were between the ages of 50 and 79 and lived independently. The sample included an equal number of men and women. Each participant completed a phone screen, an in-person screening visit, and two 5-h experimental sessions at Wake Forest School of Medicine, receiving a maximum of $225 for completing all three visits to compensate for their time.

PRESCREENING AND LOST TO FOLLOW-UP

A telephone screen was administered to interested individuals to determine their qualifications as potential participants. Exclusion criteria included (1) having a BMI outside our established range, (2) the presence of a systemic uncontrolled disease or psychiatric illness, (3) a binge eating disorder, (4) high alcoholic intake (more than 3 drinks per day), (5) the inability to safely undergo magnetic resonance imaging due to claustrophobia or to the presence of implanted magnetic objects/devices, (6) currently undergoing treatment for cancer, (7) active participation in another research study that might interfere with either the study's procedures or objectives, (8) need for assistance while walking, (9) being unable to read or speak English, or (10) the inability to correct eyesight to at least 20/40 in the scanner to complete the required tasks. Over 125 people were contacted by phone about the study, of these 22 were brought in for in-person screening visits, 7 participants did not complete the study due to various reasons: withdrawal due to claustrophobia and poor vision in the scanner. One participant was excluded from the data analyses due to poor quality of brain images during one functional scan that could not be corrected using computer software.

MEASURES

Food Craving Questionnaire (the FCQ$_{state}$)

The state version of the FCQ was used to measure food cravings and to obtain a measure of hunger as a manipulation check (Cepeda-Benito et al., 2000). The craving measure consists of 15 items that target preferred foods using a 5-point scale (1 = strongly disagree; 5 = strongly agree) with the mid-point being anchored by the label neutral; thus, total scores can range from 15 to 75. In our own work, we have found the FCQ state to be very sensitive to food restraint (Rejeski et al., 2010). The 15-items are averaged for a total score. In addition, three items from the FCQ can be used to derive an index of hunger. The Cronbach alpha internal consistency reliabilities in this study for both scales were excellent, $\geq$0.90.

The Interview for the Diagnosis of Eating Disorders (IDED-IV)

The semi-structured interview described by Kutlesic et al. (1998) was employed to exclude any potential participants that might have a binge-eating disorder as defined by the DSM-IV criteria Dr. Williamson, an investigator involved in the development of the IDED-IV, provided the training on how to screen for Binge Eating.

IN-PERSON SCREENING AND ASSESSMENTS

An in-person screening visit was completed to obtain an informed consent, collect biometric data, assess current states of physical activity and possible dieting, and to screen for binge eating disorders. The IDED-IV was used to identify and exclude people with possible eating disorders. Eligible individuals were scheduled for two imaging visits 7–10 days apart. If necessary, participants were fitted for MRI-safe corrective lenses to be used in the scanner during computer tasks.

EXPERIMENTAL PROTOCOL FOR THE SCANNING VISITS

Participants completed two 5-h visits beginning in the early morning around 8:00 a.m. Participants were asked to arrive in a fasting state, having not eaten breakfast or consumed anything other than water. During each visit, participants ate a prepared breakfast containing 350 calories for females and 450 calories for males. The meals were designed by a staff nutritionist to provide a heart healthy balance of macronutrients containing approximately 25% fat, 15% protein, and 60% carbohydrates. Participants were allowed to choose macronutrients from a menu. Following the consumption of at least 75% of their breakfast, participants completed a baseline FCQ$_{state}$. The participants then fasted for 2.5 h under the supervision of research center nursing staff.

Approximately 45 min before the imaging procedure, the research staff then administered the MRI safety form and led each participant in a practice session of the tasks to be completed during the fMRI. About 30 min before the scan time, participants either consumed a can of the Nestle MR beverage BOOST® (short

term energy surfeit containing 240 calories, vanilla flavor) or an equivalent volume of water. They then completed a second round of the FCQ_{state}. The assignment of the MR and water condition was randomized.

RESTING STATE SCANNING TASKS

Participants wore goggles (Resonance Technology, www.mrivideo.com) in the scanner that were directly interfaced with a computer screen. The MRI consisted of a resting-state session where individuals viewed a cross on the computer screen interfaced with their goggles for a period of 5 min.

SCANNING PROTOCOL

All scans were performed on a 1.5 GTE scanner using an 8 channel neurovascular head coil (GE Medical Systems, Milwaukee, WI, USA) and included anatomic imaging, perfusion, and one resting state fMRI. All fMRI data was used to evaluate differences in brain networks between each individual's MR and water treatment condition.

Functional images for the network analyzes measured changes in the T2*-relaxation rate that accompany changes in blood oxygenation. The T2* signal is sensitive to changes in blood oxygen content. As brain activity changes, the oxygen content of the blood in the same area also changes. Thus, the T2* signal is an indirect measure of changes in neural activity (Ogawa et al., 1990). Functional imaging was performed using multi-slice gradient EPI ($TR = 2000$ ms; $TE = 40$ ms; field of view = 24 cm (frequency) × 15 cm (phase); matrix size = 3.75 × 3.75 × 5 mm).

IMAGING PROCESSING AND NETWORK ANALYSES

In preparation for generating brain networks, all scanning images were realigned and normalized to standard space using FSL (Smith et al., 2004). The time courses were extracted for each voxel in gray matter based on the Automated Anatomical Labeling atlas (Tzourio-Mazoyer et al., 2002) and band-pass filtered to remove signals outside the 0.009–0.08 Hz range (Biswal et al., 1995). To account for physiological noise, mean white matter, CSF, and motion correction parameters were regressed from the filtered time series. This regression procedure removes signal fluctuations that are unlikely to be from neuronal activity (Fox et al., 2005). A correlation matrix was then created by computing Pearson correlations between all possible pairs of voxels (~21,000 voxels). This produced a 21,000 × 21,000 matrix which each cell (ij) representing the correlation coefficient between nodes i and j. A threshold was then applied to the correlation matrix and all cells that surpassed this threshold were assigned a value of 1 while remaining cells were assigned a value of zero. The threshold was defined so that the relationship between the number of nodes and average number of connections at each node was consistent across subjects to produce an adjacency matrix. Specifically, the relationship $S = \log(N)/\log -(K)$ was the same across subjects as described above (Hayasaka and Laurienti, 2010). The threshold $S = 2.5$ was used for this paper. This resulted in networks with connection densities meeting expected values based on the size of the networks (Laurienti et al., 2011). All remaining analyses were completed using the binary $21,000 \times 21,000$ adjacency matrix.

To define hubs-of-interest, we generated degree (K) maps for each individual. Degree is the number of functional connections linked to a node. For each individual, we generate a degree (K) map which gives a degree value (i.e., number of connections) for each voxel in the network. In order to compare regions-of-interest (ROIs) between the two conditions, we generated the average number of connections for each ROI for each individual. This allowed us to run a paired t-test to test the difference in degree or number of connections for each ROI between the MR and water conditions. The same ROIs defined below were used to evaluate average degree for each individual.

To assess network organization, first order (direct) and second order (indirect) connection analyses were performed for ROIs that qualified as hubs-of-interest. First order connections are the immediate network neighbors of the hubs identified because they share direct connections. In this paper, we calculate the direct connections as the number of direct connections between two ROIs (i.e., the insula and the amygdala). Second order connections are areas that have direct connections to the immediate neighbors of the hubs (**Figure 1**). Indirect connections between two ROIs can be asymmetric because the ROIs may or may not share the same number of connections to those neighbors. This asymmetry for indirect connections between two hubs does not reflect a difference in directionality of information flow; instead, it reflects a difference in the complexity of the connections between the two ROIs. For instance, a large number of indirect connections from ROI-A to ROI-B indicates a large number of connections from ROI-A's neighbors to ROI-B; whereas, a small number of indirect connections from ROI-A to ROI-B indicates that there are a small number of connections from ROI-B's neighbors to ROI-A.

For our analysis, the following ROIs were used: insula, defined by the Automated Anatomic Labeling (AAL) atlas; amygdalae, defined by the AAL atlas; the superior temporal pole, defined by the AAL atlas; anterior cingulate cortex, defined as a sphere with a radius of 10 mm with the MNI coordinates ($x = 4, y = 38, z = 0$); the right hippocampus, defined as a sphere with a radius of 6 mm and the MNI coordinates ($x = 21, y = -7, z = -18$) based on the region with the highest degree in the degree map. The ACC and the right hippocampus ROIs used were determined by drawing spheres over the region using the WFU pick-atlas

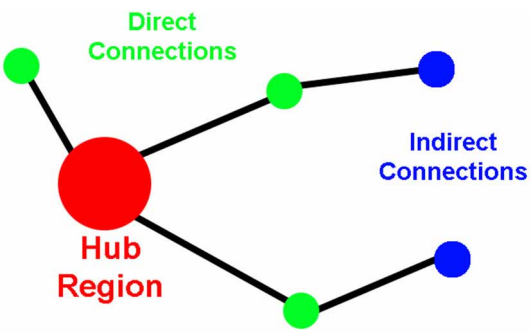

FIGURE 1 | Cartoon demonstrating the properties of the direct and the indirect connections to network hubs.

software (Tzourio-Mazoyer et al., 2002; Maldjian et al., 2003). For each ROI created with the exception of the hippocampus, both the left and right corresponding regions were included. The ROI used for the hippocampus was unilateral because the right side was the only side that differed between MR and water conditions.

The ROIs generated in pick-atlas software are at a $2 \times 2 \times 2$ mm resolution. All ROIs were resliced to $4 \times 4 \times 5$ mm, the resolution of the imaging data, for this analysis. Standard reslicing practices combine a subset of original voxels into a new larger sized voxel. This process causes adjacent ROIs to overlap and to share common voxels. To correct for this issue, every resliced voxel is assigned to a single ROI. This assignment is based on the most frequent assignment of the original $2 \times 2 \times 2$ mm voxels in the AAL atlas.

ANALYTICAL STRATEGY AND STATISTICAL ANALYSES

In this paper, resting brain network data collected during the water condition was used to provide empirical support for the existence of a hot-state brain network for appetite (HBN-A). We relied on both extant research and an empirical approach to locate "hubs of interest" in the HBN-A during our first-stage, exploratory analysis. Thus, we used a data-drive approach to the identification of our hubs. The hubs were then used as seeds for further analysis of connectivity within the network, which was our primary outcome. It is important to note that we used node degree to inform our connectivity analyses, and the connectivity analyses to inform our identification of hubs. We defined hubs-of-interest as regions-of-interest (ROIs) that had a larger degree (i.e., greater number of connections) in the water than MR condition. For our first-stage, exploratory analysis of hubs of interest, we set the alpha level for hubs-of-interest at $p < 0.10$ which allowed us to capture ROIs that may have important connectivity profiles despite not reaching a conventional level of significance using the degree metric.

In addition, a second phase for validating the conceptual import of each hub was to examine first order (direct) and second order (indirect) connections between the hubs-of-interest. To be included in the HBN-A, hubs not only had to pass the first level of screening based on degree, but also had to show evidence of either first or second order connectivity to one of the other hubs-of-interest. If the hub met these two criteria, it was then considered a node in the HBN-A. Once the nodes of the HBN-A were established, we then compared connectivity in this network for the water condition vs. the MR treatment condition. It is important to note that all hypothesis tests conducted were based on paired *t*-tests and a 0.05 pairwise level of significance.

We used a mixed model ANCOVA to test the effect of the MR manipulation on the food craving questionnaire. In this analysis, we controlled for the assessment taken at the time of the post-breakfast feeding as well as the random subject effect. The outcome of interest was the craving responses taken just prior to conducting the fMRI scans.

RESULTS

Participants (7 males and 7 females) had a mean (*SD*) age of 71.35 (4.92) years with a Body Mass Index of 30.43 (2.09). Two of our participants were African American and the remaining 12 were Caucasian. The leading comorbidities included arthritis ($n = 8$) and hypertension ($n = 4$); these health conditions were followed by cancer ($n = 2$) and cardiovascular disease ($n = 1$).

The efficacy of the MR manipulation was supported by data showing that ratings of hunger from the food craving inventory were higher on the day that participants consumed water prior to the scanning procedure, mean (SE) = 9.00 (0.67), as compared to the day they received a MR, mean (SE) = 6.78 (0.75); $t = 2.14$ (13), $p = 0.05$.

CRAVING

As discussed above, a mixed-model ANCOVA was employed to examine how participants' level of state craving was influenced by the two treatment conditions (MR or water). Analysis of the FCQ_{state} measure produced a significant main effect for the MR manipulation [$F_{(1,\ 12)} = 6.23$, $p = 0.028$]. Cravings were significantly higher in the water than the MR condition, respectively: LS means (SE) = 41.74 (2.41) vs. 33.62 (2.41).

NETWORK ANALYSES

Our initial analysis examined which ROIs served as hubs-of-interest for the hot-state network. This step involved a qualitative comparison of the resting brain networks in the water and MR treatment conditions that was guided by evidence from existing research. We then conducted statistical tests to empirically validate the qualitative findings. Using this method, five hubs met the criteria and were included as nodes of the HBN-A: ACC ($p = 0.053$), the right hippocampus ($p = 0.030$), superior temporal pole ($p = 0.029$), insula ($p = 0.030$), and amygdala ($p = 0.079$). **Table 1** provides results for the between condition statistical analyses of these hubs; whereas, **Figure 2** shows the location of the high-degree nodes that were spatially consistent across individuals.

Within **Figure 2** it is also important to note that the precuneus had higher degree in the MR than the water condition ($p = 0.05$). However, in defining the HBN-A, we were interested in ROIs that were hubs in the water condition; these ROIs did not include the precuneus. Moreover, the precuneus did not have a greater number of connections in the water condition compared to the MR condition or to any of the other hubs-of-interest in the HBN-A.

Figures 3–6 illustrate the number of direct (upper panel in each figure) and indirect (lower panel in each figure) connections between each of the five nodes identified in **Figure 2**. Supporting data from statistical tests can be found in **Tables 2**, **3**. In each case,

Table 1 | Summary of hubs of the HBN-A.

Degree profile	Water [Mean, (SE)]	MR [Mean, (SE)]	*p*-value
ACC	78.000 (10.689)	50.888 (6.879)	0.053
Hippocampus	127.152 (24.982)	61.800 (7.900)	0.030*
STP	120.051 (20.866)	76.790 (8.820)	0.029*
Insula	73.127 (8.321)	49.703 (5.412)	0.030*
Amygdala	48.42 (7.103)	34.485 (6.380)	0.079
Precuneus	83.348 (12.171)	133.551 (24.868)	0.050

*STP, superior temporal pole; ACC, Anterior Cingulate Cortex; *, significance.*

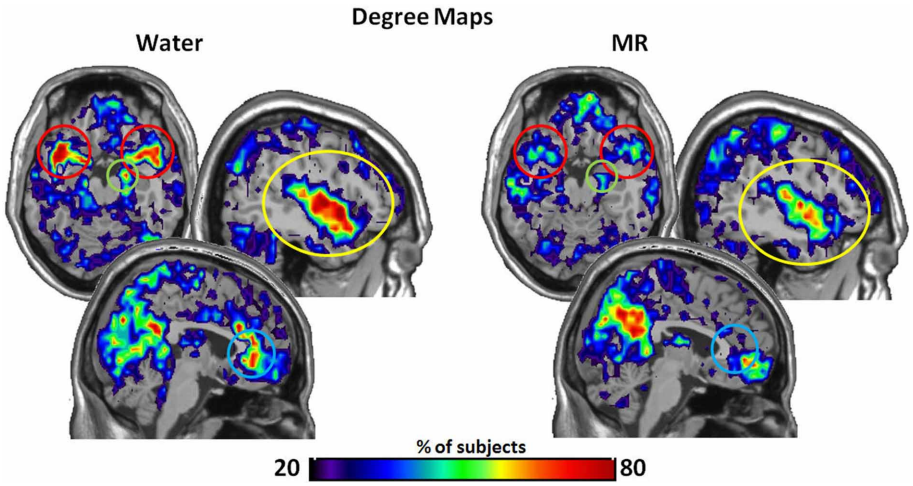

FIGURE 2 | The maps demonstrate brain areas found to consistently have high degree (i.e. number of connections). For each subject, the voxels with degree values in the top 20% were identified. The maps shown here represent the overlap of these voxels across subjects in each condition. The consistency of overlap between conditions is indicated by the color bar which represents the percentage of individuals for which each voxel was among the top 20%. On the top left are axial slices (MNI $z = 54$) through the superior temporal pole and amygdala (red circle) and the right hippocampus (green circle). On the top right are sagittal slices (MNI $x = 139$) through the insula (yellow circle). Finally the images on the bottom are a sagittal slices (MNI $x = 95$) through the anterior cingulate (blue circle). **Figures 3–7** are shown with these same slices. *The figure highlights that the superior temporal pole, right hippocampus, the ACC, and the insula have greater connectivity in the water condition than in the MR condition during resting state.*

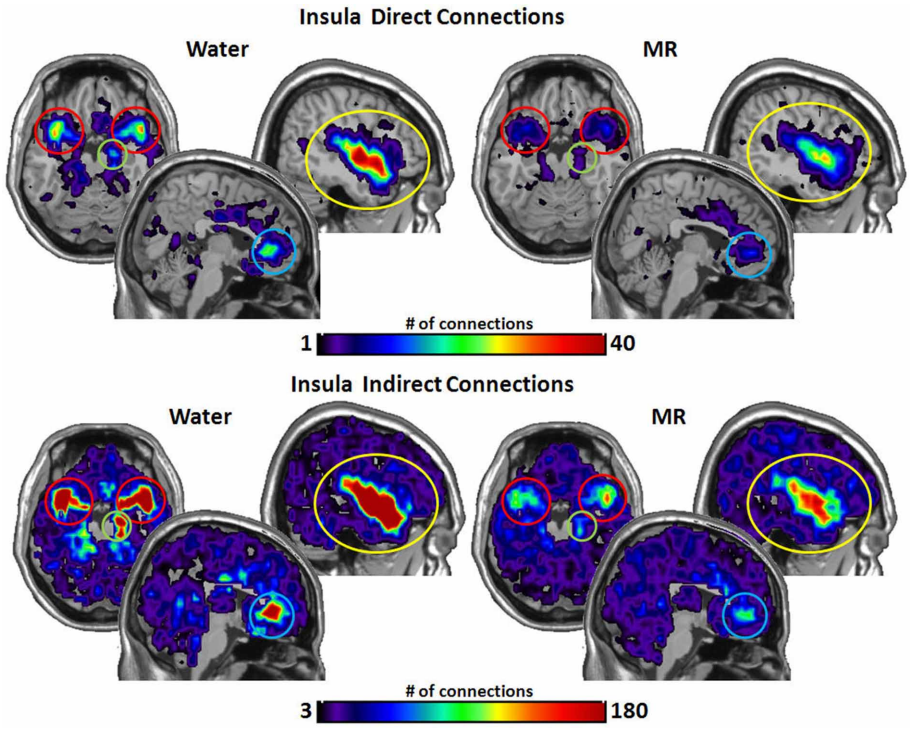

FIGURE 3 | These maps show the total number of connections from the insula during resting state averaged across individuals. The brain images on the top demonstrate the insula's average number of direct connections with other brain regions. *These images demonstrate that the insula has a greater number of direct connections with the ACC, STP, Amygdala, and the hippocampus during the water condition vs. the MR condition.* The brain images on the bottom demonstrate the total number of indirect connections from the insula averaged across participants. *These images demonstrate that during the water condition, the insula has a greater number of indirect connections with the ACC, STP, amygdala, and hippocampus than during the MR condition.*

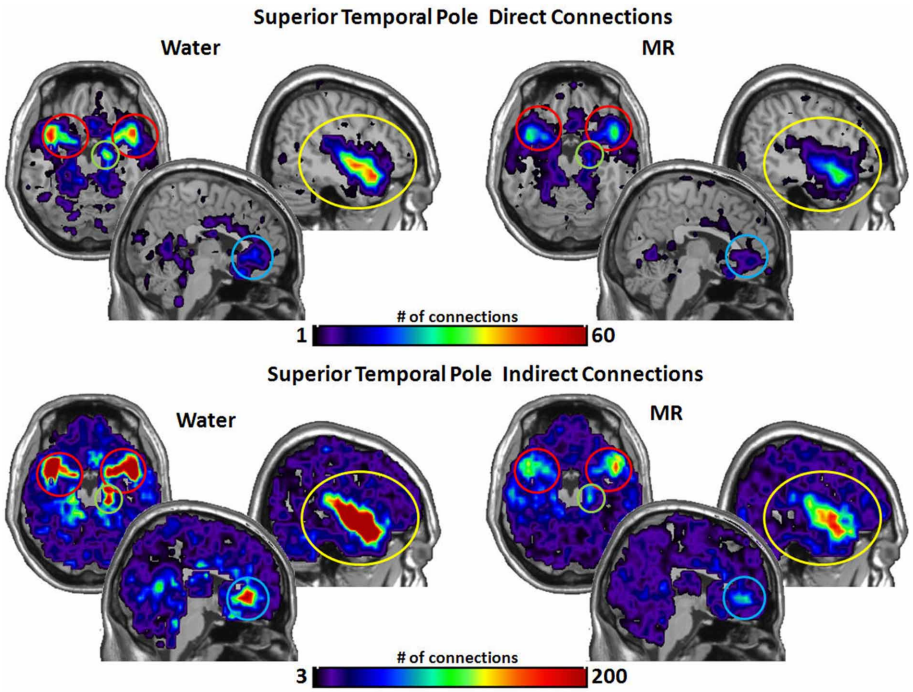

FIGURE 4 | The brain images on the top demonstrate the total number of direct connections from the superior temporal pole (STP) averaged across participants. *These images demonstrate that the STP has a greater number of direct connections with the insula and the hippocampus during the water than in the MR condition.* The difference in direct connections with the ACC and the amygdala did not reach significance ($p = 0.059$ and $p = 0.052$, respectively). The brain images on the bottom demonstrate the total number of indirect connections from the STP averaged across individuals. These images demonstrate that during the water condition the STP has a greater number of indirect connections to the insula, ACC, and the hippocampus than during the MR condition. The indirect connections to the insula were marginally significant ($p = 0.051$).

the number of connections is always higher in the water condition as compared to the MR condition. Also, a large number of direct and indirect connections can be observed within each seed ROI; however, this would be expected. For defining the HBN-A, we were interested in the interconnections from each ROI to every other ROI in the HBN-A.

Inspection of the data in **Figure 3** shows that the insula had significantly more direct connections with the superior temporal pole (STP) (red circle, $p = 0.018$), to the anterior cingulate cortex (ACC) (blue circle, $p = 0.021$), the hippocampus (green circle, $p = 0.029$), and the amygdala (red circle, $p = 0.045$) in the water than the MR condition. The insula also exhibited greater indirect connections to the same four nodes within the HBN-A during the water condition: the STP (red circle, $p = 0.032$), the ACC (blue circle, $p = 0.024$), hippocampus (green circle, $p = 0.030$) and the amygdala (red circle, $p = 0.050$).

As shown in **Figure 4**, the STP had significantly more direct connections with the insula ($p = 0.018$) and with the hippocampus ($p = 0.016$) in the water than MR condition. Although the STP appears to have had a greater number of direct connections with the ACC and the amygdala (**Figure 4**), these trends were just below an alpha level of 0.05; that is, the probability values were $p = 0.059$ and $p = 0.052$, respectively. Indirect connections from the STP were also significantly greater in the water condition with the insula ($p = 0.021$), the ACC ($p = 0.017$), the hippocampus ($p = 0.027$), and marginally significant with the amygdala ($p = 0.051$).

Figure 5 illustrates the number of connections from the anterior cingulate cortex (ACC). The ACC had significantly more direct connections with the insula ($p = 0.021$) in the water condition and marginally significant greater direct connections with the STP ($p = 0.059$). However, the ACC had significantly more indirect connections to all four nodes in the water vs. the MR condition: insula ($p = 0.009$), STP ($p = 0.024$), amygdala ($p = 0.041$), and the hippocampus ($p = 0.035$).

Figure 6 illustrates the number of connections that the amygdala had within the water vs. MR condition. Direct connections were significantly greater with the insula ($p = 0.045$) with trends for the STP ($p = 0.052$) and hippocampus ($p = 0.062$). The amygdala also had a greater number of indirect connections with the insula ($p = 0.016$), with the STP ($p = 0.022$), and with the hippocampus ($p = 0.015$) in the water condition, whereas the effect with the ACC was marginally significant ($p = 0.053$).

Figure 7 captures the direct and indirect connections from the hippocampus. The hippocampus had a greater number of direct connections in the water condition with the insula ($p = 0.029$) and STP ($p = 0.016$). Indirect connections from the hippocampus were higher in the water condition with the insula ($p = 0.022$), the STP ($p = 0.025$), and the ACC ($p = 0.048$).

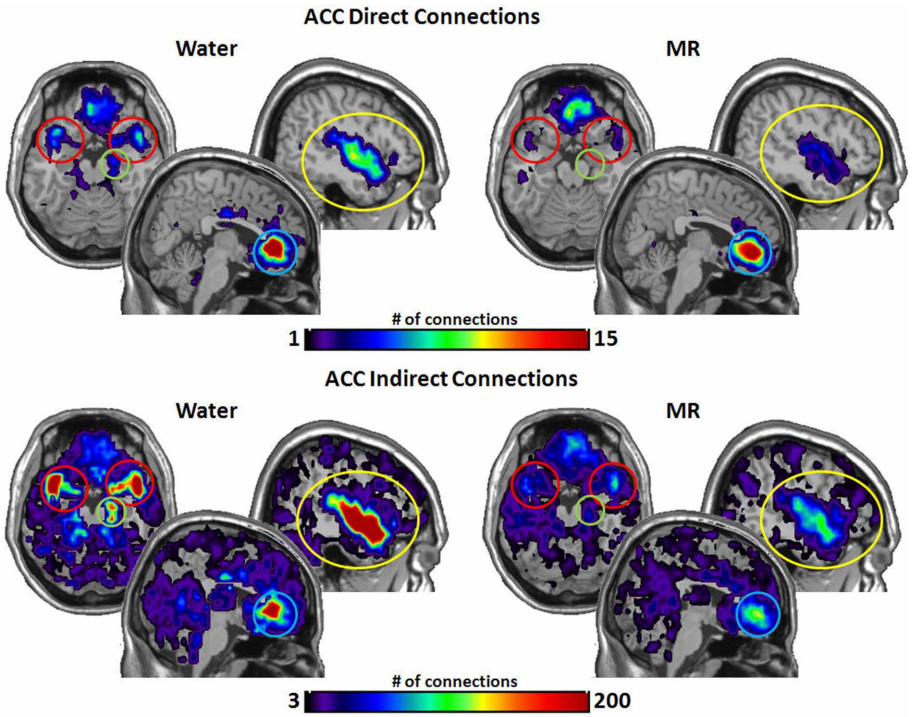

FIGURE 5 | The brain images on the top demonstrate the total number of direct connections from the anterior cingulated cortex (ACC) averaged across participants. *These images demonstrate that the ACC has a greater number of direct connections to the insula during the water than in the MR condition.* It appears that the ACC also has greater direct connections with the STP in the water condition; however, this trend did not reach statistical significance (0.059). The brain images on the bottom demonstrate the total number of indirect connections from the ACC averaged across individuals. These images demonstrate that during the water condition the ACC has a greater number of indirect connections to the insula, ACC, the amygdala and the hippocampus than during the MR condition.

Figure 8 provides a composite cartoon of both the direct and indirect connections between the nodes of the HBN-A that were greater in the water than the MR condition. Because the direct connections are first order, we refer to this circuit as the primary circuit of the HBN-A, where we use the term secondary circuit to capture the second order or indirect connections. Effect sizes are provided for each connection with both the primary and secondary circuits to provide the reader with a sense of their relative strength. For the direct connections, the effect sizes ranged from 0.38 to 0.74; whereas, for indirect connections, the effect sizes ranged from 0.53 to 0.82. It is important to note that nearly all of these effect sizes are moderate to large in magnitude.

When examining the primary circuit of the HBN-A (**Figure 8A**), there are several notable features. First, the insula is a hub in the primary circuit as it has direct connections to every other node in the HBN-A. Second, there is also a sub-circuit which includes the insula, the hippocampus, and the STP (**Figure 8A**). And third, this sub-circuit was reproduced within the secondary circuit which involved indirect connections.

Whereas the structure of the primary circuit was found to be embedded within the secondary circuit, there were many more indirect connections between the nodes of the HBN-A. The indirect connections or the secondary circuit showed high connectivity between all 4 nodes with the exception of a few connections with the amygdala. Of note, the secondary circuit revealed a greater number of indirect connections with the ACC and the amygdala with the other regions in the HBN-A. Specifically, the ACC had outgoing, indirect connections to every other node in the HBN-A. The amygdala has greater outgoing, indirect connections to the insula, the hippocampus, and the STP. The amygdala was the only ROI in the HBN-A that did not have reciprocal indirect connections to every other ROI in the HBN-A. However, it is important to point out that the amygdala showed a trend of significantly greater indirect connections to both the ACC ($p = 0.053$) and the STP ($p = 0.051$).

DISCUSSION

The consumption of a meal replacement (MR) beverage in a group of older, obese adults after a short-term period of food restraint lowered state cravings and hunger ratings. As we shall see, these shifts observed with the MR were most likely due to the alleviation of the "hot-state" created by the short-term restriction from food. Specifically, consistent with these self-report data, we also demonstrated that consumption of a MR blunted connectivity in the hot-state brain network of appetite (HBN-A). To our knowledge, this is the first paper to show that a MR can modulate brain networks after a short-term period of food restraint. A novel feature is that using graph theory we identified a complex network of indirect connectivity within the HBN-A.

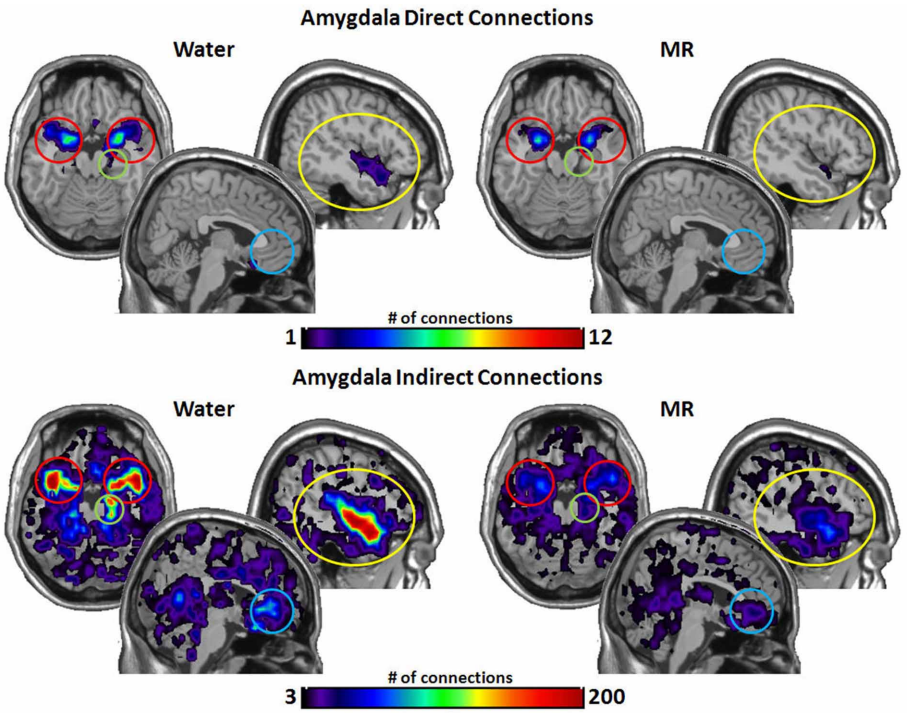

FIGURE 6 | The brain images on the top demonstrate the total number of direct connections from the amygdalae averaged across participants. *These top images demonstrate that the amygdala has a greater number of direct connections with the insula during the water than in the MR condition.* The brain images on the bottom demonstrate the total number of indirect connections from the amygdalae averaged across participants. *These images demonstrate that the amygdala has a greater number of indirect connections to the insula, the hippocampus, and the STP during the water condition than during the MR condition.* Despite visually appearing to have a greater number of connections to the ACC, this trend did not reach statistical significance ($p = 0.053$).

Table 2 | Summary of direct connections between nodes of the HBN-A.

Total number of direct connections	Water [Mean, (SE)]	MR [Mean, (SE)]	*p*-value
Insula–Amygdala	132.371 (26.765)	77.429 (18.798)	0.045*
Insula–Hippocampus	209.143 (60.357)	51.286 (12.501)	0.029*
Insula–STP	2610.571 (485.806)	1278.000 (197.122)	0.018*
Insula–ACC	707.571 (140.501)	285.214 (87.067)	0.021*
STP–Amygdala	345.857 (77.915)	178.143 (45.532)	0.052
STP–ACC	593.786 (173.683)	234.143 (83.338)	0.059
STP–Hippocampus	529.000 (105.903)	220.643 (43.129)	0.016*
ACC–Hippocampus	57.000 (25.035)	10.214 (3.598)	0.091
ACC–Amygdala	18.357 (5.990)	9.071 (3.656)	0.180
Amygdala–Hippocampus	59.286 (16.611)	27.929 (9.765)	0.062

*, *0.05.*

To define the HBN-A, we examined the resting brain network of the experimental trial in which individuals continued to refrain from eating; i.e., the water condition. Qualitatively, we observed that their brain networks had hubs in regions associated with the identification, processing, and the emotional elaboration of visceral food cues. These brain regions were defined as hubs-of-interest because they had a greater number of connections (degree) in the water vs. the MR condition. Once we defined these hubs, we were able to investigate the direct and indirect connections among these hubs between the two treatment conditions to fully characterize the network.

To our knowledge, this is the first study that uses graph theory to build and to study resting brain networks after a short-term period of food restraint. Graph theory is becoming an increasingly popular technique among neuroscientists to study human brain networks using fMRI; however, this technique is radically different from traditional fMRI methodologies as it allows the study of whole brain relationships. Thus, it is difficult to fully integrate this work with previous neuroimaging studies as we are most likely capturing a different network. As a result, we have decided to fully characterize this network and to name it the HBN-A.

In our investigation of the HBN-A, we found that when individuals continued to consume only water, their brain networks had hubs in the following regions: insula, superior temporal pole, hippocampus, ACC, and amygdala (see **Figure 2**). These hubs became the nodes of the HBN-A, having a long history of being identified with visceral sensations and hedonic attribution/elaboration (Gautier et al., 2001; Labar et al., 2001; Rothemund et al., 2007). Importantly, we do not believe the hubs defined in the HBN-A are the only regions that are active in a "hot-state." It is more than likely that these regions are the upstream result of primary, sub-cortical networks that have been well characterized as being important for eating behavior and

Table 3 | Summary of indirect connections between nodes of the HBN-A.

Total number of indirect connections	Water [Mean, (SE)]	MR [Mean, (SE)]	*p*-value
Insula–Amygdala	2107.286 (399.922)	1257.357 (306.347)	0.050*
Insula–Hippocampus	3332.143 (765.529)	1308.143 (222.055)	0.030*
Insula–STP	31331.643 (6325.345)	18182.214 (2618.435)	0.032*
Insula–ACC	8066.429 (1419.632)	3599.714 (796.690)	0.024*
STP–Amygdala	2352.071 (403.201)	1479.786 (342.118)	0.051
STP–ACC	7882.286 (1457.577)	3428.429 (585.329)	0.017*
STP–Hippocampus	3646.000 (752.764)	1599.000 (249.125)	0.027*
STP–Insula	26656.071 (3678.668)	15925.429 (2047.362)	0.021*
ACC–Insula	21480.000 (3781.479)	8227.286 (2078.500)	0.009*
ACC–Hippocampus	2429.143 (772.684)	569.857 (187.532)	0.035*
ACC–Amygdala	1180.857 (375.519)	346.571 (96.477)	0.041*
ACC–STP	24000.214 (6547.866)	8839.000 (2725.875)	0.024*
Amygdala–STP	21729.000 (5951.026)	6780.000 (1412.635)	0.022*
Amygdala–ACC	3267.643 (1089.861)	866.571 (247.474)	0.053
Amygdala–Hippocampus	2613.071 (624.028)	724.500 (192.236)	0.015*
Amygdala–Insula	12309.071 (2931.547)	3983.714 (978.878)	0.016*
Hippocampus–Amygdala	1615.429 (431.436)	775.786 (261.550)	0.068
Hippocampus–ACC	4155.786 (1491.771)	793.214 (242.229)	0.048*
Hippocampus–STP	23542.000 (6573.370)	8037.857 (2161.360)	0.025*
Hippocampus–Insula	14417.071 (3946.902)	3269.214 (795.160)	0.022*

*, *0.05.*

addiction in animal studies (Kringelbach et al., 2012; Panksepp and Biven, 2012; Stice et al., 2013). Moreover, in the current study, there was substantially greater connectivity between these nodes in the water than MR condition. As shown in **Figure 8**, nearly all of these connections had moderate to large effect sizes (Cohen, 1992).

The insula, the hub of the primary circuit (**Figure 8A**), is well-known for its association with gustation and visceral sensations in general (Critchley, 2004; Kringelbach et al., 2012; Uddin et al., 2013); however, it has also been implicated in the experience of emotions derived from bodily states (Rothemund et al., 2007; Kringelbach et al., 2012). The insula is known to have connections with the superior temporal gyrus, the temporal pole, amygdala, hippocampus and the ACC (Shelley and Trimble, 2004; Nagai et al., 2007) which was evident in our data. The centrality of the insula to the HBN-A suggests that this network is viscerally-driven and is truly embodied. In other words, visceral cues from peripheral sensatory systems literally drive activity in this network (Rejeski and Gauvin, 2013). To confirm the visceral foundation of the HBN-A, future research using this paradigm should consider evaluating hormones known to be related to appetite and monitor potential changes in the autonomic nervous system.

The temporal pole (TP), the second node of the HBN-A, is considered a paralimbic region that is important for the multisensory processing of auditory, olfactory and visual stimuli (Olson et al., 2007). It has also been implicated in the emotional processing of these multisensory stimuli, and some studies have even argued that the anterior temporal lobe is important for emotional stability (Olson et al., 2007). The temporal pole is not a region that we originally hypothesized to be a hub of the HBN-A; however, it is not surprising since the TP is an important brain region for multisensory and emotional processing.

The hippocampus, another limbic structure and the third node of the HBN-A, is classically known to be important in memory; however, it has also been implicated in eating behavior (Tracy et al., 2001; Squire, 2004; Davidson et al., 2007; Bragulat et al., 2010). There is increasing evidence that the hippocampus is important for hedonic and incentive processes and for sensing the metabolic and hormonal status of the body (Lathe, 2001; Davidson et al., 2007; Bragulat et al., 2010). It is important to note that, in our analysis of the hippocampus, connectivity differences were unilateral and localized to the right-side. Interestingly, a study by Stoeckel et al. (2009) found that obese women have significantly greater unilateral activation in the right hippocampus in response to high-calorie foods compared to controls; of note, the obese women also had greater bilateral activations in the amygdala, insula, ACC, and several other brain regions as well.

The fourth node of the HBN-A is the ACC. The ACC has traditionally been associated with regulation of attention to both cognitive (dorsal portion) and emotional processing (ventral portion) and with goal-directed behavior (Devinsky et al., 1995; Bush et al., 2000; Mohanty et al., 2007; Gasquoine, 2013). The ventral (genual or anterior) region of the ACC, the region that was found to be part of the HBN-A in this study (see **Figure 5**), has been shown to be important for emotional processing (Lindgren et al., 2012; Gasquoine, 2013). Specifically it is important for evaluating and encoding the emotional salience (pleasantness/averseness) of various stimuli including the pleasantness of fat-content in drinks and human touch (Grabenhorst et al., 2010; Lindgren et al., 2012; Gasquoine, 2013). The ACC is capable of translating its emotional processing into a behavioral consequence, in part, via the autonomic nervous system (ANS) working in conjunction with the insula (Devinsky et al., 1995; Critchley et al., 2003; Gasquoine, 2013).

The fifth and final node of the HBN-A was the amygdala. The amygdala's role in fear and aversion is well known; however, the amygdala is now considered to be important for valence attribution, as it responds to both aversive and appetitive stimuli (Ball et al., 2009). The amygdala has connections to the insula in humans (Shelley and Trimble, 2004) and to the temporal pole in marquee monkeys (Olson et al., 2007), connections we observed in our data.

The primary circuit of the HBN-A (**Figure 8A**) has a subcircuit that includes the insula, the hippocampus and the STP. This sub-circuit has the capacity to integrate visceral sensation from the insula with memory/metabolic status from the hippocampus and emotional processing of multisensory stimuli from the STP. In addition, this sub-circuit has access to emotional information from the ACC and the amygdala through the hub of the insula.

Importantly, a novel feature of this study is our ability to delineate indirect connections and thereby to create a secondary circuit. With traditional neuroimaging techniques, this would have been impossible and only the primary circuit (i.e., direct connections) could have been elucidated. However, by using graph theory to define our brain network, we were able to analyze

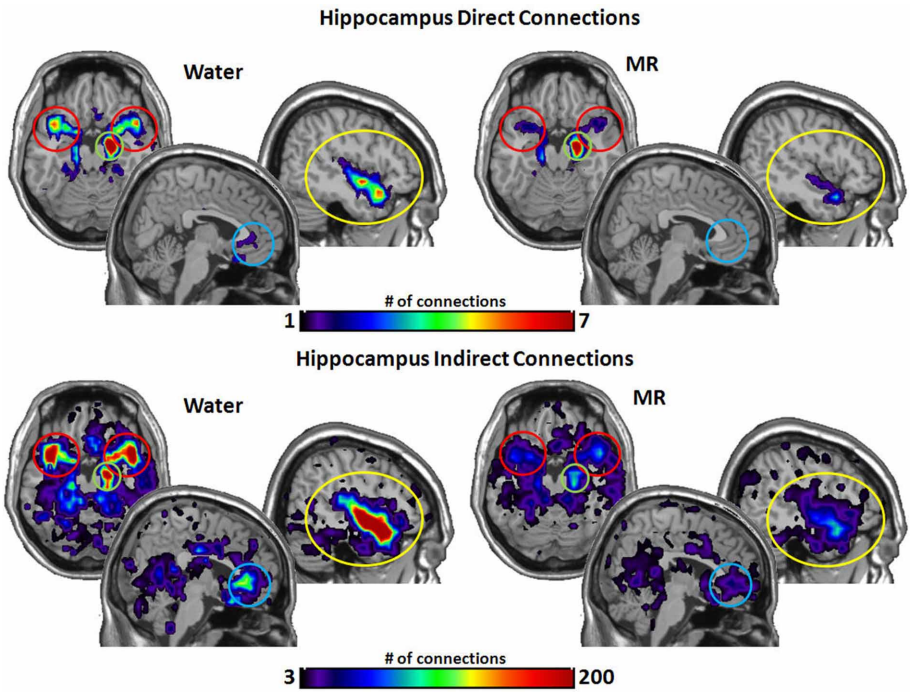

FIGURE 7 | The brain images on the top demonstrate the total number of direct connections from the right hippocampus averaged across participants. *The top images demonstrate the right hippocampus has a greater number of direct connections with the insula and to the STP in the water condition than in the MR condition.* The brain images on the bottom demonstrate the total number of indirect connections from the right hippocampus averaged across individuals. *These images demonstrate that the right hippocampus has a greater number of indirect connections to the STP, ACC, and the insula during the water condition than the MR condition.*

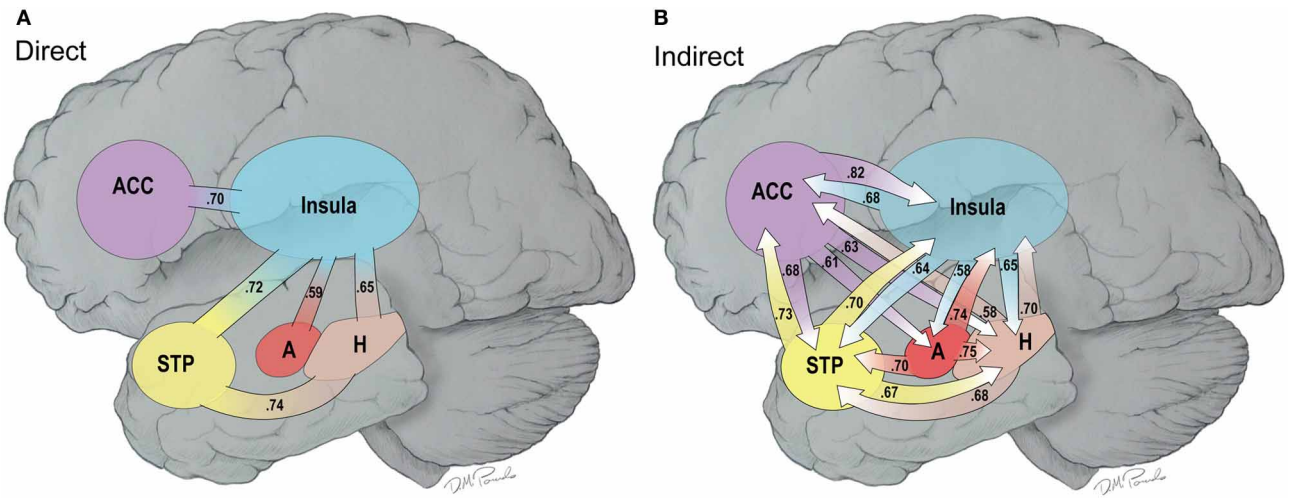

FIGURE 8 | (A,B) This is a cartoon summary of the direct **(A)** and indirect **(B)** connections among the hubs of the HNB-A that are greater in the water vs. the MR condition. The connections are weighed by effect sizes between the two conditions which are shown as part of the arrows.

indirect connections leading to the identification of the secondary circuit. This secondary circuit underscores how highly interconnected the ACC and the amygdalae are with the other regions in the HBN-A in the water condition (see **Figure 8B**). Using the direct connections only, it would appear that the ACC and the amygdalae must filter their information into the primary circuit through the hub of the insula; however, the identification of the secondary circuit suggests that this is an oversimplification. Many of the indirect connections from the ACC and amygdalae are through the insula; however, the robustness of their indirect connectivity could not have been predicted from the direct connections alone.

The secondary circuit illustrates that the indirect connections among the hubs of the HBN-A are reciprocally related to one another and highly distributed. This gives credence to the idea that brain networks are complex and do not function in a linear manner. Thus, the HBN-A is truly a circuit where sensory stimuli [visceral (insula); multisensory (STP)] and memory/metabolic status (hippocampus) are directly integrated with one another and further elaborated upon emotionally via the secondary circuit involving the ACC and the amygdala. Therefore, graph theory provides novel and important information to our understanding of functional brain network circuitry and should be further explored in future applications of functional connectivity.

Generally speaking, most regions in the HBN-A operate below the level of consciousness; thus, when people are in a "hot state" it is likely that behavior is controlled to a significant degree by automatic processes. Loewenstein (2005) has shown that when people are in "cold states" they overestimate their self-regulatory capacities; in other words, they might believe that they can control their eating behavior immediately after a noon meal, yet fail miserably at controlling consumption by mid-afternoon. Thus, future research should examine the role of the HBN-A on one's ability to control consumption. It is important to note that since MR beverages are low scoring hedonic products and are often less liked than "regular" food, it is possible that other regions may be important during active attempts at self-regulation. For instance, after a short term fast, a tasty food product may not only trigger the hubs identified in this study, but also portions of the hedonic system such as the OFC creating a more complex network. In short, at this point in time, we do not know whether the structure of the HBN-A or connectivity within this network is generalizable beyond the realm of meal replacements.

This current study is not without limitations. First, the small sample size makes us sufficiently underpowered to investigate individual differences in the HBN-A. Secondly, the target sample was restricted to an older, overweight and obese population that was not currently dieting. It is possible that differences in brain networks may have been observed if participants had been actively engaged in intentional weight loss. We also did not have a normal weight control group, meaning that these results may be limited to older adults that are overweight or obese. The results of this study should be considered specific to the population under investigation until subsequent studies confirm these effects in younger age-groups and people with more diverse biometric characteristics. Finally, the reader should be aware that we used a *per comparison* error rate for evaluating the individual connections between hubs of interest and supplemented these analyses with effect sizes. Some may view this approach as too liberal and likely to create type I errors. On the other hand, the small sample sizes often used in imaging studies makes correction for multiple comparisons challenging. We want to underscore the moderate to strong effect sizes for all comparisons conducted between the water and MR conditions providing consistent support for the HBN-A using cutting edge methods from graph theory.

In summary, our qualitative and data-drive approach was successful in defining a well interconnected HBN-A. The plausibility of the HBN-A network is supported by its significant attenuation during the consumption of a MR as compared to water. This network included many regions previous implicated in eating behavior and describes a viscerally-driven network that is rich in valence attribution and incentive-motivated processing. Furthermore, the findings of this study demonstrate that MR beverages are able to down-regulate the HBN-A, and reduce food craving/hunger following a short-term period of food restraint in older, overweight and obese adults as compared to water. In light of these findings, further research is warranted to examine how the HBN-A is related to peoples' ability to stick with daily caloric goals typically set in weight management programs. In other words, is the HBN-A a brain signature for self-regulatory failure when attempting weight loss? Does the HBN-A have important relationships with other brain regions, such as the OFC, during active attempts at self-regulation? We are beginning to address some of these questions. Currently, we are examining whether differences in the HBN-A in response to an overnight fast predicts weight loss behavior during an active weight loss intervention. Additionally, we will also investigate if weight loss blunts the HBN-A response post intervention.

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Snack food intake in ad libitum fed rats is triggered by the combination of fat and carbohydrates

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Snack food like potato chips substantially contributes to energy intake in humans. In contrast to basic food, snacks are consumed additionally to other meals and may thereby lead to non-homeostatic energy intake. Snack food is also frequently associated with hedonic hyperphagia, a food intake independent from hunger. Analysis of brain activity patterns by manganese-enhanced MRI has previously revealed that the intake of potato chips in ad libitum fed rats strongly activates the reward system of the rat brain, which may lead to hedonic hyperphagia. The purpose of the present study was to develop a two-choice preference test to identify molecular determinants of snack food triggering extra food intake in ad libitum fed rats. Different kinds of test food were presented three times a day for 10 min each time. To minimize the influence of organoleptic properties, each test food was applied in a homogenous mixture with standard chow. Food intake as well as food intake-related locomotor activity were analyzed to evaluate the effects induced by the test foods in the two-choice preference test. In summary, fat (F), carbohydrates (CH), and a mixture of fat and carbohydrates (FCH) led to a higher food intake compared to standard chow. Notably, potato chip test food (PC) was highly significantly preferred over standard chow (STD) and also over their single main macronutrients F and CH. Only FCH induced an intake comparable to PC. Despite its low energy density, fat-free potato chip test food (ffPC) was also significantly preferred over STD and CH, but not over F, FCH, and PC. Thus, it can be concluded that the combination of fat and carbohydrates is a major molecular determinant of potato chips triggering hedonic hyperphagia. The applied two-choice preference test will facilitate future studies on stimulating and suppressive effects of other food components on non-homeostatic food intake.

Keywords: snack food, food intake, macronutrients, eating behavior, rat, preference test

INTRODUCTION

Savory snacks like potato chips counted among the seven major contributors to energy intake in children and adolescents in the US during the last 21 years (Slining et al., 2013). Snack food is not part of our basic diet, but is frequently consumed additionally to other meals. Moreover, snacks show only a weak satiety effect and their calorie content is not or only partially compensated by reduced ingestion of standard meals (Whybrow et al., 2007; Chapelot, 2011). Thus, it can be concluded that snack food consumption leads to increased total energy intake. The so-called hedonic food intake is independent from hunger, may overrule the homeostatic energy balance and therefore lead to hyperphagia, i.e., food intake beyond satiety (Berthoud, 2011).

Several studies suggest that certain types of food can induce similar non-homeostatic energy intake in rats as in humans indicating the existence of a highly phylogenetically conserved neural regulation mechanism of food intake. For example, it has been shown that rats that have access to a cafeteria diet take up twice as much energy as rats with access to standard chow only. Additionally, the feeding pattern changed from meal-based food intake to snacking-based food intake (Martire et al., 2013). In a similar way, ad libitum fed rats with additional access to potato chips showed higher energy intake than rats with additional access to standard chow only (Hoch et al., 2013).

Several studies investigated the underlying physiological mechanisms which are related to non-homeostatic intake of palatable food. Recently, it was shown that a cafeteria diet affects the reward system in the rat brain (Epstein and Shaham, 2010) and that the snack food potato chips modulates the activity of brain areas that respond to cues mainly regulating reward and addiction, food intake, locomotor activity, and sleep (Hoch et al., 2013). On a molecular level, various systems are involved in the regulatory mechanisms of non-homeostatic food intake including hormones, dopamine, melanocortins or other signal molecules (Berthoud, 2011; Pandit et al., 2011; Alsio et al., 2012). For example, the hedonic intake of several snack foods seems to be regulated by the endogenous opioid system, because the opioid antagonist naltrexone attenuated the conditioned place preference induced by different solid snack foods in ad libitum fed rats (Jarosz et al., 2006). The endocannabinoid system of the gut may be an important regulator of fat intake (DiPatrizio et al., 2011).

Nevertheless, the molecular food determinants that trigger non-homeostatic food intake are not fully characterized. Several

studies used a cafeteria diet as palatable feed, which contains a selection of different articles such as cakes, pasta, potato chips, cookies, cheese, or nuts (Prats et al., 1989; Martire et al., 2013). In other studies, single food items were used, such as potato chips (Hoch et al., 2013) or Froot Loops® cereals (Jarosz et al., 2006). Excessive food intake was mostly related to the energy-, fat-, or sugar content of the food. Additionally, sensory properties were also suggested to have an influence: in well-fed rats, food intake was rather induced by the food's palatability or sensory properties, whereas the calorie content seemed to be the main contributor in rats with negative energy balance (Scheggi et al., 2013).

The aim of the current study was, therefore, to apply a two-choice food preference test that can be used to determine the activity of single components of snack food to induce food intake. Two-choice preference tests have been previously applied, for example, to test the preference of rats for food flavors, the influence of galanin administration on food choice or the relative palatability of sucrose/oil emulsions (Naim et al., 1986; Smith et al., 1996). For our purpose, a two-choice preference protocol for solid foods was modified in a way that parts of a reference powdered standard chow (STD) were replaced either by the snack food or by single components in the concentration present in the snack food. Thus, the different test foods could be tested against the STD reference and against each other. As a model for a snacking situation, the test foods were presented each time for 10 min only and the rats always had ad libitum access to standard chow pellets. This test system was then applied to analyze the effects of the macronutrients on the intake of potato chips.

MATERIALS AND METHODS

ETHIC STATEMENT

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU).

ANIMALS

Behavioral tests were conducted with 18 rats in total. Initially, the tests were conducted with eight male Wistar rats (two cages with four animals each, initial weight 210 ± 8 g, kept in a 12/12 h dark/light cycle, purchased from Charles River, Sulzfeld, Germany). The majority of experiments were reproduced with 10 male Sprague Dawley rats (two cages with five animals each, initial weight 181 ± 14 g, kept in 12/12 h dark/light cycle, purchased from Charles River, Sulzfeld, Germany). The rats had access to STD pellets (Altromin 1324, Lage, Germany) and tap water ad libitum throughout the whole study.

TEST FOODS

All test foods were prepared, mixed, and crushed in a food processor to ensure homogeneity and a similar texture. The test food PC consisted of powdered STD (Altromin 1321, Lage, Germany) in a mixture with 50% potato chips ("PFIFF Chips Salz", unflavored, salted, without added taste compounds or taste enhancers, purchased from a local supermarket; 49% carbohydrates, 35% fat, 6% protein, 4% dietary fiber, 1.8% salt). The test food ffPC contained 50% fat-free potato chips ("Lay's Light Original®", with the fat substituent olestra (OLEAN®), unflavored, salted, without added taste compounds or taste enhancers, purchased in a supermarket in the USA; 61% carbohydrates, 7% protein, 3.4% dietary fiber, 1.7% salt, 0% fat) in powdered STD. In order to test the combined influence of the macronutrients fat and carbohydrate on the palatability of potato chips, a model of the potato chips (FCH) was prepared, which consisted of 50% powdered STD and the fat and carbohydrate components of potato chips. The remaining part of the potato chips (proteins, fiber, salt, and unidentified components) was replaced by carbohydrates instead of STD in order to match the energy density of the model and PC as closely as possible. Thus, FCH consisted of 50% STD, 17.5% fat (sunflower oil, purchased from a local supermarket) and 32.5% carbohydrates (dextrin from maize starch, maltodextrine, Fluka, Taufkirchen, Germany). Additionally, the fat and carbohydrate portions of the test food FCH were tested separately. Thus, for testing the influence of the fat content (F), 17.5% fat was mixed with 82.5% STD. The effect of the carbohydrate content (CH) was tested with food consisting of 32.5% carbohydrates and 67.5% STD. The energy density of the different test foods was calculated based on the manufacturer's labeling. The calculated values and the composition of the test foods are illustrated in **Figure 1**.

EXPERIMENTAL DESIGN

For the two-choice preference tests, test foods were presented three times per day (at 9 am, 12:30 pm, and 4 pm), each time for 10 min (**Figure 2A**) in two additional food dispensers (**Figure 2B**). The test food intake was determined by the weight difference of the food dispensers before and after each access period. Energy intake

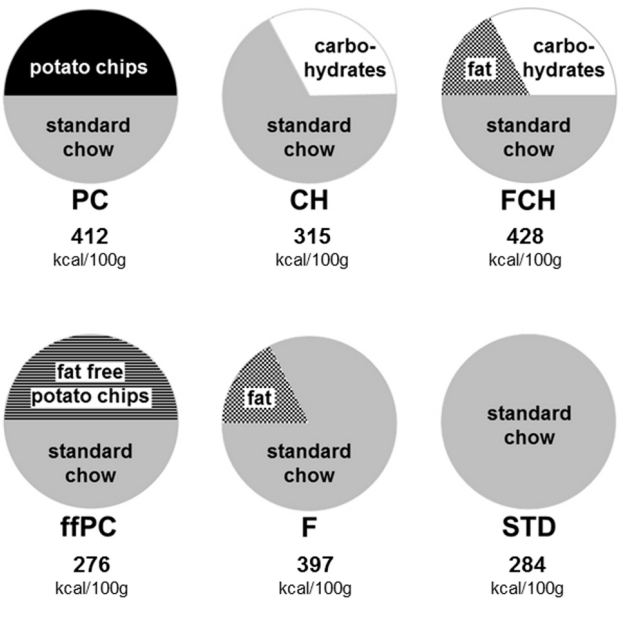

FIGURE 1 | Composition (percent by weight) and energy content (kcal/100 g) of the test foods: potato chips (PC), fat-free potato chips (ffPC), carbohydrate content of PC (CH), fat content of PC (F), fat and carbohydrate mixture (FCH), and powdered standard chow (STD).

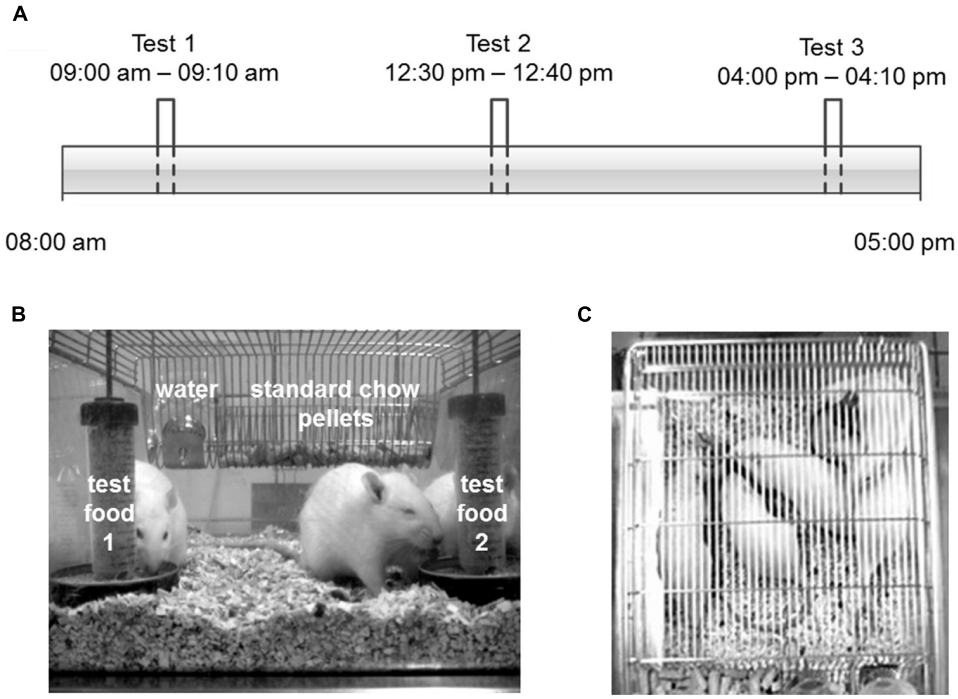

FIGURE 2 | Overview on the study design: (A) Schedule for the three separate two-choice preference tests on one day at 9 am, 12.30 pm and 4 pm. (B) Front view of the cage during the two-choice preference tests with the two additional test food dispensers (test food 1 and 2), which were presented three times a day. In the background the STD pellets as well as tap water are visible, which were constantly available ad libitum. **(C)** View from above on the cage during a preference test for the evaluation of the feeding-related locomotor activity.

was calculated by multiplying these amounts of ingested food with the respective energy contents. The relative food and energy intake were calculated by dividing the ingested amount of food or energy of the particular test food by the sum of the two test foods provided. The position of the food dispensers and the food filled into a particular dispenser were changed for every test to avoid the influence of place preferences. Additionally, the feeding-related locomotor activity of the rats was measured. For that purpose, pictures were taken every 10 s via webcams placed above the cages (**Figure 2C**). The resulting 60 pictures recorded per single period of food access were evaluated by counts: one count was defined as "one rat takes food from one food dispenser". The ingested amounts of food, energy as well as the counts were used to calculate the relative contribution of each test food to the total food intake additionally to the standard chow pellets in every single test. Each experiment was performed simultaneously in two cages on two consecutive days with three tests per day. Selected food combinations were repeated on up to six days. The following experiments were performed with two different animal cohorts: PC vs. CH, PC vs. F, PC vs. FCH, F vs. CH, FCH vs. CH, FCH vs. F, ffPC vs. PC, ffPC vs. CH, ffPC vs. F, and ffPC vs. FCH.

STATISTICAL ANALYSIS

For statistical analysis, we calculated the percentage of the test foods, which were ingested in one cage during every single 10 min preference test, related to the total intake from both test food containers. The preference tests were performed as 6–50 single tests (10 min each) with 2–4 independent animal groups (cages) comprising 4–5 individuals each. A one-way repeated measures analysis of variance (ANOVA) with the variable "test days" did not reveal any significant influence of this variable ($p < 0.05$) for the majority of the test conditions (see Results and Discussion for exceptions). For the tested combinations of PC vs. FCH ($p = 1.06 \times 10^{-7}$) and PC vs. F ($p = 4.13 \times 10^{-5}$) ANOVA showed a significant influence of the variable "test days". Consequently, we analyzed these data separately for each day.

Significances of food intake for a given test food combination were calculated by a paired, two-sided Student's t-test using Analysis ToolPak, Microsoft Excel 2013. The mean values of the single tests were calculated for the independent groups (cages) and used for statistical testing ($n = 2$–4). The data are presented in **Figures 3–5** and in **Tables 1–4**. A p-value < 0.05 was considered to be significant.

Statistical analysis regarding the energy intake and feeding-related locomotor activity was performed accordingly. The overall correlation between food intake and feeding-related locomotor activity was determined by a linear regression analysis between food intake [g] and the feeding-related locomotor activity [counts] of every single test over all tested conditions.

RESULTS

It is well-established that snack food like potato chips is able to trigger non-homeostatic food intake. The purpose of the present study was to develop a test system for the identification of the

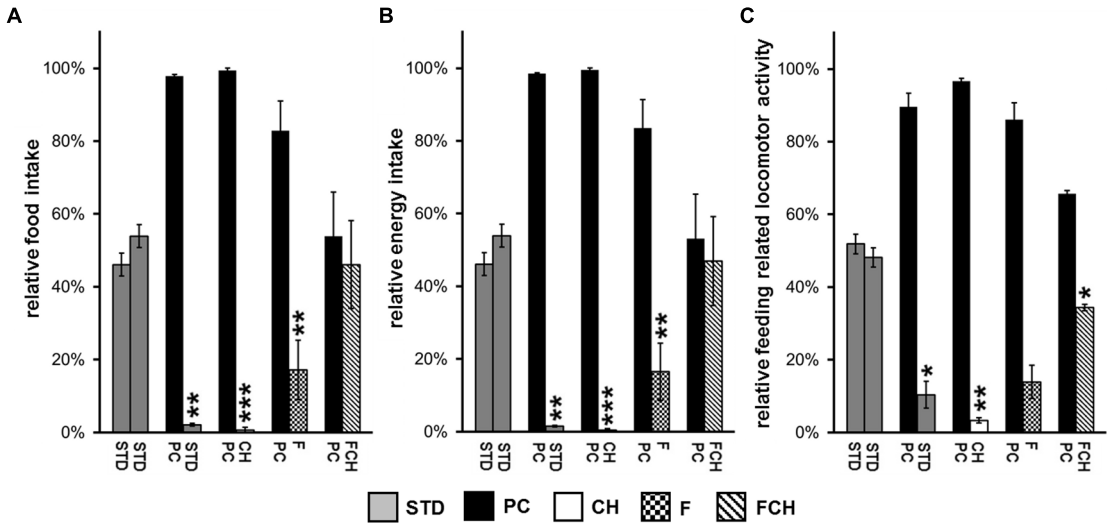

FIGURE 3 | Two-choice preference tests between the different test foods: (A) Relative food intake, (B) relative energy intake, and (C) relative feeding-related locomotor activity testing standard chow (STD) in both food containers or potato chips (PC) vs. STD as well as their major macronutrients carbohydrates (CH), fat (F), and fat and carbohydrates (FCH). Mean ± standard deviation of the relative food/energy intake or locomotor activity of the independent animal groups (cages) are shown. $***p < 0.001$, $**p < 0.01$, $*p < 0.05$.

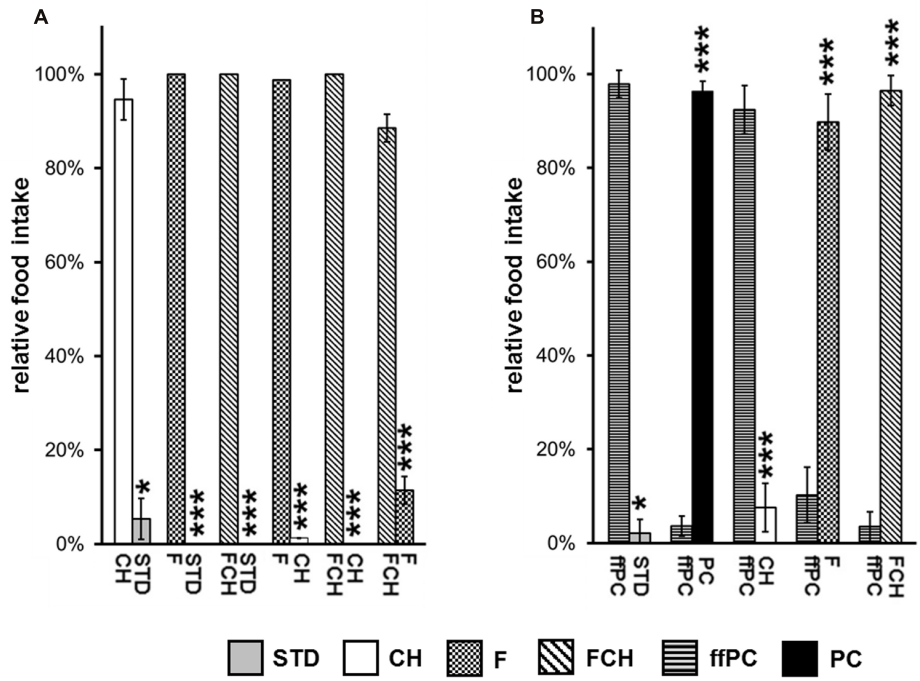

FIGURE 4 | Relative food intake during two-choice preference tests (A) applying the major macronutrients of potato chips (PC), carbohydrates (CH), fat (F) as well as fat and carbohydrates (FCH), and standard chow (STD). (B) Two-choice preference test of fat-free potato chips (ffPC) vs. STD, PC, and the macronutrients (CH, F, FCH) of PC. Mean ± standard deviation of the relative food intake of the independent animal groups (cages) is shown. $***p < 0.001$, $*p < 0.05$.

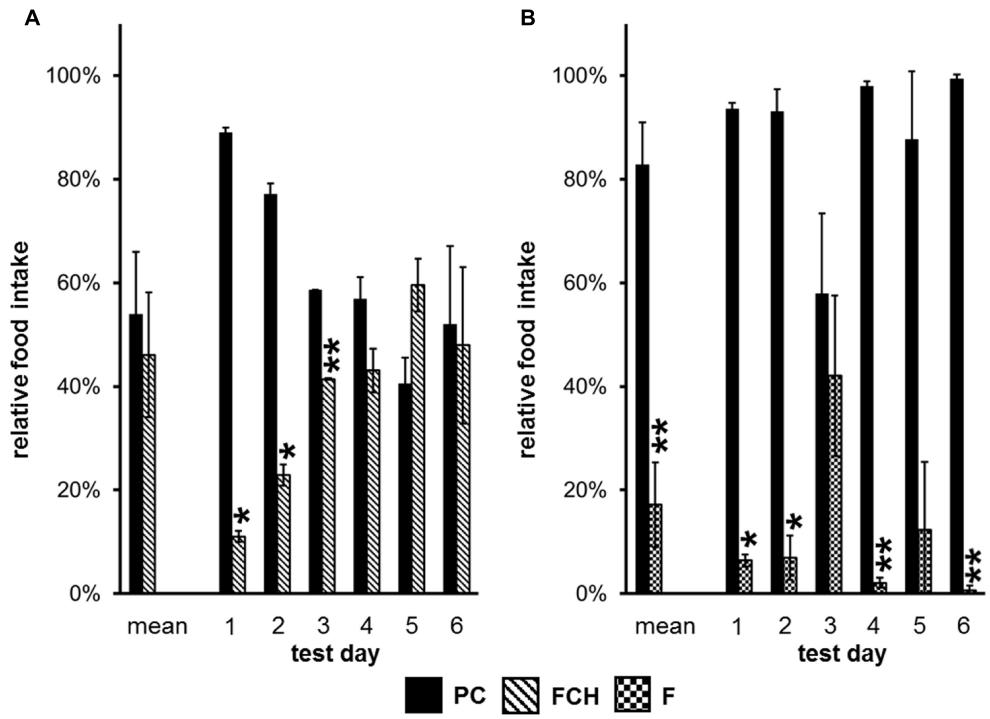

FIGURE 5 | (A) Relative food intake (mean and single values of six different test days) during two-choice preference tests of potato chips (PC) vs. the mixture of fat and carbohydrates (FCH), and (B) PC vs. the fat content of potato chips (F). Mean ± standard deviation of the relative food intake of the independent animal groups (cages) is shown. $^{**}p < 0.01$, $^{*}p < 0.05$.

particular snack food components which are responsible for these processes. The developed test system was then applied to investigate the contribution of the main macronutrients (carbohydrates and fats) to the intake of snack food.

To develop a screening assay, the potential of a test food to induce food intake in non-deprived ad libitum fed rats was used as readout. The feeding activity was recorded by two independent parameters. First, the amount of ingested food was weighed. Additionally, feeding-related locomotor activity was recorded by a camera. Both methods showed a very high correlation between all tested conditions ($r = 0.9204$, $R^2 = 0.8471$, $p < 0.001$). Feeding activity displayed as relative food intake or as relative energy intake provided similar results, which only differed by ≤3 percentage points as exemplified in **Figures 3A,B**.

Since the absolute amount of test food intake varied from day to day and was, for example, dependent on the age of the animals (data not shown), a two-choice preference test was applied (**Figure 2B**), which recorded the food intake in relation to a reference food. Although the feeding experiments were performed during the light cycle of the day, i.e., the resting phase of the rats (Hoch et al., 2013), considerable additional food intake was observed, which was dependent on the composition of the test food. A lack of side- or place-preference was observed when powdered STD was provided in both of the food dispensers resulting in a similar food and energy intake from both dispensers without significant difference ($p = 0.3311$, **Figures 3A,B**; **Tables 1A,B**). Additionally, a similar feeding-related locomotor activity on both food dispensers was observed ($p = 0.5089$, **Figure 3C**; **Table 1C**). No significant variance ($p < 0.05$) of the relative preferences for one of the two presented test foods between the test days could be observed for any of the test conditions, except for PC vs. FC and PC vs. F. These exceptions are described below in more detail.

The first experiment, when PC was tested against STD, resulted in an almost exclusive ingestion of PC (**Figures 3A,B**; **Tables 1A,B**). Next, the contribution of the two major macronutrients of PC, namely carbohydrate and fat, on the food intake was studied. For this purpose, the carbohydrate (test food CH) or fat (test food F) content as described above was added to STD. Both test foods CH and F induced a significantly (CH: $p < 0.05$, F: $p < 0.001$, **Figure 4A**; **Table 2**) higher intake than STD, whereby F prevailed against CH ($p < 0.001$, **Figure 4A**; **Table 2**), but neither CH nor F were able to induce food intake similar to PC (**Figures 3A,B**; **Tables 1A,B**). The results indicate that the activity of potato chips to induce food intake in non-deprived rats cannot be explained by the fat content or the carbohydrate content of potato chips alone.

However, when the combined fat- and carbohydrate-fractions of potato chips were added to standard chow, the intake of this test food FCH was similar (**Figures 3A,B**; **Tables 1A,B**) and the feeding-related locomotor activity only slightly lower compared to PC (**Figure 3C**; **Table 1C**). Similar to PC, FCH was also almost exclusively ingested when presented in a preference test against F or CH (**Figure 4A**; **Table 2**).

Table 1 | Statistical data for "food intake" (A) "energy intake" (B) and "locomotor activity" (C) of preference tests with two of the following test foods: powdered standard chow (STD), potato chips (PC), carbohydrates (CH), fat (F), and the mixture of fat and carbohydrates (FCH).

Food	Mean (%)	SD (%)	df	*p*-Value	*t*-Value	Number of single tests	Number of animals	See figure
(A) Food intake								
STD	46	3	1	0.3311	–1.746	12	8	3A
STD	54	3						
PC	98	0	1	0.0035	181.0	12	10	3A
STD	2	0						
PC	99	1	3	8.8×10^{-7}	135.7	36	18	3A
CH	1	1						
PC	83	8	3	0.0040	8.050	48	18	3A
F	17	8						
PC	54	12	3	0.5634	0.647	50	18	3A
FCH	46	12						
(B) Energy intake								
STD	46	3	1	0.3311	–1.746	12	8	3B
STD	54	3						
PC	98	0	1	0.0022	291.0	12	10	3B
STD	2	0						
PC	100	1	3	3.4×10^{-7}	186.9	36	18	3B
CH	0	1						
PC	84	8	3	0.0034	8.555	48	18	3B
F	16	8						
PC	53	12	3	0.6458	0.509	50	18	3B
FCH	47	12						
(C) Locomotor activity								
STD	52	3	1	0.5089	0.973	6	8	3C
STD	48	3						
PC	90	4	1	0.0421	15.08	12	10	3C
STD	10	4						
PC	97	1	1	0.0070	90.58	24	10	3C
CH	3	1						
PC	86	5	1	0.0568	11.19	36	10	3C
F	14	5						
PC	66	1	1	0.0262	24.31	36	10	3C
FCH	34	1						

Mean and SD of the relative food/energy intake or locomotor activity of the independent animal groups (cages) are presented. The p- and t-values are calculated via two-sided paired Student's t-test with df as the degree of freedom. Furthermore, number of single tests and number of animals are given.

Thus far, the present results indicate that the effect of potato chips to increase food intake in non-deprived rats is caused by its calorie content, which is essentially mediated by the fat and carbohydrate content. For a further test of this hypothesis, the feeding activity of ffPC was compared to the other test foods (STD, PC, FCH, F, and CH). As expected, ffPC showed a lower activity compared to PC, FCH and F (**Figure 4B**; **Table 2**). However, it induced a significantly higher intake compared to STD ($p < 0.05$) and CH ($p < 0.001$), despite the higher calorie content of these two test foods (**Figures 1** and **4B**). Thus, it can be concluded that other determinants trigger the intake of PC in addition to the energy density.

A one-way repeated measures ANOVA was performed to evaluate the influence of the particular test days on the results. Only two experiments showed significant influence of the test days, namely the preference tests PC vs. FCH ($p = 1.06 \times 10^{-7}$) and PC vs. F ($p = 4.13 \times 10^{-5}$) (**Figure 5**; **Tables 3** and **4**). During the first three test days, the FCH intake by rats, which were naïve to FCH, but had contact with PC in previous tests PC vs. STD, PC vs. F and PC vs. CH, was significantly lower than the PC consumption

Table 2 | Statistical data for "food intake" of preference tests with two of the following test foods: carbohydrates (CH), powdered standard chow (STD), fat (F), the mixture of fat and carbohydrates (FCH), fat-free potato chips (ffPC), and potato chips (PC).

Food	Mean (%)	SD (%)	df	*p*-Value	*t*-Value	Number of single tests	Number of animals	See figure
CH	95	4	1	0.0439	14.46	12	10	4A
STD	5	4						
F	100	0	1	2.7×10^{-5}	23997	12	10	4A
STD	0	0						
FCH	100	0	1	5.3×10^{-5}	11997	12	10	4A
STD	0	0						
F	99	0	3	1.8×10^{-7}	229.6	24	18	4A
CH	1	0						
FCH	100	0	1	5.3×10^{-5}	11997	12	10	4A
CH	0	0						
FCH	89	3	3	0.0001	26.27	24	18	4A
F	11	3						
ffPC	98	3	1	0.0278	22.86	12	10	4B
STD	2	3						
ffPC	4	2	3	2.7×10^{-5}	–43.25	24	18	4B
PC	96	2						
ffPC	92	5	3	0.0005	16.33	24	18	4B
CH	8	5						
ffPC	10	6	3	0.0009	–13.36	24	18	4B
F	90	6						
ffPC	4	3	3	8.8×10^{-5}	–29.21	24	18	4B
FCH	96	3						

Mean and SD of the relative food intake of the independent animal groups (cages) are presented. The p-and t-values are calculated via two-sided paired Student t-test with df as the degree of freedom. Furthermore, number of single tests and number of animals are given.

($p < 0.05$). On test days 4–6, no significantly higher intake of PC compared to FCH could be observed ($p > 0.05$, **Figure 5A**; **Table 3**). Changes were caused by a clear increase of FCH intake accompanied by a decrease of PC intake over the course of time, whereas the total food intake of both test foods ranged constantly between 70 and 94 g/day during the tests.

In contrast, no clear trend became apparent when the food intake of PC vs. F was compared on different test days (**Figure 5B**; **Table 4**).

DISCUSSION

It was previously shown that snack food such as potato chips is able to modulate brain circuits in rats associated with reward, food intake, satiety, and locomotor activity in comparison to standard chow (Hoch et al., 2013). These modulations of the activity patterns might be responsible for the non-homeostatic intake of snack food.

In studies dealing with non-homeostatic food intake or food addiction, a variety of palatable foods were applied, such as sugar solutions, shortening, cake, potato chips, cookies, or cheese (Prats et al., 1989; Avena et al., 2009; Martire et al., 2013). Usually, food items rich in sugar, fat or both were selected. However, it can be assumed that different types of food and different food components trigger different physiological processes related to food intake. Therefore, it is important to define the exact molecular determinants of a food item that are responsible for the excessive intake and to identify the physiological pathways that are triggered by different food components.

Thus, it was the purpose of the present study to develop a two-choice preference test for screening snack food components for their ability to trigger non-homeostatic food intake. The test system was then applied to investigate how the main macronutrients (carbohydrates and fats) of potato chips contribute to triggering the hedonic intake of this particular snack food.

The induced feeding activity was recorded by two independent readouts. On the one hand, the amount of ingested food or energy (**Figures 3A,B, 4A,B** and **5A,B**; **Tables 1A,B, 2–4**) and, on the other hand, the feeding-related locomotor activity were registered (exemplified in **Figure 3C**; **Table 1C**). The readout parameters food intake and feeding-related locomotor activity showed a very high correlation ($r = 0.9204$, $R^2 = 0.8471$, $p < 0.001$). Therefore, it could be excluded that, for example, eventual spillage of the test food biased results.

The absolute amount of consumed food varied from day to day across the different individuals and was also dependent on various further parameters like the age of the animals. Additionally, it had

Table 3 | Statistical data of the time dependence of "food intake" for preference tests with the test food combination potato chips (PC) vs. the mixture of fat and carbohydrates (FCH) mean and on test days 1–6.

Food	Mean (%)	SD (%)	df	*p*-Value	*t*-Value	Number of single tests	Number of animals	See figure
Mean								
PC	54	12	3	0.5634	0.647	50	18	5A
FCH	46	12						
Day 1								
PC	89	1	1	0.0118	53.84	6	10	5A
FCH	11	1						
Day 2								
PC	77	2	1	0.0335	19.00	6	10	5A
FCH	23	2						
Day 3								
PC	59	0	1	0.0057	111.8	6	10	5A
FCH	41	0						
Day 4								
PC	57	4	1	0.2593	2.318	6	10	5A
FCH	43	4						
Day 5								
PC	40	5	1	0.2300	–2.647	6	10	5A
FCH	60	5						
Day 6								
PC	52	15	1	0.8793	0.192	6	10	5A
FCH	48	15						

Mean and SD of the relative food intake of the independent animal groups (cages) are presented. The p-and t-values are calculated via two-sided paired Student t-test with df as the degree of freedom. Furthermore, number of single tests and number of animals are given.

been shown that the reward sensitivity for palatable food is dependent on the development stage of the rats (Friemel et al., 2010). Therefore, a differential two-choice preference test was applied (**Figure 2B**), which recorded the relative food intake of two test foods at a given feeding session. Under these conditions, a training effect could occur due to the presentation of unknown test food versus the known reference food. Therefore, each preference test was performed at least on two different days, i.e., six times. Moreover, the position of the food dispensers containing the test foods was changed after each single test to avoid the development of a place preference. The lack of side- or place-preference was observed by testing STD vs. STD by six consecutive repetitions of a test setting on two consecutive days. Here, no significant difference between the two identical test foods regarding food/energy intake ($p = 0.3311$, **Figures 3A,B**; **Tables 1A,B**) or feeding related locomotor activity ($p = 0.5089$, **Figure 3C**; **Table 1C**) was revealed. Finally, in order to minimize the influence of sensory parameters, such as consistency and flavor, the test foods were offered after homogenization in a mixture with powdered STD. Under the applied test conditions, it can, therefore, be concluded that solely differences in the composition of the test foods were responsible for differences in food intake. In summary, the established two-choice preference test seemed to provide reliable results, and could be used to screen for food components related to non-homeostatic food intake.

The developed behavioral test was then applied to investigate the influence of the major components fat and carbohydrates on the potato chip-induced hedonic food intake in ad libitum fed rats. The first experiment confirmed that PC induced a higher food and energy intake than STD indeed (**Figures 3A,B**; **Tables 1A,B**). As expected, a higher food intake compared to STD was also observed when the isolated potato chip components fat and carbohydrates were offered in similar concentrations as present in potato chips (**Figure 4A**; **Table 2**). It is worth noting that the fat component was more active than the carbohydrate component. Consequently, it can be concluded that fat seems to be one contributor to the palatability of a test food. It is reported that the preference of rats for fat is learned and leads to a preference for fatty food: rats fed a high-fat diet showed an enhanced intake of oil emulsions compared to rats which received a diet high in carbohydrates (Reed and Friedman, 1990). Beside this influence on food preference, fat is a strong contributor to an enhanced food intake by additionally increasing the meal size (Warwick and Synowski, 1999).

However, the effects of fat intake seem to be rather complex. Fat (corn oil) in the oral cavity of mice likely led to activation of

Table 4 | Statistical data of the time dependence of "food intake" for preference tests with the test food combination potato chips (PC) vs. fat (F) mean and on test days 1–6.

Food	Mean (%)	SD (%)	df	*p*-Value	*t*-Value	Number of single tests	Number of animals	See figure
Mean								
PC	83	8	3	0.0040	8.050	48	18	5B
F	17	8						
Day 1								
PC	94	1	1	0.0121	52.68	6	10	5B
F	6	1						
Day 2								
PC	93	4	1	0.0447	14.23	6	10	5B
F	7	4						
Day 3								
PC	58	15	1	0.6017	0.723	6	10	5B
F	42	15						
Day 4								
PC	98	1	1	0.0092	69.00	6	10	5B
F	2	1						
Day 5								
PC	88	13	1	0.1539	4.057	6	10	5B
F	12	13						
Day 6								
PC	99	1	1	0.0082	78.00	6	10	5B
F	1	1						

Mean and SD of the relative food intake of the independent animal groups (cages) are presented. The p-and t-values are calculated via two-sided paired Student t-test with df as the degree of freedom. Furthermore, number of single tests and number of animals are given.

the dopaminergic system through dopamine D1 receptor, which seemed to be a mediator of its reinforcing effects (Imaizumi et al., 2000). Possibly, the fatty acid transporter CD36 is involved in the detection of dietary fats in the oral cavity of rats or mice. This early detection of fats might lead to a quick preference for fatty foods (Laugerette et al., 2005).

Additionally, post-ingestive effects are responsible for an increased intake of fat. It was shown in a self-regulated intragastric infusion paradigm that rats take up a higher amount of a high-fat diet compared to a high-carbohydrate diet via intragastric infusion (Warwick et al., 2003). Such post-ingestive effects of fats are possibly mediated by fatty acid sensors such as CD36, GPR40, and GPR120 in the small intestine leading to a post-oral stimulation of appetite (Sclafani and Ackroff, 2012; Sclafani et al., 2013).

However, in the present study, neither the fat component, nor the carbohydrate component alone was able to induce food intake similar to PC. Only the combination of both components (FCH) led to a food/energy intake comparable to PC suggesting a synergistic effect of fats and carbohydrates (**Figures 3A,B**; **Tables 1A,B**). Consequently, FCH induces higher food intake than F, CH, or STD (**Figure 4A**; **Table 2**). A previous study with two different groups of rats showed that the group which had access to a mixed food consisting of fat and carbohydrates ingested a larger quantity of food compared to a group of rats which were provided with food solely with high fat content (Ramirez and Friedman, 1990). This result is in accordance with the present outcome of our two-choice preference test on solid snack food. Preference tests with liquid test food already showed that rats preferred an emulsion with fat and sugar over the single components as well as over standard chow (Lucas and Sclafani, 1990).

From these findings, it can be hypothesized that the combination of the macronutrients, fat and carbohydrates, triggers additional effects compared to the administration of only one of the components. One study showed, for example, that in rats, the administration of the GABA-B receptor agonist baclofen stimulated binge-eating of sweet-fat food, suppressed binge-eating of fat, but had no effect on binge-eating of sucrose (Berner et al., 2009). These findings clearly indicate the presence of specific mechanisms related to excessive intake of different macronutrients or their combination. Moreover, a study with rats by la Fleur et al. (2010) observed that a mixture of fat and sugar, but not the single components, led to hyperphagia-induced obesity. Additionally, the mixture of fat and sugar altered hypothalamic neuropeptide expression in a different way compared to fat or sugar alone (la Fleur et al., 2010).

Since the test foods were tested against each other in different combinations, situations could occur that animals were familiar with test foods from previous preference tests, but naïve to a newly introduced test food. Thus, the novelty or the familiarity of a test food could influence the food intake. Therefore, preference tests were performed at least six times, so that the animals were familiar with both test foods already after the first test. Subsequent ANOVA analysis revealed that the variable "test day" did not have a significant influence except for the preference tests PC vs. FCH and PC vs. F. Interestingly, a clear trend was observed in the PC vs. FCH combination: the rats, which were familiar with PC from previous preference tests during this study (PC vs. STD, F or CH), significantly preferred PC over FCH in the first three test days ($p < 0.05$). In the following test days, the preference for PC diminished (**Figure 5A**; **Table 3**). Thus, it can be concluded that FCH and PC have similar capability to induce food intake in ad libitum fed rats, but PC were preferred when rats were naïve to FCH but not to PC. In contrast, no clear trend was observed when PC was tested against F. Instead, a high and constant preference of PC against F was observed on five out of six test days. Therefore, the novelty of a particular test food did not seem to influence the feeding preference in general, but only when PC was tested against FCH.

Additionally to novelty effects, the order of food presentation could influence the feeding behavior. For example, food fatigue or acclimation could occur. Therefore, some preference tests, which had been performed at the beginning of the study, were repeated at the end of the whole sequence (e.g., PC vs. F, PC vs. CH). The repetitions provided results very similar to the initial tests. However, it cannot be fully excluded that food fatigue or acclimation effects occur under the applied conditions.

The capability of the test foods STD, CH, F, and FCH to induce food intake may be an effect of their respective energy density, because the test foods that induced higher food intake often had higher calorie content (**Figure 1**). However, the experiments with ffPC indicate that the energy content is apparently not the only trigger of food intake in non-deprived animals. The presentation of ffPC led to a significantly lower additional food intake compared to regular PC ($p < 0.001$, **Figure 4B**; **Table 2**). These results suggest that appetent fat intake is less related to textural fat properties, such as mouth feeling, but rather to the caloric content or the chemoreception of free fatty acids in the digestive tract or the gustatory system (Pittmann, 2010). In contrast to this finding, it has been reported previously that no preference could be observed in non-deprived rats for high-fat cake compared to no-fat cake. Only food-deprived rats highly preferred the high-fat cake (Sclafani et al., 1993). Notably, ffPC were highly preferred over STD and CH despite of the lower energy density of ffPC (**Figure 4B**; **Table 2**). Hence, other components or properties of ffPC beyond the energy content seem to have an additional influence on the activity of snack food to induce food intake. For example, salt or fiber may affect the food intake (Beauchamp and Bertino, 1985; Vitaglione et al., 2009). The two-choice preference test that has been applied in the present study may now provide a useful screening system to further investigate the (minor) components of potato chips which contribute to their non-homeostatic intake. The conclusion that the energy content is not the only parameter inducing food intake is supported by a previous study in which the addition of saccharin to a fat emulsion had a similar enhancing effect on food intake as the addition of sucrose (Lucas and Sclafani, 1990).

In conclusion, the present study established a behavioral screening tool that has been optimized to investigate the ability of different test foods to induce food intake in ad libitum fed rats. The assay was used to examine how the main macronutrients of potato chips, namely fat and carbohydrates, contribute to trigger hedonic food intake. It was shown that fat has a high impact on additional food intake, but the combination of both macronutrients was identified as the main contributor to the palatability of potato chips. The energy density is not the sole factor responsible for the increased food intake, since ffPC triggered higher food intake than other test foods with higher energy content. The two-choice preference test used in this study will be applied in future investigations to disentangle the influence of minor components of potato chips so that the molecular determinants of their intake can be understood in more detail. Additionally, it should be investigated if a mixture of fat and carbohydrates is able to induce similar changes in brain activity patterns as snack food.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: Tobias Hoch, Monika Pischetsrieder, Andreas Hess. Performed the experiments and analyzed the data: Tobias Hoch. Interpreted the data: Tobias Hoch, Monika Pischetsrieder, Andreas Hess. Contributed reagents/materials/analysis tools: Monika Pischetsrieder, Andreas Hess. Wrote the paper: Tobias Hoch, Monika Pischetsrieder, Andreas Hess. Finally approved of the version to be published: Tobias Hoch, Monika Pischetsrieder, Andreas Hess. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Tobias Hoch, Monika Pischetsrieder, Andreas Hess.

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Considerations about rodent models of binge eating episodes

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INTRODUCTION

A binge eating episode is defined as an uncontrolled event of hyperphagia, in which people quickly eat a large amount of food while feeling a sense of loss of control over eating (Wolfe et al., 2009). Binge eating episodes are observed in a variety of human disorders including bulimia nervosa (BN), binge eating disorder (BED), and the binge/purge subtype of anorexia nervosa (AN) (Berger and Tanofsky-Kraff, 2012). Binge eating episodes are also present in overweight and obese people, as well as non-clinical populations under specific circumstances such as stress. The etiology of this behavior is currently unknown. The use of rodent models has been essential for understanding the pathogenesis of many human diseases; however, it is challenging to mimic all features of human binge eating in rodent models (Corwin and Buda-Levin, 2004; Perello et al., 2010a). In particular, these models should not only display the objective characteristics of a binge eating episode, namely the consumption of a large amount of food in a short period of time, but also the subjective characteristics of the feeling of loss of control.

Recently, we examined the neuronal circuitries activated in naïve mice allowed to spontaneously eat a high fat diet (HFD) pellet for 2 h (Valdivia et al., 2014). We found that satiated mice with free access to regular chow rapidly consume a significant amount of HFD when exposed to it, and that HFD intake recruits centers of the mesolimbic pathway, which are known to be activated in human beings displaying binge eating behavior (see below). Experts in the field agreed that our simple model of HFD overconsumption could be relevant for studying neuronal aspects of binge eating behaviors. However, some reviewers argued that it was misleading to describe our model as a model of binge eating. Some criticisms were that our model lacked indications of feelings of loss of control, repeated feeding episodes, escalation of intake over time, a significant level of hyperphagia, and evidence that bingeing occurred in the face of aversive consequences. The notable divergence in the opinion of the journal's reviewers made evident that a comprehensive debate about rodent binge eating models is needed. Here, we briefly present our opinion about the features that a rodent model should fulfill in order to be considered a reasonable model of binge eating episodes and its implications in terms of the neuronal circuits involved.

RODENT MODELS OF BINGE EATING

The features that a model of binge eating episodes should define include the amount of calories eaten and the duration of the event. An empirically based consensus indicates that a 2 h-duration for a binge episode is a reasonable guideline for human studies (Wolfe et al., 2009). This period of time has been extensively used in rodent studies, including ours, since it comprises the entire event of hyperphagia (Berner et al., 2008; Valdivia et al., 2014). In our experience, however, this period of time could be shorter as mice eat ∼70% of the total binge intake during the first hour of food exposure. The importance of the amount of calories eaten in the definition of a binge episode has been a very controversial issue for human studies (Wolfe et al., 2009). In contrast, food intake is easily measurable in rodents, and a significant increase in caloric intake is judged as an essential feature in most studies of binge eating in rodents. In our opinion, at least a 2-fold increase of calorie intake, as compared to the control group, appears to be a reasonable criterion to decide whether a test group has experienced hyperphagia (Valdivia et al., 2014). Rapid hyperphagia in a binge eating episode should occur in an uncontrollable manner; however, perceived loss of control is a challenging concept to measure because it is an inherently subjective experience. The emotional state in rodents is an even harder notion to conceptualize, and there is currently no recognized method to assess it. Thus, we consider that the loss of control during an event of hyperphagia cannot be a requirement for a rodent model of binge eating episodes and that this is the most critical limitation in the search of accurate binge eating models.

Given the inability to include a measurable parameter to confirm that hyperphagia is uncontrollable, other features need to be considered in order to define an event of hyperphagia as a binge eating episode in a rodent model. These features include the presence or absence of previous caloric deprivation and/or limited access to palatable diets. In rodents, caloric deprivation promotes compensatory hyperphagia and also entrains the animals to shift their dietary patterns (Corwin et al., 2011). Food deprivation increases the rewarding value of palatable foods, and hyperphagia persists even after animals have reached their energy needs if fasting episodes are sufficiently

severe (Perello et al., 2010b; Kim, 2012). Despite the fact that food deprivation-induced hyperphagia in rodents displays some features observed in human binge eating, its use as a model remains controversial because it is considered that binge eating episodes in humans are not usually driven by hunger (Corwin and Buda-Levin, 2004). Indeed, non-clinical populations or BED patients may display binge eating episodes with sufficient or even excess of energy stores; however, AN and BN patients display binge eating episodes under negative energy balance conditions suggesting that some events may involve hunger (Mathes et al., 2009). Also, dieting or food restriction increases the risk of binge eating episodes not only in BN but also in non-clinical populations and BED (Stice et al., 2001). Thus, we think that food deprivation could be included as an experimental manipulation when trying to mimic particular aspects of human binge eating. Secondly, human beings normally prefer bingeing palatable foods (Avena, 2007). *Ad libitum* fed rodents exposed to a palatable food, such as HFD, display a robust event of hyperphagia (Valdivia et al., 2014). Rodents exposed to glucose or sucrose solutions also show binge intake (Avena, 2007). Thus, time-limited access to palatable diets to induce events of hyperphagia in rodents can be useful to study human binge eating. Interestingly, rodent models of carbohydrate- or fat-bingeing show notable behavioral differences during and after the hyperphagia events suggesting that the diet composition is an important factor affecting the implications of the model (Avena et al., 2009).

The frequency of binge eating episodes is a landmark feature of some human eating disorders that can be controlled in rodent models. In particular, repetitive events of hyperphagia can be induced by exposing animals to either repetitive fasting/refeeding events or daily short-term exposure to palatable foods (Corwin et al., 2011). Thus, the use of repetitive events of hyperphagia can be considered when trying to model particular aspects of BN or BED eating behavior in mice. Binge eating episodes are sensitive to stress, and its impact on food intake depends on the nature and duration of the stressor and individual susceptibility (Corwin and Buda-Levin, 2004). Therefore, the use of environmental stressors together with calorie restriction and/or limited access to palatable diets is another important feature that determines the applicability of the model (Corwin and Buda-Levin, 2004). Of note, the above mentioned features are not specified in the definition of a binge eating episode. Thus, we think these features should not be a requirement for models of binge eating in rodents, but relevant to mimic events of hyperphagia found in particular pathologies.

NEURONAL CIRCUITS UNDERLYING BINGE EATING EPISODES

Food intake is regulated by an integrated system involving both homeostatic brain circuits that drive food intake depending on energy store levels and hedonic brain circuits that drive consumption based on rewarding properties of foods (Berthoud, 2012). Homeostatic-driven eating occurs under negative energy balance conditions, when circulating factors of energy availability signal to the brain that energy stores are depleted; in contrast, hedonic-driven eating involves cognitive, reward, and emotional factors that induce the consumption of pleasurable foods even when calories are unnecessary (Berthoud, 2012). Importantly, both homeostatic and hedonic brain circuits that drive food intake are sensitive to peripheral factors, including the hormones leptin and ghrelin (Schwartz and Zeltser, 2013).

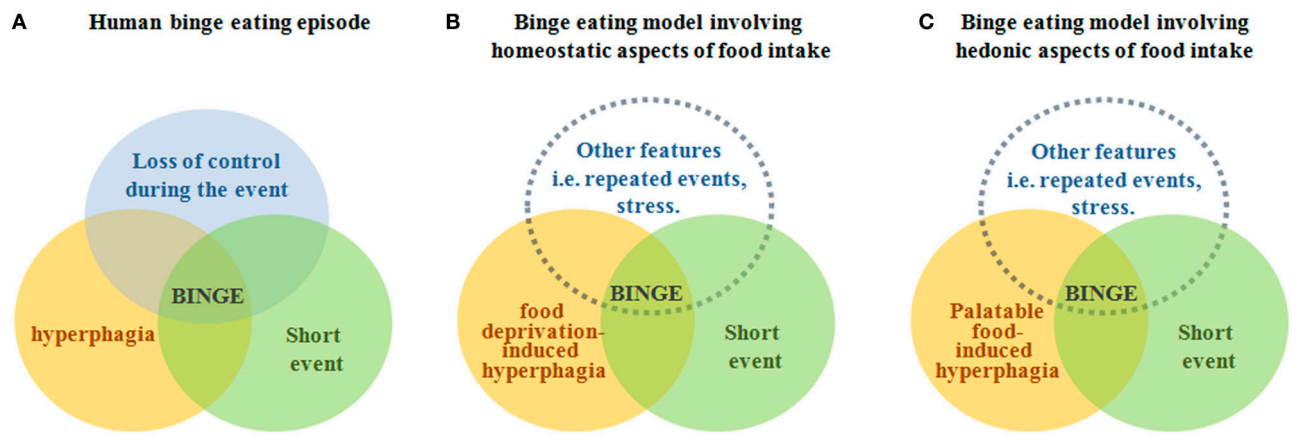

FIGURE 1 | The figure depicts our point of view of the rodent models of binge eating episodes in relation to the definition of binge eating in humans. Panel **(A)** displays a diagram representing the definition of an episode of binge eating, which is characterized by a conjunction of hyperphagia of a large amount of food (orange circle), in a discrete period of time (green circle), and with a sense of lack of control over eating (blue circle). Panel **(B)** displays a diagram representing binge eating models in which hyperphagia is induced by food deprivation and presumably involves neuronal circuits controlling homeostatic eating, which are mainly located in the hypothalamus and brainstem (Sohn et al., 2013). Panel **(C)** displays a diagram representing binge eating models in which hyperphagia is induced by the exposure to a palatable food and presumably involves neuronal circuits controlling hedonic eating, which are mainly located in the mesolimbic system (Kenny, 2011). In panels **(B,C)**, circles representing "hiperfagia" and "short event" are drawn with solid lines because they can be clearly defined for rodent models. In contrast, the blue circle representing "other features" is drawn with dotted lines because we think that a "loss of control during the event" circle cannot be required to a rodent model and, instead, other features need to be considered in order to mimic events of hyperphagia found in particular human pathologies.

Neuronal systems controlling homeostatic eating are located mainly in the hypothalamus and brainstem. The hypothalamic arcuate nucleus (ARC) plays an essential role in the regulation of eating as it is highly sensitive to peripheral signal molecules of energy status (Sohn et al., 2013). The ARC contains a set of neurons that express orexigenic factors, including the neuropeptide Y, and another set of neurons that express anorexigenic factors, including pro-opiomelanocortin-derived peptides (Sohn et al., 2013). The ARC neurons likely act as first order neurons sensing peripheral factors and then regulate second order neurons located within the hypothalamus, hindbrain, and the brainstem dorsal vagal complex, which integrates neuronal inputs from the hypothalamus with peripheral hormones and visceral sensory information (Sohn et al., 2013). Thus, homeostatic regulation of food intake involves hypothalamic systems governing intake on a meal-to-meal basis and also brainstem systems regulating meal size and/or frequency. Neuronal circuits controlling homeostatic eating are presumably involved in food deprivation-induced hyperphagia as food deprivation increases and decreases ARC gene expression of orexigenic and anorexigenic neuropeptides, respectively (Schwartz et al., 2000). In contrast, no changes in ARC neuropeptides are observed prior to scheduled-feeding of a palatable food in rodents (Bake et al., 2013).

Hedonic eating involves the dopaminergic pathways emanating from the midbrain ventral tegmental area (VTA), which project to the nucleus accumbens (NAc) in the ventral striatum and other areas such as the amygdala, medial prefrontal cortex, hippocampus, and hypothalamus (Kenny, 2011). The VTA receives projections from many mesolimbic brain nuclei and also taste information from afferent sensory fibers (Kenny, 2011). Acute rewarding stimuli activate dopaminergic VTA neurons, and dopamine release in the NAc potently enhances the drive to obtain palatable foods (Palmiter, 2007). Several studies have implicated the central dopamine system in a variety of human eating disorders (Bello and Hajnal, 2010). In rodents, NAc dopamine signaling increases in response to hyperphagia induced by either food deprivation or limited access to palatable diets (Yoshida et al., 1992; Hajnal and Norgren, 2001). The endogenous opioid system has also been shown to be involved in binge eating in humans, and food deprivation-induced hyperphagia in rodents has been reduced by opioid receptor antagonists (Boggiano et al., 2005; Corwin et al., 2011). In addition, ingestion of palatable foods increases opioid receptor binding within the NAc in rodents (Kelley et al., 2003). In contrast, acetylcholine release from NAc interneurons is involved in meal satiation, and a deregulation of this system may be related to sugar-binge eating in rodent models (Avena, 2009).

The homeostatic and hedonic circuits regulating eating are presumably integrated in the lateral hypothalamic area. This area contains orexin neurons highly innervated by hypothalamic and mesolimbic circuits that project widely within the brain (Schwartz and Zeltser, 2013). In rodents, orexin increases food intake depending on the hunger and palatability of the diet (Mahler et al., 2012). Moreover, orexin neurons are activated in response to food deprivation, in anticipation of palatable foods as well as after acute HFD consumption (Mahler et al., 2012). In human binge eating disorders, the role of the orexin system remains unexplored.

CONCLUSIONS

Clinical reports stress the complexity of assessing if an event of hyperphagia is actually a binge eating episode in humans (Wolfe et al., 2009). Thus, it is not surprising that there is currently not a general consensus in terms of which criteria a rodent model should fulfill to be considered accurate for the study of neurobiological aspects of binge eating episodes. **Figure 1** summarizes our opinion in a simple diagram. To conclude, we think that the use of rodent models displaying rapid events of hyperphagia induced by previous caloric deprivation and/or limited access to palatable diets can be useful to investigate the molecular mechanisms and neuronal circuits recruited during binge eating episodes in humans. Importantly, the experimental strategy used to induce the event of hyperphagia and its features determine the main neuronal circuits regulating food intake involved in the model.

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Eating behavior and stress: a pathway to obesity

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Stress causes or contributes to a huge variety of diseases and disorders. Recent evidence suggests obesity and other eating-related disorders may be among these. Immediately after a stressful event is experienced, there is a corticotropin-releasing-hormone (CRH)-mediated suppression of food intake. This diverts the body's resources away from the less pressing need to find and consume food, prioritizing fight, flight, or withdrawal behaviors so the stressful event can be dealt with. In the hours following this, however, there is a glucocorticoid-mediated stimulation of hunger and eating behavior. In the case of an acute stress that requires a physical response, such as a predator-prey interaction, this hypothalamic-pituitary-adrenal (HPA) axis modulation of food intake allows the stressful event to be dealt with and the energy used to be replaced afterward. In the case of ongoing psychological stress, however, chronically elevated glucocorticoids can lead to chronically stimulated eating behavior and excessive weight gain. In particular, stress can enhance the propensity to eat high calorie "palatable" food via its interaction with central reward pathways. Activation of this circuitry can also interact with the HPA axis to suppress its further activation, meaning not only can stress encourage eating behavior, but eating can suppress the HPA axis and the feeling of stress. In this review we will explore the theme of eating behavior and stress and how these can modulate one another. We will address the interactions between the HPA axis and eating, introducing a potential integrative role for the orexigenic hormone, ghrelin. We will also examine early life and epigenetic modulation of the HPA axis and how this can influence eating behavior. Finally, we will investigate the clinical implications of changes to HPA axis function and how this may be contributing to obesity in our society.

Keywords: corticotropin-releasing hormone (CRH), ghrelin, glucocorticoids, hypothalamic-pituitary-adrenal (HPA) axis, insulin, leptin

INTRODUCTION

The body's stress response is a highly adaptive phenomenon allowing an organism to divert resources to cope with actual or anticipated danger and to restore expended energy that it may fight another day. However, if the stressor is excessive or chronic, responses can become maladaptive. Excessive or chronic stress can trigger or exacerbate a huge variety of diseases and disorders, including mood disorders such as post traumatic stress disorder, anxiety, and depression (McEwen, 2008). Stress, either acute mild stress or prolonged chronic stress, can also influence our appetite, including our drive to eat and the types of food we are likely to select. In this review we will discuss the effects of stress on appetite regulation and how stress may influence our propensity to become obese.

THE ACUTE EFFECTS OF STRESS ON APPETITE

When an organism encounters a stressful event, a number of steps occur to divert resources appropriately and to assist coping mechanisms (reviewed in, Sapolsky et al., 2000; Papadimitriou and Priftis, 2009). In terms of acute appetite regulation, corticotropin-releasing hormone (CRH) is released from the medial parvocellular (mp) paraventricular nucleus of the hypothalamus (PVN) in response to the stressor. In addition to stimulating adrenocorticotropic hormone (ACTH) release from the pituitary and the cascade of events leading to glucocorticoid release, CRH is also released into the arcuate nucleus of the hypothalamus (ARC) to inhibit neuropeptide Y (NPY)/agouti-related peptide (AGRP) neurons there (Heinrichs et al., 1993; Currie, 2003). This population of cells is normally responsible for stimulating feeding behavior and suppressing energy expenditure; thus CRH released after acute stress inhibits appetite (Heinrichs and Richard, 1999; Richard et al., 2002).

Other molecules from the CRH family, such as urocortins, also play a role in appetite suppression (Weninger et al., 1999; Richard et al., 2002). Thus, early studies from Weninger et al. (1999) showed CRH-deficient mice can have normal stress-induced suppression of food intake, implicating other CRH-like molecules. More recently, Tanaka and colleagues demonstrated both CRH and urocortins suppress food intake, but the urocortins, particularly urocortin 1, do this more effectively (Tanaka et al., 2009). It is likely urocortins 1, 2, and 3 influence appetite suppression by acting on the CRHR2 receptor in the hypothalamus (Richard et al., 2002). Centrally administrated urocortins are also able to suppress ghrelin secretion, potentially preventing ghrelin-induced stimulation of appetite (Yakabi et al., 2011). On the other hand, peripherally administered urocortins act at CRHR2 receptors in the

gut to *stimulate* an increase in circulating ghrelin (Wang et al., 2013). These mechanisms likely interact to accurately fine-tune feeding.

In addition to acting on the NPY neurons of the ARC, CRH-induced appetite suppression also involves other regions of the hypothalamus: the PVN, supraoptic nucleus, perifornical and ventromedial hypothalamus; as well as brain regions further afield, the lateral septum, parabrachial nucleus, and the dorsal portion of the anterior bed nucleus of the stria terminalis (BNST; Richard et al., 2002; Ciccocioppo et al., 2003; Fatima et al., 2013). Thus, CRH injected directly into the dorsal anterior BNST (but not the ventral part or other brain regions such as the central amygdala or locus coeruleus) significantly reduces food intake in already food-deprived rats (Ciccocioppo et al., 2003).

THE CHRONIC EFFECTS OF STRESS ON APPETITE

Ethologically, the appetite suppressive response is useful for diverting energy away from food-seeking behavior and eating toward more pressing concerns, such as escaping the predator or rehearsing the speech. With longer-term stressors, however, the energy used coping needs to be replaced. In the hours to days after the onset of an ongoing stressful event (e.g., infection, bereavement), glucocorticoids in the bloodstream are elevated. Peripherally, glucocorticoids enhance the activity of lipoprotein lipase in adipose tissue, leading to an increase in fat storage (Bjorntorp, 1996, 2001). This occurs particularly in visceral fat where lipoprotein lipase activity is higher (Marin et al., 1992a). Thus, chronically elevated glucocorticoids contribute to visceral fat accumulation (Marin et al., 1992b; Rosmond et al., 1998; Epel et al., 2000). Other mechanisms by which glucocorticoids stimulate excess fat deposition are reviewed in (Spencer and Tilbrook, 2011).

In terms of feeding behavior, glucocorticoids also act on the hypothalamus to stimulate appetite (Santana et al., 1995; Dallman et al., 2004). Thus, in humans, a peripheral injection of CRH leads to increased food intake 1 h later but the amount of food consumed is directly correlated with the magnitude of the cortisol response to the injection (George et al., 2010). Glucocorticoids stimulate food intake by interacting with several appetite-regulating targets. They increase AMP-activated protein kinase signaling in the ARC to up-regulate NPY and AGRP expression in this region and stimulate the actions of these orexigenic peptides (Savontaus et al., 2002; Konno et al., 2008; Shimizu et al., 2008). Glucocorticoids also influence the function of leptin, whose normal role is to signal satiety thus suppressing appetite. Although glucocorticoids 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). Thus, adrenalectomized rats respond to intracerebroventricular (icv) leptin with a larger reduction in food intake and body weight than intact rats and the addition of glucocorticoids reduces leptin's anorexigenic effects (Zakrzewska et al., 1997).

Insulin is another appetite-regulatory hormone that is influenced by glucocorticoids, although the role of glucocorticoids here is more complex. Insulin usually acts at the hypothalamus to reduce food intake and at the ventral tegmental area (VTA) to reduce the dopaminergic neuron-mediated rewarding nature of food (Figlewicz et al., 2008). Acutely, glucocorticoids stimulate insulin secretion from the pancreas (Strack et al., 1995), having an appetite-suppressant effect. However, chronically activated glucocorticoids also contribute to insulin resistance. Thus, as is seen with leptin, glucocorticoids contribute to a reduced ability of insulin to inhibit NPY/AGRP neurons in the ARC, which has the converse effect of lessening appetite suppression (Asensio et al., 2004). The intermediate role of glucocorticoids in the connection between insulin sensitivity and increased appetite is typically observed in patients with Cushing's syndrome. Glucocorticoid excess in these patients leads to an increase in appetite, weight gain and insulin resistance (Anagnostis et al., 2009).

Glucocorticoids also influence food intake by enhancing the preference for "comfort foods." Insulin's suppressive effect on reward pathways likely means the food needs to be more "rewarding" to achieve the same effect; hence under stressed conditions rats prefer foods that are high in fat and sucrose when a choice is available (la Fleur et al., 2004; Warne et al., 2006, 2009). Chronically stressed animals thus prefer calorically dense foods (Pecoraro et al., 2004; Foster et al., 2009). This enhanced caloric intake has been proposed to correspond with the increased brain energy demand and thus preferential glucose allocation to the brain under the conditions of stress (Peters et al., 2011). Remarkably, this highly palatable food also leads to a reward-mediated negative feedback onto the hypothalamic-pituitary-adrenal (HPA) axis to suppress it. In this way, a junk food diet or a stress-induced ice-cream binge may actually alleviate the symptoms of stress (Pecoraro et al., 2004; Foster et al., 2009). Rats given chronic restraint stress for 3 h per day for 5 days voluntarily eat more lard and sucrose than control rats, and the plasma ACTH and glucocorticoid response to this restraint is suppressed in those rats that were given free access to these "comfort" foods. Unsurprisingly, these rats also become heavier than their restraint-stressed counterparts given normal chow (Pecoraro et al., 2004).

Another mechanism by which glucocorticoids can influence appetite during stress is via its interaction with ghrelin. Ghrelin is a peptide derived principally from the gut. It is released as a signal of hunger or just prior to the usual meal time to stimulate feeding (Hosoda et al., 2006). Circulating ghrelin is increased in response to stress (Kristenssson et al., 2006) and probably acts at the level of the anterior pituitary as well as higher brain regions, such as the centrally projecting Edinger Westphal nucleus (EWcp), to modulate ACTH release from the pituitary and regulate glucocorticoid negative feedback (Spencer et al., 2012). Chronic or severe stress resulting in elevated glucocorticoid secretion will also lead to elevated circulating ghrelin levels, culminating in increased ghrelin-mediated stimulation of NPY/AGRP and increased food intake (Asakawa et al., 2001; Kristenssson et al., 2006; Lutter et al., 2008; Ochi et al., 2008). Interestingly, while stress-induced elevation of ghrelin corresponds with exacerbation of social avoidance and increased food intake in wild-type animals, deletion of ghrelin receptor (growth hormone secretagogue receptor; *GHSR*−/−) results in even more pronounced social avoidance than stress does, but it does not increase food intake (Lutter et al., 2008). Activation of ghrelin signaling in response to stress may thus represent a coping mechanism, where combatting the effects of the

stressor is prioritized at the expense of increased food intake. Acute psychosocial stress in human subjects has been also documented to induce increased release of ghrelin (Rouach et al., 2007).

The consequences of a chronically stimulated HPA axis response to stress are easy to imagine. Excessive glucocorticoid production and/or elevated basal glucocorticoids, as can occur with chronic stress and mood disorders (McEwen, 2008; Lupien et al., 2009), leads to energy conservation and appetite stimulation. Excessive high calorie foods are consumed and excess weight gain and eventually obesity ensue (De Vriendt et al., 2009). However, exposure to chronic stress may also suppress appetite in some individuals, particularly in unrestrained eaters, as opposed to restrained eaters who voluntarily restrict their diet to maintain proper weight, but tend to increase their food intake when stressed (Greeno and Wing, 1994). Depression, which can often be triggered by chronic exposure to stressful events, is also frequently associated with reduced appetite (Nestler et al., 2002). It is likely ghrelin plays a principal role in determining if an individual responds to stress with an increase or a decrease in appetite. Individuals classified as "emotional eaters" (those who consume more highly palatable food during stress) have lower basal ghrelin than "non-emotional eaters" (those whose food intake is suppressed or unchanged by stress; Raspopow et al., 2010). Lower basal ghrelin levels are also associated with binge-eating, an emotional eating disorder (Geliebter et al., 2005). Stress-induced ghrelin levels remain unaltered by food intake in emotional eaters but are rapidly restored to baseline by food in non-emotional eaters (Raspopow et al., 2014). Thus, emotional eaters may require relatively more palatable food to suppress stress-induced ghrelin to the same degree as non-emotional eaters.

EARLY LIFE HPA AXIS DEVELOPMENT AND ITS EFFECTS ON EATING BEHAVIOR

Lifetime experience, whether acute or chronic, clearly shapes both HPA axis and eating behavior. However, how an individual responds to each experience can be influenced at times outside the immediately pertinent event. It is now well accepted that the early life period is one of significant vulnerability to programming influences. For instance, central pathways governing feeding and metabolism start to develop at specific stages of early life and, at this time, the animal is particularly vulnerable to influences from the environment.

An initial critical window of vulnerability occurs in prenatal life, when HPA axis and feeding-regulatory pathways begin to develop. For instance, both stress (or synthetic glucocorticoids) and poor nutrition *in utero* can have significant long-term consequences for feeding and behavior. Excessive stress during pregnancy can lead to HPA axis dysfunction (Henry et al., 1994; Rossi-George et al., 2009) and a long-term susceptibility to mood disorders in the offspring (Vallee et al., 1997), as well as impaired learning and memory (Lordi et al., 1997; Entringer et al., 2009), changes to reward pathways that lead to addictive behaviors (Morley-Fletcher et al., 2004; Thomas et al., 2009), and also, obesity (Li et al., 2010). The effects of prenatal stress on long-term feeding biology have been elegantly reviewed in (Entringer et al., 2012; Entringer and Wadhwa, 2013). Conversely, obesity during pregnancy, or even a pregnancy diet high in fat and sugar, can influence metabolic phenotype long-term as well as central reward processing, altering the way the rewarding aspects of food are perceived throughout life, leading to a preference for fatty, sugary foods (Ong and Muhlhausler, 2011).

This type of vulnerability in the developing individual continues postnatally.

In the rodent the hypothalamic connectivity involved in feeding develops during the second week postnatally (Bouret et al., 2004a,b). Leptin is one critical trophic factor in stimulating this growth. Thus, insufficient leptin available in the dam's milk while these pathways are developing can disrupt the formation of these connections (Bouret and Simerly, 2007). A premature leptin surge or excessive leptin, such as can occur with *in utero* growth restriction or with obese or hyperleptinemic dams, can also disrupt this connectivity and result in a subsequent insensitivity to satiety signals (Yura et al., 2005; Kirk et al., 2009). Similarly, ghrelin normally counteracts leptin's trophic effects on these regions and a change in the timing or magnitude of the expected progressive elevation in plasma ghrelin can also disrupt this development (Grove and Cowley, 2005). The ultimate effect of such developmental influences on the animal is a disruption of central responses to nutritional status and disrupted feeding behavior.

It is interesting to note that development of the HPA axis occurs in the rodent at similar times to the development of feeding-regulatory pathways. An animal's ability to respond to stress is immature at birth and the lifespan is characterized by a stress-hyporesponsive period that lasts from approximately the first to second weeks of life (Sapolsky and Meaney, 1986). Excessive stress, exposure to glucocorticoids, or prolonged absence from the dam can permanently terminate this stress hyporesponsive period, leading to life-long hypersensitivity to stress (Lehmann et al., 2002a,b; Barna et al., 2003; Xu et al., 2011). Certainly, early life stressful events such as maternal separation in the rodent, or child abuse/loss of a parent in humans can cause disruption of the HPA axis in this way (Koch et al., 2008; D'Argenio et al., 2009). However, neonatal developmental influences can also be fairly subtle and still have pronounced effects. For instance, Meaney's group has shown rat pups given high-intensity nursing and grooming by their dams grow up to have attenuated HPA axis responses to psychological stress and reduced vulnerability to anxiety (Liu et al., 1997; Champagne and Meaney, 2001).

In addition to, or perhaps as a result of, disrupting the HPA axis, the parental influence at this time is also crucial for establishing feeding patterns long-term. Thus, maternal separation can lead to the offspring having lower voluntary food intake and a preference for foods low in carbohydrates (Penke et al., 2001), while social isolation in previously maternally separated rats elevates food intake and weight gain (Ryu et al., 2009). It is likely this effect of the early environment on feeding patterns long-term is somewhat adaptive for the animal. Thus, early maternal separation in the wild rat likely occurs when food is scarce and foraging difficult. Thus, the offspring is brought into a world of food scarcity and high stress and its physiology adjusts accordingly to become hypersensitive to the effects of stress and to overeat. Essentially the neonatal environment thus imposes a drive to make the most of feeding opportunities when they are available (Meaney, 2001).

MECHANISMS OF EARLY LIFE INFLUENCE ON HPA AXIS FUNCTION

Early life events are able to disrupt HPA axis function in a variety of ways (**Figure 1**). Prior to birth, the fetus is remarkably well protected from the effects of stress. The placenta produces 11β hydroxysteroid dehydrogenase 2 (11βHSD2), which converts active glucocorticoids from the mother into the inactive form, ensuring maternal glucocorticoids are prevented from reaching fetal circulation (Lucassen et al., 2009). Central changes also occur in the mother to ensure she responds to stress by secreting less glucocorticoids; for instance, allopregnanolone-mediated inhibition of the noradrenergic input to the PVN is enhanced as progesterone levels increase with pregnancy, meaning HPA axis activation is suppressed (Brunton et al., 2005, 2009). However, severe or prolonged stress or synthetic glucocorticoid exposure can over-ride these protective mechanisms and influence the development of the fetal HPA axis. For instance, excess maternal glucocorticoids can increase fetal circulating glucocorticoid levels and can alter fetal 11βHSD2 (Clifton et al., 2006) and glucocorticoid receptor (GR) expression (Edwards et al., 1993). Excess fetal glucocorticoids can also interfere with normal brain growth and development at this time, with restraint stress to the dam during pregnancy leading to reduced levels of proteins such as growth-associated protein of 43 kDa (GAP-43) that are involved in synaptic pruning (Pfenninger et al., 1991; Larsson, 2006; Jutapakdeegul et al., 2009).

Postnatally there are fewer mechanisms to protect the animal from the effects of stress and excessive glucocorticoids. The presence of the dam, in rodents, is essential for the maintenance of attenuated sensitivity to stress in the stress hypo-responsive period, but the neonatal HPA axis is still very vulnerable at this time. As with fetal glucocorticoids, postnatal glucocorticoids or stress can alter synaptic pruning and can also lead to reduced GR expression in brain regions important for glucocorticoid negative feedback, the hypothalamus and hippocampus (Liu et al., 1997).

These effects of the perinatal environment on GR can be imposed long-term via changes to the epigenome. For instance, even something as subtle as the style of attention imparted by the dam to her offspring can induce pronounced epigenetic changes to GR expression. When rat pups are groomed by the dam it induces a rise, in the pup, of nerve growth factor inducible factor A (NGFI-A) expression (Hellstrom et al., 2012). The increase in NGFI-A expression in turn leads to increases in histone acetylation of the GR, demethylation of the GR promoter and increased GR activity (Hellstrom et al., 2012). Thus, pups that experienced a paucity of grooming in early life have reduced NGFI-A expression and suppressed GR activity and expression in glucocorticoid negative feedback regions. The long-term effect of this early under-grooming is a hypersensitivity to the effects of stress (Champagne and Meaney, 2001). Elevations in GR expression due to early life influence have also been linked to excess weight gain throughout life (Stevens et al., 2010; Begum et al., 2012).

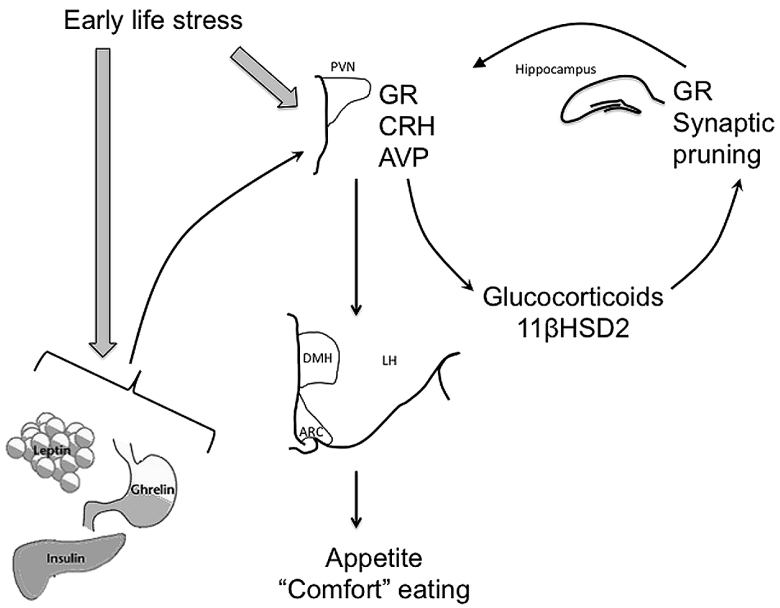

FIGURE 1 | Early life stress can influence development of the HPA axis, as well as regulation of satiety-related hormones, leptin, insulin, and ghrelin to alter feeding behavior long-term. Thus, early life stress can lead to epigenetic modification of glucocorticoid receptor (GR) expression in the hypothalamus and hippocampus and arginine vasopressin (AVP) and corticotropin-releasing hormone (CRH) in the hypothalamus, resulting in suppressed GR and increased AVP and CRH activity in response to stress later in life. Synaptic pruning in the hippocampus and circulating 11βHSD2 are also affected leading to elevated circulating glucocorticoid (GC) concentrations both under basal conditions and in response to stress. These effects of early life stress are ultimately seen in altered outputs from the paraventricular nucleus of the hypothalamus (PVN) to feeding-related nuclei such as the arcuate nucleus (ARC) and the dorsomedial nucleus of the hypothalamus (DMH). Early life stress can also potentially induce increased release of trophic/satiety hormones such as leptin, insulin, and ghrelin, again influencing appetite, feeding behavior, and metabolism throughout life.

Arginine vasopressin (AVP) regulation of the HPA axis response to stress is also subject to epigenetic modification by early life events. Thus, in the mouse, early separation from the dam leads to changes in DNA methylation, resulting in increased PVN AVP expression and changes in coping responses to stress (Murgatroyd et al., 2009; Murgatroyd and Spengler, 2011). While the early life period is one of particular vulnerability to environmental influences, epigenetic modification can occur in response to the environment at any time. Thus, chronic social stress in adult mice can induce lasting demethylation of the CRH gene, resulting in heightened anxiety-like behavior (Elliott et al., 2010).

In addition to the early influence of stress and glucocorticoids directly on the HPA axis, stress and glucocorticoids can also independently influence development of the feeding circuitry discussed above. For instance, perinatal glucocorticoids, in rodents and humans, can lead to elevations in plasma leptin (Bruder et al., 2005; Marinoni et al., 2008). Given what we know about the sensitivity of the developing hypothalamic connectivity to circulating leptin at this time, it is highly likely this glucocorticoid-mediated increase in leptin interferes with the normal leptin-induced establishment of connections between the ARC, PVN, dorsomedial nucleus of the hypothalamus (DMH), and lateral hypothalamus (LH). Glucocorticoids can also influence levels of other crucial trophic hormones at this time, increasing insulin release from the pancreas (Moyer-Mileur et al., 2011) and ghrelin release from the gut (Hosoda et al., 2006; Kristenssson et al., 2006). There is even recent evidence maternal insulin sensitivity during pregnancy can influence fetal brain activity and may contribute to prenatal programming of long-term insulin sensitivity (Linder et al., 2014). Again, it is likely these changes are able to interfere with appropriate establishment of feeding-related circuitry in the hypothalamus. It is also worth noting these trophic factors may also contribute to HPA axis development, further consolidating the link between the HPA axis and feeding. Thus, elevated neonatal leptin levels (independent of other environmental stimuli) can lead to an increase in GR in the hypothalamus and hippocampus and resulting changes in HPA axis sensitivity to glucocorticoid negative feedback (Proulx et al., 2001).

CONCLUSION AND CLINICAL IMPLICATIONS

The discussed data make it clear that the HPA, stress, axis and feeding regulation are inextricably linked, with the early life developmental environment being critical in establishing both. The challenge now will be to ensure we achieve the appropriate balance when influencing these systems with parental care and neonatal medical treatments. There is no doubt that several current perinatal treatments, while crucial for their immediate purpose, have far-reaching side-effects on systems such as the HPA axis and feeding circuitry. For instance, synthetic glucocorticoid, administered prenatally to assist in lung development, may elevate plasma leptin (Marinoni et al., 2008), stimulate epigenetic modifications in GR and elevate 11βHSD2 (Clifton et al., 2006). Similarly, the current practice of intensively feeding premature and small for gestational age babies to accelerate brain and lung development has the negative side-effect of predisposing these babies to long-term excess weight gain (Ong et al., 2000; Stettler et al., 2005). While these strategies may be essential in the immediate term to ensure the newborn's survival, consideration should be given to how we can mitigate the long-term negative effects. Understanding of the mechanisms by which stress interacts with eating behavior in the developed adult is also essential for behavioral and pharmaceutical treatments to prevent excess weight gain in at-risk patients.

AUTHOR CONTRIBUTIONS

Luba Sominsky and Sarah J. Spencer conceived of, researched, drafted, and finalized this review. They give final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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The role of alpha-7 nicotinic receptors in food intake behaviors

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Nicotine alters appetite and energy expenditure, leading to changes in body weight. While the exact mechanisms underlying these effects are not fully established, both central and peripheral involvement of the alpha-7 nicotinic acetylcholine receptor (α7nAChR) has been suggested. Centrally, the α7nAChR modulates activity of hypothalamic neurons involved in food intake regulation, including proopiomelanocortin and neuropeptide Y. α7nAChRs also modulate glutamatergic and dopaminergic systems controlling reward processes that affect food intake. Additionally, α7nAChRs are important peripheral mediators of chronic inflammation, a key contributor to health problems in obesity. This review focuses on nicotinic cholinergic effects on eating behaviors, specifically those involving the α7nAChR, with the hypothesis that α7nAChR agonism leads to appetite suppression. Recent studies are highlighted that identify links between α7nAChR expression and obesity, insulin resistance, and diabetes and describe early findings showing an α7nAChR agonist to be associated with reduced weight gain in a mouse model of diabetes. Given these effects, the α7nAChR may be a useful therapeutic target for strategies to treat and manage obesity.

Keywords: α7 nicotinic receptor, nicotine, obesity, eating behaviors, food intake

INTRODUCTION

Nicotine has long been known to affect energy balance and weight. Smokers, for example, weigh less than age- and sex-matched non-smokers (Albanes et al., 1987), while smoking cessation is associated with increased food intake and weight gain (Stamford et al., 1986; Williamson et al., 1991; Filozof et al., 2004). Given the strong link between smoking and reduced weight, many report using smoking for weight control, or avoid cessation due to fear of weight gain (Camp et al., 1993; Wiseman et al., 1998; Fulkerson and French, 2003). Experimentally, nicotine has been shown to suppress appetite, increase energy expenditure, and alter feeding patterns, which can lead to weight loss (Jo et al., 2002; Zoli and Picciotto, 2012). Despite these known effects, however, the mechanisms underlying nicotine's effects on eating behaviors and obesity remain unclear. Nicotine acts on both high-affinity nicotinic cholinergic receptors, such as the α4-β2 receptor, and low-affinity receptors, such as the α7 receptor, both centrally and peripherally. Recent studies suggest that the alpha-7 nicotinic acetylcholine receptor (α7nAChR) may play a particularly prominent role in nicotinic effects on eating behaviors. As such, this review focuses on neuronal effects of nicotinic agents, especially those involving the α7nAChR, how stimulation of this receptor influences eating behaviors and weight, and the potential utility of α7nAChR agonists as a novel treatment strategy for obesity.

ALPHA-7 NICOTINIC ACETYLCHOLINE RECEPTORS

Neuronal nicotinic acetylcholine receptors consist of ligand-gated ion channels that are activated by acetylcholine, but also respond to nicotine and similar compounds. These receptors are comprised of five transmembrane subunits arranged around a central pore (Paterson and Nordberg, 2000; Dani and Bertrand, 2007). These subunits include αβ combinations (α2–α6 and β2–β4), homomeric nAChRs (α7–α9), and a heteromer α combination (α9 with α10) (McGehee et al., 1995; Jones et al., 1999; Dani and Bertrand, 2007). The two main types of nAChRs found in the brain are α4–β2 receptors and α7 receptors (Jensen et al., 2005; Changeux, 2010). While different nAChR subtypes may affect circuits involved in feeding behavior (Jo et al., 2002; Mineur et al., 2011a,b; Zoli and Picciotto, 2012), this review will focus on α7nAChRs, which are receiving increased research attention for their involvement in eating behaviors and food intake.

CENTRAL EFFECTS OF α7nAChRs ON EATING BEHAVIORS

Previous reviews have described peripheral effects of nicotine and other α7nAChR agonists on obesity and eating behaviors (Bencherif et al., 2011; Lakhan and Kirchgessner, 2011). As such, while recent evidence for peripheral effects will be briefly examined, the primary focus of this review will be on central effects. Overall, nicotine and other α7nAChR agonists appear to suppress appetite through numerous complex, interacting central pathways, particularly those in the hypothalamus, which plays a fundamental role in energy balance. When various interactions are jointly considered, activation of hypothalamic α7nAChRs is thought to result in overall increased inhibition of appetite circuits, resulting in decreased food intake (Jo et al., 2002). Stimulation of α7nAChRs may also reduce food intake via effects on reward pathways or cortical networks involved in eating behaviors.

α7nAChR EFFECTS ON HYPOTHALAMIC NEUROPEPTIDES

Hypothalamic nuclei most associated with energy balance and feeding regulation include the lateral hypothalamus (LH), ventromedial hypothalamus (VMH), arcuate nucleus (ARC), and paraventricular nucleus (PVN). The LH is often simplistically described as the "hunger center" and the VMH the "satiety center" (Schwartz et al., 2000; Zoli and Picciotto, 2012). The ARC is a primary center for peripheral feeding signal integration (e.g., leptin, insulin) and contains neurons that stimulate feeding and those that inhibit feeding when activated, with projections to the PVN and LH (Schwartz et al., 2000; Kageyama et al., 2012; Zoli and Picciotto, 2012).

A primary potential pathway for α7nAChR mediation of eating behaviors involves hypothalamic cholinergic input. The hypothalamus contains rich cholinergic innervation and some of the highest levels of α7nAChR expression in the brain (Sargent, 1993). Appetite-related circuits within the hypothalamus can be modulated by nAChR activation, with a complex network of hormone and neuropeptide signals exerting neuronal effects to regulate eating behaviors. A number of studies have demonstrated effects of nicotine on these signals. Here, we will discuss α7nAChR involvement in cholinergic effects on proopiomelanocortin (POMC), neuropeptide Y (NPY), and melanin-concentrating hormone (MCH), all of which are involved in feeding regulation (**Figure 1**).

POMC AND NPY

Nicotine may suppress appetite via activation of POMC neurons. POMC is produced in the hypothalamus (Huang et al., 2011; Zoli and Picciotto, 2012) and is a precursor for melanocortins, such as α-melanocyte-stimulating hormone (α-MSH), associated with suppressed food intake (Schwartz et al., 2000; Zoli and Picciotto, 2012). Electrophysiologically, Huang et al. (2011) demonstrated that nicotine excites mouse hypothalamic POMC neurons and that α7nAChRs are present on these neurons. Nicotine effects were reduced by the α7nAChR antagonist methyllycaconitine (MLA), suggesting at least partial mediation by α7nAChRs. As such, POMC stimulation is a potential mechanism through which α7nAChR agonism may suppress appetite. It should be noted, however, that MLA is not as selective an antagonist for α7nAChRs as α-bungarotoxin (Klink et al., 2001; Mogg et al., 2002), which should be considered when MLA is used to assess α7nAChR effects.

Neuropeptide Y, also produced in the hypothalamus, is associated with increased food intake (Schwartz et al., 2000). NPY neurons in the ARC project to the PVN to stimulate feeding (Morris, 1989; Kageyama et al., 2012). Thus, POMC and NPY have opposing effects on food intake. Smokers show reduced NPY levels compared to non-smokers, and smoking cessation is associated with increased NPY (Hussain et al., 2012), suggesting NPY inhibition as a mechanism for appetite suppression. However, nicotine effects on NPY are complex. As with POMC, NPY neurons in the hypothalamus are stimulated by nicotine and express α7nAChRs. Excitation of NPY neurons by nicotine is partially mediated by α7nAChRs, as MLA reduces excitation. Although nicotine reduces hypothalamic NPY mRNA in rats acutely (Frankish et al., 1995), NPY mRNA *increases* with chronic administration (Frankish et al., 1995; Li et al., 2000), which is accompanied by *decreased* food intake (Li et al., 2000). This is counterintuitive, as NPY stimulates food intake. However, nicotine also reduces hypothalamic NPY receptor density (Kane et al., 2001), which could explain the decreased intake. Another explanation for the net appetite-inhibiting effect of nicotine is that the depolarizing effect of nicotine on POMC neurons (anorexigenic) is significantly greater than that on NPY neurons (orexigenic). Furthermore, in addition to NPY neuron excitation

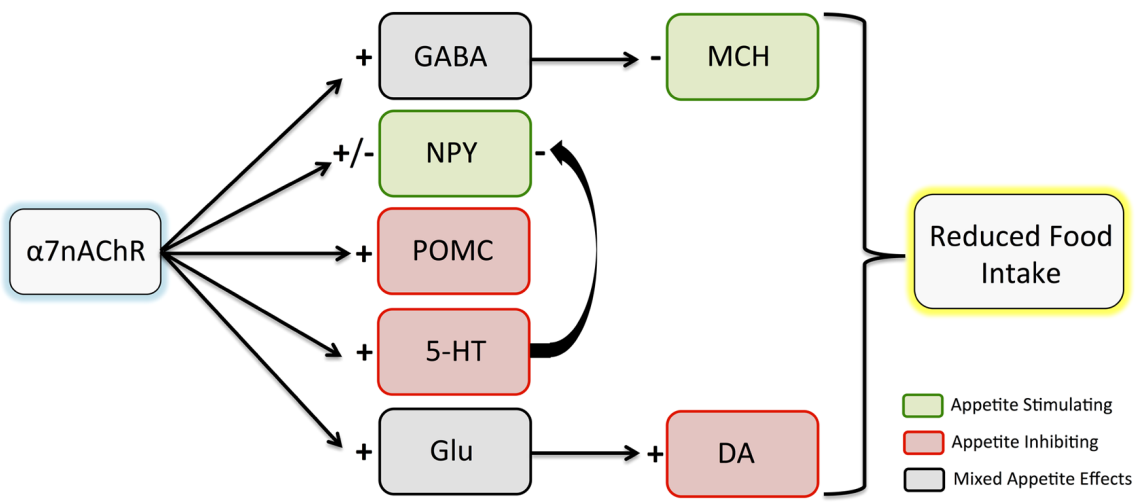

FIGURE 1 | Effects of α7nAChR stimulation on hypothalamic neuropeptide and neurotransmitter release. Although there are complex interactions among pathways, it is hypothesized that the net effect of α7nAChR stimulation leads to reduced food intake. Inhibitory effects are indicated with a minus sign, while excitatory effects are indicated with a plus sign. Note that both inhibitory and excitatory effects of α7nAChR stimulation have been observed on NPY. While appetite effects of dopamine elsewhere in the brain are mixed, studies suggest that hypothalamic dopamine release contributes to appetite inhibition. α7nAChR, α7 nicotinic acetylcholine receptor; GABA, gamma aminobutyric acid; NPY, neuropeptide Y; POMC, proopiomelanocortin; 5-HT, serotonin; Glu, glutamate; MCH, melanin-concentrating hormone; DA, dopamine.

by nicotine, inhibition of excitatory synaptic activity (glutamate release) on NPY neurons was also observed, an effect not seen in POMC neurons (Huang et al., 2011). Thus, although nicotine can excite NPY neurons, the greater direct excitation of appetite-inhibiting POMC neurons compared to appetite-stimulating NPY neurons, in addition to indirect inhibition of NPY neurons (via reduced glutamate release) may contribute to the net effect of appetite inhibition by nicotine and other α7nAChR agonists.

MELANIN-CONCENTRATING HORMONE

Melanin-concentrating hormone (MCH) neurons are primarily located in the LH (Zamir et al., 1986) and also stimulate food intake (Qu et al., 1996). MCH may have a particular role in reward-related aspects of food, as MCH neurons project to the nucleus accumbens (NAC) and the ventral tegmental area (VTA), brain areas involved in reward processes (Schilstrom et al., 1998; Jo et al., 2005). MCH knockout mice are excessively lean and demonstrate reduced food intake (Shimada et al., 1998; Marsh et al., 2002). α7nAChRs may mediate gamma aminobutyric acid (GABA)-related inhibition of MCH neurons in the LH, leading to this appetite suppression (Jo et al., 2005).

α7nAChR MODULATION OF NEUROTRANSMITTERS INVOLVED IN FOOD INTAKE BEHAVIORS

In addition to hypothalamic neuropeptides, nicotine modulates effects of multiple other neurotransmitter systems in the brain. The following section describes the impact of nicotine on GABA, glutamate, dopamine (DA), and serotonin, focusing on how α7nAChRs may inhibit appetite by modulating these neurotransmitter systems.

GAMMA AMINOBUTYRIC ACID

Release of GABA, the main inhibitory neurotransmitter in the brain, is influenced by nAChRs (McGehee et al., 1995; Jones et al., 1999). Nicotine effects on appetite reduction may be associated with decreased excitability of MCH neurons in the LH via increased GABAergic inhibitory tone. Jo et al. (2005) found nicotine administration to facilitate GABAergic transmission in adult mice, and prenatal nicotine exposure to enhance postnatal GABAergic transmission. Specific involvement of α7nAChRs was also demonstrated, as an α7nAChR-specific antagonist (α-bungarotoxin) blocked these effects. As such, activation of α7nAChRs on GABAergic terminals in the hypothalamus may contribute to the anorexigenic effects of nicotine.

GLUTAMATE AND DOPAMINE

Glutamate is the main excitatory neurotransmitter in the brain and plays a role in rewarding effects of nicotine, as nicotine increases glutamate release in the VTA and NAC, brain regions central to reward mechanisms (McGehee et al., 1995; Reid et al., 2000; Schilstrom et al., 2000). High concentrations of α7nAChRs are observed in the VTA (Clarke and Pert, 1985; Dominguez del Toro et al., 1994; Schilstrom et al., 1998; Jones and Wonnacott, 2004) and are thought to mediate nicotine-associated glutamate release (McGehee et al., 1995; Schilstrom et al., 2000). α7nAChR-mediated glutamate release plays a large role in nicotine's effects on DA, a neurotransmitter critical in the reinforcing effects of nicotine (Schilstrom et al., 1998; Fowler et al., 2008). α4-β2nAChRs are sufficient for these reinforcing effects (Besson et al., 2012), likely via direct effects on DA neurons (Wooltorton et al., 2003; Besson et al., 2012). However, stimulation of α7nAChRs activates DA neurons via glutamatergic inputs (Yoshida et al., 1992; Schilstrom et al., 2000, 2003; Garzon et al., 2013). Thus, α7nAChR activation ultimately increases DA, but this is largely mediated via glutamatergic effects. Additionally, α7nAChRs may be important in dopaminergic function following long-term nicotine exposure, as they are more resistant to desensitization at usual levels for smokers than nAChR subunits containing β2 receptors, and may prevent dopaminergic hypoactivation resulting from chronic β2 desensitization (Besson et al., 2007, 2012).

The role of α7nAChR-mediated glutamate release in food consumption remains unclear. Administration of a glutamate *antagonist* has been found to increase food intake in rats (Maldonado-Irizarry et al., 1995; Stratford et al., 1998). As such, glutamate release stimulated by an α7nAChR *agonist* could decrease food intake. Increased DA release, amplified by α7nAChR-mediated glutamate release, increases the reward value of food (Yoshida et al., 1992; Schilstrom et al., 1998). Quarta et al. (2009) observed striatal DA release in mice following administration of an α7nAChR agonist (choline), an effect not observed in mice lacking α7nAChRs. Food-induced DA release is attenuated by an α7nAChR antagonist (MLA), implicating α7nAChRs in eating-related reward (Schilstrom et al., 1998). However, the role of DA in feeding behaviors is complex and varies by brain region. Although DA contributes to rewarding aspects of food intake in areas such as the VTA and NAC, hypothalamic DA release is though to contribute to nicotine-related reductions in food intake (Meguid et al., 2000; Schwartz et al., 2000). Thus, further study is needed to determine if effects of α7nAChRs on DA lead to overall increased or decreased consumption.

SEROTONIN

Serotonin inhibits food intake (Waldbillig et al., 1981; Jo et al., 2002), likely by promoting satiety (i.e., meal stopping; Shor-Posner et al., 1986). One mechanism may be via NPY, as evidence suggests serotonin inhibits NPY release (Dryden et al., 1995, 1996a,b). Nicotine-induced nAChR activation can increase serotonin release, contributing to appetite suppression (Summers and Giacobini, 1995; Jo et al., 2002). Activation of α7nAChRs is thought to influence serotonin release, as α7nAChRs have been identified on serotonergic neurons (Galindo-Charles et al., 2008) and α7nAChR stimulation increases serotonin release in the dorsal raphe nucleus (Li et al., 1998).

CORTICAL α7nAChR INVOLVEMENT IN FOOD INTAKE BEHAVIORS

Cortically, α7nAChR activation may affect limbic and paralimbic brain systems such as the insula and cingulate cortex, which also play a role in reward aspects of eating behaviors (Volkow et al., 2010) and contain rich cholinergic innervation (Nyback et al., 1989).

INSULA/SALIENCE NETWORK

The insula, containing primary taste cortex, is involved in eating behavior regulation, including involvement in rewarding aspects of food and food-related arousal (Tataranni et al., 1999; Hinton et al., 2004; Cornier et al., 2009). The insula is also a central component of the salience network, an intrinsic brain network involved in assessing relevance of internal and external stimuli (Seeley et al., 2007; Bressler and Menon, 2010), in which altered response has been observed in obese, compared to lean, individuals (Garcia-Garcia et al., 2012; Kullmann et al., 2013). The insula is associated with urges and cravings related to both food and drugs of abuse (Pelchat et al., 2004; Naqvi and Bechara, 2009; Forget et al., 2010). Indeed, smokers sustaining insula damage following a stroke showed little subsequent difficulty quitting smoking, suggesting a role for the insula in effects of nicotine (Naqvi et al., 2007). However, the role of α7nAChRs in the insula is not yet known. Via α-bungarotoxin binding, studies have found α7nAChRs in the insula in both rats (Fuchs, 1989) and monkeys (Han et al., 2003). Presence of α7nAChRs in the human insula has been suggested by detection of α7nAChR mRNA (Wevers, 2011), but insular α7nAChR protein levels have not yet been studied in humans. As such, further study of α7nAChRs in the insula, and how activation of these receptors relates to eating behaviors, is needed.

POSTERIOR CINGULATE/DEFAULT MODE NETWORK

The posterior cingulate cortex may also be involved in eating behaviors, having been associated with neuronal responses to visual food cues and taste (Tataranni et al., 1999; DelParigi et al., 2005; Cornier et al., 2009). The posterior cingulate is also a key component of the default mode network (DMN), an intrinsic brain network involved in self-referential thoughts and attention to internal stimuli (Buckner et al., 2008). DMN activity may play a role in eating behaviors, as overactivity of this network has been observed in obese, compared to lean, individuals (Tregellas et al., 2011a). Furthermore, this activity, which was associated with measures of appetite, was shown to change in response to feeding in lean, but not obese individuals. Nicotine can reduce resting-state DMN activity, including the posterior cingulate (Tanabe et al., 2011). α7nAChRs are present in high concentrations in the cingulate cortex, as assessed by α-bungarotoxin binding (Breese et al., 1997; Marutle et al., 2001). A study of DMN activity in schizophrenia patients observed reduced response following treatment with an α7nAChR partial agonist [3-2,4-dimethoxybenzylidene anabaseine (DMXB-A)], specifically in the posterior cingulate (Tregellas et al., 2011b). As with non-mentally ill obese individuals, DMN overactivity has been observed in schizophrenia patients (Garrity et al., 2007; Whitfield-Gabrieli et al., 2009), who are obese are rates twice those observed in the general population. Given these findings, it is possible that activation of α7nAChRs could be a mechanism to normalize DMN hyperactivity in obesity.

α7nAChRs AND PERIPHERAL FACTORS INVOLVED IN EATING BEHAVIORS AND OBESITY

Recent studies have discovered a key role for α7nAChRs in peripheral factors related to obesity. In a mouse model of diabetes, Marrero et al. (2010) found that an α7nAChR-selective agonist (TC-7020) reduced weight gain and food intake, as well as glucose and triglyceride levels and expression of proinflammatory cytokines. These effects were reversed by an α7nAChR antagonist (MLA), supporting α7nAChR involvement. In humans, Cancello et al. (2012) have also found evidence supporting α7nAChR involvement in obesity. In addition to identifying α7nAChR expression in human mature adipocytes, they found that expression was downregulated in obese compared to lean adults, and that weight loss partially restored α7nAChR expression.

A potential mechanism through which peripheral α7nAChRs may exert weight and food intake effects is by mediating anti-inflammatory effects. Inflammation is a key feature of obesity, associated with increased proinflammatory cytokine production, insulin resistance, and development of type 2 diabetes (Marrero et al., 2010; Wang et al., 2011). Activation of α7nAChRs on cytokine-producing cells, such as macrophages, mediates this inflammatory response by inhibiting inflammatory cytokine production (Wang et al., 2011). A number of studies have demonstrated anti-inflammatory effects of nicotine (Wang et al., 2003; Lakhan and Kirchgessner, 2011) and smokers may have a reduced risk of some inflammatory diseases such as ulcerative colitis (Lakhan and Kirchgessner, 2011). The "cholinergic anti-inflammatory pathway" can be activated by α7nAChR agonists (Cheng et al., 2007). Supporting this, nicotine-induced cytokine inhibition can be blocked by α7nAChR-specific antagonists (Cheng et al., 2007), and α7nAChR knockout mice show increased LPS-induced proinflammatory cytokine production, including TNFα and IL-1β (Wang et al., 2003). Wang et al. (2011) found adipose tissue and macrophages in mice to express α7nAChRs, and while nicotine suppressed proinflammatory cytokine production, this effect was not observed in α7nAChR knockout mice. Additionally, nicotine reduced adipose tissue inflammation and improved insulin sensitivity in obese mice. Xu et al. (2012) observed improved insulin sensitivity in rodents following treatment with either nicotine or an α7nAChR agonist (PNU-282987), an effect not observed in α7nAChR knockout animals. These studies suggest that α7nAChRs are critical in anti-inflammatory effects of nicotine. Given this, therapeutics targeting α7nAChRs are increasingly being explored for diseases involving inflammation, such as diabetes, arthritis, and ulcerative colitis (Wang et al., 2003; Marrero et al., 2010; Bencherif et al., 2011; Lakhan and Kirchgessner, 2011).

CONCLUSION

The α7nAChR plays an important role in both central and peripheral mechanisms involved in eating behaviors and energy balance. Studies have found links between α7nAChR expression and obesity, insulin resistance, and diabetes. Centrally, α7nAChRs modulate hypothalamic neuropeptides and neurotransmitters involved in feeding regulation and play a role in cortical processes affecting intake behavior. Overall, although the circuits involved are complex, it appears that net effects of nicotine and other α7nAChR agonists result in appetite suppression, which could lead to weight loss. Peripherally, and perhaps also centrally, α7nAChRs are also an important mediator of inflammation, a key contributor to health problems in obesity.

Although α7nAChR agonists have not yet been investigated for eating behavior effects in humans, preliminary animal work supports this idea, finding peripheral effects such as improved insulin sensitivity (Wang et al., 2011; Xu et al., 2012) and reduced weight gain and metabolic changes in a model of diabetes (Marrero et al., 2010). Further support for extending α7nAChR studies to humans lies in the observation that α7nAChRs are downregulated in human obesity, but normalize with weight loss (Cancello et al., 2012). In conclusion, given nicotine's effects in humans, experimental support for α7nAChR involvement in eating behavior regulation, and early evidence of α7nAChR agonist effects in animal studies, the α7nAChR may represent a promising new therapeutic target for weight management and the treatment of obesity in humans.

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Compulsivity in anorexia nervosa: a transdiagnostic concept

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The compulsive nature of weight loss behaviors central to anorexia nervosa (AN), such as relentless self-starvation and over-exercise, has led to the suggestion of parallels between AN and other compulsive disorders such as obsessive–compulsive disorder (OCD) and addictions. There is a huge unmet need for effective treatments in AN, which has high rates of morbidity and the highest mortality rate of any psychiatric disorder, yet a grave paucity of effective treatments. Viewing compulsivity as a transdiagnostic concept, seen in various manifestations across disorders, may help delineate the mechanisms responsible for the persistence of AN, and aid treatment development. We explore models of compulsivity that suggest dysfunction in cortico-striatal circuitry underpins compulsive behavior, and consider evidence of aberrancies in this circuitry across disorders. Excessive habit formation is considered as a mechanism by which initially rewarding weight loss behavior in AN may become compulsive over time, and the complex balance between positive and negative reinforcement in this process is considered. The physiological effects of starvation in promoting compulsivity, positive reinforcement, and habit formation are also discussed. Further research in AN may benefit from a focus on processes potentially underlying the development of compulsivity, such as aberrant reward processing and habit formation. We discuss the implications of a transdiagnostic perspective on compulsivity, and how it may contribute to the development of novel treatments for AN.

Keywords: anorexia nervosa, compulsivity, obsessive–compulsive disorder, addiction, neurobiology, habit formation, reward

Anorexia nervosa (AN) is a severely debilitating psychiatric disorder characterized by relentless self-starvation with dramatic physiological and psychological effects. It is associated with low rates of recovery (Berkman et al., 2007), and has the highest mortality rate of any psychiatric disorder (Arcelus et al., 2011). Individuals with AN place extreme over-importance on the control of weight and shape, which becomes central to their self-evaluation, and often have disturbed body image perception (Fairburn et al., 2003). These distorted beliefs and perceptions are accompanied by a lack of concern over extreme emaciation, a perpetual drive for thinness and continuous lowering of weight goals (Barbarich-Marsteller et al., 2011; see **Figure 1** for DSM-V diagnostic criteria). Characteristic behaviors seen in AN to achieve these goals, such as extreme dietary restriction and driven over-exercise, have been described as evidence of compulsivity and aberrant reward processing (Park et al., 2011, 2012; Cowdrey et al., 2013; Kaye et al., 2013a). Indeed the stereotyped and often ritualistic behaviors seen in AN have been compared to that of obsessive–compulsive disorder (OCD; Steinglass and Walsh, 2006), with the two disorders often being reported as comorbid (Halmi et al., 1991), leading to the suggestion that they may share common underlying neurobiological mechanisms (Steinglass and Walsh, 2006). Simultaneously, parallels with addictive disorders such as substance dependence have been increasingly suggested (Zink and Weinberger, 2010; Barbarich-Marsteller et al., 2011; Kaye et al., 2013a), with similarities in the inability to cease behaviors despite adverse consequences.

The aim of this review is to explore how a transdiagnostic view of compulsivity, as a dimension on which several psychiatric disorders may fall, can further our understanding of persistent weight loss behavior in AN. Neurobiological and behavioral correlates of compulsivity will be discussed, with particular focus on how these relate to AN, and parallels with OCD and substance dependence. Better understanding of neurobiological and behavioral processes underpinning compulsive weight loss behavior may aid development of much needed novel treatment strategies for AN.

COMPULSIVE BEHAVIOR IN AN

Compulsivity can be defined as a trait leading to behavior that is inappropriate to the situation, persists despite having no relationship with any overall goal, and results in undesirable consequences (Dalley et al., 2011). In individuals with AN, dietary restriction tends to take on a driven and compulsive quality. This behavior may be motivated by an aberrant sense of reward, specifically the perceived reward of extreme dietary control and thinness (Fladung et al., 2010, 2013; Park et al., 2011, 2012). In some individuals, such extreme control of eating and weight cannot be sustained, and AN may then be complicated by the development of binge eating and compensatory purging such as self-induced vomiting and/or laxative abuse (Fairburn et al., 2003), which also appears to have an element of compulsivity. Compulsive over-exercising is also a common feature, and is reported to be more prevalent in restrictive AN (80%), compared to the binge/purging subtype

To be diagnosed as having AN a person must display:

- Persistent restriction of energy intake leading to significantly low body weight (in context of what is minimally expected for age, sex, developmental trajectory, and physical health)–.
- Either an intense fear of gaining weight or of becoming fat, or persistent behaviour that interferes with weight gain (even though significantly low weight).
- Disturbance in the way one's body weight or shape is experienced, undue influence of body shape and weight on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight.

Subtypes:

Restricting Type – During the last three months, the person has not engaged in recurrent episodes of binge eating or purging behaviour (i.e., self induced vomiting or the misuse of laxatives, diuretics, or enemas).

Binge/Purge Type – During the last three months, the person has engaged in recurrent episodes of binge eating or purging behaviour (i.e., self induced vomiting or the misuse of laxatives, diuretics, or enemas).

FIGURE 1 | The DSM-V diagnostic criteria for anorexia nervosa (American Psychiatric Association, 2013).

(43%; Dalle Grave et al., 2008). The presence of compulsive exercise in AN is extremely challenging to manage and can contribute to medically dangerous degrees of weight loss.

The compulsive behaviors seen in AN have often been compared to those of OCD, but with the obsessional focus being on eating, weight and shape. The compulsions that characterize OCD are defined as repetitive, purposeful actions, which are often performed to reduce the anxiety caused by persistent, intrusive thoughts (Steinglass and Walsh, 2006). In the same way, individuals with AN have persistent, intrusive thoughts regarding food and weight gain, and may develop compulsive, ritualized behaviors in an attempt to neutralize the anxiety associated with these thoughts (Steinglass and Walsh, 2006).

Comorbidity is found to be high between AN and OCD (Halmi et al., 1991) OCD is reported to be most prevalent in the restrictive subtype of AN (Fornari et al., 1992; Lilenfeld et al., 1998), although reported prevalence has been inconsistent across studies (Godart et al., 2002). The presence of obsessive–compulsive symptoms is a risk factor for developing AN (Anderluh et al., 2008); and the level of such symptoms remains elevated to some extent even after recovery (Holtkamp et al., 2005). Familiality is reported, with the first degree relatives of individuals with AN showing an elevated risk for OCD (Bellodi et al., 2001). Candidate gene studies suggest common genetic liability between the two disorders (Mas et al., 2013). Comorbidity with obsessive–compulsive personality disorder (OCPD) is also high (Lilenfeld et al., 2006), and the excessive self-control (Pinto et al., 2014), perfectionism and rigidity seen in OCPD (Ansell et al., 2010) may parallel AN more closely.

Aspects of the compulsive behaviors characteristic of AN have increasingly been compared to the compulsive drug-seeking behavior seen in substance dependence (Scheurink et al., 2010; Zink and Weinberger, 2010; Barbarich-Marsteller et al., 2011; Kaye et al., 2013a). The developmental period of onset is similar, with an initial phase of reward seeking, in the form of weight loss in AN, which is experienced as rewarding and pleasurable (Scheurink et al., 2010; Park et al., 2011, 2012), as if it were a drug. This is followed by a narrowing of the behavioral repertoire and the lack of ability to cease behaviors despite their adverse consequences (Kalivas and Volkow, 2005). The compulsive drug-seeking behavior of addicts may parallel the relentlessness with which individuals with AN pursue weight loss. Individuals find it increasingly difficult to refrain from weight loss behavior such as restriction and compulsive exercise despite adverse consequences, and even describe symptoms of withdrawal similar to those experienced in drug addiction (Allegre et al., 2006). In terms of comorbidity, there is a higher incidence of substance dependence in ED than the general population: for example, the US National Centre on Addiction and Substance Abuse found that up to 50% of individuals with an eating disorder abuse substances compared with 9% of the general population, and up to 35% of individuals with substance abuse have an eating disorder compared with 3% of the general population (Casa, 2003; Baker et al., 2013). However, a lower incidence of substance use has been reported in restrictive AN than in other types of eating disorders such as bulimia nervosa (BN) (Holderness et al., 1994; Kaye et al., 2013a). This suggests that there may be a specific relationship between the binge-purge cycle of behavior, which may itself take on an addictive quality, and higher rates of substance abuse (O'Brien and Vincent, 2003).

Nevertheless, there are notable distinctions in information processing between AN, OCD, and substance dependence. In AN, an increased focus on delayed gratification and long term goals is seen (Kaye et al., 2013a). This is reflected in marked differences in the ability to delay reward in AN as compared to substance dependence. Substance dependent individuals and those with binge eating disorder show a preference for smaller immediate reward (Davis et al., 2010), whereas individuals with AN favor delayed larger reward (Steinglass et al., 2012b). That said, starvation in those vulnerable to AN may produce an immediately rewarding

sense of control (Park et al., 2012), acting as a positive reinforcer of behavior. Equally, avoiding negative consequences such as dysphoric mood during refeeding, which some individuals with AN experience as "withdrawal symptoms" from starvation, may be important short term goals. Perhaps as a consequence of these immediate reinforcers, the long term goal of weight loss becomes irrationally overvalued (Barbarich-Marsteller et al., 2011). Interestingly, individuals with OCPD are more able to delay reward than those with OCD, and this ability to delay reward is associated with perfectionism and rigidity (Pinto et al., 2014). This supports the suggestion that AN may parallel OPCD more closely than OCD. That said, studies looking at decision making processes in AN, OCD, and substance dependence suggest in all three disorders a tendency to make disadvantageous decisions when choosing between immediate or long terms gains (Lawrence et al., 2006; Tchanturia et al., 2007b; Verdejo-Garcia et al., 2007). This impairment in decision making is suggested to be linked to the compulsive and self-destructive behavior seen across these disorders (Tchanturia et al., 2007b). Whilst individuals with AN may show the ability to delay reward in general, their impairment in decision making may lead them to engage in compulsive weight loss behaviors despite adverse outcomes.

COMPULSIVITY AS A TRANSDIAGNOSTIC CONCEPT

The core feature that unites AN, OCD, and substance dependence is the compulsive nature of disorder-specific behavior. Compulsive weight loss behavior, such as persistent food restriction and over-exercise, is a prominent feature of AN, and is parallel to the compulsive behaviors characteristic of OCD and substance dependence; with inevitable comorbidities. If such parallels reflect similarities in the underlying mechanisms that drive this behavior, there should be some agreement about the neurobiological correlates of aberrant behavior across these disorders. Robbins et al. (2012) have suggested a transdiagnostic approach to compulsivity, arguing that it has cross-diagnostic significance, as evidenced by commonalities and comorbidities in behaviors across a range of disorders. They posit that a transdiagnostic approach to compulsivity may aid in the development of novel treatment avenues relating to specific behaviors, rather than focusing on diagnosis. This focus on constructs of behavior, as opposed to symptoms and disorder categories, reflects the recent RDoC (Research Domain Criteria) research strategy adopted by the National Institute of Mental Health (NIMH). This strategy emphasizes the need to break away from the use of symptoms and diagnostic categories for classification. Instead, research should focus on the variables that define certain dimensions of behavior, or constructs, seen transdiagnostically across psychiatric disorders. In line with this, we suggest that focusing on compulsivity as a transdiagnostic concept may help in understanding commonalities in the compulsive behaviors seen not only in AN but also in other disorders, such as OCD and substance dependence, without categorizing them together unnecessarily.

There is a huge unmet need for translational research in AN to develop novel treatments, especially for adults with severe AN. Effective treatments require an optimal understanding of processes underlying AN, so that the correct treatment targets are identified. The use of a transdiagnostic approach to compulsive weight loss behaviors in AN may begin to address this problem. This review will now focus on the neurobiological and behavioral correlates of compulsivity across disorders and how this can guide novel avenues for the development of treatments for AN.

SUMMARY

- Parallels are seen between the compulsive nature of behavior in AN, OCD, and substance dependence.
- Despite similarities, important distinctions in disorder-specific compulsivity and information processing are seen across disorders.
- A transdiagnostic view of compulsivity, seen in varying manifestations across disorders, may aid the development of treatments targeting compulsive behavior.

WHAT IS THE NEURAL BASIS OF COMPULSIVITY?

Models of the neurocircuitry involved in compulsive behavior suggest the involvement of a cortico-striatal circuit, consisting of a striatal and prefrontal component (Robbins, 2007; Brewer and Potenza, 2008; Fineberg et al., 2010). The striatal component is seen as responsible for driving compulsive behavior, while the prefrontal component controls or inhibits this behavior. Abnormalities in either of these components (hypoactivity/hyperactivity) may result in an increase in compulsive behavior. The caudate nucleus (the striatal component) is suggested to drive compulsive behaviors, with the orbitofrontal cortex (OFC, the prefrontal component) exerting inhibitory control over these (see **Figure 2**). This is distinct but related to an "impulsive" cortico-striatal circuit, involving the ventral striatum (VS)/nucleus accumbens (NAc) (striatal component), and the anterior cingulate cortex (ACC)/ventromedial prefrontal cortex (vmPFC; prefrontal component). These compulsive and impulsive circuits are suggested to be intercommunicating, with the possibility that abnormalities in one circuit lead to abnormalities in the other (Fineberg et al., 2010). This is relevant for theories of dependence in which it is suggested that what may begin as impulsive behavior may eventually become compulsive with repetition of behavior, with a corresponding shift in control from impulsive to compulsive neural circuitry (Everitt and Robbins, 2005). This may relate to eating disorders involving binge/purge behaviors, in which the behaviors may initially be driven by impulsivity rather than compulsivity.

This compulsive cortico-striatal circuit can be illustrated using tasks which are thought to measure compulsivity. Failures in reversal learning, the ability to adapt behavior following negative feedback, have been suggested to reflect cognitive inflexibility, meaning a rigid cognitive style, which may contribute to compulsivity (Fineberg et al., 2010). Reversal learning is impaired by lesions to the OFC (Dias et al., 1996; Berlin et al., 2004; Remijnse et al., 2006), an area selectively activated during reversal learning tasks (Hampshire and Owen, 2006). Lesions to the medial striatum have also been shown to produce impairments in reversal learning in monkeys (Clarke et al., 2008), supporting the suggestion that a cortical and striatal component is involved in compulsive behavior. Set-shifting tasks, in which attention is required to switch between multiple tasks, or elements of a task (Miyake et al., 2000), is also thought to measure cognitive inflexibility (Fineberg et al., 2010). Lesions to the lateral PFC in primates (Dias et al., 1996) and the

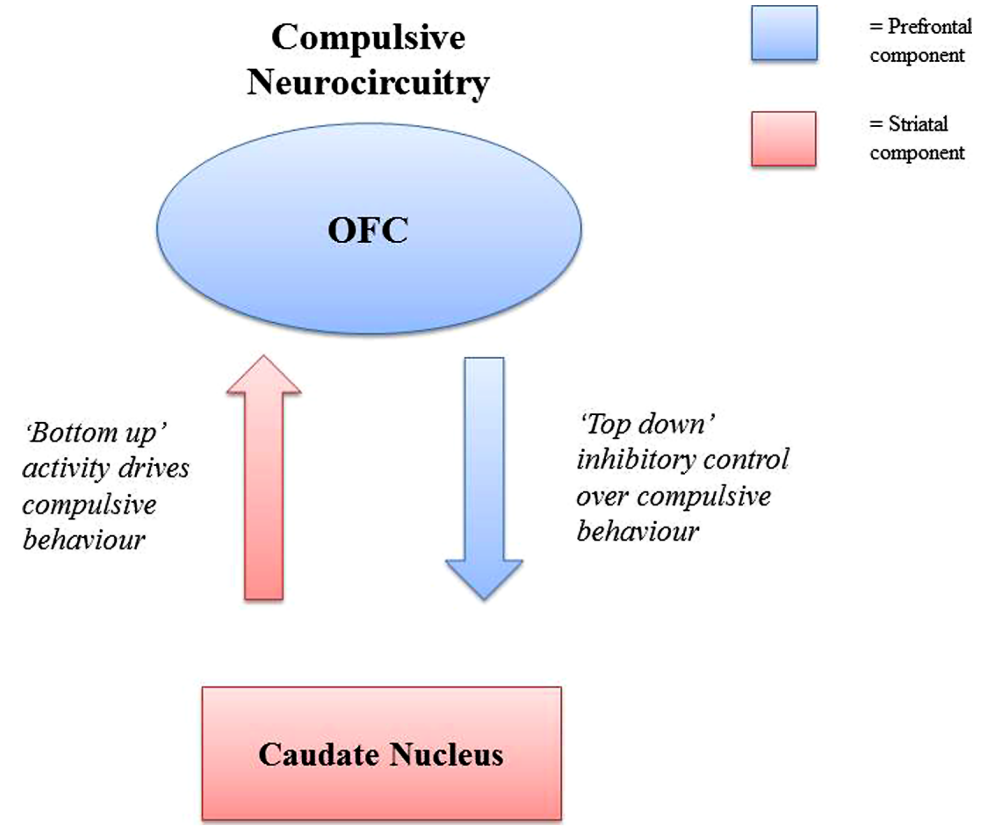

FIGURE 2 | Representation of the cortico-striatal circuitry suggested to be involved in the development compulsive behavior (Robbins, 2007; Brewer and Potenza, 2008; Fineberg et al., 2010). In the circuit, activity in the striatal component drives compulsive behavior, whilst activity in the prefrontal component inhibits compulsive behavior. Both failures in "top down" control of the prefrontal components, and over activity of "bottom up" striatal activity can result in increases in compulsive behavior. Abbreviation: OFC, orbitofrontal cortex.

ventrolateral PFC in humans (Hampshire and Owen, 2006) impair performance on set-shifting tasks. Failures in both these types of task, and the corresponding neuroanatomy, suggest that impairments in "top down" cortical control may underpin compulsive behavior, and over activity of striatal regions may also underpin, or exacerbate compulsivity.

ARE NEURAL AND BEHAVIORAL CORRELATES OF COMPULSIVITY OBSERVED IN AN, OCD, AND ADDICTION?

Dysfunction of the neurocircuitry implicated in compulsivity can be seen across AN, OCD, and addiction (see **Table 1**). In terms of a dysfunctional striatal component, AN has been associated with increased caudate function, measured both directly during a monetary reward task (Wagner et al., 2007) and indirectly during exposure to aversive food stimuli (Cowdrey et al., 2011). In OCD, increased functional connectivity within the cortico-striatal circuitry has been shown to correlate positively with Y-BOCS score, together with increased volume and activity in the caudate nucleus (Hou et al., 2013). Evidence of increased activity in the caudate nucleus (Saxena et al., 1998) and alterations in caudate volume have also been reported in OCD (Scarone et al., 1992). In contrast to AN and OCD, chronic substance use has been associated with decreased activation of the caudate during reward anticipation (van Hell et al., 2010). However, high scores on self-reported food addiction have been associated with increased activation in the caudate (Gearhardt et al., 2011), suggesting there may be differences in caudate activation between behavioral and substance addictions.

Decreased activation of a prefrontal component can also be seen across disorders. Reduced activity in the OFC whilst viewing disorder-related stimuli has also been found in AN (Uher et al., 2004), and alterations in OFC volume has been reported (Frank et al., 2013). Reduced activation of the OFC during reversal learning has been found in individuals with OCD and their unaffected relatives (Chamberlain et al., 2008), and abnormalities in OFC gray matter have been found in both OCD (Menzies et al., 2008), and substance dependence (Franklin et al., 2002) Reduced functional connectivity of the right inferior and superior OFC has been related not only to self-reported compulsivity in OCD but also to compulsive drug taking in substance dependence (Meunier et al., 2012).

Abnormalities in the "compulsive" circuit are implied by deficits in neuropsychological tasks thought to tap this construct (see **Table 1**). Individuals with AN are often described as having low cognitive flexibility (Tchanturia et al., 2004), and consistently show

Table 1 | Evidence of abnormalities in the neurocircuitry, neurotransmitters and behavioral correlates of compulsivity, across AN, OCD, and Addictions.

	Model of compulsivity	Evidence in AN	Evidence in OCD	Evidence in addictions
Neurocircuitry	• Fronto-striatal circuit involving the OFC (prefrontal component) and the caudate nucleus [striatal component; Robbins (2007), Brewer and Potenza (2008), Fineberg et al. (2010)]. • Abnormalities result from impairments in prefrontal inhibitory control OR over activity of the striatal component. Robbins (2007), Brewer and Potenza (2008), Fineberg et al. (2010).	• Increased caudate function during reward task Wagner et al. (2007), and during exposure to aversive food stimuli Cowdrey et al. (2011). • Decreased OFC activity during disorder related stimuli Uher et al. (2004), decreased OFC volume Frank et al. (2013). • Deactivation of fronto-striatal circuitry predicted by levels of compulsivity Rothemund et al. (2011).	• Increased functional connectivity of CTSC circuits correlated with Y-BOCS scores Hou et al. (2013). • Increased volume and activity in caudate Scarone et al. (1992), Saxena et al. (1998), Hou et al. (2013). • Reduced activation of OFC during reversal learning Chamberlain et al. (2008); abnormalities in OFC gray matter Menzies et al. (2008), reduced functional connectivity of OFC Meunier et al. (2012).	• Increased functional connectivity of CTSC circuits correlated with Y-BOCS scores Hou et al. (2013). • Increased volume and activity in caudate Scarone et al. (1992), Saxena et al. (1998), Hou et al. (2013). • Reduced activation of OFC during reversal learning Chamberlain et al. (2008); abnormalities in OFC gray matter Menzies et al. (2008); reduced functional connectivity of OFC Meunier et al. (2012).
Neurotransmitters	• Role for serotonin and 5-HT receptors: impairments in reversal learning linked to reduced serotonin Clarke et al. (2004), Evers et al. (2005); antagonizing 5HT-2A receptors impairs reversal learning Boulougouris et al. (2008). • Role for the mesolimbic dopamine system: DA tone in the ventral loops important in regulating reward and reinforcement Wise (2002), Fineberg et al. (2010); DA D2 receptor agonists associated with impairments in reversal learning Cools et al. (2006), and increased compulsive behavior in Parkinson's disease Voon et al. (2009). • Modulation of glutamate by DA involved in reward reinforcement processes Guardia et al. (2011), glutamate involvement in set-shifting Nicolle and Baxter (2003).	• Increased 5HT-1A and decreased 5HT-2A binding potential Kaye et al. (2005b), Bailer et al. (2007). • Altered striatal DA release in recovered AN: increased binding of DA D2/3 receptors in anterior VS Frank et al. (2005), Bailer et al. (2013); DA release experienced as anxiogenic Bailer et al. (2012). • DA D2 receptor polymorphism linked to AN Bergen et al. (2005). • Reduced HVA in CSF in recovered AN Kaye et al. (1999), increased levels in acute AN Castro-Fornieles et al. (2008). • Increased CSF glutamate in AN Nakazato et al. (2010).	• Agonizing 5HT-2C and 1B receptors increases compulsivity in OCD Hollander et al. (1991), Gross-Isseroff et al. (2004), Tsaltas et al. (2005). • DA D2 receptor agonists linked to repetitive checking behavior in rodents Pitman (1989), Tizabi et al. (2002). • Successful use of antipsychotics in treating OCD patients Cavedini et al. (2004). • Increased levels of DA in the striatum Perani et al. (2008). • Increased CSF glutamate in OCD patients; efficacy of drugs that act on GABA and glutamate pathways Pittenger et al. (2011).	• Antagonizing 5HT-2A and agonizing 5HT-2C receptors prevents relapse in rodents Fletcher et al. (2002), Pentkowski et al. (2010); antagonizing 5HT-1B receptors inhibits cocaine-seeking in rodents Miszkiel et al. (2011). • Initial reinforcing effect of drugs associated with release of extracellular DA in striatum Koob and Volkow (2010). • Decreased activity in mesolimbic DA system with chronic use Volkow et al. (2007a); Decreased DA D2 receptors and DA release in striatum Volkow et al. (2002). • Efficacy of drugs that act on GABA and glutamate pathways Kenny and Markou (2004).

(Continued)

Table 1 | Continued.

	Model of compulsivity	Evidence in AN	Evidence in OCD	Evidence in addictions
Behavioral tasks	• Impairments in cognitive flexibility tasks such as reversal learning Fineberg et al. (2010), associated with OFC Dias et al. (1996), Berlin et al. (2004), Remijnse et al. (2006) and medial striatal abnormalities Clarke et al. (2008), and set-shifting Fineberg et al. (2010) associated with PFC abnormalities Dias et al. (1996), Hampshire and Owen (2006).	• Low in cognitive flexibility Tchanturia et al. (2004). • Impairments on set-shifting tasks Holliday et al. (2005), Steinglass et al. (2006), Bühren et al. (2012). • Subtle impairments in reversal learning Sarrar et al. (2011).	• Impairments on set-shifting Fontenelle et al. (2001), Lawrence et al. (2006) and reversal learning tasks Remijnse et al. (2006), Chamberlain et al. (2008), Valerius et al. (2008).	• Impairments on set-shifting Saraswat et al. (2006) and reversal learning tasks Izquierdo and Jentsch (2012).

poor set-shifting abilities (Holliday et al., 2005; Steinglass et al., 2006; Bühren et al., 2012). Poor set-shifting has been suggested as an endophenotype of both AN and BN, and is associated with both longer illness duration and increased disorder-related rituals (Roberts et al., 2010). Subtle impairments in reversal learning have also been reported in AN, and are shown to occur before and after weight gain (Sarrar et al., 2011), suggesting this is a trait rather than state related impairment. OCD and substance dependence have also both been associated with deficits in reversal learning (Remijnse et al., 2006; Chamberlain et al., 2008; Valerius et al., 2008; Izquierdo and Jentsch, 2012), and set-shifting (Fontenelle et al., 2001; Lawrence et al., 2006; Saraswat et al., 2006).

NEUROTRANSMITTER INVOLVEMENT IN COMPULSIVITY

The role of serotonin

Serotonin has widespread effects on satiety, impulse control and mood, with a range of evidence indicating a specific role for 5-HT receptors in compulsive behavior (see **Table 1**). A rodent model of OCD indicates that 5-HT-2C receptor agonists increase compulsivity or the persistence of response in these rats (Tsaltas et al., 2005). This result has been replicated in exacerbating compulsive symptoms in human OCD patients (Hollander et al., 1991), in whom 5-HT-1B receptor agonists also exacerbate OCD symptoms (Gross-Isseroff et al., 2004). Impairments in reversal learning have been linked to reduced brain serotonin (Evers et al., 2005), particularly in areas such as the OFC (Clarke et al., 2004), and antagonism of 5-HT-2A receptors impairs reversal learning (Boulougouris et al., 2008), indicating their involvement in the development of compulsive behavior.

The 5-HT system has been extensively studied in AN, with much evidence of dysfunction in this system (for a recent review see Kaye et al., 2013b). Imaging studies have consistently shown increased 5HT-1A and decreased 2A receptor binding potential in individuals both currently ill and recovered from AN (Kaye et al., 2005a,b; Bailer et al., 2007), suggesting a trait and not state related alteration. Interaction between 5HT-1A and 2A receptors in the medial prefrontal cortex (mPFC) have been suggested to modulate impulsivity and compulsivity (Carli et al., 2006), although there is yet to be evidence of this mechanism in the development of compulsive behavior in AN.

The 5-HT system is modulated by many drugs of abuse (Kirby et al., 2011). Research in this area is extensive, the scope of which cannot be covered in this review. However, evidence to date points to a particular role for 5HT-2A and 2C receptors in relapse during withdrawal, and in the persistence of compulsive drug-seeking in addiction (Fletcher et al., 2002; Pentkowski et al., 2010). Furthermore, there is an inhibitory effect of 5-HT-1B receptor antagonists on cocaine-seeking behavior (Miszkiel et al., 2011). In terms of behavioral addictions, peripheral measures suggest reduced serotonin levels in pathological gamblers (Nordin and Sjödin, 2006). This has been hypothesized to be linked to maladaptive decision making in these individuals (Kirby et al., 2011), and treatment with SSRIs has been reportedly effective (Hollander and Rosen, 2000). Although aberrancies in the 5-HT system are seen across compulsive disorders, these do not appear to be universal in nature, suggesting that the serotonergic neurotransmitter system alone is not driving compulsive behavior.

The role of dopamine

Dopamine (DA) is considered key to the rewarding effects of both natural and drug-derived reward (Volkow et al., 2012b), and the mesolimbic DA pathways in particular play a crucial role in reward and reinforcement processes (Wise, 2002). Models of compulsivity have emphasized the importance of DA tone in the ventral loops that link the ventral ACC and the VS/NAc in regulating reward and reinforcement behaviors (Fineberg et al., 2010; see **Table 1**). DA D2 receptor agonists such as levodopa have been associated with deficiencies in reversal learning (Cools et al., 2006), and increased compulsive behavior in Parkinson's disease (Voon et al., 2009). Studies in animals have also shown that the administration of DA agonists induces stereotyped behaviors associated with OCD (Pitman, 1989). Specifically, the use of a selective D2 receptor agonist in rats was associated with the development of repetitive checking behavior (Tizabi et al., 2002). DA dysfunction in OCD patients is also indicated by the successful use of antipsychotics when SSRI treatment is unsuccessful (Cavedini et al., 2004). There have also been reports of increased levels of DA in the striatum in OCD (Perani et al., 2008).

A key process in the initial reinforcing effect of drugs is their ability to produce increases in extracellular DA in limbic regions such as the NAc/VS (Koob and Volkow, 2010). This release of DA is associated with a feeling of euphoria, and is experienced as rewarding (Volkow et al., 1996), thus positively reinforcing their use. The expectation of drug reward (i.e., in certain contexts) is also important in reinforcing drug use (Volkow et al., 2003), and is likely to depend on neurotransmitters such as glutamate, which is known to modulate DA release in the NAc (Kalivas and Volkow, 2005). These DA- dependent effects cause drugs themselves and drug-associated stimuli to gain incentive salience and promote further drug-seeking behavior (Everitt and Robbins, 2005). This repeated and prolonged DA increase results in synaptic changes in DA pathways, and these changes may be responsible for the formation of compulsive habits that persist despite adverse consequences in substance dependence (Wolf, 2002).

In substance dependent individuals, activity in the mesolimbic DA system is decreased, a deficiency that persists for months following detoxification (Volkow et al., 2007a). Chronic cocaine abusers are shown to have decreased levels of D2 receptors and a decrease of DA release in the striatum (Volkow et al., 2002), although initial drug use is associated with synaptic increases in DA (Volkow et al., 2007a). Evidence of decreased DA activity following chronic drug use has led to the suggestion that this deficiency in DA may cause an increase in the compulsion to seek further drug reward to order to increase deficient DA levels, with repetition of this behavior leading to a dependence on the substance (Fineberg et al., 2010). In contrast, individuals recovered from AN have increased binding of DA D2/D3 receptors in the anterior VS while currently ill AN patients has shown increased levels of the DA metabolite homovanillic acid (HVA) in cerebrospinal fluid (Castro-Fornieles et al., 2008). The increased DA receptor binding in recovered AN patients contrasts with the reduced striatal DA binding found in those with substance abuse (Volkow et al., 2009, 2012a), which is paralleled by those with BN (Broft

et al., 2012), and obesity (Kenny, 2011). Striatal DA response has been negatively associated with binge eating and vomiting (Broft et al., 2012), suggesting that individuals who engage in overeating may have reduced DA function, similar to that found in substance dependence. DA release in the dorsal putamen has been associated with anxiety in recovered AN, which contrasts with the euphoria reported by controls (Bailer et al., 2012). Palatable food ingestion is associated with DA release in the striatum (Bassareo and Di Chiara, 1999), suggesting that individuals with AN may experience this DA release as aversive, as opposed to the hedonic response seen in healthy controls. This aversive reaction to food may explain the relentless food restriction seen in AN (Kaye et al., 2013a).

Glutamate is the principal excitatory neurotransmitter in the brain and is involved in many cognitive functions such as memory and learning (Jamain et al., 2002). The mesolimbic DA system, central to brain reward processes and compulsivity, has a variety of excitatory glutaminergic and inhibitory gamma-amino butyric acid (GABA, of which glutamate is the precursor) inputs (Guardia et al., 2011). The NAc, a region heavily implicated in reward processes, has glutaminergic inputs from limbic regions, and GABAergic projections to other reward-related areas (Guardia et al., 2011). There is extensive physiological and neurochemical evidence to suggest that reward-related learning requires an interaction between DA and glutamate, and occurs as the result of modulation by DA of glutamate synapses in the striatum (Beninger and Gerdjikov, 2005). Alterations in glutamate receptor binding in the cingulate cortex and dorsal striatum has been associated with impairments in set-shifting ability (Nicolle and Baxter, 2003), further suggesting a role for glutamate in cognitive flexibility and compulsivity.

Dopamine modulation of glutamate has been implicated in the development and expression of addictive behaviors, and there is evidence suggesting that modulation of GABA and glutamate pathways may be effective in the treatment of substance use disorders (Olive et al., 2012). Animal studies have shown the efficacy of GABA agonists in attenuating the positive reinforcing effects of drugs, and glutamate antagonists may be effective in preventing relapse (Kenny and Markou, 2004). Drugs modulating glutaminergic and GABAergic pathways have also been shown to act on binge eating, purging and weight loss in eating disorders (Guardia et al., 2011). Interestingly, both OCD and AN are associated with an increase in cerebrospinal fluid glutamate (Nakazato et al., 2010; Pittenger et al., 2011). Although successful treatment with glutaminergic drugs has yet to be reported in AN, some improvement has been found in the treatment of OCD (Pittenger et al., 2011).

SUMMARY

- Models of compulsivity indicate a bottom up striatal component driving compulsivity, and a top down prefrontal component inhibiting compulsive behavior.
- Dysfunction of this neurocircuitry, and impairments in tasks reflecting activity in these regions, can be seen in AN, OCD, and substance dependence.
- Abnormalities in neurotransmitter activity linked to impulse control and reward, such as DA, glutamate and serotonin, are also seen across disorders.

DOES COMPULSIVITY REFLECT DYSREGULATED HABIT FORMATION?

Compulsivity, as previously defined, can also be described as a tendency to carry out repetitive acts in a habitual manner (Fineberg et al., 2010). Habits are described as behaviors that are not innate, are engaged in repeatedly and become fixed, occur without conscious effort and can be elicited by external stimuli (Graybiel, 2008). Two distinct types of learning are involved in the development of behavior that is not innate or is outside of conscious awareness: action-outcome learning and stimulus-response learning (Robbins et al., 2012). Action-outcome learning (also referred to as goal-directed learning) occurs when a particular action leads to a rewarding outcome. If at any point the action no longer leads to reward, the frequency of that action will decrease (Balleine and O'Doherty, 2010). However, if these new actions are engaged in repeatedly (over-trained), they may become insensitive to the outcome, and will be repeated even when they do not result in reward (stimulus-response learning; Graybiel, 2008). Thus, behavior can become a habitual response to environmental stimuli associated with the rewarding outcome (Steinglass and Walsh, 2006). Despite the suggested distinction between goal-directed and habit learning, early description of habits describe them as a form of automatic goal-directed behavior. Bargh (1989) suggests that habits form as the instrumental link between goals and actions, and are automatically activated when a relevant goal is present. This may be particularly relevant for disorders such as AN, in which automatic habits, which take the form of compulsive weight loss behaviors, may occur unconsciously in the persistent presence of long term weight loss goals.

Research indicates neural distinctions between goal-directed and habit learning. In humans, the ventromedial PFC has been linked to goal directed learning (Daw et al., 2005), while the putamen has been linked to habit learning (Tricomi et al., 2009). Cortico-striatal connectivity as indexed by diffusion tensor imaging (DTI), which measures the strength of white matter tracts has been associated with differences in habit and goal-directed control of actions (de Wit et al., 2012). In a behavioral learning task, the tendency to rely on habits was associated with white matter tract strength between both premotor cortex and posterior putamen, and gray matter density in the posterior putamen; while the tendency to use goal directed control was associated with tract strength in the ventromedial PFC from the caudate (de Wit et al., 2012). The cortico-striatal circuitry implicated in habit vs. goal directed behavioral control is similar to that suggested in models of the neurocircuitry of compulsivity (see **Figure 2**), supporting the suggestion that compulsive behavior may be underpinned by habitual control of behavior, as these constructs appear to be indexed by overlapping neurocircuitry. Investigating abnormalities in habit learning and related brain areas may thus further understanding of the development of compulsive behavior across disorders.

IS EXCESSIVE HABIT FORMATION OBSERVED IN AN, OCD, AND SUBSTANCE DEPENDENCE?

Emergent evidence suggests that dysregulation of habit formation may provide a mechanism by which the development of well-entrenched, compulsive behaviors can occur: for example,

once drug taking is engaged in repeatedly, it becomes associated with a number of environmental stimuli or cues, which thereby become triggers for compulsive drug-seeking and drug taking, despite knowledge of the negative consequences of engaging in this behavior (Belin et al., 2011). Habit learning may also play a role in disorders such as AN and OCD, in which certain compulsive, and sometimes ritualistic, behaviors may persist despite a lack of reward or intermittent reward, and a wide variety of negative consequences (Steinglass and Walsh, 2006).

The development of substance dependence has been described in terms of a transition from initially goal-directed drug taking, driven by the reinforcing properties of the drug, to progressively more compulsive drug-seeking controlled by the habit system (Everitt and Robbins, 2005), and ultimately driven by environmental stimuli associated with the drug (Belin et al., 2011). Animal models of compulsive drug-seeking have investigated habit formation using outcome devaluation paradigms, in which previously rewarding action-outcome contingences are devalued, and persistence of behavior despite this devaluation is measured. These demonstrate that reinforcement with cocaine, in comparison to a natural reward such as lemon-sucrose solution, resulted in accelerated habit learning, and was subsequently less sensitive to devaluation (Miles et al., 2003). Similar results have been found with other drugs of abuse, such as alcohol (Dickinson et al., 2002), suggesting that the reinforcing effects of drugs may promote dominance of the habit system in learning and result in the development of compulsive drug-seeking behaviors. Everitt and Robbins (2005) support this idea with a neural systems model of substance dependence, in which this transition from goal directed to habitual control represents a change from prefrontal cortical to striatal control, and a progression from ventral to dorsal areas of the striatum (Everitt and Robbins, 2005). This is supported by rodent studies (Ito et al., 2000, 2002), and data from human cocaine addicts showing increased dorsal striatum activity during presentation of drug cues (Volkow et al., 2006). Drugs of abuse may also decrease the ability of the individual to exert control over these habits, even when presented with persistent negative and aversive consequences (Deroche-Gamonet et al., 2004). A parallel can be drawn here with the compulsive behaviors seen in AN: individuals with AN often report wanting to recover from their disorder, despite persisting with compulsive behavior that maintains their emaciation.

Compulsive behavior in OCD may also be associated with over-reliance on habits in learning; Gillan et al. (2011) showed that individuals with OCD had a selective impairment in goal directed control of behavior which forced them to rely on previously learned habits. Although individuals with OCD showed no overall impairment in using feedback to guide learning, they showed weaker knowledge of the direct causal relationship between actions and outcomes, leading to errors. Symptom severity was predictive of persistent responding to devalued stimuli, suggesting the continued use of previously learned habits may be related to the severity of compulsions (Gillan et al., 2011). Using a shock avoidance paradigm, Gillan et al. (2013) also demonstrated that individuals with OCD have a tendency to develop excessive avoidance habits, further supporting the idea that a reliance on habit formation in learning at the expense of goal directed control may underlie compulsive behaviors in OCD.

Walsh (2013) elegantly outlines the mechanisms by which aberrant habit formation may contribute to the maintenance of AN. It is suggested that restrictive eating may begin as the result of goal-directed weight loss behavior, in which behavior becomes associated with a rewarding outcome (weight loss). If this restrictive eating behavior is repeated enough it may become relatively insensitive to reward. In this way, weight loss behavior becomes highly practiced and over trained, and weight loss as a rewarding outcome may be needed only intermittently, or even no longer necessary for this behavior to continue. The fact that habitual behavior is outcome-independent makes it highly resistant to change, reflecting the treatment resistance often seen in individuals with AN (Walsh, 2013). It is further suggested that during the process of habit formation in AN, restrictive behavior itself becomes rewarding through conditioned reinforcement (Walsh, 2013), in which a set of cues begin to develop, and take on rewarding properties themselves (Everitt and Robbins, 2005). Individuals with AN may thus start to find that the reward of weight loss is no longer required as the now habitual weight loss behaviors themselves and associated cues have become rewarding or reinforcing. This process is also seen in substance dependence, in which cues associated with drug taking become associated with craving for the drugs and drug taking (Wikler, 1973).

Rothemund et al. (2011) reported that high levels of compulsivity in severely low weight AN patients predicted deactivation of the fronto-striatal circuitry, a finding that was interpreted as reflecting the transition from goal-directed actions to the development of habitual compulsive behavior, similar to that described in substance dependence (Everitt and Robbins, 2005). During the high calorie picture conditions in this study, individuals with AN showed increased activation in the right caudate and right precuneus, suggested to reflect differences in automatic habit learning processes compared to control participants. Furthermore, reduced gray matter volume in the bilateral OFC and right middle and superior frontal gyrus was found in individuals with AN, indicative of dysfunctional goal-directed behavioral control. The results of this study suggest aberrancies in the neurocircuitry associated with goal directed actions and habits in AN, which may reflect the persistence of compulsive weight loss behaviors in this disorder.

SUMMARY

- Excessive reliance on stimulus-response habits in learning may underpin the development of compulsive behaviors, with evidence of this in both OCD and substance dependence.
- Models of addiction suggest a transition over time from initially goal directed to habitual behavior, reflected by a shift in corresponding neurocircuitry.
- Recent theory implicates habit formation in the development of compulsive weight loss behaviors in AN, but to date there is no published evidence testing this theory.

THE ROLE OF POSITIVE AND NEGATIVE REINFORCEMENT IN COMPULSIVITY

Whilst AN and OCD have often been conceptualized as disorders driven by avoidance and fear of weight gain in AN, and of disorder related obsessions in OCD- substance dependence is commonly

thought to be driven by an approach response, to gain the rewarding effects of drugs. If compulsive behavior is driven by a common process in these disorders, there should be some similarities in the reinforcement processes maintaining compulsive behavior.

POSITIVE AND NEGATIVE REINFORCEMENT IN AN

AN has often been associated with anhedonia and a negative mindset, with the suggestion that compulsive self-starvation serves to reduce the anxiety and negative affect that becomes associated with food (Zink and Weinberger, 2010). Indeed, food ingestion may be experienced as anxiogenic for individuals with AN (Bailer et al., 2012). This may provide one mechanism through which individuals with AN experience self-starvation as a relief from this food associated anxiety, and indicates that negative reinforcement plays a big role in the maintenance of AN. Furthermore, compulsive exercise behavior has been associated with high levels of anxiety and depression in AN, and may serve as a means of reducing these negative states (Meyer et al., 2011).

However, the maintenance of restrictive behavior in AN may also involve positive reinforcement processes. Selby et al. (2014) suggest a model in which positive emotions associated with successful weight loss, such as feelings of pride and accomplishment, reinforce and drive further weight loss behavior. Over time, weight loss behavior is conditioned to elicit positive emotions regardless of whether further weight loss is attained (Selby et al., 2014). The underlying premise of this theory is consistent with that of Walsh's habit formation theory (Walsh, 2013). Evidence that weight loss behavior in AN may be driven by positive reinforcement comes from a series of functional magnetic resonance imaging (fMRI) studies. Fladung et al. (2010) found that positive feelings and increased activation in the VS were associated with underweight pictures in individuals with AN, with reduced VS activation during the normal weight pictures. This was in comparison to healthy individuals, who showed the opposite pattern of activation in the VS. In a further study, Fladung et al. (2013) found that this VS signal may change over time, in the same way that neural activation evolves over time in substance dependence. In this second study, individuals with AN, whose illness duration was five times shorter than those in the first study, showed the same increased activation to underweight pictures and decreased activation to normal weight pictures in comparison to healthy controls. However, the signal did not differ between categories, suggesting that the signal in the VS may evolve over time, as the illness duration increases. The authors suggest that over time cues associated with self-starvation are linked to strong motivational value, which further reinforces starvation and weight loss behavior (Fladung et al., 2013). Increased positive feelings and a pronounced attentional bias to stimuli depicting physical activity has also been found in AN (Giel et al., 2013).

A SHIFT FROM POSITIVE TO NEGATIVE REINFORCEMENT?

Selby et al. (2014) suggest that positive emotion/reinforcement may be particularly relevant in the initial stages of AN, with negative reinforcement becoming more important in maintaining behavior over time, where weight loss behavior is used to reduce negative emotions. It may be that the later stages of AN and drug dependence are similar, in that the early rewarding effects of food restriction and drug-taking may both cause these behaviors to be repeated and become habitual/compulsive in nature (Zink and Weinberger, 2010). This could be reflected by synaptic changes in both substance dependence and AN (Everitt and Robbins, 2005; Fladung et al., 2013).

The synaptic changes seen over time in drug dependence are well studied. The initial DA-dependent reinforcing effect of drugs is associated with a feeling of euphoria, and is experienced as rewarding (Volkow et al., 1996), thus positively reinforcing their use. These DA-dependent effects cause drugs themselves and drug-associated stimuli to gain incentive salience and promote further drug-seeking behavior (Everitt and Robbins, 2005). The repeated and prolonged DA increase results in synaptic changes in DA pathways, and these changes may be responsible for the formation of compulsive habits in substance dependence (Wolf, 2002). These later stages of habitual drug-seeking and taking may be negatively reinforced by the withdrawal symptoms experienced in absence of the drug, during which individuals can experience both physical and emotional negative states (Koob and Volkow, 2010). Protracted withdrawal is associated with hypofunctioning of the DA pathways and anhedonia (Volkow et al., 2007b), which may result in an increased drive for further drug-seeking to counteract this. Avoiding these withdrawal symptoms is likely to play a big role in reinforcing drug use, suggesting that the later stages of compulsive drug use may also involve negative reinforcement.

The complex interplay of positive and negative reinforcement in the development of compulsive behavior is also emphasized by habit formation studies in OCD. Whilst theories of habit formation in AN and substance dependence have been largely focused on appetitive habits resulting from initially rewarding outcomes, evidence suggests that OCD may be underpinned by the formation of avoidance habits. The compulsions seen in OCD do not seem to develop from an initially rewarding outcome, but rather are typically used to avoid some undesirable consequence (Gillan et al., 2013). These avoidant habits may be related to increased punishment sensitivity reported in OCD (Fullana et al., 2004), in which patients' compulsions reduce distress, and as such may be experienced as an immediate avoidance of punishment (Salkovskis, 1999). If avoidance is important in the development of OCD, it may also have an impact on habit formation in AN and substance dependence. Individuals with AN have been shown to have both heightened reward and punishment sensitivity, suggested to reflect the avoidant and anxious behaviors seen in AN, which may be used as a means of avoiding increased emotional reactivity to reward and punishment (Jappe et al., 2011). As such, habits in AN may develop as an avoidance of the negative emotions associated with food and weight gain. However, substance dependence is associated with decreased reward and punishment sensitivity (de Ruiter et al., 2009). Whilst compulsive habit behaviors in OCD, appear to begin as the avoidance of something negative, initial drug taking in substance dependence may be much more dependent on increasing the experience of reward. This process is less clear in AN, in which behavior may begin as the pursuit of something rewarding (weight loss) and the avoidance of negative states and anxiety associated with food.

SUMMARY

- Whilst AN is often associated with avoidance behaviors, it may in part involve the pursuit of the rewarding aspects of weight loss.
- Theories of substance dependence suggest a shift over time from positive to both positive and negative reinforcement, corresponding to a shift from goal directed to habitual behavior.
- Compulsive behavior in AN may reflect the later stages of substance dependence, in which compulsive habits are driven by both positive and negative reinforcement.
- Negative reinforcement appears to dominate the development of persistent compulsions in OCD.

THE ROLE OF STARVATION IN COMPULSIVE BEHAVIOR

The evidence reviewed so far suggests that compulsive weight loss behaviors seen in AN are associated with aberrant neurocircuitry and dysfunctional mechanisms of learning and reward that promote compulsive behavior. Crucially, there is evidence that the physiological consequences of starvation promote compulsivity and habit learning, and directly affect positive reinforcement (see **Figure 3** for a representation of the effects of starvation on the development of compulsive weight loss habits in AN). For example, in animal models DA (Verhagen et al., 2009b) and serotonin are released in the NAc during starvation-induced hyperactivity, while DA antagonism inhibits anorectic behavior (Verhagen et al., 2009a).

Behavioral evidence in humans suggesting that starvation is associated with the development of compulsive behaviors derives from the Minnesota Experiment (Keys et al., 1950), in which previously healthy males were restricted to half their average food intake for 6 months. These individuals developed food rituals and obsessions, some of which persisted after food restriction ceased, and

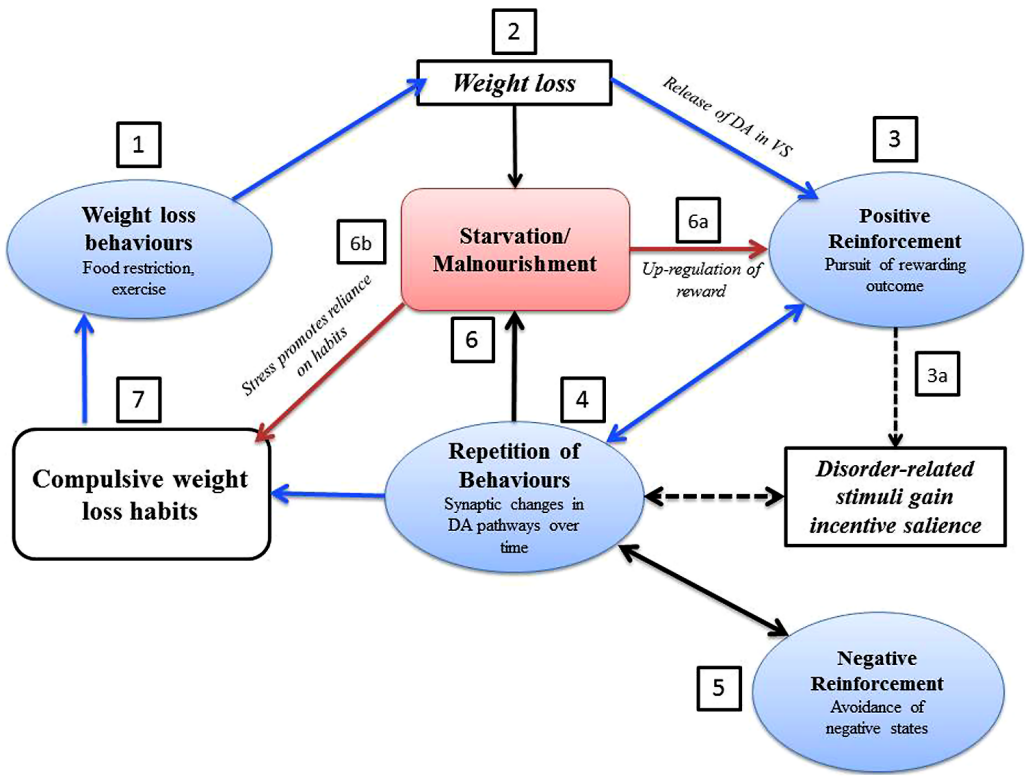

FIGURE 3 | The vicious circle of self-starvation in anorexia nervosa: representation of the development of compulsive weight loss habits in AN. This can be conceptualized as a vicious circle, augmented by starvation. (1) Goal-directed weight loss behaviors, such as food restriction and exercise, lead to weight loss. (2) Weight loss is experienced as rewarding, which may be positively reinforced by an increase of DA in the VS. (3) The pursuit of the rewarding aspects of weight loss leads to a repetition of weight loss behaviors: (a) disorder related cues may gain incentive salience through conditioned reinforcement, and may themselves trigger weight loss behavior (Walsh, 2013). (4) Repetition of rewarding weight loss behavior may result in synaptic changes in DA pathways (Fladung et al., 2013) and result in DA hypofunctioning in a similar way to substance dependence (Everitt and Robbins, 2005). (5) The avoidance of negative states and the anxiety associated with food may become more important in reinforcing weight loss behaviors in the later stages of AN (Selby et al., 2014). (6) Starvation and malnourishment further promote weight loss behavior in the following ways: (a) the up regulation of reward associated with starvation may lead to disorder-related cues gaining incentive salience (Fladung et al., 2010) through a molecular cascade in which starvation induced reductions in glucose and insulin lead to an increase in phasic DA transmission in the VS (Zink and Weinberger, 2010). Furthermore, AN is associated with increased sensitivity to both reward and punishment (Jappe et al., 2011) which may enhance both positive and negative reinforcement of weight loss. (b) Stress is associated with a reliance on habits in learning (Schwabe et al., 2007), and the stress associated with starvation may promote the compulsive weight loss behaviors seen in AN. (7) Once weight loss behavior is repeated enough, a rewarding outcome may no longer be needed for behavior to continue, and weight loss behaviors may become compulsive and habitual (Walsh, 2013). Abbreviations: DA, dopamine; VS, ventral striatum.

some engaged in binge eating or excessive exercise. They also experienced cognitive impairment and periods of low mood during the study. These individuals were psychologically and physically healthy prior to the experiment, suggesting that these symptoms were due to the food restriction imposed during the experiment. It is possible that starvation effects impact on aberrant neurocircuitry, and dysfunctional mechanisms of learning and reward, to promote compulsive behavior in a vicious circle.

In the animal model of AN, activity based anorexia (ABA) compulsive hyperactivity becomes intrinsically linked to food restriction. Rats on a restricted diet given free access to a running wheel will increase activity to the point of death (Routtenberg and Kuznesof, 1967; Adan et al., 2011). Interestingly, young female rats, as in human AN, show particular susceptibility to this effect (Hancock and Grant, 2009). These rats also show food anticipation activity (FAA), in which an increase in physical activity precedes a meal (Mistlberger, 1994). This increase in activity preceding a meal has also been documented in AN patients (Scheurink et al., 2010). These phenomena have been suggested to reflect an evolutionary advantage of increased activity (foraging) during times of famine (Illius et al., 2002), and appear to show a link between food restriction and increased physical activity. Evidence from the ABA model also suggests a role for DA and serotonin dysfunction in the development of hyperactivity during food restriction. Antagonism of DA receptors is shown to increase food intake and decrease overall physical activity in ABA rats (Verhagen et al., 2009a). Similarly, DA depletion and DA receptor blockade in the NAc decreases FAA (McCullough and Salamone, 1992; Barbano and Cador, 2006). Serotonin has a suppressive effect on food intake (Simansky, 1996) and a decrease in food intake is seen in rats treated with serotonergic agonists (Clifton et al., 2000; Lee et al., 2002). Furthermore, a decrease in 5HT release in the NAc is seen in ABA rats (Verhagen et al., 2009b). Given that AN is associated with altered DA and serotonin levels (see Neurotransmitter Involvement in Compulsivity), this evidence suggests that alterations in these neurotransmitters during the chronic food restriction of AN may increase vulnerability to compulsive exercising.

Food restriction has also been linked to an increase in reward sensitivity. Chronic food restriction, resulting in significant body weight loss, has been shown to increase reward effectiveness when electrically stimulating brain reward circuitry in rats (Fulton et al., 2004). Moreover, body weight correlates with the stimulation threshold for reward, in that a lower body weight leads to a weaker stimulation threshold (Abrahamsen et al., 1995). This effect can also be seen in drug reward. Indeed, chronic food restriction in rats has been found to enhance the rewarding properties of drugs by up-regulating synaptic plasticity in the NAc (Carr, 2007). This increased sensitivity to reward during food restriction may underpin reports of increased salience and attention for disorder-related cues in AN (Fladung et al., 2010, 2013; Giel et al., 2013). Zink and Weinberger (2010) suggest that these disorder-related cues gain incentive salience through a molecular cascade in which starvation induced reductions in glucose and insulin lead to an increase in phasic DA transmission in the VS, a process which conditions associated cues to become highly motivationally salient. This salience increases the likelihood that the behavior will continue, in the same way as drug-associated cues in substance dependence (Zink and Weinberger, 2010). In this way, the neurobiological changes associated with chronic food restriction may enhance the experience of reward in AN, and positively reinforce disorder related compulsions.

The reliance on habits or stimulus-response learning suggested to lead to compulsive behavior may be potentiated in times of stress. Stress is shown to modulate cognitive memory systems in favor of neo-striatum dependent habit systems (Schwabe et al., 2007). Furthermore, participants exposed to an experimental stressor have been shown subsequently to rely on habits during an instrumental learning task, and to show reduced knowledge of the action-outcome associations needed for goal-directed behavior (Schwabe and Wolf, 2009). Food shortage has also been associated with impairments in memory in animals (Plaçais and Preat, 2013), and even brief food restriction is shown to lead to alterations in gene expression of stress hormones (Guarnieri et al., 2012). Thus in AN, psychological and physical stressors associated with the disorder, as well as chronic food restriction, may promote reliance on habits.

SUMMARY

- Starvation and weight loss is associated with physiological changes that promote compulsive behavior.
- The ABA animal model of AN links food restriction to hyperactivity, and alterations in DA and serotonin are seen in ABA rats.
- Food restriction is associated with an increase in reward sensitivity and positive reinforcement.
- An increase in stress prior to learning is associated with dependence on stimulus-response habits in humans.

IMPLICATIONS FOR TREATMENT

The behavioral and neurobiological research reviewed suggests that compulsivity is a construct that can be seen in varying degrees across disorders such as AN, OCD, and substance dependence. Conceptualizing compulsive behavior across diagnostic categories opens the way for transdiagnostic treatment strategies. These may target common processes underpinning the compulsivity seen in disorders such as AN, OCD, and substance dependence. The dysregulation of habit formation and reward processes suggested to underpin the development of compulsivity is a potential target for these novel treatment strategies. Treatments targeted at disrupting habitual behavior may have transdiagnostic efficacy.

Functional magnetic resonance imaging findings suggest that the neural areas associated with habit learning and goal directed behavior may be dysfunctional during symptom provocation in AN, providing a potential neural underpinning for an overreliance on habit formation at the expense of goal directed actions. A model of habit formation in AN may thus provide a testable explanation of the development of the persistent compulsive behaviors that develop during the disorder.

Re-patterning habitual behaviors, alongside reversing starvation, has been emphasized as an important component in the treatment of AN (Park et al., 2011, 2012); some concepts previously aimed at behavioral change and habit breaking in other disorders have been translated for use in AN. Given the effects of starvation on neurobiological maintaining mechanisms reviewed

above, it is likely to be of crucial importance to reverse starvation effects in tandem. Examples of habit breaking strategies are components of cognitive remediation therapy (CRT) and exposure response therapy (ERT). CRT was originally developed for use in the treatment of psychosis, and has also been adapted for use in eating disorders to improve cognitive flexibility and break cognitive habits. Preliminary research into CRT has found improvements in self-reported cognitive flexibility in individuals with AN (Tchanturia et al., 2007a). ERT, which targets conditioned fear responses and conditioned reward, has been used in the treatment of OCD (Foa et al., 2005) and addiction (Kaplan et al., 2011), and some success has been found with graded exposure to food cues in AN, reducing meal-related anxiety post-treatment (Steinglass et al., 2012a).

Given evidence of neural circuitry underpinning compulsivity across disorders, neuromodulatory interventions such as deep brain stimulation (DBS), and the less invasive repetitive transcranial magnetic stimulation (rTMS), may benefit from common neural targets for treatment across disorders. DBS is a reversible, adjustable neurosurgical treatment that involves implanting electrodes that send electrical impulses to chosen locations in the brain (Rauch, 2003). DBS has been effective in the treatment of OCD when targeting the NAc, and is suggested to normalize excessive connectivity between the NAc and PFC (Figee et al., 2013). The reported efficacy of DBS to the NAc in OCD and addictions (Kuhn et al., 2007; Liu et al., 2008; Muller et al., 2009; Denys et al., 2010; Figee et al., 2013) is consistent with the involvement of fronto-striatal circuits across compulsive disorders. Both animal studies (Liu et al., 2008; Henderson et al., 2010) and DBS in substance dependent individuals (Kuhn et al., 2007; Muller et al., 2009) have found decreases in addictive behaviors when stimulating the NAc. Given that the NAc area is suggested to be involved in the transition from voluntary to compulsive drug use (Everitt and Robbins, 2005), it is possible that NAc stimulation may disrupt this process and thereby reduce compulsive behaviors. Case reports of DBS to the NAc in AN patients have also reported symptom alleviation both in the presence and absence of comorbid OCD (Wu et al., 2012; Lipsman et al., 2013; McLaughlin et al., 2013). RTMS, a non-invasive brain stimulation technique targeting the dorsolateral prefrontal cortex, has been shown to have some efficacy across disorders, reducing cravings and consumption in substance dependence (Barr et al., 2011), decreasing compulsions and obsessions in OCD (Blom et al., 2011), and reducing anxiety and potentially the urge to exercise in AN (Van den Eynde et al., 2013).

Novel pharmacological treatments targeting addictive or compulsive behaviors may also prove useful in the treatment of compulsivity across disorders. The DA dysfunction seen in many compulsive disorders may be a potential target for pharmacological intervention. Given animal and human evidence that DA modulation of glutaminergic transmission plays a role in reward and reinforcement, glutaminergic medications may also prove useful in compulsive disorders such as addictions (Kenny and Markou, 2004). The use of drugs that target GABA and glutamate pathways has shown some benefit in the treatment of substance use disorders (Clarke et al., 2004; Olive et al., 2012), binge eating (Guardia et al., 2011), and OCD (Pittenger et al., 2011), and may also prove beneficial in other disorders in which compulsive behavior develops.

CONCLUDING REMARKS

Conceptualizing compulsivity as a transdiagnostic concept, uniting separately classified disorders through common pathological processes, may help in the development of novel behavioral and neural interventions, which could be effective across diagnostic boundaries. These are urgently needed, given the poor outcome and limited evidence base for treatment of AN, especially in adulthood. Future research should aim to test this concept directly, for example by looking at the behavioral and neural basis of habit formation in relation to compulsivity in AN, OCD, and substance dependence, as well as other disorders that exhibit compulsive behaviors.

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AT_1 receptor blockade alters nutritional and biometric development in obesity-resistant and obesity-prone rats submitted to a high fat diet

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Obesity is a chronic metabolic condition with important public health implications associated with numerous co-morbidities including cardiovascular disease, insulin resistance, and hypertension. The renin angiotensin system (RAS), best known for its involvement in cardiovascular control and body fluid homeostasis has, more recently, been implicated in regulation of energy balance. Interference with the RAS (genetically or pharmacologically) has been shown to influence body weight gain. In this study we investigated the effects of systemic AT_1 receptor blockade using losartan on ingestive behaviors and weight gain in diet induced obese (DIO) rats. Prior to losartan administration (30 mg/kg/day) body weight gain remained constant within the DIO animals (3.6 ± 0.3 g/day, $n = 8$), diet resistant (DR) animals (2.1 ± 0.6 g/day, $n = 8$) and in the age-matched chow fed control (CHOW) animals (2.8 ± 0.3 g/day, $n = 8$), Losartan administration abolished body weight gain in animals fed a high fat diet (DIO: −0.4 ± 0.7 g/day, $n = 8$; and DR: −0.8 ± 0.3 g/day, $n = 8$) while chow fed animals continued to gain weight (2.2 ± 0.3 g/day, $n = 8$) as they had previously to oral administration of losartan. This decrease in daily body weight gain was accompanied by a decrease in food intake in the HFD fed animals. Following the removal of losartan, both the DIO and DR animals again showed daily increases in body weight gain and food intake which were similar to control values. Our data demonstrate that oral losartan administration attenuates body weight gain in animals fed a HFD whether the animal is obese (DIO) or not DR while having no effect on body weight gain in age-matched chow fed animals suggesting a protective effect of losartan against body weight gain while on a HFD.

Keywords: obesity, angiotensin, losartan, diet induced obesity, angiotensin receptor

INTRODUCTION

Obesity is a chronic metabolic condition with important public health implications associated with numerous co-morbidities including cardiovascular disease, insulin resistance, and hypertension. Adipose tissue, once thought solely as a storage depot for excess triglycerides, is now known to be an important endocrine organ. Adipocytes produce and release a number of adipokines, which have been shown to act centrally to influence food intake and energy metabolism (see Woods et al., 1998; Galic et al., 2010; Harwood, 2012; Williams and Elmquist, 2012 for review).

The diet induced obesity (DIO) animal model is commonly used to study obesity and its numerous co-morbidities. In contrast to genetic models of obesity, DIO develops as a consequence of consuming a high fat diet (HFD) or high fat/high sugar diet for a period of time and, thus, more closely mimics the etiology of obesity in the majority of humans that display this phenotype. Interestingly, not all animals or people who prefer/consume the HFD become obese, and these subjects are described as diet resistant (DR; Levin et al., 1989; Smith and Ferguson, 2012). The reason for this divergence is unknown (Levin et al., 1989). The DIO phenotype is only partially explained by increased food intake (Levin et al., 1989); obese animals on a HFD diet develop a resistance to the actions of leptin (El-Haschimi et al., 2000; Lin et al., 2000; Wang et al., 2001; Boyle et al., 2011), an adipokine shown to be a key player in the control of food intake and energy metabolism (see Friedman and Halaas, 1998; Jequier, 2002; Galic et al., 2010 for review).

While the renin angiotensin system (RAS) is best known for its involvement in cardiovascular control and body fluid homeostasis, the RAS has more recently been implicated in the regulation of energy balance. Not only does adipose tissue have a local RAS (for review see Cassis et al., 2008; de Kloet et al., 2010; Frigolet et al., 2013) but serum levels of all the components of the RAS [renin, angiotensinogen (AGT), angiotensin (ANG) converting enzyme] are elevated in obesity (Cooper et al., 1998; Yasue et al., 2010). In addition, ANG receptors (Burson et al., 1994; Crandall et al., 1994; Cassis et al., 1996) and all the components of the RAS have been localized and been shown to be fully functional in adipose tissue (Cassis et al., 1988; Engeli et al., 1999;

Fowler et al., 2009), and expression of these components has been shown to positively correlate with adiposity (Hainault et al., 2002; Engeli et al., 2005; see Kalupahana and Moustaid-Moussa, 2012 for review). The RAS has also been implicated in energy expenditure, a key component in energy balance. Recent studies have demonstrated that AGT deficient ($Agt^{-}/^{-}$) mice or mice lacking the AT1a receptor ($Agtr1a^{-}/^{-}$) have attenuated body weight and adiposity when fed a HFD (Massiera et al., 2001; Kouyama et al., 2005). These changes were accompanied by increased locomotor activity (Massiera et al., 2001) and rectal temperature and O_2 expenditure (Kouyama et al., 2005). The fact that both the $Agt^{-}/^{-}$ and $Agtr1a^{-}/^{-}$ animals and their wild type counterparts consumed the same amount of the HFD suggests that attenuation of diet-induced weight gain and adiposity of the $Agt^{-}/^{-}$ and $Agtr1a^{-}/^{-}$ animals may be due to the increased energy expenditure. Lastly, pharmacological manipulations of the RAS have demonstrated an involvement for the RAS in body weight regulation and composition in both obese (Stucchi et al., 2009; Miesel et al., 2012; Premaratna et al., 2012) and non-obese animal models (Santos et al., 2008; Weisinger et al., 2008). As such, recent attention has been directed toward the use of antagonists of the RAS in the treatment of obesity (see Weisinger et al., 2009a for review).

In light of these observations, the present study was undertaken to determine the influence of AT_1 receptor blockade on nutritional behavior and biometric development in obesity-resistant and obesity-prone rats submitted to a HFD.

MATERIALS AND METHODS

All procedures were conducted in accordance with the Canadian Council on Animal Care regulations and approved by Queen's University Animal Care Committee.

DIET INDUCED OBESITY

Upon arrival, male Sprague–Dawley rats (125–150 g) were housed in pairs in a temperature controlled room on a 12 h light–dark cycle and exposed to either a HFD (Research Diets, New Brunswick, NJ #D12451, composition 45% kcal% fat, 35% kcal% carbohydrate, and 20% kcal% protein) or standard chow diet (LabDiet 5001, composition 13.5% kcal fat, 58% kcal carbohydrate, and 28.5% kcal% protein) with water provided *ad libitum*. Weight gain was measured on a weekly basis from time of arrival until week 10 at which time animals exposed to the HFD were divided into DIO or DR based on those who gained the greatest and least weight, respectively. Animals that were greater than 700 g were placed in the DIO group while animals that weighed less than 600 g were considered DR. Animals that were of intermediate weights (600–700 g) were eliminated from this study as they could not be reliably classified as DR or DIO rats. The DIO and DR phenotypes were validated in accordance with the DIO model of others (Levin and Dunn-Meynell, 2002; Hyland et al., 2007; Li et al., 2011) and in our own colony (Smith and Ferguson, 2012).

Rats were continued on their respective diets and body weight, food intake and water intake were measured daily between 8 am and 10 am from week 17. After a control period of 14 days, the ANG type 1 receptor (AT1R) antagonist, losartan (Sigma Chemical Company), was administered in the drinking water for 2 weeks at 30 mg/kg (Konno et al., 2012; Oliveira-Junior et al., 2014). Losartan concentration was calculated daily for each animal based on that day's weight and the previous 24 h water consumption. Following the 2 week losartan treatment, animals were returned to normal tap water for an additional 10 days.

DATA ANALYSIS

A one way analysis of variance (ANOVA) was used to determine if body weights were different between animals in the DIO, DR, and CHOW groups. Mean body weight gain, food intake/100 g body weight, and water intake/100 g body weight were obtained for each group (DIO, DR, and CHOW) for 7 days immediately prior to losartan administration, days 1–7 and days 8–14 of losartan treatment, and days 3–10 post losartan treatment. A repeated measures ANOVA and *post hoc* Tukey multiple comparison tests ($p < 0.05$ was considered as significant) was used to determine whether body weight gain, food intake, or water intake was altered as a result of losartan treatment in each of the three groups (DIO, DR, CHOW).

RESULTS

A total of 24 rats were used in this study of which 8 were in the DIO group, 8 were in the DR group and 8 animals were age-matched chow fed controls (CHOW). Rats classified as DIO, based on weight gain after 10 weeks on the HFD, had a mean body weight of 717.1 ± 12.4 g ($n = 8$). DR rats weighed the same as age-matched chow fed controls (DR mean body weight = 571.8 ± 16.5 g, $n = 8$; chow fed mean body weight = 570.9 ± 18.4 g, $n = 8$; ANOVA $p < 0.0001$, chow vs DR, ns Tukey *post hoc* analysis) and weighed significantly less than the rats classified as DIO (DIO vs DR, $p < 0.001$ Tukey *post hoc* analysis; see **Figure 1**).

At week 17, the beginning of daily body weight, food intake, and water intake measurements, DIO rats weighed significantly more (DIO 892.6 ± 11.3 g, $n = 8$, $p < 0.001$ one way ANOVA) than both the HFD fed DR rats (658.8 ± 16.4 g, $n = 8$, $p < 0.05$ Tukey *post hoc* analysis vs DIO) and chow fed control (CHOW)

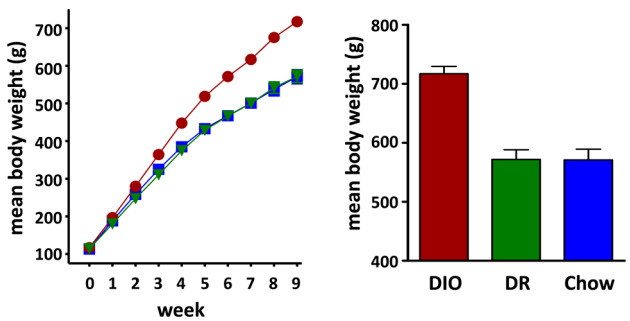

FIGURE 1 | Diet Induced Obesity (DIO) Phenotype: mean weekly body weight of rats classified as DIO (red circle) or diet resistant (DR, green triangle) after 8 weeks of *ad libitum* feeding on a HFD and age-matched chow fed controls (Chow, blue square) Bar graph on the right shows the mean body weights of rats considered DIO or DR based on those who gained the greatest and least weight, respectively, and age-matched chow fed control animals at time of group assignment.

animals (692.5 ± 25.2 g, $n = 8$, $p < 0.001$ Tukey *post hoc* analysis vs DIO) while body weights of the DR and CHOW animals were not significantly different ($p > 0.05$ DR vs CHOW, Tukey *post hoc* analysis).

Body weight increased steadily in all three groups (DIO, DR, CHOW) during the 14 day control period (see **Figure 2**). However, during losartan administration rats consuming the HFD (DIO and DR) no longer demonstrated an increase in body weight while age-matched chow fed control animals continued to gain weight as they had previously to losartan administration (see **Figure 2**). As illustrated in **Figure 3**, mean daily body weight gain remained constant within all groups during the control period with age-matched chow fed control animals (CHOW) demonstrating a mean body weight gain of 2.8 ± 0.3 g/day ($n = 8$), DIO animals gaining 3.6 ± 0.3 g/day ($n = 8$) and DR animals gaining 2.1 ± 0.6 g/day ($n = 8$). Losartan administration abolished body weight gain in animals fed a HFD (DIO and DR, $p < 0.001$, repeated measures ANOVA) while chow fed animals continued to gain weight as they had prior to oral administration of losartan ($p = 0.12$, repeated measures ANOVA; see **Figures 2** and **3**). This decrease in daily body weight gain was accompanied by a decrease in food intake (see **Figure 4**) in the HFD fed animals (DIO and DR, $p < 0.001$, repeated measures ANOVA) while water consumption was not altered ($p > 0.05$, repeated measures ANOVA; see **Figure 4**). Following the removal of losartan both the DIO and DR animals again showed daily increases in body weight gain and food intake which were similar to control values (see **Figures 2–4**). Losartan administration was without effect on daily body weight gain, food intake or water consumption in the chow fed animals (see **Figures 2–4**).

DISCUSSION

Here, we assessed the effect AT1R blockade with losartan on body weight and food intake in the rat DIO model of obesity. In line with previous findings (Machado et al., 2012; Sagae et al., 2013; Oliveira-Junior et al., 2014), we show that oral administration of

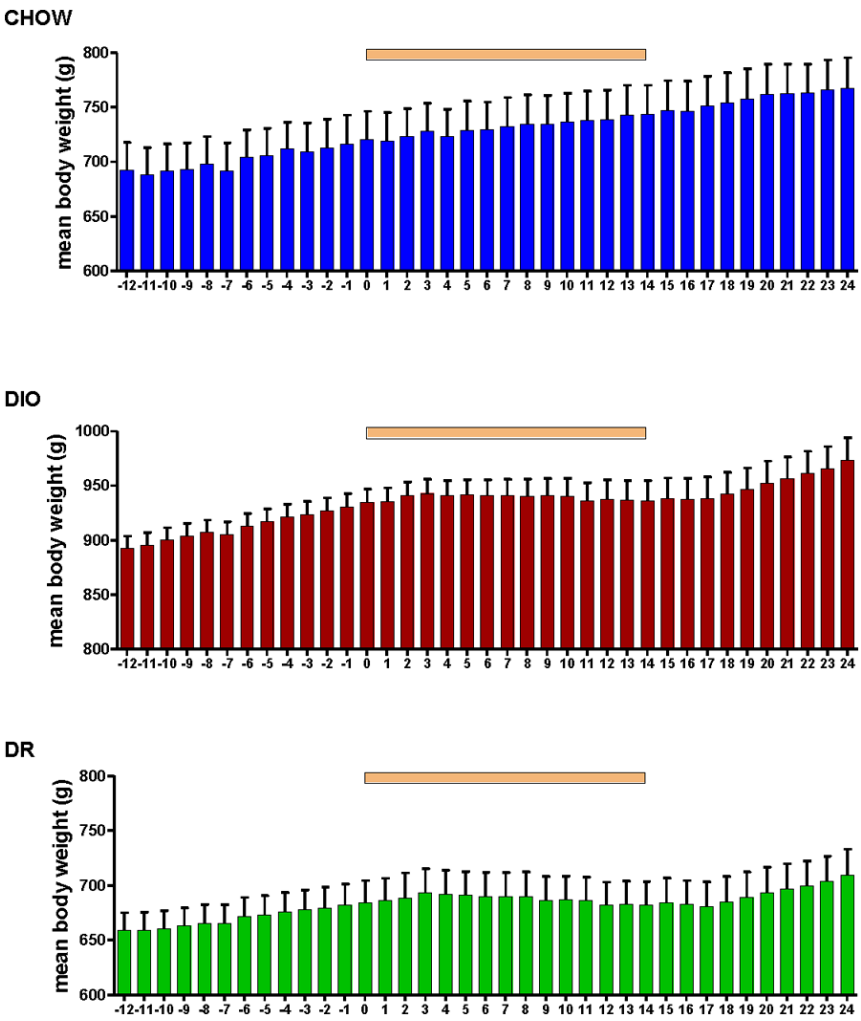

FIGURE 2 | Mean daily body weight in age-matched control rats fed standard rat chow (CHOW, upper panel), DIO rats (middle panel), and DR rats (lower panel) before, during and after oral administration of losartan. Time of losartan administration indicated by the orange bar over the graphs.

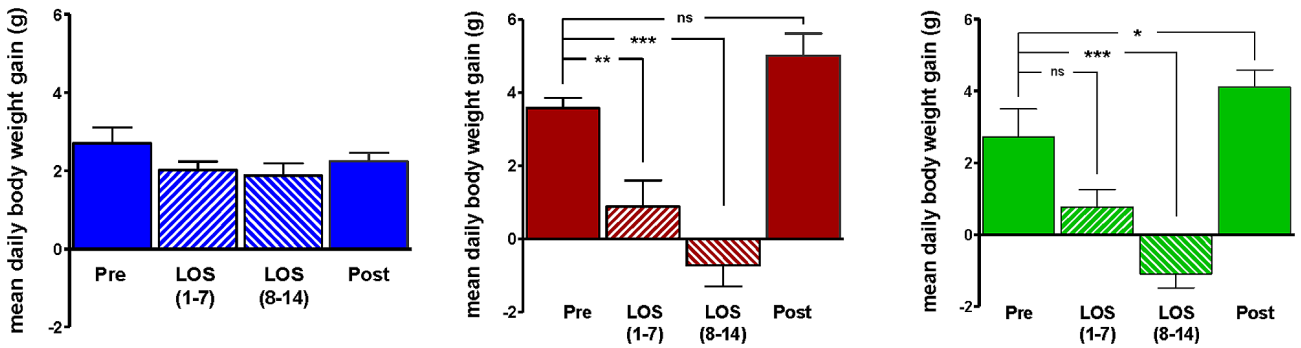

FIGURE 3 | Average daily weight gain in age-matched control rats fed standard rat chow (CHOW; left) or in DIO rats (middle) or DR rats (right), immediately prior to losartan treatment (Pre), days 1–7 (LOS 1–7) and days 8–14 (LOS 8–14) of losartan treatment and the final 7 days (days 18–24) following losartan treatment (Post). Losartan attenuated body weight gain in DIO and DR animals as compared to control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; Tukey *post hoc* analysis).

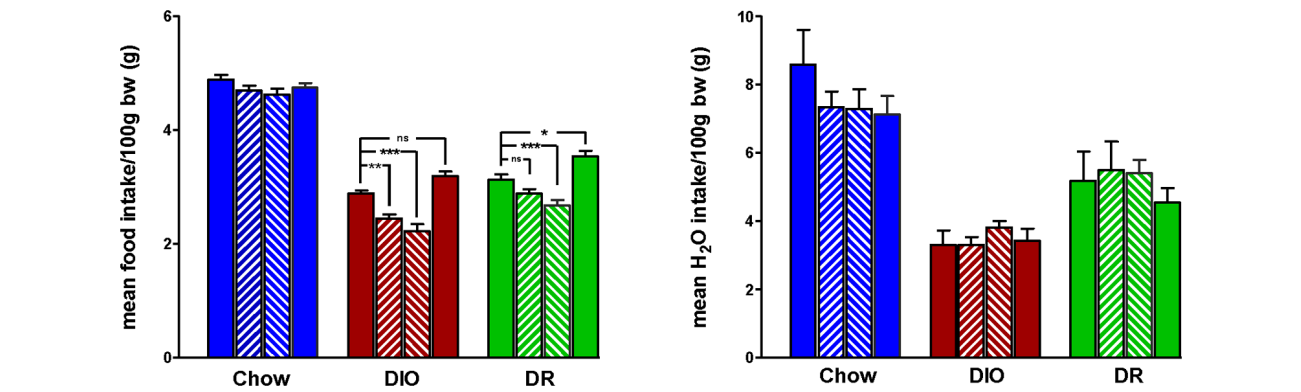

FIGURE 4 | Mean daily food (left panel) and water (right panel) intake for 7 days immediately prior to losartan treatment (Pre), days 1–7 (LOS 1–7) and days 8–14 (LOS 8–14) of losartan treatment and the final 7 days (days 18–24) following losartan treatment (Post). Food intake was decreased in DIO and DR animals during losartan treatment compared to control values (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; Tukey *post hoc* analysis) while losartan treatment had no effect on food intake in age-matched chow fed controlanimals compared to control food intake. Water intake was unaffected in all groups.

losartan abolishes weight gain in DIO rats fed a HFD without influencing body weight in age-matched chow fed, animals. Our study, however, extends these findings to show that AT1R receptor blockade also prevents weight gain in animals fed a HFD but who were not obese (DR animals).

A role for AT1R in the development of DIO is supported by studies in AT1R knockout mice (Agtr1a$^{-/-}$) that show an attenuation of diet induced body weight gain as compared to their wild type counterparts (Kouyama et al., 2005). Blockade of AT1R by a variety of pharmacological agents have also been shown to prevent HFD induced body weight gain (Sharieh Hosseini et al., 2014) or to impair body weight gain in DIO animals (Machado et al., 2012; Miesel et al., 2012; Sagae et al., 2013; Oliveira-Junior et al., 2014). An interesting finding of the present study is that AT1R blockade had a similar effect on weight gain in the DR animals but not in age-matched chow fed controls suggesting a role for the RAS on body weight gain only in animals receiving a HFD regardless of body weight and adiposity. This finding may in part be explained by the losartan-induced decrease in food intake, however, further studies to elucidate the mechanism(s) by which blockade of the RAS with losartan is able to prevent body weight gain in animals fed a HFD are warranted, though decreased leptin and increased adiponectin concentrations have already been proposed (Zorad et al., 2006; Weisinger et al., 2009b; Premaratna et al., 2012). In the present study, losartan administration did not change body weight gain in age-matched chow fed (CHOW) animals a finding that is at odds with previous work where administration of AT1R antagonists attenuated body weight gain in rats fed a normal diet (Zorad et al., 2006). These apparently contradictory findings may be due to the differences in duration of AT1R antagonist administration (14 days in the current study vs 18 week administration in the Zorad et al. (2006), study differences in strain of rat, or in the AT1R antagonist used. The fact that water consumption was not altered by losartan suggests that the losartan-induced drop in food intake observed in our study is not due to any secondary effect such as the satiety that results from increased water consumption.

The data we present here raises important questions that will require further studies to elucidate the mechanism(s) by

which losartan exerts its protective effects on body weight gain in the response to a HFD. Future studies investigating alterations in energy expenditure as well as body composition may provide insights regarding specific mechanisms underlying losartan's ability to inhibit weight gain. Analysis of circulating concentrations of the RAS and subsequent vascular and adipose reactivity to angiotensin II may also provide important information. Finally, measurement of circulating metabolic hormones (leptin, adiponectin,. . ..) and hypothalamic neurotransmitters (αMSH, NPY, ...) prior to, during, and post losartan treatment may uncover mechanisms through which the AT1 receptor modulates these systems. Collectively, such future studies will likely elucidate the complexity through which the AT1 receptor activation participates in the pathophysiology of adiposity.

CONCLUSION

Our data clearly demonstrates that oral losartan administration attenuates body weight gain in animals fed a HFD whether the animal is obese (DIO) or not (DR) while having no effect on body weight gain in age-matched chow fed animals. We hypothesize that angiotensin system blockade has a protective effect on body weight gain while on a HFD. Our data suggests that the value of losartan may extend beyond that of the treatment of hypertension and may be indicated as a suitable treatment for metabolic syndrome, the confluence of cardiovascular risk factors including hypertension, abdominal obesity, dislipidemia, and type 2 diabetes.

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Leptin and insulin signaling in dopaminergic neurons: relationship between energy balance and reward system

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The central actions of leptin and insulin are essential for the regulation of energy and glucose homeostasis. In addition to the crucial effects on the hypothalamus, emerging evidence suggests that the leptin and insulin signaling can act on other brain regions to mediate the reward value of nutrients. Recent studies have indicated the midbrain dopaminergic neurons as a potential site for leptin' and insulin's actions on mediating the feeding behaviors and therefore affecting the energy balance. Although molecular details about the integrative roles of leptin and insulin in this subset of neurons remain to be investigated, substantial body of evidence by far imply that the signaling pathways regulated by leptin and insulin may play an essential role in the regulation of energy balance through the control of food-associated reward. This review therefore describes the convergence of energy regulation and reward system, particularly focusing on leptin and insulin signaling in the midbrain dopaminergic neurons.

Keywords: leptin, insulin, midbrain, dopamine, reward, energy homeostasis

INTRODUCTION

Obesity, a multifactorial metabolic disorder that leads to many adverse health consequences, has reached epidemic proportions globally with more than 500 million adults being obese as of 2008 (Frühbeck et al., 2013). Obesity occurs as a result of genetic, behavioral, environmental, physiological, social, and cultural factors. Among the listed causes, behavioral and environmental factors have been described as the major contributors to the dramatic increase in obesity in the past two decades. The fundamental etiology of obesity is an energy imbalance between calorie consumption and energy expenditure with relatively higher food consumption (Racette et al., 2003; Nguyen and El-Serag, 2010). Drive for food consumption is a multiple process which is not only caused by nutritional status of the body but is also affected by the food palatability (the rewarding aspect of food) and other environmental and social factors. Increased energy intake due to excessive consumption of palatable food has contributed to the rise of obesity. It is well established that the hypothalamus plays a central role in regulation of energy balance and food intake to maintain the body's physiological requirements. An extensive body of evidence has demonstrated that endocrine regulators such as insulin and leptin mainly act on the hypothalamus of the central nervous system (CNS) to regulate food intake and body weight. In addition, expression of leptin and insulin receptors in other regions of the brain such as the doparminergic (DA) neurons suggests that the two hormones exert their effects in other areas outside of the hypothalamus. The neuronal circuit of DA neurons mediating reward, motivational and hedonic mechanisms in the CNS is also involved in the regulation of many aspects of feeding behavior and energy homeostasis. Indeed, accumulating evidence has indicated that leptin and insulin act on the midbrain DA neurons mediating feeding behaviors and therefore affecting energy balance (Fulton et al., 2006; Homme et al., 2006; Figlewicz et al., 2008; Leinninger et al., 2009, 2011; Morton et al., 2009; Opland et al., 2010; Bruijnzeel et al., 2011; Domingos et al., 2011; Mebel et al., 2012). In this review, we seek to focus on the energy homeostasis role of insulin and leptin particularly in the midbrain DA reward circuit system.

INSULIN AND LEPTIN IN CONTROL OF ENERGY BALANCE IN CNS

Studies on the infusion of insulin into the brain have opened the view that peripheral hormones can act on the brain to regulate food intake and body weight (Woods et al., 1979; Porte and Woods, 1981; Brief and Davis, 1984; Schwartz et al., 1992; Chavez et al., 1995, 1996; Air et al., 2002). Leptin, the adipose-derived hormone, was identified in the mid-1990 and it was shown to exert its actions mainly in the CNS (Zhang et al., 1994; Halaas et al., 1995). Since then, various studies have been carried out to elucidate the role of leptin in energy homeostasis particularly in the brain giving further insight into its role in obesity. Moreover, expression of insulin and leptin receptors throughout the brain confirmed, at least partially, the functional signaling of these hormones in the CNS (Havrankova et al., 1978; Gammeltoft et al., 1984; Zahniser et al., 1984; Werther et al., 1987; Unger et al., 1991; Kar et al., 1993; Huang et al., 1996; Couce et al., 1997; De Matteis and Cinti, 1998; Elmquist et al., 1998; Shioda et al., 1998; Burguera et al., 2000; Funahashi et al., 2003; Leshan et al., 2006). Various studies have

also demonstrated the role of insulin and leptin signaling on glucose homeostasis in the brain. These studies employed different experimental models such as insulin receptor knock out and db/db mice, and Zucker fa/fa rats which lack leptin receptors in both CNS and periphery (Chua et al., 1996; Bruning et al., 2000; Koch et al., 2008). In addition neuron-specific leptin receptor knockout mice provided obvious evidence on the role of leptin action in the CNS (Balthasar et al., 2004; van de Wall et al., 2008).

The hypothalamic nuclei where both insulin and leptin receptors are strongly and widely expressed have been described as the key site for insulin and leptin actions in the CNS (McGowan et al., 1992; Satoh et al., 1997; Ring and Zeltser, 2010). It has been suggested that both insulin and leptin act on two functionally opposite groups of neurons in the arcuate nucleus (ARC) of the hypothalamus to provide negative feedback for food intake and energy balance. Leptin and insulin inhibit orexigenic neurons expressing neuropeptide Y (NPY)/agouti-related protein (AgRP), neuropeptides that are known to stimulate food intake and reduce energy expenditure. Conversely, they activate pro-opiomelanocortin (POMC)/cocaine and amphetamine related transcript (CART) neurons. Anorexic neurons expressing POMC, a protein precursor which is processed to melanocortins including α-melanocyte stimulating hormone (α-MSH), reduce food intake and increase energy expenditure (Schwartz et al., 2000; Morton et al., 2006; Belgardt and Bruning, 2010; Figlewicz and Sipols, 2010).

Other hypothalamic nuclei such as paraventricular nucleus (PVN) and lateral hypothalamic area (LHA) may directly or indirectly mediate the effects of insulin and leptin since these regions receive innervations from both NPY/AgRP and POMC/CART neurons and also express insulin and leptin receptors. The melanocortin receptors 3 and 4 (MC3/4R) and NPY receptors which respond to the anorexigenic effects of α-MSH and the orexigenic effects of NPY/AgRP, respectively, are expressed abundantly in the PVN and LHA (Mountjoy et al., 1994; Parker and Herzog, 1999). In addition, these neurons project to other brain regions that mediate the perception of satiety (e.g., the nucleus of the solitary tract, NTS, in the hindbrain) and the reward system (the mesolimbic DA system; Morton et al., 2006, for review). Recent studies showed that neurotensins-containing neurons in the LHA innervate to the local orexin neurons and the ventral tegmental area (VTA) of the DA system (Leinninger et al., 2011). Leptin was shown to act on the leptin receptor-expressing neurons in the LHA to control orexin and the mesolimbic DA system and contribute to the control of energy balance (Leinninger et al., 2009, 2011).

INSULIN AND LEPTIN ACTIONS ON THE REWARD SYSTEM TO MODULATE ENERGY HOMEOSTASIS

The broad expression of insulin and leptin receptors in several CNS regions raised the question about their functions beyond the hypothalamus (Havrankova et al., 1978; Unger et al., 1991; Huang et al., 1996; Guan et al., 1997; Elmquist et al., 1998; Figlewicz et al., 2003; Funahashi et al., 2003; Fulton et al., 2006; Homme et al., 2006). Among these regions, the DA neuron system, which plays an important role in the regulation of reward and motivational behaviors, emerged as a potential target for insulin and leptin actions. The mesolimbic DA neurons project from the VTA and substantia nigra (SN) to the nucleus accumbens (NAc) and have been implicated in the rewarding and motivating aspects of food intake (Berridge, 1996; Saper et al., 2002; Kelley et al., 2005b; Wise, 2006). One of the factors contributing to increased incidences of obesity is diet composition especially in this modern era where most people opt for processed or instant foods. Given that the reward system directly or indirectly regulates feeding behaviors, there is therefore an increased interest in studies focusing on the role of the reward circuit and the DA neurons in modulating feeding behaviors and energy homeostasis (Figlewicz and Sipols, 2010).

Intra-cerebroventricular insulin injection decreased lever rates for sucrose solution, decreased sucrose self-administration (Figlewicz et al., 2006, 2008) and reversed conditioned place preference (CPP) with high fat diet (Figlewicz et al., 2004). CPP measures the ability of an animal to respond to the rewarding aspects of food and reduced CPP by insulin hence suggests that this hormone can modulate reward-related feeding behavior (Palmiter, 2007). Specifically, a recent study showed that direct administration of insulin into the VTA reduced food intake and repressed feeding of sweetened high-fat diet in the sated condition (hedonic feeding; Bruijnzeel et al., 2011; Mebel et al., 2012). Importantly, deletion of the insulin signaling in the catecholaminergic neurons resulted in increased sucrose sensitivity and an obese phenotype (Könner et al., 2011). However, catecholaminergic neurons represent both the dopaminergic and the norepinephrinergic neurotransmitter activities. Therefore, the exact mechanism underlying the effect of insulin signaling on hedonic and reward feeding behavior cannot be deduced solely from the observations made in catecholaminergic neurons. In an attempt to identify the mechanism of insulin signaling in DA system, the dopamine re-uptake transporter (DAT) has emerged as a potential cellular target for insulin action. DAT transports DA from the synapse back to the nerve terminal, hence decreasing dopamine activity (Jaber et al., 1997). Insulin increased DAT mRNA level and activity, this could lead to enhanced clearance of dopamine from the synapse and therefore reducing DA signaling (Figlewicz and Sipols, 2010, for review). To gain further mechanistic insight into the effect of insulin on the DA signaling, there is need to carry out more studies using an experimental model in which the insulin signaling has been disrupted specifically in the DA neurons.

Pharmacological studies have indicated that leptin also modulates behaviors associated with dopamine reward circuit. Leptin decreased lateral hypothalamic self-stimulation as well as sucrose self-administration and sucrose CPP (Figlewicz et al., 2001, 2004, 2006; Shalev et al., 2001). Moreover, leptin declined drug seeking behaviors caused by food deprivation (Shalev et al., 2001; Hao et al., 2004). In addition, direct leptin injection into the VTA reduced food intake (Homme et al., 2006; Morton et al., 2009; Bruijnzeel et al., 2011). These findings imply that leptin provides negative effects on DA reward neurons resulting in food intake reduction. Further evidence shows that leptin suppressed the mesolimbic DA signaling by decreasing the DA neuronal firing frequency and subsequently reducing DA levels in the NAc

(Krügel et al., 2003; Homme et al., 2006). In addition, presynaptic leptin action can suppress excitatory synaptic transmission into DA neurons in VTA (Thompson and Borgland, 2013). Moreover, similar to the effects observed in insulin signaling, decreased DA concentrations by leptin could be attributed to increased DAT activity (Perry et al., 2010). However, investigations on the function of mesolimbic DA system in leptin-deficient animals showed opposite findings in that the DA signaling originating from the VTA was reduced. *Ob/ob* mice contained less tyrosine hydroxylase, the rate-limiting enzyme for DA synthesis, and showed decreased DA content in the VTA and NAc (Fulton et al., 2006; Roseberry et al., 2007). In addition, dopamine 2 (D2) receptor binding decreased in the VTA of these mice and this was reversed by leptin treatment (Pfaffly et al., 2010). It is difficult to explain the discrepancies observed in *ob/ob* mice but it is possible that chronic leptin deficiency stimulates other compensatory mechanisms, for example, chronic leptin deficiency might lead to changes in normal intracellular signaling pathways and activate a feedback regulatory loop that might be responsible for regulating DA content and function and ultimately decreasing the function of DA neurons (Opland et al., 2010, for review).

Genetic techniques using viral-mediated RNA to knock down the leptin receptor in the VTA also showed increase in food intake and sensitivity for highly palatable food highly suggesting the crucial role of leptin in VTA in modulation of feeding behavior and energy homeostasis (Homme et al., 2006; Davis et al., 2011). Consistently, recent studies using optogenetic approach to activate DA neurons and quantify the reward value of nutrients strongly confirmed the negative effects of leptin on the reward value via reduction in DA signaling (Domingos et al., 2011). However, DA neurons-specific knockdown of leptin receptor using cre-loxP system ($Lepr^{DAT-Cre}$) showed no change in body weight or food intake (Liu et al., 2011). This could be because disrupting the leptin signaling only in a small subset of leptin receptor-expressing neurons in VTA in $Lepr^{DAT-Cre}$ mice might not be sufficient to affect energy balance and this loss might be compensated by other leptin receptor neurons in the other brain regions. Therefore, further investigation using different genetic approaches with higher sensitivity such as tissue-specific re-activation of leptin receptor signaling only in dopaminergic neurons might be helpful to assess the role of leptin receptor signaling in this reward circuit.

As mentioned above, LHA has been suggested as a target for leptin action to modulate the reward circuit. In addition, it has been suggested that the group of neuron in the LHA project to the mesolimbic regions to control DA action and reward (DiLeone et al., 2003; Harris et al., 2005; Kelley et al., 2005a; Opland et al., 2010). Among these, two populations of neurons have been identified: melanin concentrating hormone (MCH) and orexin expressing neurons. These neurons are known to project to the NAc and VTA, respectively, to promote feeding and modulate reward (Qu et al., 1996; Mieda and Yanagisawa, 2002; Georgescu et al., 2005). However, leptin is known to inhibit orexin and MCH activities in this circuitry (Qu et al., 1996; Yamanaka et al., 2003). Interestingly, LHA also consists of neurons expressing leptin receptors which are distinct from MCH and orexin neurons and innervate to the VTA. Moreover, leptin acts on these neurons to modulate the mesolimbic DA system and decrease feeding (Leinninger et al., 2009). Recent studies demonstrated that majority of leptin receptor neurons in LHA contain neurotensins (Nts) and leptin receptors in Nts neurons project to the VTA and local orexin neurons but not MCH neurons to mediate the physiological action of leptin on orexin neurons and the mesolimbic DA system (Leinninger et al., 2011).

INSULIN AND LEPTIN SIGNALING PATHWAYS IN CNS

Studies in the hypothalamus have provided a basis for understanding the molecular mechanism of insulin and leptin in the CNS even though the entire mechanism remains to be elucidated. The CNS insulin signaling is quite similar to that in peripheral organs. Insulin binds to and activates its receptor, a tyrosine kinase with autophosphorylating activity, and receptor activation leads to phosphorylation of insulin receptor substrate (IRS)/phosphatidylinositol 3-OH kinase (PI3K) pathway (Hadari et al., 1992). The catalytic subunit p110 of PI3K complex converts phosphatidylinositol-4,5-biphosphate (PIP2) into phosphatidylinositol-3,4,5-triphosphate (PIP3) to phosphorylate and activate downstream Akt/PKB (Niswender et al., 2003). This Akt activation in turn phosphorylates forkhead transcription factor O1 (FoxO1) which functions, especially in ARC, as a transcriptional suppressor of POMC gene and as a transcriptional activator of AgRP gene (Kitamura et al., 2006; Ren et al., 2012). Phosphorylated form of FoxO1 is subsequently excluded to the cytoplasm, allowing binding of transcriptional stimulators such as pSTAT3 to the POMC promoter. At the same time, FoxO1-mediated AgRP expression is inhibited (Plum et al., 2006; Varela and Horvath, 2012, for reviews; **Figure 1A**). However, unlike the peripheral insulin signaling in which IRS1 protein plays an essential role in insulin signal transduction, it has been suggested that the IRS2 is a major player involved in CNS insulin action on energy homeostasis regulation (Davis et al., 2004; Kubota et al., 2004; Porte et al., 2005, for review). IRS1 is sparsely expressed in the ventral hypothalamus and IRS1-deficient mice do not express abnormal metabolic phenotype (Araki et al., 1994; Tamemoto et al., 1994). On the other hand, IRS2 is abundant in the ARC and tyrosine phosphorylation of IRS2 is associated with increased PIP3, indicating the activation of PI3K/Akt downstream pathway mainly through IRS2 (Niswender and Schwartz, 2003; Torsoni et al., 2003). In support of this notion, mice lacking IRS2 showed increased food intake and fat mass and impaired reproductive activity (Burks et al., 2000).

Leptin binding to its receptor triggers IRS phosphorylation and also activate PI3K activity (Niswender and Schwartz, 2003). However, leptin receptor does not have intrinsic tyrosine kinase activity and requires JAK-STAT binding for full activation (Sweeney, 2002, for review). Leptin binding to its receptor allows JAKs in juxtaposition to phosphorylate and activate each other. In addition, recent report revealed that Rho-kinase 1 (ROCK1) plays a critical role in leptin signaling by phosphorylating JAK2 via a direct ROCK1-JAK2 interaction (Huang et al., 2012; **Figure 1A**). Phosphorylation of leptin receptor allows association of STAT, a substrate for JAK. After its dissociation form leptin receptor, STAT is phosphorylated and forms active dimers. Activated

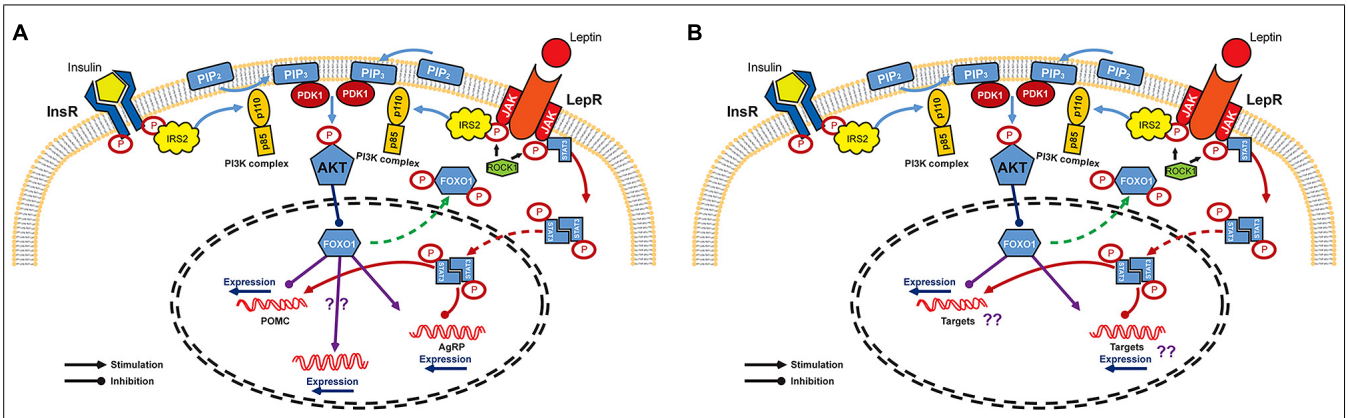

FIGURE 1 | Signaling pathways of leptin and insulin in the CNS. **(A)** Leptin and insulin pathways converge on IRS/PI3K/Akt axis in arcuate nucleus of the hypothalamus. Binding of insulin and leptin to their receptors leads to the phosphorylation of IRS2 which activates PI3K to facilitate the phosphorylation of Akt by PDK. Akt activation finally phosphorylates and excludes FoxO1 from nucleus to inactivate it. FoxO1 is known as a repressor of POMC expression, but as an activator of AgRP expression. Expression of POMC and AgRP is also regulated by JAK/STAT3 pathway. Once leptin binds to its receptor, STAT3 is phosphorylated by JAK and is dimerized. The active dimeric form of STAT3 translocates to the nucleus where it inhibits AgRP and activates POMC expression. **(B)** Schematic diagram of insulin and leptin signaling in DA neurons. InR, insulin receptor; LepR, leptin receptor; IRS, insulin receptor substrate; PIP2, phosphatidylinositol-4,5-biphosphate; ROCK1, Rho-kinase 1; PIP3, phosphatidylinositol-3,4,5-triphosphate; PI3K, phosphatidylinositol 3-OH kinase; PDK1, phosphoinositide-dependent kinase-1; Akt, protein kinase B; FoxO1, forkhead transcription factor O1; JAK, Janus kinase; STAT3, signal transducer and activator of transcription 3; POMC, proopiomelanocortin; AgRP, agouti-related protein.

pSTAT3 translocates to the nucleus leading to transcriptional events such as stimulating POMC and inhibiting AgRP expression (Leshan et al., 2006; Mesaros et al., 2008; Ernst et al., 2009; **Figure 1**).

Although leptin and insulin mediate somewhat independent neuronal responses, there seems to be a crosstalk between these two hormones in energy homeostasis in the CNS (Niswender and Schwartz, 2003; Benomar et al., 2005; Porte et al., 2005). Specifically, it has been demonstrated that the IRS/PI3K/Akt axis is important for both insulin and leptin action in CNS (Niswender et al., 2003; Xu et al., 2005, 2010; Hill et al., 2008; **Figure 1**). Moreover, this overlap might also exist in the molecular pathways that provide negative effects to the insulin and leptin signaling such as the phosphatase protein tyrosine phosphatase 1B (PTP1B) and the suppressor of cytokine signaling 3 (SOCS3). PTP1B inhibits both insulin and leptin signaling and mice lacking PTP1B are more sensitive to both leptin and insulin and resistant to diet-induced obesity (Elchebly et al., 2000; Zabolotny et al., 2002). SOCS3 is a known negative regulator of leptin (cytokine in general) signaling (Sweeney, 2002). However, SOCS3 can also cause insulin resistance by modifying insulin receptor and IRS proteins leading to impaired insulin signaling (Rieusset et al., 2004; Ueki et al., 2004). The FoxO1, a nuclear transcriptional factor downstream of the PI3K/Akt axis which is known to mediate insulin action, might also be a potential crosstalk in the insulin and leptin signaling (Altomonte et al., 2004; Barthel et al., 2005). A recent study pointed out the crucial role of FoxO1 in the mediation of IRS2/PI3K signaling in LepR-expressing neurons to control energy balance (Sadagurski et al., 2012). The functional signaling of insulin and leptin, together with the presence of insulin and leptin receptors, have been confirmed in the VTA (Fulton et al., 2006; Homme et al., 2006; Iñiguez et al., 2008). PI3K activity is increased under direct administration of insulin and leptin into the VTA (Figlewicz et al., 2007). Moreover, IRS2/Akt pathway in VTA has been shown to modulate rewarding and psychomotor activating effects of cocaine and opiates (Russo et al., 2007; Iñiguez et al., 2008). Direct leptin administration into the VTA increased JAK-STAT signaling and this is essential for the effect of leptin in the VTA to decrease food intake (Morton et al., 2009). Therefore, studies on the molecular crosstalk occurring downstream of leptin and insulin in DA neurons may also be important to understand specific roles of these signals in mediating energy homeostasis and reward value of food (**Figure 1B**).

CONCLUSION

Taken together, both pharmacological and genetic studies demonstrate that insulin and leptin not only act on hypothalamic regions but also play important roles in the DA reward system to regulate feeding behavior and energy balance. Further, leptin and insulin in DA neurons seem to mediate several neuronal projections to the other brain regions such as hypothalamus and NAc that are potentially important for the regulation of feeding and mood behaviors. At a cellular level, establishing whether leptin and insulin act on the same or different populations of DA neurons would be important to distinguish their specific functions in the DA neurons and in other neuronal projections. Therefore, studies using more advanced techniques such as optogenetics and pharmacogenetic tools will be beneficial to further understand the neuronal and molecular mechanisms underlying the effects of insulin and leptin on this reward system.

AUTHOR CONTRIBUTIONS

Doan V. Khanh: drafted and edited the manuscript. Yun-Hee Choi, Ann W. Kinyua and Sang Hyun Moh: reviewed the manuscript and finalized figure. Ki Woo Kim: drafted, edited and finalized the manuscript.

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Combined compared to dissociated oral and intestinal sucrose stimuli induce different brain hedonic processes

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The characterization of brain networks contributing to the processing of oral and/or intestinal sugar signals in a relevant animal model might help to understand the neural mechanisms related to the control of food intake in humans and suggest potential causes for impaired eating behaviors. This study aimed at comparing the brain responses triggered by oral and/or intestinal sucrose sensing in pigs. Seven animals underwent brain single photon emission computed tomography (^{99m}Tc-HMPAO) further to oral stimulation with neutral or sucrose artificial saliva paired with saline or sucrose infusion in the duodenum, the proximal part of the intestine. Oral and/or duodenal sucrose sensing induced differential cerebral blood flow changes in brain regions known to be involved in memory, reward processes and hedonic (i.e., pleasure) evaluation of sensory stimuli, including the dorsal striatum, prefrontal cortex, cingulate cortex, insular cortex, hippocampus, and parahippocampal cortex. Sucrose duodenal infusion only and combined sucrose stimulation induced similar activity patterns in the putamen, ventral anterior cingulate cortex and hippocampus. Some brain deactivations in the prefrontal and insular cortices were only detected in the presence of oral sucrose stimulation. Finally, activation of the right insular cortex was only induced by combined oral and duodenal sucrose stimulation, while specific activity patterns were detected in the hippocampus and parahippocampal cortex with oral sucrose dissociated from caloric load. This study sheds new light on the brain hedonic responses to sugar and has potential implications to unravel the neuropsychological mechanisms underlying food pleasure and motivation.

Keywords: cognition, food motivation, reward, brain metabolism, sugar sensing

INTRODUCTION

Sugars, and especially sucrose, are known to be very attractive in many species. In humans, non-human primates and rats, newborn infants and pups spontaneously respond to sweet taste stimulation with sucrose by specific positive facial expressions (for review, see Berridge, 2000; Steiner et al., 2001). Capaldi and Privitera (2007) also reported that mixing sour or bitter food with the sweet taste of sucrose increased liking for these foods in infant and adult humans, while pigs and rats show spontaneous preferences for sucrose solutions over water (Glaser et al., 2000; Ackroff and Sclafani, 2011). Additionally, adjunction of sucrose as a positive taste reinforcement in a flavored solution induces strong conditioned flavor preferences in rats (Warwick and Weingarten, 1996; Gilbert et al., 2003; Bonacchi et al., 2008), humans (Mobini et al., 2007), and pigs (Clouard et al., 2012a). Interestingly, some studies also suggested that post-oral effects of sucrose, acting independently to oral signals, act as physiological reinforcers and can lead to increased sugar intake. Clear-cut flavor preferences for unsweetened solutions previously paired with intragastric sucrose infusion have indeed been reported in rats (Azzara and Sclafani, 1998; Sclafani and Glendinning, 2005), suggesting that visceral (i.e., gastro-intestinal) sucrose infusion might also be sufficient in itself to induce positive hedonic (i.e., pleasure-related) responses even in the absence of sweet taste. But interactions between the exteroceptive (e.g., taste, odor, etc.) and interoceptive (i.e., visceral) perceptions of food are also important to modulate hedonism, as demonstrated for the alliesthesia phenomenon (Cabanac and Duclaux, 1970; Cabanac, 1971), a phenomenon whereby the pleasantness of an external stimulus depends on the internal state of the organism. Metabolic signals, including gastrointestinal hormones, can modulate central functions that are not only associated with homeostatic regulation of food intake, but also with pleasure, reward and emotion (Shigemura et al., 2004; Uher et al., 2006; Zheng and Berthoud, 2007). As a consequence, exploring sugar-induced pleasure and motivation, and eventually sweet cravings, requires a focus on what is at stake in the brain when an individual is exposed to oral and/or visceral sugar stimulations.

It is now well known that, in addition to the homeostatic regulation of food intake, mainly integrated at the medullo-hypothalamic level, reward-related brain areas have a major role in the hedonic processing and regulation of food/sugar

intake. Functional magnetic resonance imaging (fMRI) studies have shown that processing of oral sucrose signals involved several pleasure-related brain regions, including the insular cortex, striatum (caudate, nucleus accumbens, and putamen), globus pallidus, amygdala, hippocampus, as well as the prefrontal and anterior cingulate cortices in humans (Frank et al., 2008; Smeets et al., 2011; Stice et al., 2013). Recently, Boubaker et al. (2012) investigated the brain responses triggered by duodenal (the duodenum being the proximal part of the small intestine) glucose sensing in pigs using the single photon emission computed tomography (SPECT) and reported metabolic changes in the orbitofrontal and dorsolateral prefrontal cortices, the caudate and putamen, but also in regions that participate in odor processing, including the prepiriform area and the anterior entorhinal cortex. Altogether, these findings emphasize that the processing of both oral and duodenal sugar signals are mediated by both homeostatic and non-homeostatic factors and involve extra-hypothalamic regions that participate in the hedonic regulation of food intake.

Only a few studies compared the brain responses triggered by sweet taste and/or calories during carbohydrate sensing. Chambers et al. (2009), however, reported that oral ingestion of glucose (combining sweet taste and calories), saccharin (sweet taste but no calorie), and maltodextrin (calories but no sweet taste) all induced differential activations in brain structures involved in taste identification and cognitive processes, including the insula and the dorsolateral prefrontal cortex. Only oral glucose and maltodextrin ingestion, however, induced changes in activation in reward-related brain regions, including the orbitofrontal and anterior cingulate cortices and the caudate. Using microdialyse techniques, Ferreira et al. (2012) reported that dopamine levels in dorsal and ventral striatum are associated with the amount of calories ingested in mice, and that striatal dopamine levels fluctuate in proportion to the caloric density of nutrients infused in the gut. Moreover, Ren et al. (2010) also showed in mice that hormonal signaling, rather than glucose utilization, is the main stimulus regulating striatal dopamine release during glucose ingestion, and that intravenous glucose infusions promote the increase of dopamine levels.

All these studies strongly support the assumption that both oral and post-oral signals during carbohydrates ingestion/infusions are processed in pleasure and reward-related brain structures in humans, rodents or pigs. However, as far as we know, no study has yet compared the brain metabolism triggered by oral and/or duodenal sensing of the same sugar (e.g., sucrose) in the aforementioned extra-hypothalamic regions. Both the hedonic oral properties and the post-oral signals arising from sucrose are able to strongly promote food intake and preference. However, little is known about the ability of sucrose intake to stimulate brain reward circuits in the absence of oral signals.

In western countries and, increasingly, in developing countries, modern diets contain a wide array of manufactured and processed foods consisting of mixtures of starch and sugars (for review, Tappy and Le, 2010), with sucrose, fructose, glucose and lactose as the most common dietary sugars. Sucrose remains the leading added sugar consumed in the American diet and the leading source of fructose, although a rapid increase in fructose consumption has been noticed in the last decades (Tappy and Le, 2010). Yet, chronic consumption of high-sugar diets has been proved to induce metabolic, neurophysiological and brain alterations leading to eating disorders and obesity in humans, pigs and rats (Zhao et al., 2005; Lomba et al., 2009; Benton, 2010; Val-Laillet et al., 2010, 2011). Consequently, the characterization of the brain networks that contribute to the processing of oral and/or post-oral sugar signals in animal models, by leading to a better understanding of impaired eating behaviors like the onset of exacerbated sugar preferences in humans, might fulfill the current needs in human nutrition and health research. The pig appears has a very good model for exploring food preferences and brain mechanisms related to nutrition, as stated in previous review papers (Sauleau et al., 2009; Clouard et al., 2012b).

The aims of the present study are (i) to investigate whether the combination (or congruence, i.e., stimulation of two different sensory pathways by similar/consistent stimuli) or dissociation between oral and post-oral sucrose perception influence the brain activity in reward-related brain structures, and (ii) to determine whether duodenal infusion of sucrose in the absence of sweet taste induce specific activity in the brain reward circuit.

MATERIALS AND METHODS

ETHICS STATEMENT

The experiments presented in this paper were conducted in accordance with the current ethical standards of the European Union (Directive 2010/63/EU), Agreement No. A35-622 and Authorizations No. 01894 and No. 35-88. The whole experimental procedure presented in this paper has been reviewed and validated by the regional ethics committee in animal experiment of Brittany, France.

ANIMALS AND HOUSING

A total of seven 30-kg Large White × Landrace female pigs were used in this study. The pigs were housed in individual pens (150 × 60 × 80 cm) and had free access to water. A chain was suspended in each pen to enrich the environment of the animals and fulfill their natural disposition to play. The room was maintained at ∼24°C with a 13:11-h light–dark cycle. The animals were fed daily a pelleted diet composed of 40% pea, 15% corn, 14.46% barley, 13.92% wheat, 13.56% soybean meal, 0.68% calcium carbonate, 0.58% mono-calcic phosphate, 0.3% vegetable oil, 0.3% vitamin complement, 0.24% salt (net energy: 2.15 Mcal/kg of food). The animals received 1 kg of diet per day.

SURGERY

After a 24-h fasting period, the pigs were preanesthetised with an intramuscular injection of ketamine (15–20 mg/kg, Mérial, Lyon, France). Suppression of pharyngotracheal reflex was obtained by inhalation of isoflurane (3–5% v/v, Baxter SAS, Maurepas, France) immediately before tracheal intubation. A surgical level of anesthesia was maintained by isoflurane (2–3% v/v) delivered by a mechanical ventilator and analgesia was obtained by an intravenous injection of a morphinic agent (Fentanyl 4 ml, 1.4 ml/min, Renaudin, Paris, France). Heart and respiratory rates were continuously monitored throughout surgery using a

pulse oximeter (Ohmeda oximeter, GE Healthcare Clinical Systems, Limonest, France) and an infrared capnometer (Amstrong capnometer, Gambo Engström, Bromma, Sweden). A midline laparotomy was performed under aseptic conditions. A catheter was fixed into the proximal duodenum, tunneled under the skin and exteriorized between the shoulders for intraduodenal (ID) infusions of 0.9% NaCl (saline) or 16% sucrose. After surgery, all the animals had 3 weeks of recovery before the start of the brain imaging sessions.

PRELIMINARY DETERMINATION OF PLASMA GLUCOSE KINETIC

Three 30-kg Large White × Landrace female pigs different from those used in the present study were used to measure plasma levels of glucose further to a 30-min ID infusion of 16% sucrose (300 ml, 197 kcal). The animals were implanted a duodenal catheter following the same procedure as described above (see Surgery). A jugular catheter was also inserted into a collateral vein surrounding the jugular vein in the neck, tunneled under the skin and exteriorized at the nape level. After surgery, all the animals had at least a week of recovery before blood sampling. The day of blood sampling, the pigs were anesthetized after fasting overnight for 16–18 h and intubated following the same procedure as described above (see Surgery). Ears and eyes of the animals were sealed with cotton and surgical tape respectively, in order to minimize auditory and visual stimulations. Light and noise were set to a minimum at least 15 min after the anesthesia and pigs were subjected to a 30-min ID infusion of 16% sucrose (300 ml, 197 kcal). The solution was injected for 30 min with a peristaltic pump connected to the duodenal catheter with an injection rate of 10 ml per min. Blood samples were collected 10 min (−10 min) and immediately before (0 min) the start of the sucrose infusion, every 2 min from 2 to 60 min after the start of the infusion, every 5 min from 60 to 100 min after the start of the infusion and every 10 min from 100 to 120 min after the start of the infusion. Blood samples were collected in tubes containing 5 μl of ethylenediaminetetraacetic (EDTA 0.8 M, Sigma Aldrich, Saint Quentin, France). Blood samples were immediately centrifuged at 4000 g during 10 min at 4°C and the resulting plasma samples were conserved at −20°C until assaying. Plasma glucose was measured in duplicate by an automated spectrophotometric method (Konélab 20i, Thermo Fisher Scientific Inc., Waltham, MA, USA) using the enzymatic assay Glucose RTU™ (BioMérieux® SA, Marcy l'Étoile, France). The intra-assay coefficient of variation was <3%.

PRELIMINARY EXPOSURE TO ORAL AND/OR DUODENAL SWEET STIMULATIONS

In order to habituate the animals to oral and/or duodenal sucrose stimulations, all the animals were exposed to three different situations twice a week and during the two consecutive weeks before the beginning of the brain imaging sessions. The three experimental situations induced at the moment of the daily meal were: (i) exposure to the standard diet (1 kg, control), (ii) exposure to the standard diet (1 kg) added with 5% sucrose (the added sucrose represented 50 g and 196.9 kcal, which provided oral and visceral exposure to sucrose), and (iii) exposure to the standard diet (1 kg) with concomitant duodenal infusion of a sucrose solution (16%, 10 mL/min during 30 min, 196.9 kcal, i.e., duodenal sucrose stimulation without oral exposure). We chose two different sucrose concentrations for the oral and duodenal stimulations on purpose. A 5% sucrose addition to the feed is sufficient to increase palatability and therefore to induce putative pleasure-related brain activations. Even though 16% sucrose drinking solutions are attractive to pigs (Clouard et al., 2014), a too high sucrose concentration in solid feed might have reverse effects (i.e., aversion) in some animals and would have compromised the feed granulation process and altered the feed texture. For the duodenal stimulation, the aim was to maximize the metabolic effect and homeostatic response (e.g., increased glycemia and insulinemia). A 5% sucrose solution might have been insufficient to induce a significant homeostatic response with clear brain metabolism changes. In contrast, the literature has described clear responses with 16% sucrose infusions.

BRAIN IMAGING PROCEDURE

During 4 weeks, the pigs underwent four brain-imaging sessions each to investigate the brain metabolism following oral and/or duodenal stimulations with sucrose. The brain imaging modality used to investigate cerebral blood flow (CBF) was the SPECT of technetium-99m (^{99m}Tc, CIS Bio International, France) coupled with hexamethyl-propylene-amine-oxime (HMPAO, Ceretec, GE Healthcare, Velizy, France).

Animal preparation and oral/duodenal stimulations

After fasting overnight for 16–18 h, the animals were anesthetized in a quiet room and subjected to a tracheal intubation following the same procedure as described above (see Surgery). A venous catheter was inserted into their right ear in order to inject the radiolabel. Light and noise were set to a minimum at least 15 min before the start of the oral and duodenal stimulations. Ears and eyes of the animals were sealed with cotton and surgical tape respectively, in order to minimize auditory and visual stimulations.

The animal underwent oral (OS) and duodenal (DS) stimulations before brain image acquisition. The experimental devices are illustrated in **Figure 1**. The OS was originally described in Gaultier et al. (2011). It consisted in irrigating the pig's tongue (24 mL/min) with an unsweetened artificial saliva (OS–) or a sweetened artificial saliva (OS+, 5% sucrose; see Hellekant et al., 1997 for the saliva composition). A tube was positioned on the middle of the tongue and connected to the computer-operated automat developed in our laboratory (Gustautomat, INRA, St Gilles, France) and inspired by the Taste-o-Matic by Hellekant's group (Danilova et al., 2002). The DS was obtained by ID infusions of solutions. The DS+ corresponded to an ID infusion of 300 ml of 16% sucrose (197 kcal) and the control treatment corresponded to an ID infusion of 300 ml of saline (DS–). The choice to use 16% sucrose for ID infusion was based on the will to obtain a rapid and marked effect of ID sucrose on plasma glycaemia (**Figure 2A**). The solutions were injected with a peristaltic pump connected to the duodenal catheter, and the injection rate was 10 ml per min.

The schematic representation of the experimental paradigm used for the oral/duodenal stimulations before brain imaging is described in **Figure 2B**. The delay between OS and DS was chosen in such a way that the oral sucrose sensing (OS+) and the

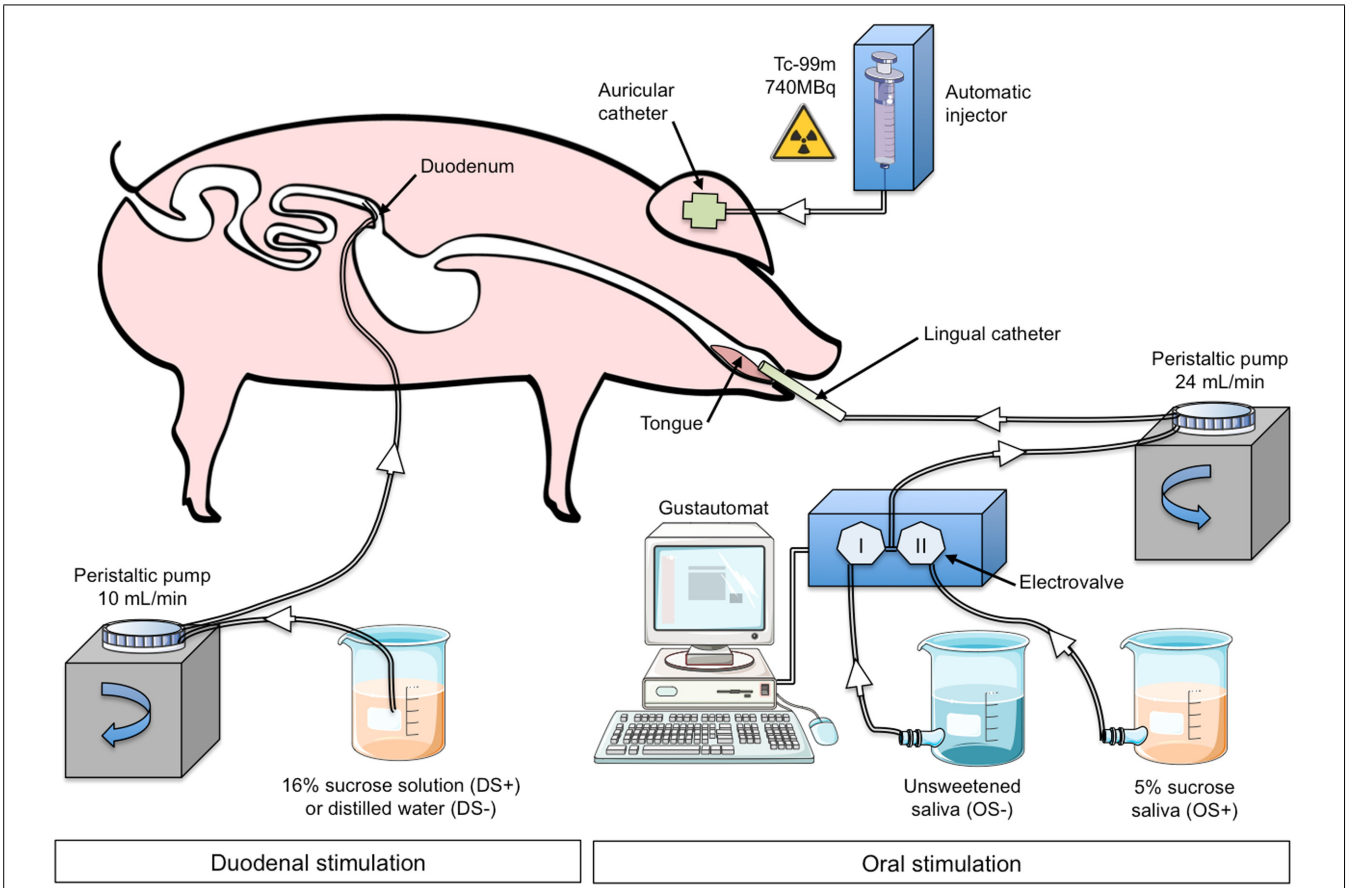

FIGURE 1 | Experimental device and paradigm designed to perform oral (OS) and duodenal (DS) stimulations for brain imaging in anaesthetized pigs. The illustrations used to make this figure were obtained from the "Servier Medical Art" website, http://www.servier.fr/servier-medical-art. OS–: oral non-sweet stimulation with artificial saliva; OS+: oral stimulation with 5% sucrose artificial saliva; DS–: duodenal stimulation with saline; DS+: duodenal stimulation with 16% sucrose. Injection of ^{99m}Tc-HMPAO was performed 50 min after the start of the DS but was concomitant to the OS.

peak of maximum glycaemia after the ID sucrose infusion (DS+) were synchronized. Briefly, at least 15 min after the anesthesia, the animals were subjected to the DS for 30 min. The OS was performed 47 min after the start of the DS. The OS+ consisted in a 3-min neutral oral stimulation (i.e., unsweetened saliva) to accommodate the mucosa thermoreceptors and mechanoreceptors to the stimulation, preceding the diffusion of sweetened saliva for 2 min. The OS+ was ended by a 1-min neutral stimulation. The OS− consisted in a 6-min neutral stimulation with unsweetened saliva. The order of the four different stimulation treatments (OS+DS+, OS+DS−, OS−DS+, and OS−DS−) was alternated for each pig according to a Latin square procedure to prevent any order effect.

Radiolabel administration and image acquisition

As the plasma glucose peak occurs ∼50 min after the start of the DS+ (**Figure 2A**), the radiolabel (^{99m}Tc-HMPAO, 740 MBq) was injected 50 min after the start of the DS, that is 3 min after the start of the OS. The radiolabel enters the neurons very quickly after injection (i.e., in less than a minute), proportionally to the neuronal activity, and stays within the neurons, meaning that brain images acquired after the injection of the radiolabel are representative of the brain activity at the moment of the injection.

At least 15 min after the radiolabel injection, the anesthetized animals were transferred and placed in a Head First Prone (ventral decubitus) position on the bed of a gamma-camera (APEX SP-6, Elscint, Tel-Aviv, Israel) fitted with a fan-beam collimator (50-cm focus). SPECT brain image acquisitions were performed at least 20 min after the radiolabel injection, when complete brain–blood equilibrium is reached (Thomsen et al., 2008). Sixty projections with a 120-s exposition were acquired at different projection angles (6°per step). Transaxial images were reconstructed using the filtered back projection method (FBP) applying a Metz filter (power parameter $q = 3$). An acquisition matrix size of 128 × 128 was used and spatial resolution of the final images was 0.76 mm per pixel for x- and y-axis and 1.47 mm per pixel in z-axis.

Image processing

The images were processed with statistical parametric mapping (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK) implemented in MATLAB 7.9 R2009B (The Mathworks Inc., Natick, MA, USA). SPM8 software was adapted to the characteristics of the pig brain. Template images based on 16 female pigs of same

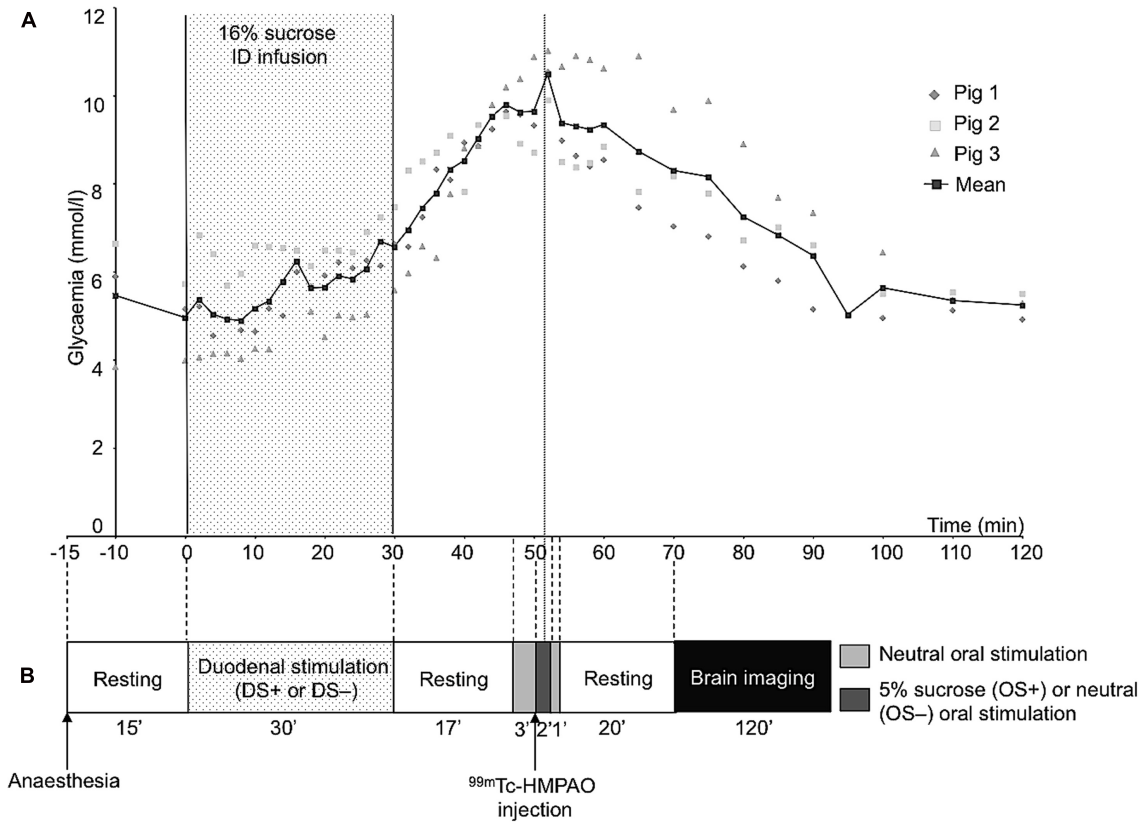

FIGURE 2 | (A) Plasma concentrations of glucose before and after a 30-min duodenal infusion of 16% sucrose (10 ml/min, 300 ml corresponding to 197 kcal). Blood samples were collected on three animals different from that used for behavioral tests and cerebral imaging. **(B)** Schematic representation of the experimental paradigm used for the oral and duodenal stimulations before brain imaging. This experiment aimed at exploring the brain metabolism (single photon emission computed tomography of ^{99m}Tc-HMPAO) further to oral and/or duodenal sucrose sensing. As the ^{99m}Tc has a fast fixation time, the initial tracer uptake reflects cerebral blood flow (CBF) at a fast time window after injection (Kapucu et al., 2009). Consequently, the CBF recorded during imaging, that is 20 min after the radiolabel injection, corresponded to the CBF measured at the time of the radiolabel injection, that is during the oral sensing of artificial saliva added with 5% sucrose (OS+) or unsweetened artificial saliva (OS–, control), and during the peak of maximal glycaemia resulting from the ID infusion of 16% sucrose (DS+) or after ID infusion of saline (DS–, control).

age and breed, as described in Gaultier et al. (2011), were used as reference images for the processing of the SPECT images acquired in the present study.

In the present study, the actual original DICOM files were converted in NIfTI files with the IMAGEJ software (Wayne Rasband NIH, Bethesda, MA, USA) and the images were manually reoriented. The spatial coordinates were centered compared to a reference point (x_0 y_0 z_0) set at CP (*commissura posterior*) in the CA–CP (*commissura anterior–commissura posterior*) plane according to the stereotaxic reference defined by Saikali et al. (2010). The reoriented images were masked using a large brain mask image to remove the extracerebral matter. The four images of each animal were realigned to the mean using a 6-parameter spatial transformation. The realigned images were spatially normalized using the SPECT template image. Normalization consisted in a 12-parameter affine transformation, followed by a non-linear normalization in 16 iterations. Following normalization, tri-linear interpolation was performed with a final voxel size of 1 mm × 1 mm × 1 mm. Finally, the spatially normalized images were smoothed using a Gaussian smoothing kernel with the full width at half maximum (FWHM) set at 8 mm × mm 8 × 8 mm, and masked using a brain mask image to remove the extracerebral matter. The brain mask image was obtained by thresholding the MRI template, and smoothing this image with a Gaussian kernel filter with FWHM set at 8 mm × 8 mm × 8 mm.

Statistical brain imaging analysis

The regional ^{99m}Tc-HMPAO uptake was standardized to the mean global uptake using proportional scaling. The SPECT images acquired after oral and/or duodenal infusions were analyzed with SPM8 implemented in MATLAB 7.9 R2009B. Paired *t*-tests were performed to compare the effects of oral and/or duodenal sucrose infusions on brain metabolism. A total of three contrasts were analyzed: (1) oral and duodenal sucrose infusions vs. control (OS+DS+ vs. OS–DS–), (2) oral sucrose infusion vs. control (OS+DS– vs. OS–DS–) and, (3) duodenal sucrose infusion vs. control (OS–DS+ vs. OS–DS–). The contrast OS+DS− vs. OS−DS+ was not investigated because it was not possible to dissociate the site effect (oral vs. duodenal) from the concentration effect (respectively 5% and 16% sucrose). A small volume

correction (SVC) analysis based on regions of interest (ROIs) selected upon *a priori* hypotheses was performed. This analysis allowed for voxel to voxel comparisons within restricted ROIs corresponding to the cerebral regions in which differential activations were found in previous studies described in the introduction. With this analysis, we managed to identify within specific ROIs the voxels for which the activity was statistically different between treatments. The ROIs included the putamen, the caudate, the globus pallidus, the nucleus accumbens, the amygdala, the insular cortex, the anterior prefrontal cortex, the dorsolateral prefrontal cortex, the orbitofrontal cortex, the cingular cortex (anterior/posterior, dorsal/ventral), the hippocampus and parahippocampal cortex (**Figure 3**). An uncorrected value of $P = 0.01$ was set as the threshold for the clusters' peak. Clusters comprising a minimum of 25 contiguous voxels were considered significant.

The statistical analysis with SPM8 produced a listing of voxels that corresponded to peaks of maximum intensity for which the CBF differed between treatments in each ROI. Each voxel/peak was associated with a set of coordinates (x y z) corresponding to its spatial location in the CA–CP plane with CP set as the origin. The ROIs chosen for the SVC analysis were anatomically identified using a 3D-digitized pig brain atlas developed in our laboratory (Saikali et al., 2010), and representation of the clusters with different metabolism was performed using 3DSlicer (http://www.slicer.org/).

RESULTS

The results from the preliminary determination of the glucose plasma kinetics are illustrated in **Figure 2A**.

The regions of differential CBF values obtained with the SVC analyses on the three contrasts are summarized in **Table 1** and illustrated in **Figure 4**.

ORAL AND DUODENAL SUCROSE INFUSION (OS+DS+) VS. CONTROL (OS−DS−)

Paired oral and duodenal sucrose infusion compared with saline treatment induced peaks of significant activation in the basal

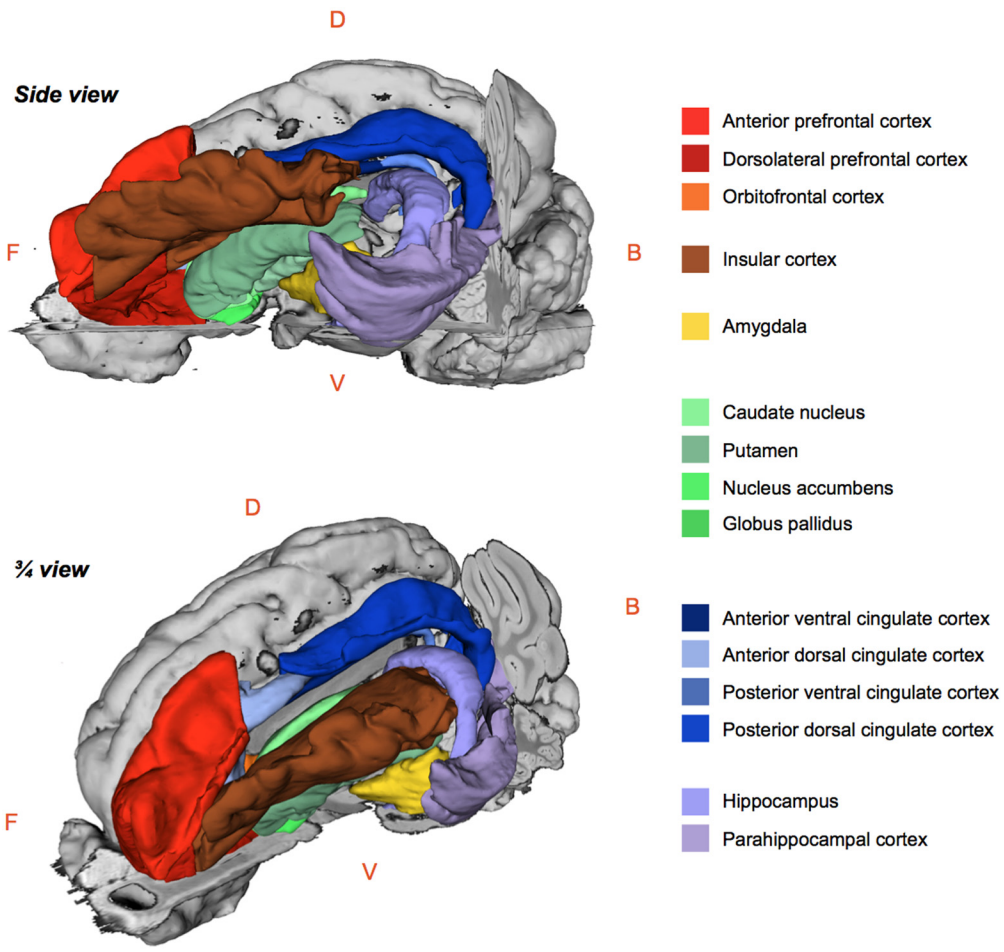

FIGURE 3 | Schematic 3D-representations of a pig's brain showing the whole right hemisphere (gray) and a skinned left hemisphere with the regions of interest selected for the brain metabolism small volume correction (SVC) analysis using statistical parametric mapping (SPM). Images were obtained with 3DSlicer (http://www.slicer.org/) and the 3D-digital pig brain atlas developed in our lab by Saikali et al. (2010). F, Front; B, Back; D, Dorsal; V, Ventral.

Table 1 | Summary of the brain activations (bold) and deactivations (italic) identified in the regions of interest selected for the small volume correction (SVC) analysis using the SPM (statistical parametric mapping) software ($t > 3.14$; $P < 0.01$ uncorrected) and for the following contrasts: OS+DS+ (oral and duodenal sucrose stimulation), OS+DS− (oral sucrose stimulation only), and OS−DS+ (duodenal sucrose stimulation only) respectively, vs. control.

		OS+DS+ vs. control		OS+DS− vs. control		OS–DS+ vs. control	
Structures	**Side**	**Peak *t***	***x,y,z* (mm)**	**Peak *t***	***x,y,z* (mm)**	**Peak *t***	***x,y,z* (mm)**
APFC	L	*4.95*	*−6 37 −1*	*4.82*	*−6 36 3*	*4.43*	*−4 33 −4*
APFC	R	*3.62*	*8 42 4*	*3.40*	*4 43 5*		
DLPFC	L	*3.17*	*−10 40 0*	*3.81*	*−2 40 5*		
DLPFC	R	*3.61*	*8 40 3*	*3.84*	*0 41 5*		
				4.22	**6 33 14**		
OFC	L						
OFC	R						
VACC	L	**3.78**	**−2 4 15**			**5.49**	**0 11 13**
VACC	R	**5.25**	**2 5 14**			**6.74**	**2 9 13**
DACC	L			*3.83*	*−2 39 5*	**4.57**	**0 11 14**
DACC	R			*3.43*	*0 40 4*	**4.69**	**0 11 14**
VPCC	L						
VPCC	R						
DPCC	L			**5.90**	**−2 −12 14**		
DPCC	R			**12.98**	**2 −12 14**		
IC	L	*4.28*	*−20 10 11*	*5.08*	*−18 11 10*		
IC	R	**3.60**	**14 17 10**				
HIP	L			**3.57**	**−14 4 −6**		
HIP	R	**3.95**	**6 −1 11**			**10.75**	**4 −1 11**
PHC	L			*4.09*	*−12 −9 0*		
				4.90	**−16 4 −6**		
PHC	R	*4.92*	*22 8 5*	*5.99*	*22 10 3*	*4.68*	*22 4 1*
				4.05	**2 −14 14**		
AMY	L						
AMY	R						
CAU	L			*3.18*	*−8 18 8*		
CAU	R	**4.47**	**8 7 10**	**3.95**	**6 8 10**	**6.97**	**6 18 8**
PUT	L			*4.33*	*−12 16 7*		
PUT	R	**4.36**	**12 15 9**			**8.97**	**10 19 8**
NAcc	L						
NAcc	R						
GP	L						
GP	R						

APFC, anterior prefrontal cortex; DLPFC, dorsolateral prefrontal cortex; OFC, orbitofrontal cortex; VACC, ventral anterior cingulate cortex; DACC, dorsal anterior cingulate cortex; VPCC, ventral posterior cingulate cortex; DPCC, dorsal posterior cingulate cortex; IC, insular cortex; HIP, hippocampus; PHC, parahippocampal cortex; AMY, amygdala; CAU, caudate nucleus; PUT, putamen; NAcc, nucleus accumbens; GP, globus pallidus. The peak t-value and coordinates in the CA–CP (commissura anterior–commissura posterior) reference plane are indicated for each significant cluster, for the left (L) and right (R) hemispheres.

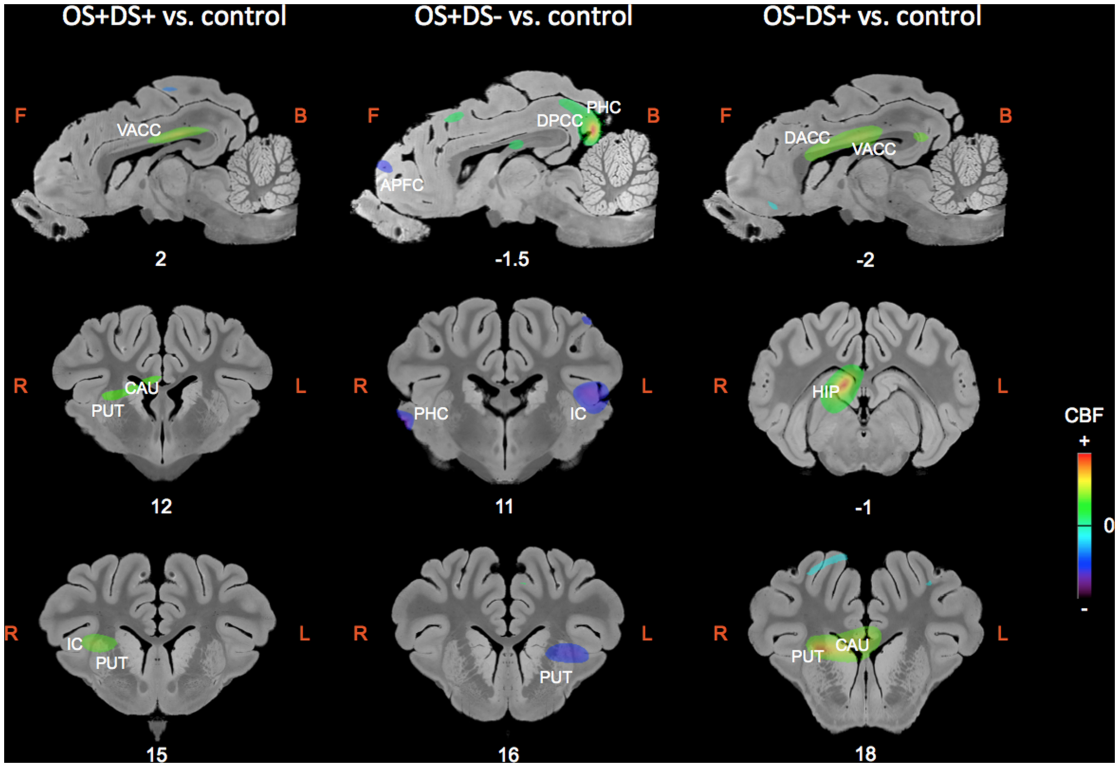

FIGURE 4 | Sagittal and coronal MRI sections showing clusters of differential cerebral blood flow identified during the small volume correction (SVC) analyses in different regions of interest chosen upon *a priori* hypotheses for the three following contrasts: OS+DS+ (oral and duodenal sucrose stimulation) vs. control, OS+DS− (oral sucrose stimulation) vs. control, and OS−DS+ (duodenal sucrose stimulation) vs. control. The *x* or *y* coordinates in the CA–CP (*commissura anterior–commissura posterior*) plane are indicated below the images. The threshold for significance was set at $P < 0.01$ (uncorrected). Positive cerebral blood flow (CBF) values indicate a brain metabolism activation and negative CBF values indicate a brain metabolism deactivation compared to control. L, left; R, right; APFC, anterior prefrontal cortex; CAU, caudate nucleus; PUT, putamen; HIP, hippocampus; IC, insular cortex; DACC, dorsal anterior cingulate cortex; VACC, ventral anterior cingulate cortex; PHC, parahippocampal cortex.

nuclei and especially in the right putamen ($t = 4.36$, $P_{unc} = 0.002$, 12 15 9) and right caudate ($t = 4.47$, $P_{unc} = 0.002$, 8 7 10). Peaks of significant activation were also found bilaterally in the ventral anterior cingular cortex (L: $t = 3.78$, $P_{unc} = 0.005$, −2 4 15; R: $t = 5.25$, $P_{unc} = 0.001$, 2 5 14), the right insular cortex ($t = 3.60$, $P_{unc} = 0.006$, 14 17 10), and right hippocampus ($t = 9.51$, $P_{unc} < 0.001$, 6 −1 11). The paired oral and duodenal sucrose infusion compared with saline treatment also induced peaks of significant deactivation bilaterally in the anterior prefrontal cortex (L: $t = 4.95$, $P_{unc} = 0.001$, −6 37 −1; R: $t = 3.62$, $P_{unc} = 0.006$, 8 42 4) and dorsolateral prefrontal cortex (L: $t = 3.17$, $P_{unc} = 0.01$, −10 40 0; R: $t = 3.61$, $P_{unc} = 0.006$, 8 40 3). This contrast was also associated with deactivated clusters in the left insular cortex ($t = 4.28$, $P_{unc} = 0.003$, −20 10 11) and the right parahippocampal cortex ($t = 4.92$, $P_{unc} = 0.001$, 22 8 5).

ORAL SUCROSE INFUSION (OS+DS−) VS. CONTROL (OS−DS−)

The oral sucrose infusion compared with control treatment induced activated clusters of which the peaks were in the right caudate ($t = 3.95$, $P_{unc} = 0.004$, 6 8 10), right dorsolateral prefrontal cortex ($t = 4.22$, $P_{unc} = 0.003$, 6 33 14), left hippocampus ($t = 3.57$, $P_{unc} = 0.004$, −14 4 −6−), dorsal posterior cingulate cortex (L: $t = 5.90$, $P_{unc} = 0.001$, −2 −12 14; R: $t = 12.98$, $P_{unc} < 0.001$, 2 −12 14) and bilateral parahippocampal cortex (L: $t = 4.90$, $P_{unc} = 0.001$, −16 4 −6; R: $t = 4.05$, $P_{unc} = 0.003$, 2 −14 14). Oral sucrose infusion compared with control also induced peaks of significant deactivation in the left putamen ($t = 4.33$, $P_{unc} = 0.002$, −12 16 7), left caudate ($t = 3.18$, $P_{unc} = 0.010$, −8 18 8), left insular cortex ($t = 5.08$, $P_{unc} = 0.001$, −18 11 10), and bilaterally in the dorsal anterior cingular cortex (L: $t = 3.83$, $P_{unc} = 0.004$, −2 39 5; R: $t = 3.43$, $P_{unc} = 0.007$, 0 40 4), anterior prefrontal cortex (L: $t = 4.82$, $P_{unc} = 0.001$, −6 36 3; R: $t = 3.40$, $P_{unc} = 0.007$, 4 43 5), dorsolateral prefrontal cortex (L: $t = 3.81$, $P_{unc} = 0.004$, −2 40 5; R: $t = 3.84$, $P_{unc} = 0.004$, 0 41 5), and parahippocampal cortex (L: $t = 4.09$, $P_{unc} = 0.003$, −12 −9 0; R: $t = 5.99$, $P_{unc} < 0.001$, 22 10 3).

DUODENAL SUCROSE INFUSION (OS−DS+) VS. CONTROL (OS−DS−)

Duodenal sucrose infusion compared with saline infusion induced clusters of significant activation with peaks in the right basal nuclei, including the caudate ($t = 6.97$, $P_{unc} < 0.001$, 6 18 8) and the putamen ($t = 8.97$, $P_{unc} < 0.001$, 10 19 8). Peaks of significant activations were also found bilaterally in the ventral (L: $t = 5.49$, $P_{unc} = 0.001$, 0 11 13; R: $t = 6.74$, $P_{unc} < 0.001$, 2 9 13) and

dorsal anterior cingular cortex (L: $t = 4.57$, $P_{unc} = 0.002$, 0 11 14; R: $t = 4.69$, $P_{unc} = 0.002$, 0 11 14), as well as in the right hippocampus ($t = 10.75$, $P_{unc} < 0.001$, 4 −1 11). The contrast was also characterized by deactivated clusters of which the peaks were in the left anterior prefrontal cortex ($t = 4.43$, $P_{unc} = 0.002$, −4 33 −4) and right parahippocampal cortex ($t = 4.68$, $P_{unc} = 0.002$, 22 4 1).

DISCUSSION

The major findings of our study were that oral and/or duodenal sucrose sensing induced CBF changes in brain regions known to be involved in memory, reward processes and hedonic evaluation of sensory stimuli, and that combined (or congruent) oral and duodenal sucrose perception processing differed from that of dissociated oral and duodenal stimulations. Moreover, our results showed that sucrose duodenal stimulation only, which increased blood glucose levels, significantly activated the anterior cingulate cortex, right putamen and hippocampus, similarly to the congruent bimodal sucrose stimulation and contrary to the oral sucrose stimulation only, suggesting that visceral signals can modulate hedonic processes without oral perception. Some brain deactivations in the prefrontal cortex and insular cortex were only detected in the presence of oral sucrose stimulation.

The modulation of the hedonic circuit showed in our study is in accordance with previous work in both fasted and sated humans that revealed metabolism changes in these regions further to sugar ingestion (glucose, Chambers et al., 2009) or during oral taste stimulation paired with caloric load (sucrose, Haase et al., 2009, 2011; Smeets et al., 2011). It is also consistent with previous studies in humans or animal models that highlighted differential brain responses in the aforementioned structures further to separated oral or visceral sugar sensing. In pigs, duodenal glucose infusions in the absence of oral glucose sensing induced differential CBF responses compared to a control stimulation in the prepiriform area, the anterior entorhinal cortex, the orbitofrontal, dorsolateral and anterior prefrontal cortices, the hippocampus and the dorsal striatum (Boubaker et al., 2012). Compared to a control situation, oral sucrose sensing triggered differential activation in the insula and anterior cingulate cortex, the nucleus accumbens and the caudate of sated women (Frank et al., 2008). In fasted humans, ingestion of maltodextrin, a caloric non-sweet compound, triggered activation in the insular, the orbitofrontal and dorsolateral prefrontal cortices and the caudate (Chambers et al., 2009).

The originality and novelty of our work rests upon the comparison of the oral, duodenal and combined stimulations with the same sugar (sucrose) in a controlled experimental model, demonstrating that gustatory and visceral signals can independently or synergistically modulate the hedonic circuit. Some modulations were common to the three contrasts studied (oral/duodenal sucrose vs. control, oral sucrose vs. control, and duodenal sucrose vs. control), such as activation of the right caudate nucleus, or deactivation of the left anterior prefrontal cortex and right parahippocampal cortex. Some other modulations were detected only when duodenal sucrose stimulation was performed, such as the activation of the ventral anterior cingulate cortex, right putamen and right hippocampus. Conversely, some other modulations were detected only when oral sucrose stimulation was performed, such as the deactivation of the dorsolateral prefrontal cortex and left insular cortex. Finally, there was a synergetic effect of combined oral and duodenal stimulation with a specific activation of the right insular cortex.

The dorsal striatum (caudate and putamen) and the prefrontal cortex are both involved in reward processing (Schultz, 2000). The prefrontal cortex, which closely interacts with the striatum, is also known to be involved in the processing of food-related stimulations (Ramnani and Owen, 2004) and to participate in motivation, memory and cognitive functions in humans (Cardinal et al., 2002; Kouneiher et al., 2009). Simons et al. (2005) reported that the anterior prefrontal cortex might participate in memory processes, and more specifically in "the recollection of context detail," that is in the association between a past event (e.g., food intake) and the contextual information relating to that event (e.g., internal state, subjective feelings, etc.). In the present study, changes in the prefrontal cortex metabolism might be due to the retrieval of contextual information arising from the preliminary exposure to sucrose in awake animals, or simply to sucrose sensing and pleasantness. The anterior prefrontal cortex also shares connections with the dorsolateral prefrontal cortex, which is known to play a major role in the regulation of food intake, notably through its inhibitory inputs to the orexigenic network (Gautier et al., 2000; Del Parigi et al., 2002; Le et al., 2006). Consequently, the perception of the sweet taste of sucrose in the mouth might stimulate appetite through the deactivation of the dorsolateral prefrontal cortex. Further behavioral studies in the pig model or human are necessary to confirm this hypothesis, but it is already well known that sweet taste increases food palatability and appetence in pigs and humans (Kampov-Polevoy et al., 2006; Clouard et al., 2012b).

The activation of the putamen was only visible when duodenal sucrose infusion was performed (with or without concomitant oral sucrose), suggesting that sugar ingestion rather than sweet taste in itself is probably responsible for the significant activation of the putamen further intake of high-sugar beverages in humans (Stice et al., 2013). The fact that oral perception of sugar, without calorie ingestion or duodenal sugar sensing, activates the dorsal striatum differently from the other treatments raises the question of the congruence between oral and visceral sweet perceptions. It is well known that non-caloric sweeteners activate the brain reward circuit differently from caloric sweeteners like sucrose, and that chronic consumption of sweeteners can alter the brain processing of sweet taste (Green and Murphy, 2012). Frank et al. (2008) demonstrated that sucrose elicits a stronger brain response in the anterior insula and striatum compared to sucralose, a non-caloric sweetener. Moreover, only sucrose engaged dopaminergic midbrain areas in relation to the behavioral pleasantness response, and the taste pleasantness predicted left insula response (Frank et al., 2008). This is consistent with our own data that showed (i) a consistent activation of the dorsal striatum only when sucrose sensing was accompanied by a caloric intake (and elevated plasmatic glycemia), and (ii) metabolism changes in the left insular cortex only when sucrose was perceived orally. In a nutshell, these results suggest that even if sweet taste only might induce pleasantness, its combination with a congruent visceral signal (caloric load and elevated glucose plasma levels) is necessary to trigger the activation of anterior cingulate cortex and putamen. This could

have important implications on how effective non-caloric sweeteners are in their ability to substitute sugar intake. Their impact on appetite, food intake and motivation should be explored more precisely, both in humans and animals models.

The absence of brain metabolism differences between treatments in the nucleus accumbens is quite surprising though. The nucleus accumbens is a well-known hotspot for food liking and wanting (Berridge, 2009), and functional anomalies of this nucleus, that are characteristic of drug-addicted patients, were described in obese humans (Wang et al., 2001; Volkow et al., 2008) as well as in minipigs fed a high-fat and high-sugar diet (Val-Laillet et al., 2011). Avena (2007) and Avena et al. (2008) consistently and elegantly demonstrated the neurochemical addictive-like responses to sugar in rodents, and especially the patterns of dopamine release in the nucleus accumbens induced by intermittent and excessive sugar intake. Interestingly, Avena et al. (2006) also demonstrated *via* a sucrose sham feeding procedure that dopamine is repeatedly released in the nucleus accumbens in response to sweet taste, as previously showed by Mark et al. (1991) with saccharin, and that acetylcholine is released while drinking sugar in real-feeding rats, but not in sham-feeding rats. The fact that dopamine/opioid stimulation of nucleus accumbens can amplify the reactivity of mesocorticolimbic circuits and consequently magnify incentive salience of cues associated with sucrose reward might explain the brain functional anomalies and increased "wanting" observed further chronic sugar intake. Of course, the absence of CBF differences in the nucleus accumbens in our animals does not preclude the possibility of differences in term of neurotransmitters metabolism and activity, that could be investigated *via* PET in pigs (Alstrup and Smith, 2012). All these results, including ours, suggest that the ventral and dorsal striatum have specific roles in integrating taste and metabolic signals related to sugar consumption, and that further functional and molecular brain imaging studies in rodent and pig models should help disentangling their respective roles in the onset of addictive-like behavior and deregulation of food intake control further to chronic overconsumption of sugars.

The hippocampus and parahippocampal cortex are known to be involved in the integration of subjective internal states with relevant sensory cues (LaBar et al., 2001). It is interesting to notice that, if no difference of metabolism was observed in these regions between the two contrasts including duodenal sucrose stimulation, the perception of oral sucrose only induced many specific changes of metabolism. The same applies to the dorsal posterior cingulate cortex. These findings support the idea that the hippocampus and parahippocampal cortex might contribute to the integration of congruent/incongruent nutrient sensing, as suggested by Haase et al. (2009), i.e., in our study, a sweet taste in the mouth but with no calorie and no sucrose receptors activation in the intestine. In rodents, the perirhinal cortex, which is part of the parahippocampal cortex, sends sensory information to the hippocampus (Furtak et al., 2007; Kerr et al., 2007). The hippocampus supports memory and cognitive functions, while participating in emotional processes (for review, see Fanselow and Dong, 2010). Consequently, the CBF changes observed in the hippocampus and parahippocampal cortex showed that brain regions involved in memory and emotion mediated the processing of oral and/or duodenal sucrose sensing differently. The fact that the metabolism changes in the hippocampus and parahippocampal cortex were similar between combined oral/duodenal sucrose and duodenal sucrose, but different from the activation/deactivation pattern induced by oral sucrose only, comforts the idea that these two structures are probably important to recognize the congruence of a sweet oral stimulation according to the internal state, or that recall processes related to sucrose sensing might be independent of duodenal signals. In a further study, it would be interesting to homogenize the sucrose concentrations used for oral and intestinal stimulations because in the present work, the sucrose solution infused in the duodenum was more concentrated than that infused in the mouth. This methodological limitation prevented us to compare oral sucrose only vs. duodenal sucrose only, and we cannot exclude that some brain metabolism changes observed with the combined stimulation are perhaps due to the perception of incongruence in terms of solutions strength.

The large overlapping of brain metabolism between the two conditions involving a visceral stimulation are probably partly related to the internal state of the subjects, since brain imaging was performed when glucose plasma levels (that mediate early satiation) were at a maximum. Numerous studies reported that brain activation is modified by the internal state of the subjects (hunger or satiety) at the time of imaging. Using PET, Tataranni et al. (1999) found an increased CBF in regions involved in the homeostatic regulation of food intake (e.g., hypothalamus), in taste recognition and hedonic evaluation (e.g., insula and orbitofrontal cortex), in emotion and memory processing (e.g., parahippocampal and anterior cingulate cortices, caudate, putamen and hippocampus) in a hunger state compared to a situation where the subjects had ingested a caloric meal before imaging (satiety state). Using fMRI, Haase et al. (2011) reported that, in hungry and sated humans, sucrose ingestion induced changes in activation in the cingulate, orbitofrontal and parahippocampal cortices, the amygdala, the insula and the hippocampus. Haase et al. (2009, 2011) also reported that there were significantly greater responses in the hunger than in the satiety condition during sucrose stimulation in taste regions (the insula and the orbitofrontal cortex), in regions that participate in emotion processing (the amygdala, the caudate and the cingulate cortex) and memory (the hippocampus and the parahippocampal cortex). Altogether, these findings suggest that internal state plays a major role on brain activation during food-related stimulations.

CONCLUSION AND PERSPECTIVES

In conclusion, we demonstrated that oral, duodenal and the bimodal perception of sucrose induced different patterns of brain metabolism in structures involved in memory, reward processes and hedonic evaluation of sensory stimuli. Using controlled conditions in a pertinent animal model for human nutrition and nutrient sensing, we managed to demonstrate that some brain metabolism changes are specific to oral sensing or duodenal sensing, respectively, and that bimodal sucrose stimulation can even have a synergetic effect in some brain areas such as the insular cortex. We identified brain areas that are probably involved in the congruence between the sweet perception and internal

state. All these results have important implications for discussions related to caloric vs. non-caloric sweeteners consumption and impact of sugars on the brain hedonic circuit and motivational processes.

To disentangle the respective roles of oral and post-oral signals in reward-related responses and their anomalies, further work would benefit from combining different brain imaging modalities to investigate brain activity (assessed *via* CBF or glucose consumption) in conjunction with molecular explorations (e.g., brain dopamine and opioids). Also, because sugar consumption is frequently associated with fat in western diets, it is important to examine the relative role of sugar and fat-food contents in the activation of brain reward regions, as well as their possible interaction/synergy in promoting brain anomalies and at-risk eating behaviors. From a recent study performed in humans (Stice et al., 2013), it appears that sugar more effectively recruits reward and gustatory brain regions than fat, which justifies conducting controlled studies with brain imaging in animal models to confirm these findings and explore the underlying mechanisms.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: David Val-Laillet, Marie-Christine Meunier-Salaün and Caroline Clouard. Performed the experiments: Caroline Clouard. Analyzed the data: Caroline Clouard, Paul Meurice and David Val-Laillet. Contributed reagents/materials/analysis tools: Charles-Henri Malbert and Paul Meurice. Wrote the paper: Caroline Clouard and David Val-Laillet. Revised the paper before submission: David Val-Laillet, Marie-Christine Meunier-Salaün, Charles-Henri Malbert and Paul Meurice.

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Mood, food, and obesity

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Food is a potent natural reward and food intake is a complex process. Reward and gratification associated with food consumption leads to dopamine (DA) production, which in turn activates reward and pleasure centers in the brain. An individual will repeatedly eat a particular food to experience this positive feeling of gratification. This type of repetitive behavior of food intake leads to the activation of brain reward pathways that eventually overrides other signals of satiety and hunger. Thus, a gratification habit through a favorable food leads to overeating and morbid obesity. Overeating and obesity stems from many biological factors engaging both central and peripheral systems in a bi-directional manner involving mood and emotions. Emotional eating and altered mood can also lead to altered food choice and intake leading to overeating and obesity. Research findings from human and animal studies support a two-way link between three concepts, mood, food, and obesity. The focus of this article is to provide an overview of complex nature of food intake where various biological factors link mood, food intake, and brain signaling that engages both peripheral and central nervous system signaling pathways in a bi-directional manner in obesity.

Keywords: mood, depression, anxiety, food, obesity

INTRODUCTION

It is hypothesized that individuals engage in a variety of behaviors to regulate their mood (Morris and Reilly, 1987). Important among mood regulating behaviors is food consumption. The interaction between mood, emotional state, and feeding behaviors is complex and it is hypothesized that individuals regulate their emotions and mood by changing both food choices and quantities. It is also apparent that mood can affect the self-rewarding mechanisms of food consumption (Morris and Reilly, 1987). Specific types of food tend to be preferred under certain psychological conditions due to the influence of foods on the activity of brain reward centers (**Figure 1**) (Rangel, 2013; Jauch-Chara and Oltmanns, 2014; Weltens et al., 2014). Positive feedback loops can result in enhancement of appetite leading to obesity. Interestingly, highly palatable foods activate the same brain regions of reward and pleasure that are active in drug addiction (Volkow et al., 2012), suggesting a neuronal mechanism of food addiction leading to overeating and obesity (Davis et al., 2011, 2014; Dileone et al., 2012; Volkow et al., 2012; Dagher, 2013; Davis, 2013; Ziauddeen and Fletcher, 2013; Pai et al., 2014; Potenza, 2014). Dopamine, which directly activates reward and pleasure centers, affects both mood and food intake (Cantello et al., 1989; Diehl and Gershon, 1992; Fochtmann and Fink, 1992; Black et al., 2002; Cawley et al., 2013), further supporting the link between psychology and eating behaviors.

Mood disorders are often found in association with abnormal feeding behaviors. For example, depression and anxiety are comorbidities of obesity (Novick et al., 2005; Simon et al., 2006; Kloiber et al., 2007). Impairment in central nervous system (CNS) function has been linked to obesity that in turn impacts mental and physical health (Allison et al., 2009; Talen and Mann, 2009; Duarte et al., 2010). Obese individuals are at increased risk of developing depression (25, 26), and this risk is doubled in the presence of diabetes (Anderson et al., 2001; De Groot et al., 2001; Labad et al., 2010). Depressed mood is also associated with abdominal obesity and poor diet (Roberts et al., 2003; Dong et al., 2004; Simon et al., 2006; Luppino et al., 2010; Zhao et al., 2011; Hamer et al., 2012). A link between obesity and depression has been found in animal models of mood disorders (Lombard, 2000; Pawels and Volterrani, 2008; Dallman et al., 2003, 2005; Singh et al., 2007, 2009, 2011; Dallman, 2010; Chuang et al., 2011; Diz-Chaves, 2011; Maniam and Morris, 2012; Spence and Courbasson, 2012; Akubuiro et al., 2013; Kumar et al., 2013), suggesting that a common signaling pathway may underlie these phenotypes in both humans and animals.

There are numerous articles on the regulation of food intake, obesity, and mood. However, further exploration of the interaction among mood, food, and obesity is much needed. The aim of this review article is to highlight the complex interplay among mood, emotional state, and eating behaviors that influence body weight. This review provides an overview of known biological factors and foods that influence appetite and mood *via* brain signaling pathways. Specifically discussed are the foods and biological factors, which override the normal physiological requirements of appetite regulation, and how these factors influence in a bi-directional manner emotion, food, food intake, and obesity (**Figure 1**).

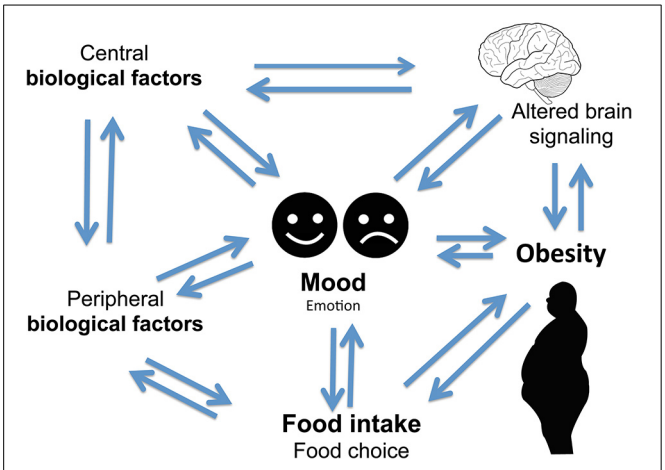

FIGURE 1 | Complex two-way relationship linking food intake, mood, and obesity.

CENTRAL NERVOUS SYSTEM IN REGULATION OF MOOD, FOOD, AND OBESITY

BI-DIRECTIONAL LINK OF FOOD AND EMOTION

In humans, eating behavior is complex and is affected by both mood and emotions (Lyman, 1982; Mehrabian, 1995; Macht, 1999; Macht and Simons, 2000). However, mood and emotions are distinct. Mood is characterized by psychological arousal in the absence of obvious stimuli that can last for several minutes or longer. In contrast, emotions are short-term affective response to reinforcing stimuli. Of all emotions, a study shows that frequent emotions such as, anger and joy have the strongest influence on appetite and food choice (Macht, 1999). Behavior based findings from human studies of questionnaires, field, and clinical studies suggest an integrative five way model that predicts five different aspects of emotional eating. These five aspects include: food choice, food intake, loss of cognitive controls, food modulating emotions, and emotion-congruent modulating eating, see review by Macht (2008). Therefore, depending on the state of negative emotions or distress, emotional eating is triggered where food intake can either increase or decrease within the same individuals (Ouwens et al., 2009). Emotional state has also been connected with addiction (Parylak et al., 2011). Sensory and psychological pathways influence food choice, the quantity, and meal frequency that may not be a part of normal physiological requirement. Many psychosomatic theories of obesity suggests that obese people overeat due to inability to perceive their physiological state, hunger, and satiety and that overeating reduce emotional discomfort and anxiety (Kaplan and Kaplan, 1957; Schachter, 1968; Bruch, 1985). The internal/external theory of obesity predicts that normal eaters alter their food intake to regulate their emotion, while obese people do not (Schachter, 1968; Canetti et al., 2002). Depending on whether an eater is restrained or emotional, stress and negative emotions could be associated with both increased and decreased motivation to eat; and under those circumstances, food choice differs (Herman and Mack, 1975). Thus, emotional distress influences emotional food choice and intake.

STRESS AND FOOD INTAKE

There is a close interaction between food, mood, and stress (Benton and Donohoe, 1999; Oliver and Wardle, 1999; Gibson, 2006; Dallman, 2010; Bast and Berry, 2014). Stress can affect feeding behavior (Greeno and Wing, 1994; Yau and Potenza, 2013), resulting in either increased or reduced food intake depending on the types of external or psychological stressors (Oliver and Wardle, 1999; Gibson, 2006; Dallman, 2010; Yau and Potenza, 2013). Similarly, chronic stress can lead to either increased consumption of palatable and rewarding foods leading to obesity or a diminished appetite leading to weight loss (Cartwright et al., 2003; Adam and Epel, 2007; Tryon et al., 2013). Furthermore, following exposure to a stressor, studies show that intake of palatable foods reduce signs of stress and anxiety (Pecoraro et al., 2004; La Fleur et al., 2005; Maniam and Morris, 2010, 2012; Ulrich-Lai et al., 2010; Finger et al., 2011, 2012). Interestingly, stress-induced preference for palatable food is often seen in humans (Souquet and Rowland, 1989; Epel et al., 2004; Pecoraro et al., 2004; Christiansen et al., 2011; Gibson, 2012; Merali et al., 2013; Sharma et al., 2013; Sharma and Fulton, 2013; Meye and Adan, 2014; Park et al., 2014; Rho et al., 2014). Notably, this behavior is extended to animals (Dallman et al., 2003, 2005; Cottone et al., 2009). This suggests that a common neurobiological pathway maybe involved in food choice and patterns of eating behavior during stress.

MOOD AND FOOD INTAKE

Mood states such as anxiety and depression affect food choice and energy metabolism. Overeating and obesity is often associated with depression and anxiety in humans which has also been reported in animal models (Novick et al., 2005; Simon and Von Korff, 2006; Kloiber et al., 2007; Singh et al., 2007, 2009; Akubuiro et al., 2013; Patterson and Abizaid, 2013; Sharma and Fulton, 2013). Both endocrine and metabolic conditions are exacerbated in major depression (Mcelroy et al., 2004; Simon et al., 2006; De Wit et al., 2010; Luppino et al., 2010; Marijnissen et al., 2011). Individuals experiencing depressed moods show preference for and consume palatable "comfort foods" as a mean to alleviate their negative feelings (Macht, 2008). Although on a short-term basis, palatable foods can provide some relief from negative emotions and mood states, chronic consumption of calorically-rich foods ultimately leads to obesity which in turn promotes vulnerability to depression and anxiety (Novick et al., 2005; Simon et al., 2006; Kloiber et al., 2007; Sharma and Fulton, 2013). Conversely, there are findings showing that prolonged high-fat feeding leads to negative emotional states, increased stress sensitivity, and altered basal corticosterone levels (Sharma et al., 2012). Thus, negative emotion impacts food choice and intake that in turns affects mood in a bi-directional manner.

Interestingly, other behaviors of reduced pleasure/reward experience, anxiety-like behavior, and heightened stress-induced hypothalamic pituitary adrenal axis (HPA) activation have been found in mice. Furthermore, after exposure to chronic high-fat diet and then switching to normal chow diet, mice showed craving for sucrose, high-fat foods, and displayed enhanced anxiety-like behavior (Sharma et al., 2012). Similar findings of increased behavioral and physiological signs of depression and anxiety have been reported in humans when switched from a high-fat sugar

diet to regular diet (Avena et al., 2008; Teegarden and Bale, 2008; Cottone et al., 2009; Pickering et al., 2009; Iemolo et al., 2012; Sharma et al., 2012; Blasio et al., 2013). All together, these findings suggest that chronic high-fat feeding promotes negative emotional states and potentiates condition for enhanced sensitivity to stress that leads to continuous repetitive cycles of overeating, weight gain, and depressed mood.

FOOD PREFERENCE AND MOOD

Hippocrates, father of modern medicine, said: "Let your food be your medicine, and your medicine be your food" (Prasad, 1998). Research from human trials and animal studies have shown that foods directly influence brain neurotransmitter systems which in turn has effects on mood and performance by altering the brain structure, chemistry, and physiology. Mood can also influence our food choices and expectations on the effects of certain foods can influence our sapiens. Some of those foods impacting mood are discussed below and summarized in **Table 1** (Spring et al., 1982-1983; Rogers and Lloyd, 1994).

Chocolate has a strong effect on mood, generally increasing pleasant feelings and reducing tension (Osman and Sobal, 2006; Parker et al., 2006b; Cartwright et al., 2007; Fletcher et al., 2007). Chocolate contains psychoactive chemicals such as andamines that stimulate the brain and result in good mood (Ottley, 2000). However, negative feelings are also associated with chocolate in some women on weight loss regimes who experience guilt after eating chocolate. The unique taste and feel from chocolate in the mouth leads to chocolate craving due to sensory factors associated with chocolate eating (Macht and Dettmer, 2006; Osman and Sobal, 2006; Parker et al., 2006b; Cartwright et al., 2007; Fletcher et al., 2007).

Caffeine, mostly consumed in the form of coffee and tea, not only has stimulant effects on enhancing alertness, vigilance, and reaction time but also increases anxiety in susceptible individuals (Acquas et al., 2002; Rossi et al., 2010). Caffeine blocks adenosine receptors in the brain and can relieve headaches, drowsiness, and fatigue. Short-term caffeine deprivation in regular users results in withdrawal symptoms (Rogers, 1995).

Omega-3 fatty acids, found in various foods can influence, mood, behavior, neuroticism, and impulse control (Van Strater and Bouvy, 2006; Conklin et al., 2007; Stahl et al., 2008). Omega-3 fatty acids play a role in major depressive disorder, bipolar disorder, schizophrenia, substance abuse, and attention deficit disorder (Young and Martin, 2003; Parker et al., 2006a; Van Strater and Bouvy, 2006; Stahl et al., 2008). Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), both members of the omega-3 fatty acid family, contribute to the fluidity of the cell membrane, and thereby play an important role in brain development and function (Pawels and Volterrani, 2008). Low blood levels of polyunsaturated omega-3 fatty acids are associated with depression, implying a role in mood disorders (Lombard, 2000; Sanchez-Villegas et al., 2007; Antypa et al., 2012; Moranis et al., 2012; Kang and Gleason, 2013; Grosso et al., 2014).

Micronutrients, such as thiamine (vitamin B1), iron, and folic acid, play a role in emotion. Thiamine containing foods influence mood states (Benton et al., 1995). Improved thiamine status increases well-being, sociability, and overall energy levels. Insufficient amounts of thiamine are associated with impaired mood and cognitive functioning (Benton et al., 1997; Benton and Donohoe, 1999).

Iron deficiency represents one of the most common nutritional problems worldwide. Iron deficiency anemia can result in depressed mood, and problems with attention and lethargy (Benton and Donohoe, 1999).

Folic acid plays an important role in the brain. Folic acid deficiency is associated with depressed mood (Coppen and Bolander-Gouaille, 2005; Young, 2007). Psychiatric patients often run the risk of developing folic acid deficiency due to loss of appetite from anticonvulsant drugs that inhibit folic acid absorption (Ottley, 2000). Collectively, these findings suggest foods influence mood.

Mood can influence food preference (Christensen and Brooks, 2006). Choice of eating palatable foods can either lead to comfort feeling or disgust. A good example of behavioral change that is observed after taking a meal is altered mood. A general effect of meal on behavior is observed from animals to humans where hunger leads to irritability and meal intake leads to arousal and alertness. Thus, a search for food is cultivated. Once satiety sets in, sedentary and calm behaviors most likely have positive rather than negative effect on mood (Macht and Simons, 2000; Macht et al., 2003; Macht and Dettmer, 2006; Macht, 2008). A potential internal information route on emotional behavior was first recognized in 2001 where nutrients from gut were relayed to the brain by the vagus nerve affecting emotions (Zagon, 2001). However, the relationship of emotions, physiological arousal, and mood in a given situation is significantly dependent upon on the subject's motivational state (Reid and Hammersley, 1999) and the individual's personality trait of neuroticism that interacts with mood and response to emotional stimuli (Dess and Edelheit, 1998).

The pathogenesis of both mood and metabolic disorders during obesity can be triggered by certain diets (Wallin and Rissanen, 1994; Sanchez-Villegas and Martinez-Gonzalez, 2013). Diets like Western diets that are rich in saturated fat and low in polyunsaturated and mono-unsaturated fatty acids tend to increase the incidences of depression (Peet et al., 1998). On the other hand, diet like the Mediterranean diet appears to reduce depression (Sanchez-Villegas and Martinez-Gonzalez, 2013; Sanchez-Villegas et al., 2013). Furthermore, many reports show the increased incidence of depression on diets that lack omega-3 polyunsaturated fatty acids (PUFA) and that depression is reduced when intake of PUFA is increased in both 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). Besides mood changes, high fat diets promote increased weight gain, 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 and Salas-Salvado, 2013; Nazare et al., 2013). The accumulation of adipose tissue in abdominal stores leads to several complications of obesity including insulin resistance leading to metabolic syndrome (Despres et al., 2006; Tchernof and Despres, 2013). These changes also lead to neurobiological impairments affecting mood disorders such as depression and anxiety (Weber-Hamann et al., 2002; Van Reedt Dortland et al., 2013a,b). It is believed that increased circulating plasma fatty acids such as

Table 1 | Summary of biological factors and food influencing mood, emotions, food intake, and brain signaling pathways.

Foods and biological factors	Influence on mood, emotion, food intake, and brain signaling pathways	References
Chocolate	Increases pleasant feeling, reduce tension, and results in good mood via serotonin and cannabinoid receptors signaling	Ottley, 2000; Osman and Sobal, 2006; Parker et al., 2006b; Cartwright et al., 2007; Fletcher et al., 2007
Caffeine	Enhances alertness and increases anxiety and results in withdrawal symptoms in some individuals *via* cannabinoid CB1 receptor signaling pathway	Rogers, 1995; Acquas et al., 2002; Rossi et al., 2010
Omega-3 fatty acids	Influences neuroticism, mood, behavior, and plays a role in mood disorders. Omega-3 fatty acids in receptor functioning, neurotransmitters levels, and monoamine metabolism are all implicated in depression (see review Parker et al., 2006a)	Lombard, 2000; Young and Martin, 2003; Parker et al., 2006a; Van Strater and Bouvy, 2006; Conklin et al., 2007; Sanchez-Villegas et al., 2007; Stahl et al., 2008; Antypa et al., 2012; Moranis et al., 2012; Kang and Gleason, 2013; Grosso et al., 2014
Micronutrients	Thiamine plays a role in emotion, mood states, and cognitive functioning. The pathway is unknown	Benton et al., 1995, 1997; Benton and Donohoe, 1999
Iron	Iron deficiency results in depressed mood and lethargy. The pathway is unknown	Benton and Donohoe, 1999
Folic acid	Folic acid deficiency is associated with depressed mood. The pathway is unknown	Coppen and Bolander-Gouaille, 2005; Young, 2007
Ghrelin	Linked to stress mediated food reward behavior, depression, and anxiety via ghrelin receptor signaling pathway	Schanze et al., 2008; Barim et al., 2009; Kluge et al., 2009, 2011; Perello et al., 2010; Chuang et al., 2011; Diz-Chaves, 2011; Kumar et al., 2013
Serotonin	Linked to food intake, depression, and anxiety via serotonin receptor signaling pathway	Wurtman and Wurtman, 1989; Benton and Donohoe, 1999; Pepino et al., 2009; Shabbir et al., 2013
Dopamine	Linked to food reward behavior and mood via dopamine receptor signaling pathway	Cantello et al., 1989; Diehl and Gershon, 1992; Fochtmann and Fink, 1992; Berridge, 1996; Black et al., 2002; Davis et al., 2009; Cawley et al., 2013
Leptin	Linked to food intake, depression, anxiety, and mood disorder via leptin receptor signaling pathway	Collin et al., 2000; Asakawa et al., 2003; Lu et al., 2006; Finger et al., 2010; Liu et al., 2010; Sharma et al., 2010; Yamada et al., 2011; Guo et al., 2012, 2013
Adiponectin	Linked to depression and mood disorder. May involve adiponectin-induced inhibition of GSK-3β pathway	Arita et al., 1999; Maeda et al., 2001; Milan et al., 2002; Cnop et al., 2003; Delporte et al., 2004; Ryo et al., 2004; Leo et al., 2006; Narita et al., 2006; Hanley et al., 2007; Weber-Hamann et al., 2007; Ye et al., 2007; Yilmaz, 2008; Zeman et al., 2009; Jeong et al., 2012; Wilhelm et al., 2013
Resistin	Indirect link to depression. The pathway is unknown	Krsek et al., 2004; Silha et al., 2004; Weber-Hamann et al., 2007; Lehto et al., 2010
Insulin	Linked to mood, depression, anxiety and negative emotion via insulin receptor signaling	Gustafson et al., 1999; Benedict et al., 2004; Koponen et al., 2008; Akbaraly et al., 2009; Almeida et al., 2009; Benoit et al., 2009; Kleinridders et al., 2009; Marks et al., 2009; Pulkki-Raback et al., 2009; Grillo et al., 2011; Chapman et al., 2013; Platt et al., 2013

palmitic acid enters the brain and impairs neurological function (Tsuboi et al., 2013). Palmitic acid impairs leptin and insulin receptor signaling in the hypothalamus and promotes weight gain (Benoit et al., 2009; Kleinridders et al., 2009). Under these circumstances, obesity is promoted, as well as a negative emotional state. In addition, leptin and insulin have been noted to influence mood (Gonder-Frederick La et al., 1989; Lu et al., 2006; Lu, 2007; Zeman et al., 2009; Ryan et al., 2012).

Furthermore, several studies have shown humans on high fat diet manifest mood disorders like depression that correlates positively with high serum palmitate (Tsuboi et al., 2013). Similarly, rats on high fat diet display increased anxiety-like

behavior, altered body weight, plasma insulin, leptin, and glucose levels when compared to rats on iso-caloric olive oil high fat diet that show no changes in body weight, glycaemia, leptin, and insulin levels (Hryhorczuk et al., 2013). Thus, saturated fats stimulate HPA disturbances and/or inflammation, leading to anxiogenic-like behavior in animals and depression in humans. All together these findings suggest an association between certain foods and improved mood.

PSYCHIATRIC AND EATING DISORDERS

The Diagnostic and Statistical Manuals of Mental Disorders (DSM-5), which was developed by the American Psychiatric Association in 1994, reported disturbed eating behaviors in psychiatric disorders (American Psychiatric Association, 2013). In humans, melancholic depression is associated with hypercortisolism, anhedonia, hypophagia, and weight loss (Fisher et al., 1997; Krishnan and Nestler, 2008; Ulrich-Lai and Herman, 2009; Hammack et al., 2010; Carroll et al., 2012; Hryhorczuk et al., 2013; Patterson and Abizaid, 2013; Schellekens et al., 2013b). In contrast to atypical depression, the most common forms of depression are characterized by reduced hypothalamic pituitary adrenal axis (HPA) activity, increased appetite, carbohydrate craving, and weight gain (Juruena and Cleare, 2007). Those with abdominal obesity are associated with hyperactive HPA axis due to an elevated response to corticotrophin releasing hormone (CRH) stimulation and increased stimulated response to stress (Pasquali, 2012).

Altered serum cortisol level is associated with depression (Parker et al., 2003; Raison and Miller, 2003; Stetler and Miller, 2011). Altered cortisol, HPA axis, and food intake have been associated with depression (Ulrich-Lai and Herman, 2009; Dallman, 2010; Schellekens et al., 2012a). The neuronal pathways that regulate food intake, and circuitries that act *via* the HPA axis are implicated in a complex two-way relationship of three concepts between mood, food, and eating behavior (**Figure 1**) (Kyrou and Tsigos, 2009; Ulrich-Lai and Herman, 2009; Dallman, 2010; Schellekens et al., 2012b, 2013b). It is noted that there is an overlap in neural circuitry of food intake and stress that likely reinforces a link between stress and feeding behavior (Maniam and Morris, 2012). These overlapping circuitries of HPA axis modulating feeding behavior and stress converge on corticosterone hormone producing neurons in the paraventricular nucleus (PVN). Thus, elevated glucocorticoid and a dysfunctional HPA axis are common to both depression and obesity.

Glucocorticoids exert multiple effects on metabolic, endocrine, immune, and behavioral functions. Glucocorticoids regulate reward and emotional processes *via* their receptors in midbrain and limbic circuits (Arnett et al., 2011; Solomon et al., 2012; Hryhorczuk et al., 2013; Patterson and Abizaid, 2013; Wang et al., 2013). Glucocorticoids not only act peripherally to maintain energy homeostasis but also centrally to modulate HPA activity, emotional, and behavioral effects of stress (Fedoroff et al., 2003; Figueiredo et al., 2003). Under physiologic acute stress, the HPA axis is activated, and glucocorticoids are released. This leads to a major restoration of energy balance by increasing insulin, increasing motivation for palatable food (Piazza and Le Moal, 1997; Dallman et al., 2006; Dallman, 2010), and mobilizing stored energy toward central stores that leads to obesity (Mann and Thakore, 1999). Thus, obesity and mood disorder are linked *via* the HPA axis. In rodents, chronic corticosterone exposure leads to increased glucocorticoid receptor (GC) expression in fore-brain and basolateral amygdala that results in depressive-like, anxiety-like behaviors, and increased locomotors (Wei et al., 2004; Boyle et al., 2005, 2006). Therefore, these findings suggest that a deficit in glucocorticoid signaling in distinct brain regions may play a role in affective disorder.

OBESITY AND MOOD

Obesity increases incidence of anxiety and mood disorders (Simon et al., 2006). Stress induced overeating and obesity is also associated with major depression in humans (Novick et al., 2005; Simon et al., 2006; Kloiber et al., 2007). Individuals under chronic stress tend to have more visceral fat due to excessive systemic cortisol levels (Brown et al., 2004; Adam and Epel, 2007; Kyrou and Tsigos, 2009). In all, there appears to be a good association between hypercortisolemic depression, abdominal fat accumulation (Weber-Hamann et al., 2002), decreased glucocorticoid-mediated negative feed back, and increased corticotropin releasing hormone (CRH) release from the paraventricular nucleus (PVN) (Holsboer, 2000). Furthermore, major depression in adolescence is linked to a higher risk for obesity in adulthood (Richardson et al., 2003). It is also noted that metabolic conditions are exacerbated in depression and vice versa (Mcelroy et al., 2004; Simon et al., 2006; De Wit et al., 2010; Luppino et al., 2010; Marijnissen et al., 2011). Like-wise, stress significantly impacts food intake in both humans and animals, thereby promoting metabolic disturbances (Block et al., 2009; Dallman, 2010; Maniam and Morris, 2012). Overeating can also be considered to be analogous to drugs of use because it reflects an addiction where individuals become physically and psychologically dependent on foods rich in fat and sugar (Avena et al., 2008, 2009; Barry et al., 2009; Parylak et al., 2011; Allen et al., 2012; Davis, 2013). Reports also show that with intake of palatable rewarding food, acute stress responses are reduced (Dallman et al., 2003; Lutter and Elmquist, 2009; Chuang et al., 2011; Kumar et al., 2013), thereby showing the potential of "comfort eating" in stress relief. All together these findings suggest that there is a reciprocal link in mood disorder and obesity.

RODENT MODELS OF MOOD AND EATING DISORDERS

Rodent studies have provided the best insight into dopamine-mediated food intake. Dopamine deficient mice die quickly due to decreased food intake (Hnasko et al., 2004). Dopamine when given in the striatum rescues deficient food intake by restarting feeding behavior. Further, when dopamine is given to the nucleus accumbens, a food preference for pleasant food vs. non-pleasant food is observed. Altered dopamine receptor expression is also associated with feeding behavior (Clifton et al., 1991; Zeng et al., 2004; Wang et al., 2009) (18). Post-transcriptional modification such as RNA editing could also play a role in altered reward circuitry mediating overeating behavior (18). It is noteworthy that altered serotonin 2C receptor ($5HT_{2C}R$) editing has been associated with dopamine production, reward, mood, feeding, and

recently obesity (Burns et al., 1997; Sodhi et al., 2001; Gurevich et al., 2002; Higgins and Fletcher, 2003; Iwamoto et al., 2005; Rosenzweig-Lipson et al., 2007; Berg et al., 2008; Olaghere Da Silva et al., 2010; Hayes and Greenshaw, 2011; Schellekens et al., 2012a). Intriguingly, both serotonergic and dopaminergic system are altered in transgenic mice with dysregulated RNA editing enzyme, ADAR2 (Singh et al., 2007, 2009, 2011) (18). These transgenic mice show significantly hyperactive brain regions implicated in reward and also behaviorally display goal oriented behavior toward food in a competitive rewarding environment (Akubuiro et al., 2013). Furthermore, altered dopamine receptor expression, food preference for high fat diet are also observed in ADAR2 transgenic mice (Akubuiro et al., 2013). Interestingly, co-morbidities of depression and anxiety behaviors and altered $5HT_{2C}R$ editing are observed in ADAR2 transgenic mice (Singh et al., 2007, 2009, 2011). Collectively, these results suggest that co-morbidities of affective disorder, overeating, and obesity could be linked *via* the modified $5HT_{2C}R$ in ADAR2 transgenic mice. However, more studies are required to provide a better understanding of the post-transcription modification of the $5HT_{2C}R$ linking to mood, food, and obesity in ADAR2 transgenic mice.

Dysfunctional serotonergic signaling has been associated with mood and obesity (Wurtman and Wurtman, 1989; Benton and Donohoe, 1999; Sodhi et al., 2001; Iwamoto and Kato, 2003; Schmauss, 2003; Kawahara et al., 2008; Morabito et al., 2010; Singh et al., 2011; Schellekens et al., 2012a; Silberberg et al., 2012; Shinozaki et al., 2013). In another rodent model of depression brain derived neurotropic factor (BDNF) was shown to have an antidepressant-like effect (Siuciak et al., 1997). BDNF has been shown to be a neurotropic factor on serotonergic neurons in BDNF heterozygous mice where dysfunctional serotonergic signaling is associated with aggression, hyperphagia, and weight gain is rescued (Lyons et al., 1999). Further exogenous BDNF application enhances serotonin signaling and modifies several behaviors regulated by serotonin feeding, body weight homeostasis, and analgesia (Siuciak et al., 1994; Pelleymounter et al., 1995). Thus, these studies suggest that dysfunctional serotonergic and dopaminergic systems play a critical role in mood, food intake, and obesity.

PSYCHOBIOLOGICAL RELATIONSHIP OF BRAIN REWARD LINKING HUNGER, ADDICTION, OVEREATING, AND OBESITY

Continuous overeating can be viewed as an addictive behavior that involve reward circuitry (Davis, 2013). Reward circuitry involved in addiction spans two key brain regions, (1) the prefrontal region and the amygdala and, (2) the limbic system integrating amygdala with hypothalamus and septal nuclei (Elliott et al., 2000; Schultz, 2000, 2002; Tzschentke, 2001; Baxter and Murray, 2002; Rolls et al., 2002; Koob and Volkow, 2010). The neural mechanism of disrupted dopamine signaling pathways being central to overeating and drugs of use and the overwhelming hallmarks of urge to seek and consume, thereby presents an addiction behavior. Another common phenomenon of compulsive intake of drugs and overconsumption of food intake seen in obesity is the loss of control due to impairments in circuits involved in decision making, self control, interoception, and regulation of mood and stress (Volkow et al., 2010).

Two hormones: ghrelin and leptin interact with the hypothalamus to regulate food intake, energy homeostasis, promote satiety, and hunger. Interestingly, both hormones have been implicated in craving behavior, eating disorder, and mood and have also been associated with the reward pathway (Kiefer et al., 2001; Opland et al., 2010; Dickson et al., 2011). Thereby, suggesting that both ghrelin and leptin are linked to mood and food intake.

There are several neurotransmitter systems involved in feeding such as serotonin, dopamine, opioids, and GABA, of which serotonin and dopamine have been the most closely linked to feeding behavior. Dopamine mediates reward specifically the "wanting" or approach behaviors toward a biologically relevant goals more so than "liking" or enjoyment aspect (Berridge, 1996; Davis et al., 2009). Opioids have been implicated more so in the "liking" or the hedonic aspect of reward processing and both neurotransmitter pathways work together in the perception of reward (Davis et al., 2009). The "wanting" behavior toward a biological relevant goal that is mediated by dopamine is probably due to how dopamine neurons receive signals and the way they are organized in the brain. Dopamine neurons are found in the midbrain region of the ventral tegmental area (VTA) and substantia nigra pars compacta projecting to striatal limbic and cortical regions. Dopamine neurons receive information from; hypothalamus and brain stem regions involved in autonomic responses, hippocampus involved in memory, amygdala involved in emotional reactivity, thalamus involved in arousal and prefrontal cortex and cingulate involved in emotional reactivity *via* neuropeptides and neurotransmitters. Neurochemistry and neuroanatomical reward circuitry involved in addiction to alcohol and drugs translate to an addiction model of overeating and obesity. Certain studies show that hunger can influence memory for food-related stimuli where the orbitofrontal cortex is specifically involved in food-related stimuli in hunger state (Morris and Dolan, 2001). In rodent studies, dopamine has been shown to play a role in feeding by determining a meal size to meal duration, and obesity (Clifton et al., 1991; Schwartz, 2000). Dopamine in the nucleus accumbens has been associated with reinforcement aspects of food and while in the hypothalamus, dopamine plays a role in initiation and duration of feeding (Wang et al., 2004b). Leptin and insulin also help to regulate dopamine production (Leinninger et al., 2009). Dopamine regulates food consumption involving the mesolimbic pathway and the hypothalamus (Volkow et al., 2011). Since dopamine levels in addiction change in these brain regions, it is conceivable that a similar mechanism of reinforcement of food may also be involved in food addiction (Wang et al., 2004b).

FOOD REWARD, ADDICTION, AND OBESITY

Food is a natural reward and has both homeostatic and hedonic characteristics (Rada et al., 2010; Volkow et al., 2011). Depending on the specific type of highly palatable food, it has the potential to engage similar brain reward pathways as drugs of abuse (Weatherford et al., 1990; Pitchers et al., 2010; Olsen, 2011). It may also arise from casual eating to compulsive eating that eventually leads to addiction (Davis, 2013). This may be from food-related brain changes that is associated with psychological changes like that seen in drug addiction (Robinson and Berridge, 2003). Both rewarding and hedonic effects of food result in

positive emotional reactions that play a major role in overeating and obesity (Fulton, 2010; Avena et al., 2013; Bongers et al., 2013; Sinha and Jastreboff, 2013; Yau and Potenza, 2013). Theoretical models support food addiction because highly palatable food activates reward pathways that lead to human and animal obesity (Finlayson et al., 2007; Berner et al., 2008; Heyne et al., 2009; Davis et al., 2011; Sampey et al., 2011; Akubuiro et al., 2013; Davis, 2013).

The American Psychological Association in the DSM-5 manual included behavioral addiction and addictions to natural rewards as a new category of "addiction and related behavior" (Volkow and O'brien, 2007). Human and rodent studies suggest that dysregulated brain reward pathways may contribute to increased intake of palatable food leading to obesity (see review by Berthoud et al., 2011). Despite the divergence in eating behavior, there is an overall increase in tasty, energy- rich foods that is independent of stress-induced hyperphagia or hypophagia (Gibson, 2006; Dallman, 2010). One hallmark of food addiction is the food craving where intense desired food consumption only compensates the craving, whereas in hunger various types of food alleviates the hunger (Martin et al., 2011). Advantages of functional magnetic resonance imaging (fMRI) and positron emission topography (PET) paradigms have been used to provide insights of neural correlates in food addiction and obesity (Wang et al., 2004a; Teegarden and Bale, 2007; Volkow et al., 2012). Interestingly, following various types of food presentation to normal healthy patients, activated brain regions of anterior cingulate cortex, orbitofrontal cortex, and insula are observed (Wang et al., 2004a; Teegarden and Bale, 2007). In contrast to obese overeating patients, neurobiological changes in the reward pathways are similar to those observed in drug addicts (Volkow et al., 2012). However, available data in humans on food addiction suggests that there is heterogeneity in the clinical definitions of food addiction, obesity, and binge eating disorder. Nonetheless through neurobiological data obtained from both human and animal studies, food cravings, overeating, and tolerance support an addiction-like model, see reviews (Albayrak et al., 2012; Volkow et al., 2012; Davis, 2013; Hone-Blanchet and Fecteau, 2014).

SOCIETY AND FOOD ADDICTION

Globally about 1 billion adults are overweight of which 475 million are obese (Organization, 2013). Obesity is a complex multifactorial disease. In the United States, increased incidence of adult obesity is on the rise. In the Westernized society, the major cause of obesity is due to reduced physical activity leading to sedentary life style and surplus of food, sodas, variety of fast food, and hyperpalatable foods, all that activate dopamine rewarding centers leading to over consumption of food (Fortuna, 2012; Granados et al., 2012; Ziauddeen et al., 2012). Hyper-palatable foods and their increased availability promote addictive and compulsive eating leading to weight gain. Addictive properties of certain types of food and addiction-like behaviors are observed in both humans and animal models. Animal studies have shown an overview of addiction-like eating behaviors when presented with foods high in sugar and fat (Avena et al., 2008, 2012). In animals, several studies of sugar-binging models support an addiction-like phenotype of tolerance, cross sensitization, withdrawal, and neurochemical changes, but does not induce obesity (Avena, 2007; Avena et al., 2008, 2009). On the other hand, several imaging studies from obese population shows that greater BMI and overeating are associated with neurobiological pathways similar to those observed in drug addicts (Stice and Dagher, 2010; Stice et al., 2010; Volkow et al., 2012, 2013). In humans, feeding behaviors are more complex but pattern of food addiction appears to parallel substance dependence (Gearhardt et al., 2011; Dileone et al., 2012). Some argue that food addiction should be included in the DSM manual (Volkow and O'brien, 2007; Taylor et al., 2010) even though food addiction is not a categorized diagnosis within DSM-5. However, recently Yale Food Addiction Scale (YFAS) has been used as a tool for diagnosis of food addiction in patients with eating disorders (Gearhardt et al., 2009; Clark and Saules, 2013). In one study, using body mass index, body fat percentage by dual-energy X-ray absorptiometry, macronutrient intake, and the YFAS scale has been used as a diagnostic tool to assess food addiction in general Newfoundland population (Pedram et al., 2013). They found that the prevalence of food addiction was significantly associated with obesity in general population. Thus, suggesting that food addiction contributes to severity of obesity in the general population and that food addiction could be a separate etiology of obesity.

In summary, findings of central mediated food intake suggest a complex two-way link between food intake and mood, emotion, reward, food, food choice, and neurotransmitters (**Figure 1**). Food addiction remains as an incomplete described phenomenon due to limited data. Overabundance of food seems to aid in food addiction specifically foods rich in fat and sugar. Although FMRI and PET imaging have been useful in providing some insights into neural correlates in food addiction and obesity, but specific food addiction phenotype in the development of obesity needs to be differentiated. Furthermore, molecular pathways or signatures that link food intake in emotion, mood, food, reward, and obesity are areas that need further investigation. These types of studies in the future will provide further insight into genetic, psychological, neuropsychiatric, and environmental risk factors associated with overeating, food addiction, and obesity.

PERIPHERAL SYSTEM IN REGULATION OF MOOD, FOOD, AND OBESITY

The gut-brain axis mediates the communication between brain and gut when it comes to appetite, satiety, and energy homeostasis (Cummings and Overduin, 2007; Ahima and Antwi, 2008; Blevins and Baskin, 2010; Gibson et al., 2010; Suzuki et al., 2010, 2012). Furthermore, peripheral hormones have also been reported to regulate mood, food intake, and obesity (Tschop et al., 2000; Nakazato et al., 2001; Olszewski et al., 2008; Blevins and Baskin, 2010; Suzuki et al., 2010; Andrews, 2011b; Dickson et al., 2011; Egecioglu et al., 2011; Skibicka and Dickson, 2011; Overduin et al., 2012; Perello and Zigman, 2012; Karra et al., 2013). Gastrointestinal signals such as cholecystokinin (CCK), bombesin, glucagon eneterostatin, insulin, resistin, somatedin, cyclohistiyl-proline, leptin, amylin, and apolipoprotein A-IV are all known to reduce food intake. The exception is ghrelin, which increases food intake. Several peripheral factors that engage the

CNS in a bi-directional manner and influence mood and food intake are summarized in **Table 1** and discussed below.

GHRELIN

A gut orexigenic hormone ghrelin is synthesized in the stomach and acts centrally to mediate increased food intake *via* central pathways (Kojima et al., 1999, 2004; Tschop et al., 2000; Nakazato et al., 2001; Andrews, 2011a; Diz-Chaves, 2011). The hypothalamus in the brain directly senses peripheral ghrelin and modifies the energy status (Schaeffer et al., 2013). Studies support that ghrelin reaches the brain *via* the vagus afferents to the nucleus solitary tract (NST), which further projects to the arcuate nucleus of the hypothalamus (Asakawa et al., 2001; Date et al., 2002; Williams and Mobarhan, 2003). Ghrelin activates downstream signaling *via* the hormone secretagogue receptor (GSH-R1a) where it is ubiquitously expressed in multiple brain regions and in peripheral tissues. Due to multiple sites of GSH-R1a expression, it is not surprising that ghrelin performs many other biological activities of growth hormone secretion, glucose and lipid metabolism, and gastrointestinal motility. However, other properties of GHS-R1a allowing dimerization with multiple G-protein coupled receptors suggest the likelihood of cross talk between many other neuropeptide systems of serotonin and dopamine (Schellekens et al., 2013a,b). Thus, ghrelin has the potential to engage multiple neuropeptide systems in mood, food, and obesity.

The ghrelinergic system also mediates the non-homeostatic hedonic rewarding and motivational aspects of food intake *via* mesolimbic dopaminergic circuitry (Dickson et al., 2011; Egecioglu et al., 2011; Skibicka et al., 2011; Perello and Zigman, 2012). Studies support ghrelin's involvement in stress mediated food reward behavior (Perello et al., 2010; Kumar et al., 2013; Chuang et al., 2011; Diz-Chaves, 2011). Numerous studies provide a link between ghrelin and affective disorders, such as depression and anxiety (Schanze et al., 2008; Barim et al., 2009; Kluge et al., 2009). Ghrelin also alleviates depression (Kluge et al., 2011). All together these studies suggest that the ghrelinergic system is an attractive system to target stress associated metabolic and mood associated eating disorders in obesity.

SEROTONIN

Serotonin has numerous functions besides regulating mood that includes regulation of sleep, appetite, and impulse control (Steiger, 2004; Daubert and Condron, 2010; Nordquist and Oreland, 2010; Mosienko et al., 2012). Serotonin levels from the gut and alimentary canal constitutes about 80–90% of the human body's total serotonin and not in the brain. This is surprising, as serotonin dictates most of our mood and happiness (Wurtman and Wurtman, 1989; Benton and Donohoe, 1999). Central serotonin pathways participate in the regulation of mood and modulate meal patterns in terms of quality and quantity. Neurotransmitter release of serotonin from serotonergic neurons in the brain is governed by food intake (Shabbir et al., 2013). The essential amino acid tryptophan that comes from food is the precursor for serotonin synthesis (Prasad, 1998). Ingestion of carbohydrates increases the plasma ratio of tryptophan to other large neutral amino acids leading to increased serotonin synthesis in the brain and alleviating depression. Such is the case for carbohydrate craving during depression that often leads to obesity and vice versa (Pepino et al., 2009; Shabbir et al., 2013). This is observed during stress, winter depression, or in people trying to give up smoking. Nicotine increases brain serotonin secretion and its withdrawal leads to depression (Wallin and Rissanen, 1994; Wurtman and Wurtman, 1996). Brain serotonin plays a role in the pathophysiology of depression, as treatments with serotonin potentiating drugs alleviates depression in seasonal affective disorder (Wurtman, 1993). Based on these findings it has been suggested that the excessive carbohydrate intake by patients with premenstrual syndrome (PMS) and seasonal affective disorder (SAD) relieves the depressive symptoms *via* an increased central serotonergic activity (Cizza et al., 2005; Miller, 2005). A diet rich in carbohydrates can relieve depression and elevate mood (Wurtman and Wurtman, 1989; Benton and Donohoe, 1999). Furthermore, research has shown that dieters tend to become depressed as the serotonin levels are reduced due to decreased carbohydrate intake (Huether et al., 1997). Thus, these studies imply that certain foods are strong mood regulators.

LEPTIN

Low leptin levels have been found to be associated with human depression and depression-like behaviors in rodents (Kraus et al., 2001; Lu et al., 2006; Guo et al., 2012; Lawson et al., 2012). Antidepressant-like effect of leptin in leptin insufficiency or leptin resistance suggests the hormone contributes to altered mood (Lu, 2007). Increased visceral fat and dyslipidemia are associated with several endocrine and metabolic changes that link to CNS control of emotional states and mood (Hryhorczuk et al., 2013). As an endocrine gland, adipose tissue secretes numerous peptide hormones that target the brain and peripheral tissues to regulate metabolism and behavior. Leptin circulates in proportion to fat mass (Maffei et al., 1995). Leptin impacts several physiological processes such as appetite, energy expenditure, and neuroendocrine function. The hormone has also been linked to human depression and has been shown in rodents to have antidepressant and anxiolytic effects (Asakawa et al., 2003; Liu et al., 2010; Yamada et al., 2011; Lawson et al., 2012). Nevertheless, there are conflicting findings of leptin levels and depression, which are discussed below.

Major depressive disorder (MDD) has been shown to be associated with lower plasma leptin levels when compared to healthy controls (Kraus et al., 2001; Atmaca et al., 2002, 2008; Westling et al., 2004; Jow et al., 2006). On the other hand, there are reports showing increased plasma leptin levels in depression (Kraus et al., 2002; Esel et al., 2005; Schilling et al., 2013), gender specific increased leptin levels in women with depressive disorder (Rubin et al., 2002; Esel et al., 2005; Zeman et al., 2009), as well as no changes of leptin by antidepressant treatment (Esel et al., 2005). In depressed individuals suffering from loss of appetite, plasma leptin levels do not differ from those of healthy controls (Deuschle et al., 1996). In another study, it was found that higher serum leptin was associated with atypical depressive patients with increased appetite (Gecici et al., 2005). In older men, a combination of elevated visceral fat and high leptin levels was associated with depression (Milaneschi et al., 2012), and high leptin correlated

positively with depressive symptoms in patients with type 2 diabetes (Labad et al., 2012). Thus, these reports suggest more studies are required to draw a better conclusion regarding the role of leptin in human depression.

Interestingly, rodent studies have provided the most conclusive findings. Leptin modulates the HPA axis and mice that lack leptin (obese *ob/ob* mice or its leptin receptor (obese *db/db* mice) show increased depression-like behavior (Collin et al., 2000; Asakawa et al., 2003; Lu et al., 2006; Finger et al., 2010; Liu et al., 2010; Sharma et al., 2010; Yamada et al., 2011; Guo et al., 2012, 2013). Furthermore, leptin deficient *ob/ob* mice have elevated corticosterone that can be reduced by leptin replacement (Garthwaite et al., 1980; Arvaniti et al., 2001). In contrast, chronic unpredictable mild stress in rats activates the HPA axis and leads to depressive-like behaviors that correlate with decreased serum leptin levels (Ge et al., 2013). Leptin receptors (LepRb) in midbrain and forebrain loci that affect emotional processes are targeted by leptin. Genetic deletion of LepRb in the hippocampus results in a depression-like phenotype, which is reduced by leptin administration to the hippocampus thereby showing an antidepressant effects (Asakawa et al., 2003; Lu et al., 2006; Finger et al., 2010; Liu et al., 2010; Guo et al., 2013). Loss of LepRb specifically in glutamatergic neurons of the forebrain elicits depressive-like behavior without affecting anxiety (Guo et al., 2012). Stress-induced dopamine release is also associated with high leptin (Burghardt et al., 2012). Leptin activates dopamine neurons in the VTA of the midbrain reducing dopamine neuronal firing and increases dopamine availability (Fulton et al., 2006; Hommel et al., 2006). Selective deletion of LepRb from midbrain dopamine neurons results in increased anxiety-like behavior, but not depressive-like behavior (Liu et al., 2011). LepRb signaling in limbic and prefrontal nuclei mediates the antidepressant action of leptin. In contrast, leptin in dopamine neurons of the ventral midbrain and in central nucleus of the amygdala leptin signaling exerts the anxiolytic actions of leptin. Thus, leptin signaling in different brain regions exerts different physiological behaviors.

In conditions of central obesity that favors insulin resistance and type 2 diabetes, leptin sensitivity is diminished. Leptin resistance is associated with high plasma leptin levels and defective LepRb signaling. These states are characteristic of obesity and increase the risk for mood disorders (Myers et al., 2012). Mice made obese by a high fat diet intake show reduced sensitivity to effects of leptin and antidepressant actions of leptin when compared to low-fat diet treated controls (Yamada et al., 2011). Further, leptin insensitivity exacerbates HPA dysregulation in obesity (Komorowski et al., 2000; Collura et al., 2009) and thereby enhances the mass of dysfunctional central adipose stores in a cortisol-dependent manner. Leptin resistance has been reported to be associated with the mid brain VTA where mesolimbic DA neurons reside (Matheny et al., 2011). Leptin resistance appears to affect multiple neural and endocrine pathways including hippocampal, mesolimbic dopamine pathways, and HPA activity ultimately affecting emotions and mood. Thus, these studies provide evidence of leptin related mechanisms underlying depression in obesity.

ADIPONECTIN

Low levels of another adipose-derived hormone, adiponectin, has been implicated in energy homeostasis, metabolic disturbances, insulin resistance (Kennedy et al., 2006; Hanley et al., 2007; Turer and Scherer, 2012; Hryhorczuk et al., 2013) and recently, depression in humans (Arita et al., 1999; Cnop et al., 2003; Ryo et al., 2004; Leo et al., 2006; Narita et al., 2006; Hanley et al., 2007; Weber-Hamann et al., 2007; Yilmaz, 2008) and rodents (Maeda et al., 2001; Milan et al., 2002; Delporte et al., 2004; Ye et al., 2007). Changes in adiponectin levels are secondary to metabolic disturbances in obesity (Morrison et al., 2011; Doumatey et al., 2012). There are conflicting reports of either positive or negative associations of adiponectins levels with mood disorder (Yilmaz, 2008; Zeman et al., 2009; Jeong et al., 2012; Wilhelm et al., 2013), or no changes in patients with major depressive disorder or with antidepressants (Lehto et al., 2010; Jeong et al., 2012). Mice exposed to chronic social defeat recapitulate the low levels of adiponectin, stress-induced depressive-like behaviors, and impaired HPA axis (Liu et al., 2012). Interestingly central administration of adiponectin has antidepressant effects (Liu et al., 2012). Thus, a link between plasma adiponectin levels and depression is observed in mice. In contrast, humans show more ambiguous results depending on the type of depressive disorder, sex, and treatment.

RESISTIN

Adipocyte-derived resistin is linked to insulin resistance in rodent models of depression-like behavior while in humans, the role of resistin is less defined (Schwartz and Lazar, 2011; Hryhorczuk et al., 2013). In genetic and diet induced obese mice circulating resistin levels are elevated (Steppan et al., 2001). In contrast, resistin is down regulated in human obesity (Way et al., 2001; Degawa-Yamauchi et al., 2003; Owecki et al., 2011; Sadashiv et al., 2012). However, there is one study that shows a positive correlation between resistin levels and atypical depression (Lehto et al., 2010). In human depression, however, resistin levels positively correlate with salivary cortisol (Krsek et al., 2004; Silha et al., 2004; Weber-Hamann et al., 2007). Conversely, resistin levels are lower in patients receiving antidepressant treatment who have remitted from depression (Weber-Hamann et al., 2007). Thus, these studies imply that resistin plays a role in affecting mood.

INSULIN

From a recent systematic review and meta-analysis there appears to be a significant cross-sectional association between depression and insulin resistance (Kan et al., 2013) and there is a bi-directional association between diabetes and depressed mood. Depression is associated with pre-diabetes insulin resistance (Anderson et al., 2001; Kan et al., 2013) and obesity (Hamer et al., 2012). However, there exists a weak association of insulin resistance and depression (Adriaanse et al., 2006; Platt et al., 2013; Shen and Bergquist-Beringer, 2013). High fat diet intake impairs the hypothalamic insulin receptor signaling (De Souza et al., 2005; Kim and Feldman, 2012) and reduced hypothalamic insulin signaling promotes weight gain and negative emotional states (Gustafson et al., 1999; Koponen et al., 2008; Akbaraly et al., 2009; Almeida et al., 2009; Benoit et al., 2009; Kleinridders et al., 2009;

Pulkki-Raback et al., 2009; Platt et al., 2013). Intranasal insulin ameliorates self-reported mood, reduce cortisol levels, and visceral obesity (Benedict et al., 2004; Chapman et al., 2013). Further treating patients with major depressive disorder and abdominal obesity, the insulin-sensitizing drug pioglitazone shows reduced sign of depression, anxiety, and reduced insulin resistance (Kemp et al., 2012).

In rodents, reduced insulin receptor signaling impacts mood when placed on a long-term 30%kcal fat diet that shows anxiolytic effects (Marks et al., 2009). Similarly, rosiglitazone administered to normal chow-fed mice and rats show an antidepressant action in behavioral despair tests (Eissa Ahmed et al., 2009; Ryan et al., 2012). Antisense RNA targeting the insulin receptor in rats results in increased depression-like behavior and anxiety-like behavior (Grillo et al., 2011). By and large, these results suggest that insulin signaling is involved in mood. However, further studies are required to determine whether intranasal insulin has antidepressant effects in depressed individuals and, if so, whether this action is maintained in obesity.

To summarize, food intake is regulated by the peripheral and central system that are engaged in a bi-directional manner. Peripheral signals mostly modulate satiety and indicate adiposity signal to the brain. Ghrelin is the only peripheral hormone that induces hunger but interestingly it is also involved in mood and hedonic aspects of food intake. There are several brain regions involved in food intake that overlaps brain areas involved in drugs of abuse and reward. Overlapping brain regions of reward, mood, and food intake suggests that molecular changes in these regions may provide further insights in to distinct and overlapping pathways that could aid in understanding clinical treatments of comorbidity of mood disorder, overeating, and obesity.

EPIGENETICS, MOOD, AND EATING DISORDER

Interaction of genes and environment has been associated with mood disorders, see review Archer et al. (2013), and eating disorders, see review Pjetri et al. (2012). Exposure to highly palatable foods rich in fat and carbohydrate induces craving. In an obesogenic environment, repetitive exposures to highly palatable food options increase the likelihood of food addiction, overeating, and obesity. There appears to be a complex interaction between genetics and environmental factors such as nutrition with neuropsychiatric, neurodevelopmental, and neurodegenerative disorders. Individual variability in numerous protein coding and non-coding regions in the genome could be related to eating disorders and affective disorders. Epigenetics mechanisms of DNA methylation, RNA editing, post-translational modification of histones, and non-coding RNAs regulate gene regulation without changing DNA sequence in response to changes in internal and external environmental variables. Epigenetics in the context of eating disorders is interesting as it has the potential to answer numerous questions including potential risk factors such as maternal nutrition and stress that alter the risk of eating disorders in the offspring. Unknown questions like how epigenetic modification responds to acute changes like malnutrition or exposure to highly palatable food needs to be answered. Furthermore, epigenetics in learning and memory could also play a critical role in development and maintenance of eating disorders. RNA editing of the $5HT_{2C}R$ has been implicated in affective disorder, stress, maternal separation, Prader Willi Syndrome, hyperphagia, and obesity (Iwamoto and Kato, 2003; Englander et al., 2005; Iwamoto et al., 2005; Bhansali et al., 2007; Kawahara et al., 2008; Morabito et al., 2010; Singh et al., 2011; Schellekens et al., 2012a, 2013b,c). RNA editing of the $5HT_{2C}R$ alters many facets of serotonin signaling *via* 24 different receptor isoforms. These edited $5HT_{2C}R$ isoforms are in a distinct ratio in different brain regions suggests an important role in linking mood, food intake and obesity *via* the $5HT_{2C}R$. Therefore, future research in defining the role of different isoforms in different brain regions is much needed to understand the regulation of RNA editing and mood disorder by the serotonergic system.

CONCLUSION

More than a third of adults and 17% of children and teenagers in the United States are obese (Ogden et al., 2014). Obesity is the second-leading cause of preventable death in the U.S. contributing to 300,000 deaths each year. In addition, the health care burden in obesity-related diseases in the U.S. could reach at staggering $861–957 billion by 2030 (Go et al., 2013a,b, 2014). This article points to biological factors engaging both central and peripheral system in a bi-directional manner linking food intake, mood, and obesity. Food intake is complex due to influence of several factors. The influence of food choice includes biological determinants of hunger, appetite, and taste. Besides these, other factors of cost, income, and availability also influence food choice. Other determinants of social and psychological factors of mood, stress, and emotion also play a critical role in food choice. Many people find it hard to stop eating a particular food even though they are not hungry. Such behaviors activate the brain reward center and alter the brain structure. Willpower has been speculated in the past to control overeating. Through neurobiological data, presence of food cravings, over eating, and tolerance support an addiction-like model by numerous signals that are involved in engaging both the central and peripheral nervous system in a bi-directional manner to regulate food intake. Genes, environment, various emotions also influence food intake, and mood states that trigger eating of palatable foods for comfort in negative emotional states. This repetitive eating of comfort foods, rich in carbohydrate, high-fats and sugar, leads to obesity. Obesity in turn regulates mood due to metabolic disturbances. Metabolic disturbances further alter brain-signaling systems leading to a bi-directional vicious cycle of mood, food, and obesity (**Figure 1**). Furthermore a complex regulation of mood and eating disorders are implied from emerging studies of epigenetics in mood and eating disorders.

FUTURE DIRECTIONS

It is recognized that animal and human findings do not entirely overlap, but animal studies have provided the most compelling neurobiological findings of addictive nature of food, overeating, food addiction, and obesity. In the future, using molecular studies toward an effort to understand the environment of plentiful food leading to obesity rather than food restriction in animal models will provide a valuable insight into the molecular mechanism of overeating and food addiction. Further, using animal models

and molecular studies in the area of withdrawal induction model in highly palatable diet are needed. Understanding how brain regions are altered with various nutrients, in depression, anxiety state may elucidate a common overlapping brain region in co-morbidities of affective and eating disorders. Epigenetic progress in relation to eating disorder has been slow. Molecular pathways of regulated non-coding RNAs in gene regulation involved in affective disorders and overeating may provide novel pathways involved in the pathogenesis. Epigenetic regulation of primary brain signaling and factors governing their metabolism needs further investigation where animal studies are likely to guide psychiatric analysis of epigenetic modification. Furthermore, next generation sequencing can be useful in finding novel long and small non-coding RNAs, alternative spliced RNAs, expression levels of coding RNAs, and RNA editing changes in the clinical treatment responders vs. non-treatment responders. It is anticipated these future studies will aid in the development of more targeted and effective therapies for preventing and treating comorbidities of mood disorder and obesity.

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Orexin-A controls sympathetic activity and eating behavior

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It is extremely important for the health to understand the regulatory mechanisms of energy expenditure. These regulatory mechanisms play a central role in the pathogenesis of body weight alteration. The hypothalamus integrates nutritional information derived from all peripheral organs. This region of the brain controls hormonal secretions and neural pathways of the brainstem. Orexin-A is a hypothalamic neuropeptide involved in the regulation of feeding behavior, sleep-wakefulness rhythm, and neuroendocrine homeostasis. This neuropeptide is involved in the control of the sympathetic activation, blood pressure, metabolic status, and blood glucose level. This minireview focuses on relationship between the sympathetic nervous system and orexin-A in the control of eating behavior and energy expenditure. The "thermoregulatory hypothesis" of food intake is analyzed, underlining the role played by orexin-A in the control of food intake related to body temperature. Furthermore, the paradoxical eating behavior induced orexin-A is illustrated in this minireview.

Keywords: body weight, orexin-A, energy expenditure, sympathetic nervous system, behavior

INTRODUCTION

Obesity and diabetes are a worldwide public health issue with extensive medical, social, and economic consequences (Yach et al., 2006; Runge, 2007). Obesity (body mass index $\geq$30 kg of body weight/m^2 of height) has negative effects on health and increases the risk of developing a variety of diseases, including cardiovascular syndromes, some cancers, and diabetes mellitus (Must et al., 1999; Field et al., 2001; Calle et al., 2003; Friedenberg et al., 2008). Over the past three decades, the prevalence of obesity has doubled in the USA and in Europe (Ogden et al., 2006; Van Vliet-Ostaptchouk et al., 2014). Although according to the most recent data published in the 2005–2006 update of the National Health and Nutrition Examination Survey (NHANES) obesity rates have stabilized, others (Wang and Beydoun, 2007) expect that the obesity "epidemic" will only continue to worsen, with as many as 75% of Americans and of Europeans potentially being overweight in the year 2020. Physicians will undoubtedly encounter obese people in clinical practice and must, then, be able to identify and address care needs specific to this patient population.

This minireview focuses on relationship between the autonomic nervous system and orexin-A in the control of eating behavior, energy expenditure, and body weight regulation. The "thermoregulatory hypothesis" of food intake (Himms-Hagen, 1995) is analyzed, underlining the role played by orexin-A in the control of eating behavior related to body temperature.

ENERGY HOMEOSTASIS

Energy homeostasis is determined by the balance between intake of calories and energy expenditure. This is regulated by interconnected neuroendocrine and autonomic pathways (Monda et al., 2008a).

Resting energy expenditure (REE) accounts for 60–75% of total daily energy expenditure. Several factors contribute to the inter-individual variability in REE such as fat-free mass (FFM; Weyer et al., 1999), sympathetic nervous system (SNS) activity (Welle et al., 1991; Messina et al., 2012), and endocrine status [e.g., thyroid hormone (Danforth and Burger, 1984)]. REE decreases with age (Roubenoff et al., 2000). This decline is due not only to the loss of FFM and an alteration in its metabolically active components, but also to the reduction in physical activity.

HYPOTHALAMUS AND OREXINS

The hypothalamus, a key component for regulation of energy homeostasis, continuously monitors signals that reflect energy status and initiates appropriate behavioral and metabolic responses (Suzuki et al., 2012). It controls glucose utilization in insulin-sensitive organs, such as skeletal muscle, as well as whole-body energy metabolism (Sudo et al., 1991; Haque et al., 1999).

Orexins A and B are hypothalamic neuropeptides, involved in the regulation of feeding behavior, sleep-wakefulness rhythm, and neuroendocrine homeostasis (Kukkonen et al., 2002; Monda et al., 2005; Viggiano et al., 2006), as reported in **Figure 1**.

These peptides derive from the prepro-orexin (preprohypocretin) gene, which encodes a precursor (130 amino acids in rodents, 131 residues in humans) that is cleaved into orexin-A (synonymous with hypocretin-1; 33 amino acids) and orexin-B (hypocretin-2; 28 residues; Sakurai et al., 1998). Orexins promote both arousal and feeding (Sweet et al., 1999). Orexin-A binds

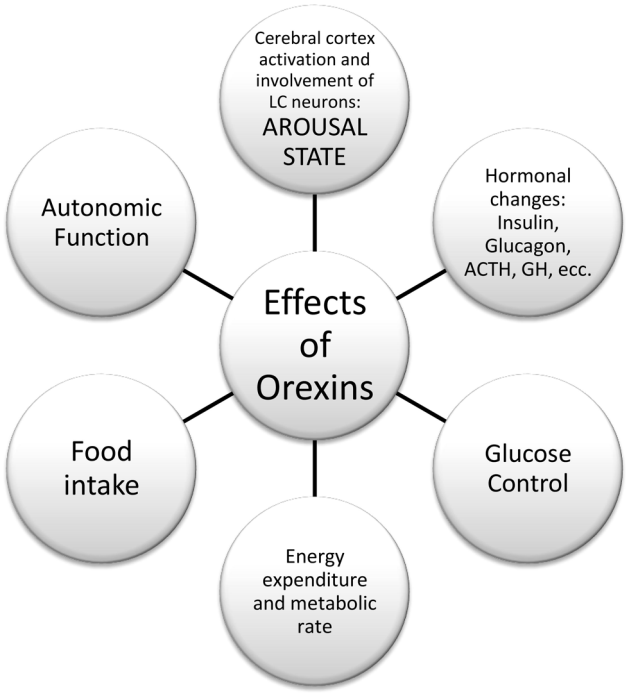

FIGURE 1 | Effects of orexins in peripheral tissue and central nervous system.

to two G-protein-coupled receptors, orexin receptor-1 (hypocretin receptor-1) and orexin receptor-2 (hypocretin receptor-2). The expression pattern of mRNA encoding two orexin receptors (OX1R andOX2R) in the rat's brain has been demonstrated (Trivedi et al., 1998; Machaalani et al., 2013). Within the hypothalamus, expression for the OX1R mRNA was largely restricted in the ventromedial (VMH) and dorsomedial hypothalamic nuclei, while paraventricular nucleus, VMH, and arcuate nucleus contain high levels of OX2R mRNA, as well as in mammillary nuclei (Zhang et al., 2005). Lu et al. (2000) have demonstrated that levels of OX1R mRNA significantly increased in the VMH of rats after 20 h of fasting. An initial decrease (14 h) and a subsequent increase (20 h) in OX1R mRNA levels after fasting were observed in the dorsomedial hypothalamic nucleus. Levels of OX2R mRNA increased in the arcuate nucleus, but they didn't change in the dorsomedial hypothalamic nucleus and paraventricular hypothalamic nucleus following fasting (Lu et al., 2000).

Orexin neurons may also functionally interact with glucose-sensitive neurons in the hypothalamus, notably the glucose-responsive cells (glucose-excited neurons: stimulated by rising glucose levels) found predominantly in the VMH, and the glucose-sensitive neurons (glucose-inhibited neurons: stimulated when glucose falls) that constitute 30% of lateral hypothalamic area (LHA) neurons. There are synaptic contacts between orexin neurons and glucose-sensitive cells in the LHA (Shiraishi et al., 2000), while orexin-A specifically stimulates the glucose-sensitive cells (Liu et al., 2001). On the contrary, orexin-A inhibits glucose-responsive neurons in the VMH (Shiraishi et al., 2000). Muroya et al. (2001) suggest that some glucose-sensitive neurons express orexins. In the medulla, orexin neurons innervate not only the ventral area (Zheng et al., 2005), but also the nucleus of the solitary tract (Ciriello et al., 2003), which is an important relay station that receives sensory signals, such as portal vein glucose availability and gastric distension from the viscera. These signals are conveyed to the hypothalamus (Horst et al., 1989).

Sugar-sensing neurons exist in restricted brain regions, such as hypothalamus and brain stem, and they are classified into two groups, called glucose-excited (GE) neurons and glucose-inhibited (GI) neurons, in terms of the mode of response to extracellular glucose changes within physiological cerebrospinal fluid (CSF) range (Burdakov and González, 2009; Gonzàlez et al., 2009). For instance, orexin neurons in the LHA and neuropeptide Y (NPY)/agouti-related peptide (AgRP) neurons in the ARC are glucose-inhibited, whereas melanin-concentrating hormone (MCH) neurons in LHA and proopiomelanocortin (POMC) neurons in the ARC are glucose-excited (Burdakov et al., 2005; Burdakov and González, 2009). The sugar sensing of orexin neurons, which is a major class of GI neurons, is metabolism-independent, since the glucose response is unaffected by glucokinase inhibitors, and mimicked by a non-metabolizable glucose analog 2-deoxyglucose (González et al., 2008), although the accurate mechanisms, particularly the functional molecules relevant to glucose-induced inhibition, have not yet been explained. Orexin neurons are not inhibited by L-glucose, galactose, α-methyl-D-glucoside, or fructose, whereas GE neurons can sense galactose. More recently, it has been suggested that orexin neurons function as a "conditional glucosensor," because the electrical activity of orexin neurons is more potently inhibited by glucose when intracellular energy levels (i.e., cytosolic levels of pyruvate, lactate, or ATP) are low, whereas high energy levels attenuate the glucose response in orexin neurons (Venner et al., 2011). Besides, Yi et al. (2009) have reported that a continuous intracerebroventricula (ICV) infusion of orexin-A (1 mmol/L, 5 μL/h) into rats fasted for 5 h brought about an increase in plasma glucose levels, and prevented a daytime decrease of endogenous hepatic glucose production (EGP). Hepatic sympathetic, but not parasympathetic, denervation blocked the orexin induced apparent enhancement of EGP.

In addition, when the γ-aminobutyric acid receptor antagonist bicuculline was administered in the perifornical area in order to activate orexin neurons, basal EGP was increased, and insulin-mediated suppression of EGP was attenuated, but the insulin-induced glucose disposal was enhanced (Yi et al., 2009).

In addition, the presence of orexin receptors in other cerebral areas suggests that orexin-A plays additional functions (Kukkonen et al., 2002). It has been demonstrated that the orexins play a role in sleep regulation (Beuckmann and Yanagisawa, 2002). Deficiency in orexin neurotransmission results in the sleep disorder narcolepsy in mice, dogs, and humans (Monda et al., 2004a). Orexin derangements in patients with narcolepsy were associated with an increased body mass index (Schuld et al., 2000) and a higher risk of type-II diabetes mellitus (Honda et al., 1996).

Orexins exert peripheral effect and this was suggested by the detection of substantial levels of orexins in plasma (Adam et al., 2002), as well as the presence of orexin receptors in several peripheral tissues, including the gastrointestinal tract (GIT), endocrine pancreas, adrenal glands, and adipose tissue (Digby et al., 2006; Heinonen et al., 2008).

Snow et al. (2002) have demonstrated that plasma orexin levels are one-fifth to one-eighth of orexin CSF values. However, the source of orexin in peripheral tissue is still unclear. Is orexin directly released into the blood stream or leaked from the CSF? One possibility is that orexin is released from the brain. The other possibility is that orexin is produced directly in peripheral tissues. Orexin-immunoreactive cells are observed in the gastrointestinal tract and pancreas. However, the question of orexin synthesis in peripheral tissue is still under discussion. Further studies are needed to better understand orexin physiology in peripheral tissues.

The influence of orexin-A on metabolic status and plasma glucose level may contribute to increase diabetics morbidity and mortality (Minokoshi et al., 1999). It has been proved that orexins affect the plasma lipoprotein profile and insulin glucose homeostasis (Muroya et al., 2001). Orexins stimulate insulin release from pancreatic cells *in vivo* and *in vitro* (Nowak et al., 2000). Several studies have focused on finding out the relationship between circulating orexin and fat mass and have proved that there is a strong correlation between low plasma orexin and obesity (Adam et al., 2002; Messina et al., 2013a). A significant issue is whether this naturally occurring biological peptide "orexin" in useful in weight management or obesity treatment. Many suggest that when orexin is peripherally injected, it activates thermogenesis, without limiting feeding or increasing physical activity. These encouraging observations have paved the way for clinical testing of the thermogenic potential of orexin (Messina et al., 2013b).

Orexin-A controls glucose production and utilization in the peripheral tissues via the autonomic nervous system (Tsuneki et al., 2010). These conclusions demonstrate that orexin is involved in the control of central and peripheral hormonal actions for the maintenance of glucose homeostasis, though it has been demonstrated that glucose control remains following decerebration (DiRocco and Grill, 1979). Existing evidence suggests that orexins induce glucose production in the liver (Stanley et al., 2010) and help glucose uptake in skeletal muscle (Yi et al., 2009). In addition it has been shown that orexins A and B differentially regulate glucagon release from pancreas (Bass and Takahashi, 2010).

In summary, there is substantial evidence in the literature that helps to define the physiological role of orexin neurons, and their connections, as reported in **Figure 2**. For instance, anatomical works by Kilduff and Peyron (2000) and Kerman (2008); physiological studies by Karnani and Burdakov (2011) and Inutsuka and Yamanaka (2013) in glucose-regulation, and Morrison et al. (2012a) in thermoregulation. More recently, opto- and pharmacogenetic tools also have been used to investigate the physiological role of these neurons (Heydendael et al., 2014; Inutsuka et al., 2014). Finally, hypothalamic orexin neurons co-express glutamate vesicular transporters (Rosin et al., 2003), suggesting an important role of this neurotransmitter in the orexinergic pathway.

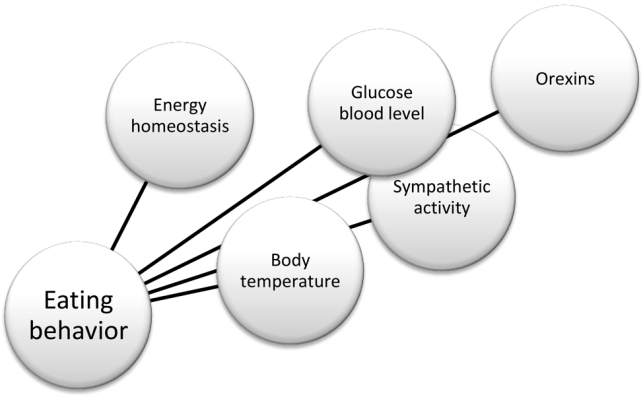

FIGURE 2 | Factors influencing eating behavior.

THE SYMPATHETIC NERVOUS SYSTEM

Eating is a complex behavior that partly involves the sympathetic nervous system. This sympathetic involvement is exerted by an influence on body temperature, in agreement with the "thermoregulatory hypothesis" of eating behavior (Himms-Hagen, 1995). Obviously, the role of the sympathetic system in controlling the eating behavior is not restricted only to changes in body temperature. For instance, the aforementioned glucose-control involves the sympathetic system.

Orexin-A also influences body temperature. In fact, an ICV administration of orexin-A induces an increase in firing rate of the sympathetic nerves to BAT, accompanied with a rise in BAT and colonic temperatures (Monda et al., 2001). The simultaneous increase in heart rate and body temperature after an ICV injection of orexin-A shows a generalized activation of the sympathetic nervous system. Few studies have been made on the topic of the roles played by different cerebral areas involved in the induction of the above-mentioned tachycardia and hyperthermia (Monda et al., 1994, 1995, 1996).

The sympathetic adjustment of thermoregulation also implies in energy expenditure. The functional organization and neurochemical influences within the CNS networks governs the level of BAT sympathetic nerve activity to produce the thermoregulatory and metabolically driven alterations in BAT thermogenesis and energy expenditure that contribute to overall energy homeostasis (Morrison et al., 2014). BAT thermogenesis contributes to the maintenance of body temperature during cold exposure and to the elevated core temperature during several behavioral states, including wakefulness, the acute phase response (fever), and stress. BAT energy expenditure requires metabolic fuel availability and contributes to energy balance.

The consequences of the "thermoregulatory hypothesis" of eating behavior are that subjects with a high set-point of body temperature and/or low sympathetic activity are induced to eat a high quantity of food to elevate the sympathetic discharge and body temperature. Many studies (Keesey and Hirvonen, 1997; Morrison et al., 2012b) indicate that some forms of obesity can be regarded as instances of regulation at an elevated set point,

while other forms seemingly result from a regulatory dysfunction, as already reported by Keesey (1988).

Conversely, subjects with a low thermal set-point and/or a high sympathetic tone need to introduce a lower quantity of food to reach a prefixed thermal set-point. Alterations of postprandial thermogenesis due to a reduced response of sympathetic activation can play an important role in inducing obesity. In other words, subjects with a low postprandial sympathetic activation need to introduce a higher quantity of foot to reach a prefixed body temperature. On the other hand, being overweight increases the sympathetic discharge that contributes to induce diseases related to abnormal body weight (Lambert et al., 2010).

Chronic sympathetic over activity is also known to be present in central obesity, and many evidences demonstrate the consequence of a high sympathetic outflow to organs such as the heart, kidneys, and blood vessels. Chronic sympathetic nervous system over activity can also contribute to a further decline of insulin sensitivity, creating a vicious cycle that may lead to the development of the metabolic syndrome and hypertension. The cause of this over activity is not clear, but may be driven by certain adipokines (Smith and Minson, 2012). In addition, the postprandial activation of the peripheral sympathetic nervous system is fundamental to maintain energy balance. A contribution of postprandial sympathetic activation to the thermic effect of food is not always evident and depends on the size and composition of the meal, with carbohydrates having the clearest effect. Signals related to food intake from various origins (e.g., gut, hepatoportal area, chemoreceptors) are integrated in the brain and result in increased peripheral sympathetic outflow. It is of interest to emphasize the role of diet composition (according to the life style of subjects) in the level of sympathetic activation during the day in view of the potential role of adrenergic over activity in the pathogenesis of obesity and its metabolic syndrome (Van Baak, 2008).

Power spectral analysis (PSA) of the heart rate variability (HRV) is considered a non-invasive method for quantitative and qualitative evaluation of the autonomic nervous system activity in various fields of research and clinical studies. In the frequency domain method of HRV, the high frequency (HF) component is associated solely with parasympathetic activity. The low frequency (LF) component is associated with both sympathetic and parasympathetic activities, but sympathetic activity is the greater contributor. LF power may correlate more with baroreflex function and/or stress that with the cardiac sympathetic innervations (Moak et al., 2007; Shah et al., 2011).

This approach should modify the interpretations about the sympathetic function in the pathophysiology of the obesity. In a study conducted in our laboratory (Monda et al., 2006a), we demonstrated that LF and HF values of premenopausal obese women were lower than values of lean women. In postmenopause, LF and HF have a comparable decline in lean and obese women, as a consequence no difference can be found. These results suggest a reduction of the vegetative modulation in obese young women and the reduction of the autonomic control regards both the sympathetic and parasympathetic components (Monda et al., 2006b). The reduction of the sympathetic branch could be an important factor in the maintenance of obesity in premenopausal age. Indeed, a reduction in the sympathetic activity could be linked to a low energy expenditure, so that a reduced energetic cost could explain the higher body weight in premenopausal women. In this experiment, the autonomic activity of postmenopausal women is lower than that of premenopausal subjects, though a better indicator of the sympathetic activity would be very low frequencies (Fleisher et al., 1996). This indicates that the modifications of the autonomic modulation cannot be included among factors related to obesity in postmenopausal subjects. Many experimental evidences have demonstrated that an increase in sympathetic and thermogenic activity reduces food intake. Therefore, the obesity can be due to an increase in food intake associated to a reduced activity of the sympathetic nervous system. On the other hand, a study revealed lower respiratory sinus arrhythmia, as evaluated by the HF-HRV spectral analysis combined with deep breathing tests, which points to the presence of cardiac vagal dysfunction in obese adolescents (Tonhajzerova et al., 2008). Importantly, autonomic imbalance with decreased parasympathetic activity maybe the final common pathway in numerous conditions associated with increased morbidity and mortality (Thayer and Lane, 2007). The evaluation of cardio respiratory interactions, in particular the heart rate variability, can provide diagnostic information about early subclinical autonomic dysfunction in obesity. Traditionally, there have been two hypotheses about the nature of the predominate abnormality in SNS behavior in human obesity. Bray (1991) used the acronym "MONA LISA" to describe his hypothesis that Most Obesities kNown Are Low In Sympathetic Activity. This vision was based principally on studies in rodents that exhibited low SNS activity and morbid obesity following lesions in the ventromedial hypothalamus. As such, low SNS activity was considered causal in the development of obesity. In contrast, Landsberg (1986) viewed SNS activation targeting the heart, blood vessels and kidneys as a critical relation to the well documented relation between obesity and hypertension (Hall, 2003; Davy and Hall, 2004).

PARADOXICAL EATING BEHAVIOR: HYPERPHAGIA AND HYPOPHAGIA BY OREXIN-A

Since orexin-A is able to induce both the activation of thermogenesis and hyperphagia, Monda et al. (2003) tested the possibility that a previous thermogenic activation induced by orexin-A can modify eating behavior. Food intake and body temperature were monitored in 24 h-fasting male Sprague-Dawley rats for 15 h after food presentation during the dark period. Orexin-A was injected into the lateral cerebral ventricle 6 h before food presentation. Food intake and body temperature were controlled also in rats receiving orexin-A at the same time of food presentation. Orexin-A caused the same elevation of body temperature in both groups, while food intake was significantly lower in the group receiving orexin-A 6 h before food presentation in comparison to the other group. This study demonstrated that the effects on food intake induced by orexin-A depend on the time of food presentation. This suggest to revise the role of orexin-A in the control of food intake. The name assigned to this peptide was due to the strong increase in food intake after an orexin-A administration, assigning a fundamental role in the induction of food intake (Wolf, 1998; Shiraishi et al., 2000). The results

of the above publication call for a re-discussion of this role, underlining the importance of orexin-A in the control of the sympathetic activity and body temperature, which in turn affects food intake. An ICV injection of orexin-A induces an increase in the sympathetic activity and in the body temperature independently of food ingestion, that is reduced in the rats with a delayed presentation of food. This suggests that the effects on body temperature are prevalent with respect to eating behavior. Then, orexin-A can induce hyperphagia, but also hypophagia, contradicting the significance of this name that assign a primary hyperphagic effect to this peptide. For this reason, orexin-A cannot be considered a substance with a primary hyperphagic effect.

Orexin-A can induce hypophagia or hyperphagia (Shiraishi et al., 2000), but it always induces an activation of thermogenesis (Monda et al., 2004b, 2008b). We believe that this peptide elevates the thermoregulatory set-point, inducing the reactions to reach the new level of body temperature. The increase in food intake, obtained in the rats with a non-delayed presentation of food, could be a reaction aimed to reach an elevated body temperature. Indeed, food ingestion induces a rise in body temperature due to postprandial thermogenesis (De Luca et al., 1987; Tentolouris et al., 2006; Monda et al., 2008a; Messina et al., 2012, 2013a). The hyperphagic effect of orexin-A disappears when the body temperature is already increased, so that a reduction in food intake can happen in this condition.

Although selective activation of orexin neurons directly can elicit eating behavior (Inutsuka et al., 2014), the above-reported demonstrations support the idea that orexin-A controls body temperature and subsequently eating behavior.

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Dopamine and glucose, obesity, and reward deficiency syndrome

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Obesity as a result of overeating as well as a number of well described eating disorders has been accurately considered to be a world-wide epidemic. Recently a number of theories backed by a plethora of scientifically sound neurochemical and genetic studies provide strong evidence that food addiction is similar to psychoactive drug addiction. Our laboratory has published on the concept known as Reward Deficiency Syndrome (RDS) which is a genetic and epigenetic phenomena leading to impairment of the brain reward circuitry resulting in a hypo-dopaminergic function. RDS involves the interactions of powerful neurotransmitters and results in abnormal craving behavior. A number of important facts which could help translate to potential therapeutic targets espoused in this focused review include: (1) consumption of alcohol in large quantities or carbohydrates binging stimulates the brain's production of and utilization of dopamine; (2) in the meso-limbic system the enkephalinergic neurons are in close proximity, to glucose receptors; (3) highly concentrated glucose activates the calcium channel to stimulate dopamine release from P12 cells; (4) a significant correlation between blood glucose and cerebrospinal fluid concentrations of homovanillic acid the dopamine metabolite; (5) 2-deoxyglucose (2DG), the glucose analog, in pharmacological doses is associated with enhanced dopamine turnover and causes acute glucoprivation. Evidence from animal studies and fMRI in humans support the hypothesis that multiple, but similar brain circuits are disrupted in obesity and drug dependence and for the most part, implicate the involvement of DA-modulated reward circuits in pathologic eating behaviors. Based on a consensus of neuroscience research treatment of both glucose and drug like cocaine, opiates should incorporate dopamine agonist therapy in contrast to current theories and practices that utilizes dopamine antagonistic therapy. Considering that up until now clinical utilization of powerful dopamine D2 agonists have failed due to chronic down regulation of D2 receptors newer targets based on novel less powerful D2 agonists that up-regulate D2 receptors seems prudent. We encourage new strategies targeted at improving DA function in the treatment and prevention of obesity a subtype of reward deficiency.

Keywords: obesity, glucose craving, dopamine release, glucoprivation, neurogentics, reward deficiency syndrome

Reward Deficiency Syndrome (RDS; Blum et al., 1996) caused by a Brain Reward Cascade dysfunction is linked to polymorphisms in the Dopaminergic system that cause hypo-dopaminergic function and result in abnormal craving behavior (Zhu and Shih, 1997). Dopamine, a very powerful neurotransmitter, controls feelings of well-being. The complex interactions of powerful neurotransmitters like serotonin, enkephalins, and GABA that ultimately regulates dopaminergic activation of the Reward Center of the brain has been characterized by Blum as "The Brain Reward Cascade" (Blum and Kozlowski, 1990; Blum et al., 2000).

While, for example, high levels of enkephalins are associated with pain suppression and low serotonin levels with depression, an individual with the *Taq1 A1* allele of the Dopamine Receptor Gene (*DRD2*), lacks enough dopamine receptor sites to release the normal amount of dopamine into the Reward Center of the brain and dopamine function is reduced (Noble et al., 1991; Delis et al., 2013). Humans possessing the A1 variant crave and seek substances and behaviors known to cause dopamine release preferentially at the nucleus accumbens (NAc) in the meso-limbic system (Stice and Dagher, 2010). They may become serious cocaine abusers or have unhealthy appetites, which lead to, eating disorders like obesity, overeating or at the other extreme, anorexia nervosa (AN), they also suffer from high levels of stress over an extended period of time. To activate their dopaminergic pathways, a self-healing process to offset their low D2 receptors, individuals are driven to engage in activities which will increase brain dopamine function (Noble et al., 1991, 1993; Delis et al., 2013). The consumption of alcohol in large quantities or carbohydrate binging stimulates the brain's production of and utilization of dopamine (Blum et al., 1996, 2000). So too does the intake of crack cocaine, cocaine, opioids, and the abuse of nicotine. Aggressive behavior has also been associated with this genetic abnormality which also stimulates the brain's use of Dopamine (Blum et al., 2000).

Reward deficiency syndrome manifests as a form of sensory deprivation the pleasure or reward mechanisms and can be relatively severe or mild, a consequence of an individual's neurochemical inability to derive pleasure from ordinary, everyday activities. The A1 variant of the *DRD2* gene generates an alteration in the reward pathways and has been associated in neurogenetic research with a spectrum of addictive, compulsive and impulsive behaviors. The RDS concept unites these disorders and may help to explain how simple genetic anomalies can give rise to complex aberrant behavior (Noble et al., 1991, 1993; Blum et al., 1996, 2000; Delis et al., 2013).

One notable study from Thanos et al. (2001) provides support for the role of the *DRD2* gene in alcohol intake in rats. Utilizing a cDNA construct, a precursor of the *DRD2* gene, was implanted into the NAc of rats. After 4 days of treatment, dopamine D2 receptors increased above pretreatment levels by 150% and alcohol drinking was halved. After 8 days in total, D2 receptor densities and alcohol drinking returned to pretreatment levels. Second injections of the same construct 24 days later, similarly increased DRD2 density, this time, with a twofold decrease in drinking (Thanos et al., 2001). This phenomenon had also been observed for cocaine dependence (Thanos et al., 2008b). In another study the same group (Wang et al., 2001) using positron-emission tomography (PET) scanning techniques, reported low D2 receptor density in the obese subjects compared to non-obese controls. The D2 receptor paucity also correlated with high body mass index (BMI).

In another study, Hamdi et al. (1992) found that dopamine D2 receptor availability in the striatum was significantly lower in obese Zucker rats than in lean non-Zucker controls. Moreover, others have shown the availability of the dopamine striatal dopamine transporter was negatively correlated with BMI in healthy volunteers (Chen et al., 2008), suggesting that the dopamine system regulates BMI. Thus, in obese subjects dopamine deficiency may promote compensatory pathological eating to activate reward circuits. Strategies with the goal of improving dopamine function may be of benefit in treating obese individuals (Curtis and Davis, 2014).

To understand the important relationship between dopamine and glucose, it is beneficial to realize that, in the meso-limbic system the enkephalinergic neurons are in close proximity, to glucose receptors. There are other important connections in the substatia nigra (SN), tuberoinfundibular neurons, globus pallidus, and other brain regions (Haltia et al., 2007).

It is well known that glucose by the actions of an ATP-sensitive potassium channel modulates GABA terminal transmitter release and SN dopamine neuronal activity. In a study, Levin et al. (2001) placed microdialysis probes into both the SN and striatum of male rats to assess the effect of altered SN glucose levels on striatal dopamine release. Striatal DA efflux transiently increased by 50% during 50 mM glucose infusion, returning to baseline after 60 min. Moreover, when GABA (A) antagonist bicuculline was added the efflux increased by a further 30%. Furthermore, nigral bicuculline alone raised striatal dopamine efflux by 31% above basal glucose levels supporting the well-known tonic GABA inhibitory input to the DA neurons. Thus changing SN glucose levels effects striatal dopamine release. Levin and associates suggest that this response may reflect the known effect of glucose on GABA axon terminals in the SN and SN Dopamine neurons, via K(ATP) channel activity and could be the mechanism by which glucose modulates the motor activity involved in food intake (Levin, 2000). Koshimura et al. (2003) found that long – term incubation with a high concentration of glucose increased the capacity of calcium uptake to enhance depolarization-induced dopamine release from Pheochromocytoma 12 (P12) cells. Taken together, these data suggest that highly concentrated glucose activated the calcium channel to stimulate dopamine release from P12 cells.

Bello et al. (2003) found that the rat dopamine transporter was up-regulated in the ventral tegmental area and the NAc of the brain when feeding was restricted with scheduled sucrose access. Moreover, Lee et al. (1988) found that dopamine can lower glucose uptake into rat white adipocytes that do not have dopaminergic receptors, by activating B3 adrenoreceptors. Glucose utilization in the direct and the indirect pathways, of the rat basal ganglia is affected by injection of intrastriatal D1 and D2 dopamine agonists (Conti et al., 2001). Depending on dosage and time after treatment fat intake in rats can be altered by dopamine receptor antagonism. In this regard, both D1 and D2 receptor co-activation significantly increased protein mass while reducing body fat, body weight, food consumption and serum concentrations of triglycerides, free fatty acid glucose, and insulin (Cincotta et al., 1997). Blood glucose studies found a significant correlation between blood glucose concentrations and cerebrospinal fluid concentrations of homovanillic acid the dopamine metabolite (Umhau et al., 2003).

The RDS hypothesis (Blum et al., 1996), embraces the concept that a genetic commonality exists between dopamine activating substances like alcohol, the opiates and even glucose. Evidence now exists that excessive sugar intake, if intermittent, can induce endogenous opioid dependence. In rats, following repeated intake of excessive sugar an opioid antagonist induced neurochemical and behavioral signs of opioid withdrawal. The indices of dopamine/acetylcholine (Ach) imbalance and anxiety were similar in quality to withdrawal from nicotine or morphine, suggesting that the rats had become sugar-dependent (Colantuoni et al., 2001).

In terms of understanding the brain reward cascade there is evidence that serotonergic activation may also influence dopamine D2 receptor function. This is of interest when we consider the so-called "sweet tooth" which has predominantly been associated with serotonin. The work by Kogan et al. (2002) confirmed that DR4004, a putative 5-HT7 receptor antagonist, has functionally activated the dopamine D2 receptor. Neuroanatomical data suggest that there may be an interactive role between NAc Ach and dopamine. There is evidence that NAc Ach is related to natural consummatory behavior, like feeding, as well as, the neural processes that elicit reward from psychostimulants. In this regard, Hajnal et al. (2000) found that cholinergic interneurons in the accumbens have a role in body weight regulation and metabolism. In this context, both stress and dopamine play an important part in the Ach response.

It is well known that in preclinical studies 2-deoxyglucose (2DG), the glucose analog, in pharmacological doses is associated with enhanced dopamine turnover and causes acute glucoprivation. In fact, indications from lines of evidence are that dopamine

release is associated with a variety of metabolic stressors that include acute glucose deprivation. Adler et al. (2000) using PET found that synaptic dopamine concentrations were enhanced by 2DG administration. In healthy volunteers, the administration of 2DG was associated with significant striatal dopamine release. These data are important because it further ties glucose levels to dopaminergic activity. Moreover, there is a relationship between dopamine release and insulin levels in the tuberoinfundibular neurons. This insulin effect is dependent on the CA++ ions, protein kinase C Na (+) – H + exchange system. Additionally, when glucose in the brain is lowered and leads to global cerebral transient ischemia, monamine release of dopamine, especially, is inhibited. In this regard, Trugman and James (1993) showed that D1 antagonists lowered glucose utilization by 24–28%; in the globus pallidus, entopeduncular nucleus, subthalamic nucleus, SN, and in the motor cortex, suggesting that by stimulation of the D1 receptor, endogenous dopamine makes a contribution in these regions to basal metabolism. In contrast with these results both D1 and D2 agonists increase glucose utilization suggesting that stimulation of D1 and D2 receptors is tied to feeding behavior. Thus, the importance of the D1 and D2 functional linkage in the brain is established by this metabolic evidence, which relates to overeating (hyperphagia).

That dopamine induces hyperglycemia in both animals and man is well known. The direct effects of dopamine on the release of glucose from primary cultured rat hepatocytes were studied in Japan by Shiroyama et al. (1998). The authors investigated the effect of dopamine on glucose release through the gluconeogenic and/or glycogenolytic pathways and found the main adrenergic receptor type beta 2, involved in glucose release. The hypothesis is that increasing the release of glucose from tissue would reduce cravings for carbohydrates and glucose. In this regard Shiroyama et al. (1998) supported this notion. Glycogen-rich and gluconeogenic-depleted hepatocytes were prepared in order to study glycogenolytic and gluconeogenic-depleted glucose release, respectively. Dopamine was shown to cause the release of glucose and the beta blocker propranolol was shown to inhibit this release. The authors conclude that mediated by beta adrenergic receptors dopamine has a direct effect on hepatocytes of increasing glucose release in the glycogenolytic and gluconeogenic pathways.

Freeman et al. (2001) studied the effect of glucose on changes induced in dopamine neuronal activity by anti-psychotic drugs and suggested antipsychotic drug-induced changes in midbrain dopaminergic neuron population activity may be influenced by caloric intake. Glucose did, in fact, reduced significantly the number of A9 and A10 dopaminergic cells that were spontaneously active per track in control rats, but attenuated significantly the chronic haloperidol- and clozapine-induced reductions in dopaminergic cells per track.

Certainly, the compulsion and the loss of control observed in the drug taking behaviors of drug-addicted subjects is similar to overeating by obese individuals. Although not well understood the mechanisms of these behaviors were studied by Michaelides et al. (2012) utilizing PET in drug-addicted subjects. Reductions in striatal DA D2 receptors were documented. In pathologically obese subjects, the same researchers found striatal DA D2 receptors reductions similar to those found in drug-addicted subjects. Moreover, DA D2 receptor levels were inversely related to the BMI of the obese subjects. Michaelides et al. (2012) postulated that decreased DA D2 receptors levels predisposed subjects to search for reinforcers; drug of choice in the case of drug-addicted subjects and food in the case of the obese subjects to compensate temporarily for a decreased sensitivity of reward circuits regulated by the activity of DA D2 receptors.

Discovery of strategies for the treatment of obesity will be assisted by better understanding of the mechanisms involved in food intake. Stice et al. (2010, 2012, 2013) and Stice and Yokum (2013) researched these mechanisms and found that carriers of the DRD2 A1 allele and other reward gene polymorphisms have a blunted response to palatable food reward and carriers of D2 and D4 polymorphisms also gained weight in a 1-year follow-up. Recent studies from Stice et al. (2013) showed an elevated brain reward response to money cues in adolescents with a parental substance use disorder, and they suggested support for the reward surfeit model rather than the reward deficit model and as such it is different from prediction of obesity. Stice et al. (2013) may not have considered the role of supersensitive D2 high receptors as suggested by Seeman and Seeman (2014). This is a very complex mechanism involving epigenetic effects in cases of substance use especially in parental substance abuse. The well-known high risk for relapse in carriers of the *DRD2* AI allele (Dahlgren et al., 2011) could be in part due to proposed dopamine receptor supersensitivity (Blum et al., 2009). Furthermore, decreased reward and negative eating behaviors in obesity are accompanied by diminished dopaminergic neurotransmission. Bariatric surgery the most successful therapy for obesity rapidly reduces hunger and improves satiety, the mechanisms are unknown and little is known about dopaminergic activity following this surgical procedure. Dunn et al. (2010) has hypothesized that after Vertical Sleeve Gastrectomy (VSG) or Roux-en-Y-Gastric Bypass (RYGB) surgery dopaminergic neurotransmission would be affected, influence eating behaviors and would contribute to the positive outcomes from bariatric surgery. The results of their study reported an expected body weight decreased and a decrease in DA D2 receptor availability after surgery. These changes were accompanied by significant decreases in plasma insulin of (62%) and plasma of leptin (41%), regional decreases in DA D2 receptors (mean ± SEM) were putamen 9 ± 4%, ventral striatum 8 ± 4%, caudate 10 ± 3%, amygdala 9 ± 3%, hypothalamus 9 ± 3%, substantia nigra 10 ± 2%, and medial thalamus 8 ± 2%. Volkow and associates (Dunn et al., 2010) point out that decreased DA D2 receptor availability following VSG and RYGB is most likely reflected in increased levels of extracellular dopamine. Although better dopaminergic neurotransmission may improve eating behavior with improved satiety and reduced hunger after bariatric procedures, in the longer term a decrease in brain D2/D3 receptor availability may enhance addiction liability and addiction transfer or even cross tolerance. The finding of decreased D2/D3 availability may explain in part the increased risk of drug seeking behavior reported following bariatric surgery. Our hypothesis that the real culprit in obesity may be RDS is supported by this finding (Blum et al., 2011a,b). Increased alcohol intake following bypass surgery was reported by Hajnal et al. (2012) and a reduced reward-related (e.g., striatal) neural activation has been observed following bariatric surgery. Studies by Ochner et al.

(2012) reveal that post-operatively reduced mesolimbic responsively was associated with reductions in wanting, high-versus low-calorie foods but not in liking for high caloric foods. These findings support the hypothesized delineation between wanting and liking; the idea that wanting, but not liking is a dopaminergic reward pathway process (Blum et al., 2012a).

Interestingly, in animal models a predisposition in offspring to food addiction was caused by feeding rat mothers fatty, sugary, and salty snacks (junk food) during pregnancy and lactation. Compared to controls rat offspring demonstrated an increase in weight and BMI, their mothers displayed binge eating and junk food overeating behaviors (Ong and Muhlhausler, 2011). These observations may be of relevance to pregnant women with eating disorders and obese women treated with bariatric surgery, in order for them to have healthy children with normal appetites and weight. One must also consider the negative consequences of the hypodopaminergic genetics involved in RDS including obesity.

In support of hypodopaminergic genetics and sugar addiction Avena et al. (2012), found clear evidence that sugar shares the characteristics of addiction neurochemicals, since, like addictive substances, it releases both opioids and dopamine. These authors Avena et al. (2013a,b) classified sugar as addictive, because it follows the typical addiction pathway consisting of binging, withdrawal, craving and cross-sensitization delineated by Blumenthal and Gold (2010) and Blum et al. (2011a,b).

In fact, cross-sensitization was observed in rats showing the movement from to sugar to drugs (Gosnell, 2005). Surprisingly work by Cantin et al. (2010) on a comparative evaluation of the large majority of rats with a history of cocaine addiction, cocaine is valued similarly to the lowest concentrations of sweet water. Additionally, all experiments from the previous 5 years were evaluated. The retrospective analysis revealed that most rats will give up cocaine use in favor of saccharine, the non-drug alternative. A minority, at the heaviest level of past cocaine use <15%, continued to take cocaine and in spite of being hungry, chose cocaine rather than a natural sugar that could relieve their need for calories. Most importantly Cole et al. (1990) suggest that initiation into addiction requires, sensitization and cross tolerance, thus, this model fits for sugar. It is of interest that the withdrawal from sugar induces imbalances in both Ach and dopamine similar to opiate withdrawal. Specifically, Avena et al. (2008) using microdialysis found an increase in extracellular Ach and a decrease in dopamine release, in the NAc shell, in rats undergoing withdrawal from sugar binging. This finding suggest that a state, that involves anxiety, an altered accumbens dopamine and Ach balance is induced by intermittent binging on sucrose and chow followed by fasting. This is similar to withdrawal from opiates following naloxone and may be a feature of some eating disorders.

While there are these similarities between the addictiveness of food and drugs, its validity as a model of obesity has been questioned based on the idea that food is not a psychoactive drug (Sansone and Sansone, 2013). With that said, at the Columbia University Seminar on Appetitive Behavior, the concept of "food Addiction" was one of various proposed causes of the obesity epidemic. This has been vigorously debated in the media (Avena, 2010), as well as in the scientific community (Michaelides et al., 2013). Moreover, the criteria in the Diagnostic & Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) pertaining to substance abuse, has been applied to food addiction in humans based on ever increasing evidence in animal and humans (Gold and Avena, 2013). In terms of sugar being considered a psychoactive substance, clinical accounts from self-identified food addicts describe using food to self-medicate; they eat in order to change a negative mood state (Blum et al., 2013). Behaviors reported by self-identified food addicts conform to the seven DSM-5 criteria for substance use disorders (Campbell et al., 2013). This notion of commonality has been confirmed by studies that show that food craving, in both normal weight and obese patients, activates similar areas of the brain to those indicated in drug seeking (Wise, 2013). Avena (2010) adequately defined *binging, withdrawal, and craving* by presenting evidence from animal models of binge eating of sucrose or glucose, in a review that summarized evidence for "food addiction." In a *PANTHER* analysis of gene array expression performed on 152 unique genes, resulting in a total of 193 multiple-factor (MF) assignments, sorted into 20 categories (Avena et al., 2010; Blum et al., 2012a,b) found gene clusters expressed significantly differently, in the *ad libitum* sucrose group compared to the sucrose binge eating group. These clusters seem to be convergent with the neurotransmitters involved in the brain reward circuitry like serotonin; endorphins; GABA; dopamine; cannabinoids; ACH and leptin, and specifically in the brain reward cascade (Yarnell et al., 2013) and RDS (Downs et al., 2013).

CONTROVERSIAL FINDINGS

Since the original finding by Blum et al. (1990) first to associate the *Taq-A1* of the dopamine D2 receptor gene polymorphism and severe alcoholism there have been controversial findings possibly due to poor control screening. One example of poor screening and negative findings relative to the role of dopaminergic gene polymorphisms and reward seeking behavior as well as parenting is observed in the work of Creemers et al. (2011) from a Dutch general population. This problem exists even in the current literature.

We have cautioned against including RDS behaviors in the control group which could lead to spurious results. Since that time there have been no less than 3738 (Pubmed-6-23-14) peer reviewed articles on many peripheral and central nervous system (CNS) behaviors and physiological processes. Understandably addiction or even the broader term RDS involves very complex gene × environment interaction and one cannot expect that a single gene like the DRD2 gene would have a powerful effect by itself, however, albeit many negative findings, there is still a plethora of evidence for the role of the DRD2 gene polymorphisms and a number (small sample of studies represented herein) of addictive and other reward dependent behaviors including: *alcohol dependence* (Pato et al., 1993; Ponce et al., 2003; Munafò et al., 2007; Smith et al., 2008; Pinto et al., 2009; Grzywacz et al., 2012; Wang et al., 2013); *drug dependence* (Li et al., 2004; Xu et al., 2004; Young et al., 2004; Barratt et al., 2006; Li et al., 2006; Hou and Li, 2009; Chen et al., 2011a,b; Al-Eitan et al., 2012; Jacobs et al., 2013; Lee et al., 2013; Ohmoto et al., 2013; Sullivan et al., 2013; Suraj Singh et al., 2013; Vereczkei et al., 2013; Wang et al., 2013; Clarke et al., 2014; Roussotte et al., 2014; Schuck et al., 2014); *mood disorders*

(Vaske et al., 2009; Huertas et al., 2010; Zhu et al., 2011; Zou et al., 2012; Hettinger et al., 2012; Jutras-Aswad et al., 2012; Tsuchimine et al., 2012; Whitmer and Gotlib, 2012; Zai et al., 2012; Peciña et al., 2013; Zhang et al., 2014); *rearing behaviors* (Mills-Koonce et al., 2007; Bakermans-Kranenburg and van Ijzendoorn, 2011; Beaver and Belsky, 2012; Masarik et al., 2014); *obesity* (Spangler et al., 2004; Fang et al., 2005; Huang et al., 2005; Epstein et al., 2007; Nisoli et al., 2007; Barnard et al., 2008; Blum et al., 2008; Eny et al., 2009; Epstein et al., 2010; Mathes et al., 2010; Stice et al., 2010; van Strien et al., 2010; Jabłoński, 2011; Anitha et al., 2012; Chen et al., 2012; Winkler et al., 2012; Ariza et al., 2013; Carpenter et al., 2013; Cameron et al., 2013; Hess et al., 2013; Alsiö et al., 2014); *Anorexia Nervosa* (Bergen et al., 2005); *motivation* (Trifilieff et al., 2013); *brain metabolism* (Noble et al., 1997); *ADHD* (Gold et al., 2014), and *pathological gambling* (Gyollai et al., 2014).

It has been argued that the significance of the *Taq 1A* polymorphism is presumed to be related to decreased nucleus accumbens neurotransmission leading to reward deficiency. While human imaging studies have reported lower levels of striatal DA D2 receptors in subjects with the *Taq 1A* polymorphism, the significance is less clear. In subjects with the *Taq 1A* polymorphism 18F 6FDOPA studies have reported significantly increased striatal uptake of 18F 6FDOPA consistent with increased DA synthesis. If there is increased DA synthesis and release, the decreased apparent levels of striatal DA D2 receptors may be due to increased extracellular DA levels. Increased synthesis may be due to a decrease in striatal D2 auto-receptors. While this may be correct the surfeit theory of drug dependence may be incorrect. In fact, surfeit concepts have been also made with regard to escalation of cocaine abuse claiming that the increased abuse is due to increased dopaminergic activity in the accumbens. However, recent clear evidence from Willuhn et al. (2014) dispels this and suggests otherwise that escalation of cocaine abuse is due to low dopaminergic function. In fact utilizing sophisticated analyses they suggested that agonistic not antagonistic intervention would be prudent in terms of treating all addictions.

In terms of BMI and dopaminergic gene polymorphisms and subsequent associations there is controversy especially with regard to the dopamine transporter gene. As stated earlier Chen et al. (2008) reported a significant negative correlation between BMI and striatal DAT1 levels. However, others did not find this association in so called obese healthy subjects (once again not very well screened for RDS behaviors), albeit a larger cohort in the van de Giessen et al. (2013). In addition this non-association was also reported by Thomsen et al. (2013) as well utilizing so called healthy obese subjects. However, there are number of other reports which support the DAT1 negative association with BMI including: Need et al. (2006), Fuemmeler et al. (2008), Wang et al. (2011), Sikora et al. (2013), and Valomon et al. (2014). With these studies in favor of an association of dopaminergic gene polymorphisms and even a study showing that methamphetamine known to block DAT1 reducing fat and carbohydrate intake (Danilovich et al., 2014), there is real controversy concerning the actual role of BMI as a biological marker for obesity relative to percent body fat, as clearly pointed out by Shah and Braverman (2012). This notion is highlighted in a study from Chen et al. (2012) that shows a significant correlation with carriers of the DRD2 *Taq-A1* and higher percent body fat compared to DRD2 *Taq-A2*.

There is some controversy concerning a conclusive statement that sugar addiction may lead to obesity (Hone-Blanchet and Fecteau, 2014). However, the evidence seems to favor a bond between Substance Use Disorders, as clinically categorized in the DSM 5, and food reward (see Brownell, 2012) including an article by Gold and Avena (2013).

In terms of eating disorders there have been a number of reports indicating the potential link between reward gene polymorphisms and binge eating (Davis et al., 2008). The finding that obese carriers of the DRD2 *Taq-A1* allele had higher reward sensitivity (binging behavior) compared to normal weight controls favors the surfeit theory rather than the deficit RDS concept. While this might be true we must caution against these findings because the controls in the study may not reflect a phenotype free of all RDS behaviors which could lead to spurious results. The suggestion of blocking Dopamine activity in the reward circuitry may be of interest in the short term but damaging in the long term as discussed in this article (Blum et al., 2012a). Interestingly, Gearhardt et al. (2011) also found differential neuro-correlates to food scores in healthy young women lean to obese. Food addiction scores correlated with greater activation in the anterior cingulate cortex, medial orbitofrontal cortex, and amygdala in response to anticipated receipt of food. Furthermore, food addiction scores showed greater activation in the dorsolateral prefrontal cortex and the caudate in response to anticipated receipt of food but less activation in the lateral orbitofrontal cortex in response to receipt of food. This particular study portrays the complexity of attempting to dissect brain reward function related to eating behavior.

While there is evidence from Stice's group that polymorphisms in both dopamine D2 and D4 result in a blunted response to palatable foods and subsequent weight gain (Stice et al., 2008a,b,c, 2010) a paper by Stice et al. (2011) showed in youth that increased striatal dopamine neurotransmission may also be a risk factor for obesity using fMRI. Certainly this supports the surfeit dopamine theory proposed by Robinson and Berridge (2000), and correctly suggests that obesity is a complex disorder and based on both genetics and environment (epigenetics) individuals having increased motivation for food may fall into two categories (based on gender, age of onset, etc.) either deficit or surfeit in terms of dopaminergic function. More research is required to carefully dissect these differences in the future in terms of "liking and wanting" (Blum et al., 2012a; Willuhn et al., 2014).

We have discussed the potential problems associated with bariatric surgery such as transfer of addiction (Blum et al., 2011a,b) and the work of Dunn et al. (2010) revealing reduced D2R availability (hypodopaminergic state) following bariatric surgery suggestive of increased requirement for self- administered drugs or behaviors linked to dopaminergic activation. Interestingly in five obese subjects Steele et al. (2010) found that pre bariatric surgery the obese subjects had a lower D2 R availability compared to post-surgery levels 6 weeks after surgery whereby it was found that D2R availability increased. This of cause would suggest reduced drug and or addictive behavioral seeking behaviors linked to enhanced dopaminergic function. The question which remains is that the findings by Dunn et al. (2010), was post 7 weeks compared to

6 weeks by Steele et al. (2010) and could this represent a downward trend leading to once again a hypodopaminergic trait. An important question as it relates to our proposed theories regarding transfer of addiction following even longer periods post-bariatric surgery seems prudent.

In addition while we have pointed out that there is evidence for a decreased availability of D2R in obese subjects (Volkow et al., 2009) there is some controversy that argues this is only true for severe obesity (Eisenstein et al., 2013; Kessler et al., 2014). Once again we evoke the concept that using BMI as a factor is not an appropriate phenotype (other RDS behaviors may be a confound variable) and that mild obesity may not indicate the real disorder. This has been underscored by the need for alcoholism severity as the true endophenotype (Blum et al., 1990; Connor et al., 2002). Importantly, Volkow's group have now published at least 13 papers supporting their original concept of low D2R availability and obesity (Tomasi and Volkow, 2013), however lowered D2R availability was not found with novelty seeking in obesity (Savage et al., 2014).

While additional studies are required to determine the link between dopaminergic function and AN as well as other eating disorders, there are a number of neurogenetic reports. Gervasini et al. (2013) showed a number of interesting associations with dopamine gene polymorphisms including: DRD4 variable number of tandem repeats (VNTR) 7R/7R was significantly associated with greater risk for AN; significant differences in asceticism scores between DAT1 VNTR genotypes; and significant differences in Drive for Thinness and Body Dissatisfaction between DRD4- C-616G genotypes. Moreover, Nisoli et al. (2007) found that independent of obesity, the A1+ allele, both in A1/A1 and A1/A2 genotypes of the DRD2 gene significantly associated with the Drive for thinness and Ineffectiveness. The A1+ allele, both in A1/A1 and A1/A2 genotypes, was not differently distributed among disease groups; on the contrary two EDI subscales (Drive for thinness and Ineffectiveness) resulted in association with A1+ allele without effect of the eating disease or obesity. This finding suggested that the A1+ allele is not simply related to body weight but the A1+ allele is a marker of a genetic psychological trait in humans with high risk to develop pathological eating behavior. In fact other work from National Institute of Alcohol Abuse & Alcoholism (NIAAA) has clearly shown significant linkage disequilibrium between the −141 Indel and two exon seven SNPs (939Y and 957Y) of the DRD2 gene was observed over a distance of >50 kbp in the AN probands but not in the controls. This further suggests that transmitted variation in D2 dopamine receptor affecting transcription and translation efficiency plays a role in vulnerability to AN.

KB220 COMPLEX

It is of interest that the complex KB220Z and variants thereof have overcome brain reward circuitry abnormalities, in protracted abstinent psychostimulant abusers observed using qEEG analysis. In fact, KB220Z following only one oral dose of 24 g resulted in an increase in alpha bands with a concomitant increase in low beta bands after 1 h, an effect which usually requires 10–20 biofeedback sessions. This is further supported by preliminary work in China using fMRI showing direct significant activation of dopaminergic pathways compared to placebo during the resting state (Blum et al., 2012b). Thus if KB220Z stimulates dopamine release then it is quite possible that the released dopamine will have an impact on glucose release, which could offset abnormal glucose or even food cravings. We must await further required research to determine the benefits induced by this putative natural D2 agonist especially investigating functional magnetic resonance imaging resting state functional connectivity (rsfMRI) in rodents.

In summary, typically, obesity is associated with abnormal eating behaviors. Brain imaging studies, in both humans and animal models, for the most part, implicate the involvement of DA-modulated reward circuits in pathologic eating behaviors. It is known that food cues increase striatal extracellular DA, providing evidence for the role of DA in the non-hedonic motivational properties of food (Wang et al., 2009). In addition, food cues also increase brain metabolism in the orbitofrontal cortex suggesting the association of this region with enhanced "wanting" of food consumption. Importantly, similar to drug-dependent subjects, striatal DA D2 receptor availability is decreased in obese subjects, which may induce them to seek food (glucose and high fat) as a means to compensate temporarily for under-stimulated (deficient) reward circuits (Thanos et al., 2008a). Reduced DA D2 receptor densities are also associated with reduced responsively in both striatal and prefrontal regions involved in inhibitory control, which may provoke their inability to control food intake and as such weight gain. Interestingly, gastric stimulation especially in obese subjects activates limbic and cortical regions. These same brain regions are activated during drug craving in drug dependent individuals (Cyders et al., 2013). Moreover, obese subjects have enhanced sensitivity to the sensory properties of food. This fact coupled with a reduction of DA D2 receptors places obese subjects at high risk for uncontrollable eating behavior. As noted, in bypass surgery when eating is not an option, there is a transfer of addictive – like behaviors and subsequently drug – seeking may become the new reinforcement (Thanos et al., 2013).

Thus, we submit that results from these on-going investigations indicate that multiple, but similar brain circuits are disrupted in obesity and drug dependence. We encourage new strategies targeted at improving DA function in the treatment and prevention of obesity a subtype of reward deficiency (Avena et al., 2013a,b).

AUTHOR CONTRIBUTIONS

The authors contributed equally to this manuscript and all provided approval.

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Conflict of Interest Statement: Kenneth Blum is the holder of a number of patents in both the US and foreign countries involved with genetic testing and natural D2 agonistic activity to treat RDS including obesity. He is currently the owner of IGENE, LLC., Synaptamine, Inc. and co-owner of Victory Nutrition, LLC., and RD Solutions LLC. He is a paid consultant from Malibu Beach Recovery Center, Path Foundation NY, USA, RDSolutions, Victory Nutrition, and Chief Scientific Advisor Dominion Diagnostics, LLC. Mark S. Gold is a paid consultant of Malibu Beach Recovery Center. There no other conflicts to report.

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A possible link between BDNF and mTOR in control of food intake

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Food intake is intricately regulated by glucose, amino acids, hormones, neuropeptides, and trophic factors through a neural circuit in the hypothalamus. Brain-derived neurotrophic factor (BDNF), the most prominent neurotrophic factor in the brain, regulates differentiation, maturation, and synaptic plasticity throughout life. Among its many roles, BDNF exerts an anorexigenic function in the brain. However, the intracellular signaling induced by BDNF to control food intake is not fully understood. One candidate for the molecule involved in transducing the anorexigenic activity of BDNF is the mammalian target of rapamycin (mTOR). mTOR senses extracellular amino acids, glucose, growth factors, and neurotransmitters, and regulates anabolic reactions response to these signals. Activated mTOR increases protein and lipid synthesis and inhibits protein degradation. In the hypothalamus, mTOR activation is thought to reduce food intake. Here we summarize recent findings regarding BDNF- and mTOR-mediated feeding control, and propose a link between these molecules in eating behavior.

Keywords: BDNF, mTOR, mTORC1, food intake regulation, body weight, AMPK, nutrients

INTRODUCTION

Several lines of evidence indicate that neurons in the hypothalamus sense nutrient sufficiency. These neurons are regulated by many factors involved in feeding control, such as leptin (Morton et al., 2006). The roles of many other feeding-related peptides, both orexigenic and anorexigenic, have also been extensively studied. For example, clear obesity and leanness phenotypes are observed in knockout mice lacking pro-opiomelanocortin (POMC; a precursor of α-melanocyte-stimulating hormone (MSH); Yaswen et al., 1999) and melanin-concentrating hormone (MCH; Shimada et al., 1998), respectively. Recently, another factor involved in regulation of feeding and metabolic regulation in the brain has come into the spotlight: brain-derived neurotrophic factor (BDNF; Ooi et al., 2012; Rios, 2013; Vanevski and Xu, 2013; Marosi and Mattson, 2014).

BRAIN-DERIVED NEUROTROPHIC FACTOR

Brain-derived neurotrophic factor is the most prominent neurotrophic factor in the central nervous system. Indeed, BDNF and its cognate high-affinity receptor, TrkB, are widely expressed in the brain from development to adulthood. BDNF promotes differentiation, maturation, and survival of neurons, and plays important roles in synaptic plasticity through the activation of TrkB, a receptor tyrosine kinase (Nawa and Takei, 2001; Park and Poo, 2013). TrkB-expressing (i.e., BDNF-responsive) neurons are distributed in the arcuate nucleus (ARC), paraventricular nucleus (PVN), lateral hypothalamus (LH), ventromedial nucleus (VMH), and dorsomedial nucleus (DMN) of the hypothalamus (Yan et al., 1997).

BDNF AND REGULATION OF FOOD INTAKE

The first evidence that BDNF is involved in body weight control came from a rather serendipitous result. While assessing the neuroprotective effects of neurotrophins, Lapchak and Hefti (1992) found that chronic intracerebroventricular (ICV) infusion of BDNF in adult rats after fimbrial lesion reduced body weight. Subsequent systematic experiments also revealed that ICV injection of BDNF suppressed appetite and promoted weight loss in rats (Pelleymounter et al., 1995). The second clear line of evidence that BDNF plays crucial role in food intake comes from studies of genetically manipulated mice. Mice heterozygously deleted for the gene encoding BDNF (*Bdnf*$^{+/-}$) produce half of the wild-type level of BDNF protein and exhibit a severely obese phenotype due to overeating (Lyons et al., 1999; Kernie et al., 2000). Furthermore, brain-specific deletion of *Bdnf* (Rios et al., 2001), deletion of dendritic BDNF mRNA (Liao et al., 2012), shRNA-mediated knockdown of BDNF using a viral vector (Unger et al., 2007), and a hypomorphic allele of *Trkb* that expresses only a quarter of TrkB all result in hyperphagia, obesity, and metabolic imbalances such as hyperglycemia (Xu et al., 2003).

Genotype–phenotype interactions indicate that BDNF–TrkB signals also play important roles in weight control in humans. For instance, a *de novo* missense mutation of the *TRKB* gene, Tyr722Cys, which leads to a defect in downstream signaling, was identified in an 8-year-old male who presented with hyperphagia, severe obesity, and developmental delay (Yeo et al., 2004). Similarly, patients with Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) syndrome, who have a truncation of chromosome 11, exhibit hyperphagia and obesity. Analysis of the genomes of WAGR patients revealed that

they are heterozygous for deletion of *BDNF*. Patients with *BDNF* haploinsufficiency were all obese, whereas only 20% of WAGR patients without *BDNF* deletion were obese (Han et al., 2008). A common single-nucleotide polymorphism (SNP) of *BDNF*, G196A, produces the amino acid substitution Val66Met in the prodomain. The Val66Met mutant exhibits defects in intracellular trafficking and activity-dependent release of mature BDNF (Egan et al., 2003). A genome-wide association study linked this SNP to susceptibility to obesity in humans (Beckers et al., 2008; Speliotes et al., 2010; Waterhouse and Xu, 2013). Likewise, in an experimental model, G196A knock-in mice exhibit increased body weight (Chen et al., 2006). Because these mutations in humans are all genomic, not somatic, the effect of BDNF deficiency on metabolic abnormalities may arise from systemic and/or developmental activities. Indeed, it has been proposed that BDNF contributes to metabolism in peripheral organs (Nakagawa et al., 2000; Hanyu et al., 2003). However, brain- or hypothalamus-specific deletion or knockdown of BDNF induces overeating and obesity. These results suggest that BDNF acts directly on the hypothalamic circuit that regulates food intake and metabolism, thereby controling body weight. In addition, BDNF injection into the brain can rescue the obese phenotype of mutant mice. These results indicate that BDNF exerts its anorexic action in an acute, temporally specific manner, and that the effects of loss of BDNF on feeding behavior are not the result of developmental defects in neural circuits. BDNF also acts on midbrain dopaminergic neurons, which are involved in hedonic eating related to the reward/addiction system (Cordeira et al., 2010; Alsiö et al., 2012). Thus, this system may contribute to overeating in individuals carrying these mutations. Future studies should investigate these issues in greater detail.

Previous studies of eating behavior have focused on extracellular cues and neural circuits in the hypothalamus, but have not looked as closely at intracellular signaling mechanisms. Considering the acute effect of BDNF on food intake, we wondered what signaling molecules play major roles in BDNF-mediated feeding control. Mammalian target of rapamycin (mTOR), a kinase that governs metabolism in peripheral cells, has attracted attention as a regulator of food intake through the brain (Cota, 2009; Wiczer and Thomas, 2010; Howell and Manning, 2011; André and Cota, 2012). BDNF is a major activator of mTOR in neurons (Takei et al., 2001, 2004; Inamura et al., 2005) therefore, we hypothesize that the anorexigenic action of BDNF is mediated by mTOR in neurons. Before elaborating on this idea, we will provide a brief introduction of mTOR and its signaling pathways.

MAMMALIAN TARGET OF RAPAMYCIN

Mammalian target of rapamycin is the mammalian ortholog of yeast TOR, which is the target molecule of rapamycin, an anti-fungal and immunosuppressant compound. mTOR is a serine/threonine kinase that forms two complexes, mTOR complex 1 (mTORC1) and 2, which have different molecular partners. mTORC1 is a key component of the nutrient-sensing network that controls cellular metabolism: it integrates various extracellular cues, such as nutrients (amino acids and glucose) and growth factors, and it regulates various biochemical processes, including translation, autophagy, transcription, and lipid biosynthesis. These biochemical reactions induce anabolic states and thereby promote cell growth. The signaling pathways upstream and downstream of mTORC1 have recently been elucidated (Laplante and Sabatini, 2012; Takei and Nawa, 2014); we have provided a simplified schematic of neuronal mTOR signaling in **Figure 1** Leucine, taken up by the system L-amino acid transporter, activates mTORC1 (Ishizuka et al., 2008). Growth factors such as BDNF (Takei et al., 2001, 2004; Inamura et al., 2005), insulin (Lee et al., 2005), and insulin-like growth factor (Quevedo et al., 2002) activate the phosphoinositide 3-kinase (PI3K)/Akt pathway through their tyrosine kinase receptors in neurons. Akt directly phosphorylates tuberous sclerosis complex 2 (TSC2), a suppressor of Rheb that activates mTORC1. When glucose levels are sufficient, AMP-activated protein kinase (AMPK) activity decreases, and thus mTORC1 becomes active (Dash et al., 2006). These inputs converge on mTORC1; therefore, the availability of amino acids and/or glucose is essential for growth factor-mediated mTORC1 activation (Hara et al., 1998; Ishizuka et al., 2013). mTORC1 phosphorylates eukaryotic initiation factor 4E-binding protein (4EBP) and thereby stimulates translation. In addition, phosphorylation of p70S6 kinase (p70S6K) by mTORC1 also promotes translation and lipid biosynthesis, whereas phosphorylation of ULK1 inhibits autophagy. All of these processes increase total protein and lipid levels in the cell and thereby increase cellular mass (Laplante and Sabatini, 2012; Takei and Nawa, 2014). It should be noted that mTORC1 signaling is regulated by a feedback mechanism: mTORC1 and p70S6K phosphorylate

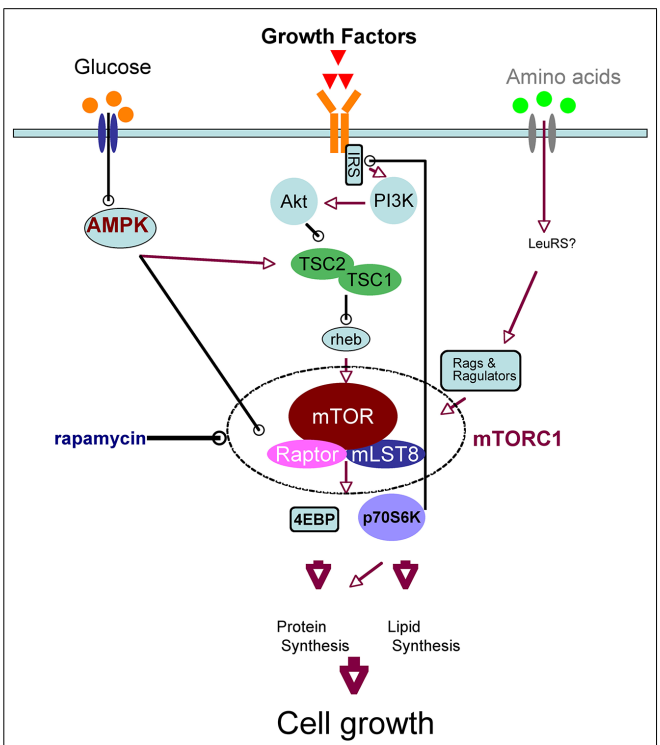

FIGURE 1 | Simplified schematic of upstream and downstream mTORC1 signaling.

and inactivate insulin receptor substrate (IRS; Tremblay and Marette, 2001; **Figure 1**), which interacts receptor tyrosine kinases such as insulin receptor and TrkB (Yamada et al., 1997). Thus, prolonged activation or overactivation of mTORC1 results in desensitization of this signaling cascade; this is thought to be one mechanism of insulin resistance (Tremblay and Marette, 2001).

mTOR AND REGULATION OF FOOD INTAKE

In the brain, the first hint of a relationship between food intake and mTORC1 signaling was provided by the involvement of AMPK on feeding. AMPK is a cellular fuel gage that senses the AMP/ATP ratio and turns off metabolic pathways that consume ATP (Kemp et al., 2003). Because AMPK itself plays a key role in metabolism, the regulation of hypothalamic AMPK was initially investigated independently of mTOR (Minokoshi et al., 2004, 2008). Leptin, insulin (Minokoshi et al., 2004), and cilliary neurotrophic factor (CNTF; Steinberg et al., 2006) reduce AMPK activity in the hypothalamus similarly to re-feeding after fasting; thus, these molecules suppress food intake and thereby reduce body weight. By contrast, adiponectin (Kubota et al., 2007), ghrelin, and AICAR (Andersson et al., 2004), a pharmacological activator of AMPK, stimulate AMPK activity, and thus increase food intake and body weight. Moreover, adenovirus-mediated expression of dominant-negative (DN) AMPK reduces food intake and body weight, whereas expression of constitutive-active (CA) AMPK increases them (Minokoshi et al., 2004). Recent findings regarding the signaling network (**Figure 1**) suggest that regulation of food intake by AMPK may converge on the mTORC1 system in the hypothalamus. AMPK activates TSC2, a suppressor of mTORC1, and thereby inhibits mTORC1 (Inoki et al., 2003). In addition, AMPK phosphorylates Raptor, a scaffold protein of mTORC1, also leading to inhibition of mTORC1 (Gwinn et al., 2008). Thus, like rapamycin, AMPK activation suppresses mTORC1 and increases food intake.

Direct evidence that mTORC1 signaling might be coupled with feeding has been reported (Cota et al., 2006). Similar to the action of leptin, ICV injection of leucine activates mTORC1 and reduces food intake. Moreover, the effects of both leucine and leptin in the brain on mTORC1 signaling and food intake are counteracted by rapamycin, a specific mTORC1 inhibitor. Fasting and re-feeding also affect mTORC1 signaling in neurons in the hypothalamus. It remains unclear which area of the hypothalamus, as well as which types of neurons, participates in mTORC1-mediated regulation of food intake. The answer to this question might be provided by precise analysis using methods such as immunohistochemistry with phospho-specific antibodies to mTOR network molecules. Studies of mTORC1 signaling on food intake using genetically modified mice have yielded seemingly paradoxical consequences. Global deletion of p70S6K ($s6k^{-/-}$), a downstream signaling molecule of mTORC1, protects against diet-induced obesity (Um et al., 2004). By contrast, injection of an adenovirus vector carrying DN-p70S6K into the mediobasal hypothalamus increases food intake and body weight, whereas overexpression of CA p70S6K reduces both parameters (Blouet et al., 2008; Ono et al., 2008). Furthermore, conditional deletion of *Tsc1*, an upstream suppressor of mTORC1 in hypothalamic neurons (and in beta cells of the pancreas), induces hyperphagic obesity, and hypothalamic POMC neuron-specific deletion of *Tsc1* results in the same phenotype (Mori et al., 2009). Because *Tsc1* deletion induces mTORC1 activation, this phenomenon seems contradictory to the anorexic effect of mTORC1.

Two issues complicate our understanding of mTORC1's action on food intake and weight control: the negative-feedback mechanism and the difference between the peripheral (or systemic) and central (brain) activities of mTORC1. Prolonged activation of mTORC1 signaling induced by gene knockouts of upstream or downstream molecules may cause inactivation of mTORC1 in the hypothalamus via negative-feedback. Thus, for example, knockout of *Tsc1* may cause orexigenic rather than anorexigenic effects due to long-lasting feedback suppression of mTORC1. Precise biochemical analysis of mTORC1 signaling in these animals may help to resolve this paradox. Systemic mTORC1 pathway activation induces cell growth in many organs, and thereby increases body weight; this may explain why global knockout of p70S6K suppresses weight gain (Blouet et al., 2008; Ono et al., 2008). Nevertheless, because the organism must maintain

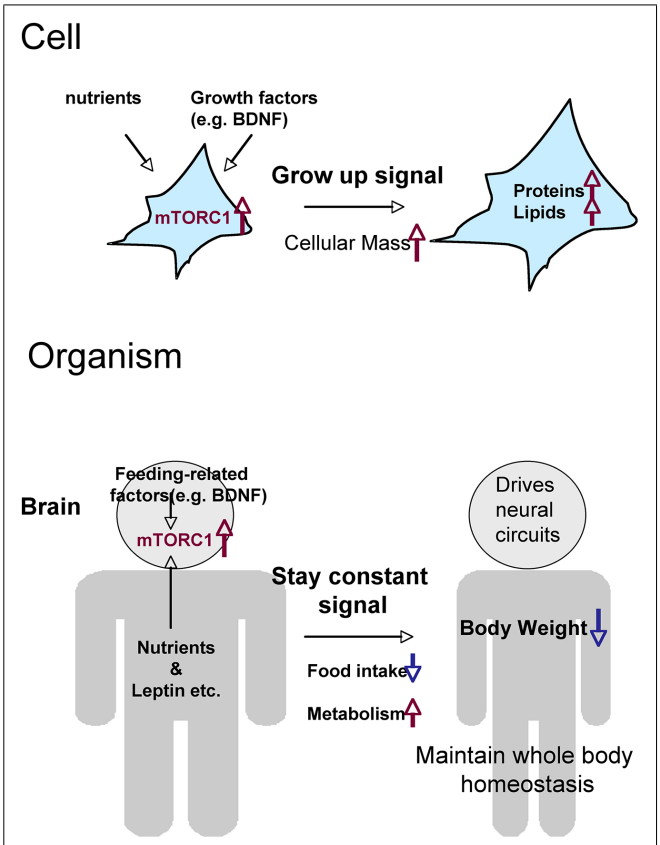

FIGURE 2 | Hypothetical roles of mTORC1 on metabolism and food intake, comparing the cellular and organismal levels. Through the activation of mTORC1, cells that receive nutrients and growth factors grow by increasing total levels of proteins and lipids. Cells (neurons) in the brain activate mTORC1 in response to nutrients and a variety of feeding-related factors. The resultant signals give the order to stop eating and maintain whole-body homeostasis.

whole-body homeostasis, it is quite likely that phasic activation of mTORC1 in hypothalamic neurons drives an anorexic state. In other words, mTORC1 senses the "satiety" signal in the brain in order to maintain an appropriate body weight (**Figure 2**).

BDNF AND mTOR

Amino acids, glucose, and growth factors induce mTORC1 activation in the hypothalamus and reduce food intake. In addition to leucine and leptin, CNTF (Cota et al., 2008) and bone morphogenic protein 9 (BMP9; Townsend et al., 2012) also suppress food intake. Culture studies have revealed that these molecules elicit mTORC1 signaling in neuronal cells. Leucine activates mTORC1 through the system L-amino acid transporter in primary cultured neurons (Ishizuka et al., 2008), whereas CNTF induces the phosphorylation of STAT3 via mTORC1 in neuroblastoma cells (Yokogami et al., 2000). These phenomena are similar to the effects of BDNF on both mTORC1 signaling and food intake. On the cellular level, BDNF is a potent activator of mTORC1 in neurons, and it stimulates anabolic responses such as protein synthesis (Takei et al., 2001, 2004). Although there is as yet no direct evidence, it is very likely that the anorexigenic action of BDNF is mediated by mTORC1 activation in the hypothalamus. Importantly, BDNF-mediated mTORC1 activation is limited by glucose availability (Ishizuka et al., 2013). Because glucose and amino acids are indispensable for the maintenance of homeostasis, this observation suggests the existence of a safeguard system in which glucose sufficiency overrides other mTORC1-activating stimuli in neurons. Therefore, it is necessary to obtain direct evidence that the anorexigenic action of BDNF is really mediated by mTORC1 signaling, either via the use of mTOR inhibitors such as rapamycin or knockdown of mTORC1 components in the hypothalamus. In addition, it is also important to determine which types of neurons in the hypothalamus are actually responsible for mTORC1-mediated feeding control. The use of a unique promoter-driven Cre-mouse (such as POMC-Cre) to make conditional knockout in mTOR or mTORC1 components in certain hypothalamic neurons may be useful in this regard.

In unicellular organisms and at the single-cell level in metazoans, nutrient uptake and subsequent mTORC1 activation lead to cell growth (i.e., increase in cellular mass and/or proliferation). In multicellular organisms, the brain regulates food intake to maintain whole-body homeostasis. Thus, mTORC1 in hypothalamic neurons senses many complex signals, both from the periphery (e.g., glucose, amino acids, insulin, leptin, and ghrelin) and from neural networks within the brain (e.g., via peptides and BDNF; **Figure 2**). The mechanisms by which the brain controls feeding behavior are complex, but mTORC1 may represent a cellular crossroads for the regulation of food intake and metabolism by nutrients and other inputs.

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Body weight status, eating behavior, sensitivity to reward/punishment, and gender: relationships and interdependencies

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Behavioral and personality characteristics are factors that may jointly regulate body weight. This study explored the relationship between body mass index (BMI) and self-reported behavioral and personality measures. These measures included eating behavior (based on the *Three-Factor Eating Questionnaire*; Stunkard and Messick, 1985), sensitivity to reward and punishment (based on the *Behavioral Inhibition System/Behavioral Activation System* (*BIS/BAS) scales*) (Carver and White, 1994) and self-reported impulsivity (based on the *Barratt Impulsiveness Scale-11*; Patton et al., 1995). We found an inverted U-shaped relationship between restrained eating and BMI. This relationship was moderated by the level of disinhibited eating. Independent of eating behavior, *BIS* and *BAS* responsiveness were associated with BMI in a gender-specific manner with negative relationships for men and positive relationships for women. Together, eating behavior and *BIS/BAS* responsiveness accounted for a substantial proportion of BMI variance (men: ∼25%, women: ∼32%). A direct relationship between self-reported impulsivity and BMI was not observed. In summary, our results demonstrate a system of linear and non-linear relationships between the investigated factors and BMI. Moreover, body weight status was not only associated with eating behavior (*cognitive restraint* and *disinhibition*), but also with personality factors not inherently related to an eating context (*BIS/BAS*). Importantly, these relationships differ between men and women.

Keywords: eating behavior, gender differences, obesity, personality traits, reward sensitivity, punishment sensitivity, Behavioral Activation System, Behavioral Inhibition System

INTRODUCTION

Body weight regulation and the development of obesity are associated with multiple interdependent factors and mechanisms. These mechanisms include, at the individual level, genetic and endocrine factors as well as behavioral and personality characteristics (e.g., Williamson et al., 1995; Bellisle et al., 2004; Provencher et al., 2004; Dina et al., 2007; Farooqi et al., 2007; Frayling et al., 2007; Klok et al., 2007; Ahima, 2008; Davis and Fox, 2008; Rosenbaum et al., 2008; Page et al., 2011). One of the most important factors contributing to body weight status is eating behavior, which is commonly assessed by the *Three-Factor Eating Questionnaire* (*TFEQ*; Stunkard and Messick, 1985). The *TFEQ* measures three dimensions of eating behavior: *cognitive restraint (CR)*, *disinhibition (DIS)*, and *susceptibility to hunger* or *hunger (HUN)*, for short. *Cognitive restraint* measures individual control over eating. Restrained eaters attempt to suppress impulses to eat in order to pursue long-term weight goals. Typical characteristics are avoidance of fattening foods and eating of small portions. The factor *disinhibition* reflects overeating tendencies. Disinhibited eaters typically initiate eating because of external environmental cues, such as palatable food. They have difficulties resisting food stimulation and/or eat under emotional distress. Considering this, *cognitive restraint* (conscious restriction of food intake) and *disinhibition* (tendency to overeat) conceptually represent antagonistic concepts. The third factor, *hunger*, characterizes the extent to which hunger feelings are experienced and evoke food intake. While *hunger* and *disinhibition* are positively associated with body mass index (BMI; e.g., Bond et al., 2001; Boschi et al., 2001; Bellisle et al., 2004; Bryant et al., 2008; Lesdéma et al., 2012), the relationship of *cognitive restraint* and BMI seems to be more complex and non-linear: In normal weight individuals they are usually positively associated, but the relationship is typically negative in overweight and obese individuals (e.g., Foster et al., 1998; Lluch et al., 2000; Bellisle et al., 2004; Provencher et al., 2004; de Lauzon-Guillain et al., 2006; Cappelleri et al., 2009). Additionally, *cognitive restraint* and *disinhibition* are not independently related to BMI, they interactively influence body weight

status (Stunkard and Messick, 1985; Westenhoefer et al., 1990; Williamson et al., 1995; Hays et al., 2002; Dykes et al., 2004). Specifically, *cognitive restraint* attenuates the effect of *disinhibition* on BMI. What is more, previous investigations indicate that eating behavior (including presumably also underlying biological mechanisms) and body weight status mutually influence each other. For example, there are alterations in the level of *cognitive restraint* as well as *disinhibition* in response to dieting (e.g., Karlsson et al., 1994; Pekkarinen et al., 1996; Foster et al., 1998; Westerterp-Plantenga et al., 1998; Dalle Grave et al., 2009; Savage et al., 2009; Tucker and Bates, 2009).

In addition to eating behavior, various personality traits are related to food consumption and weight status (Faith et al., 2001; Elfhag and Morey, 2008). One of the most popular models of personality that may explain individual variations in food intake is the *reinforcement sensitivity theory* (RST; Gray, 1970, 1982, 1987; Gray and McNaughton, 2000). Based on this theory, two general motivational systems that underlie behavior and affect have been suggested—the Behavioral Inhibition System (BIS) and the Behavioral Activation System (BAS), commonly assessed by the *BIS/BAS* scales (Carver and White, 1994). The BIS represents the aversive motivational system. It is sensitive to signals of punishment, reward omission, and novelty. The BIS is supposed to inhibit behavior that may lead to negative or painful outcomes and is associated with negative affect (negative reinforcement). The BAS reflects the appetitive motivational system. It is sensitive to signals of reward and the avoidance of punishment (positive reinforcement). High BAS responsiveness is related to enhanced approach behavior and positive affect.

As food can be both a positive or negative reinforcer, responsiveness of these systems potentially plays a substantial role in body weight regulation. However, the relationship between sensitivity to reward (as a facet of BAS responsiveness) and BMI has been almost exclusively investigated in women. Investigations showed positive associations of reward sensitivity with BMI and eating habits supporting weight gain (Davis et al., 2004, 2007; Franken and Muris, 2005). In addition, reward responsiveness has been related to neural responses. In particular sensitivity to reward was shown to be positively associated with neural responses to pictures of highly palatable food in a fronto-striatal-amygdala network (Beaver et al., 2006). Further findings indicate that long-lasting overeating and obesity account for adaptations of the reward system (Wang et al., 2001; Volkow et al., 2008; de Weijer et al., 2011). In combination with the aforementioned findings, these studies led to the development of a *hyper- vs. hyposensitivity theory* of reward in obesity (e.g., Davis and Fox, 2008). According to this theory, some individuals show an inherent heightened reward sensitivity (*hypersensitivity*) and are particularly susceptible to the rewarding properties of high-calorie food. They are thus supposed to regularly overeat on fattening food and consequently become overweight or obese. Prolonged overeating and corresponding obesity, on the other hand, are associated with alterations in the dopaminergic (DA) reward circuitry, presumably to compensate for an enhanced DA tone (Wang et al., 2001; Volkow et al., 2008; de Weijer et al., 2011). These alterations are assumed to result in *hyposensitivity* to reward in obese individuals as well as in increased hedonic eating to compensate this deficiency. This theory was explored by Davis and Fox (2008). According to their model, in both genders BMI and sensitivity to reward are non-linearly associated by an inverted U-shaped relationship. More specifically, the authors reported high reward sensitivity in overweight and mildly obese participants and low reward sensitivity in morbidly obese ones. Thus, although sensitivity to reward and sensitivity to punishment are assumed to be dispositional traits rather than transient states or symptoms (Wilksch and Wade, 2009), at least sensitivity to reward seems to be flexible to a certain extent.

To our knowledge, the association between sensitivity to punishment and BMI so far has not yet been studied directly, although several studies demonstrate a relationship between sensitivity to punishment and eating disorders. Similar to obese subjects, patients suffering from bulimia nervosa and anorexia nervosa (binge/purge subtype) are characterized by overeating. This points at possible similarities in the underlying personality structure leading to a shared decision-making profile (Brogan et al., 2010). Studies investigating eating disorders repeatedly report high punishment responsiveness in patients compared to healthy controls (e.g., Harrison et al., 2010, 2011). In addition, sensitivity to punishment has been shown to be positively associated with symptoms of binge eating (Davis, 2013). Again, these studies are almost exclusively restricted to women. Matton et al. (2013) clustered adolescents with respect to reward and punishment responsiveness. Interestingly, the cluster of subjects with both high reward sensitivity and high punishment sensitivity outscored other clusters on self-reported eating problems (i.e., data regarding concerns about eating, body shape and weight as well as emotional and external eating). Although girls were more likely to belong to this cluster, effects were similar for both girls and boys. Based on these findings, Matton et al. (2013) proposed that adolescents in this cluster are especially vulnerable to the development of eating problems.

Sensitivity to reward is regarded as one aspect of the multidimensional psychological construct *impulsivity* (e.g., Guerrieri et al., 2008). Generally, impulsive behavior is rapid and rash, characterized by a lack of planning and less forethought about consequences of spontaneous actions (Moeller et al., 2001). As the term "multidimensionality" indicates, impulsivity covers several different but related concepts. The relationship to overeating is thus not straightforward. While individual differences in some aspects of impulsivity are likely to contribute to the ability to resist overeating, others may not. Various tasks that assess aspects of impulsive behavior indicate altered decision-making in overweight and obese individuals. In *Delay Discounting Tasks* or *Delay Gratification Paradigms*, for example, obese subjects in general (Rasmussen et al., 2010) or obese women in particular (Weller et al., 2008; Weygandt et al., 2013) chose more often immediate but smaller monetary or food-related reward in comparison to normal weight control subjects. In the *Iowa Gambling Task* obese volunteers preferred high immediate reward despite long-term losses. This was shown in both genders (Pignatti et al., 2006; Brogan et al., 2011), women (Horstmann et al., 2011), or men (Koritzky et al., 2012). In addition, obese women and children of both genders

lacked appropriate inhibitory control in the non-reward related *Stop Signal Task* (Nederkoorn et al., 2006a,b). Another task measuring inhibitory control, the *Go/No-Go Task*, showed especially overweight and obese adolescent girls to have difficulties inhibiting prepotent motor responses to high-calorie food (Batterink et al., 2010). Heightened impulsivity was also reported for overweight children (Braet et al., 2007) as well as overweight and obese adults (e.g., Chalmers et al., 1990; Mobbs et al., 2010) based on different self-reported measures. For example, Mobbs et al. (2010) reported higher levels of urgency, lack of perseverance and strong sensitivity to reward in overweight and obese women. They concluded that overweight and obesity are associated with problems in inhibiting dominant behavior and intrusive thoughts. Within the obese population, there is evidence for heightened self-reported impulsivity among severely compared to less severely obese individuals (Rydén et al., 2003), and impulsivity was further related to higher food intake in women using the *Barratt Impulsiveness Scale* (BIS; Guerrieri et al., 2007).

An important factor that contributes to differences in eating behavior and personality, and probably also to body weight regulation, is gender. Women, for example, have higher scores of *cognitive restraint* and *disinhibition* compared to men (Bellisle et al., 2004; Provencher et al., 2004; Li et al., 2012). Additionally, eating disorder symptomatology is more prevalent among women (e.g., Keel et al., 2007; Matton et al., 2013; Yean et al., 2013). Furthermore, men and women differ in personality traits such as impulsivity. For example, higher sensation seeking and behavioral risk taking was observed in men compared to women (Arnett, 1992; Byrnes et al., 1999; Cross et al., 2011). Additionally, both gender-independent and gender-specific effects have been reported, for example, with respect to the *Iowa Gambling Task* and weight status (Pignatti et al., 2006; Brogan et al., 2011; Horstmann et al., 2011; Koritzky et al., 2012). The precise relationship between impulsivity, BMI and gender thus is not clear from previous data. Furthermore, women are more sensitive to both reward and punishment compared to men (Carver and White, 1994; Jorm et al., 1999; Cross et al., 2011). Yet, the relationship of these measures to weight status has not been sufficiently explored in males, as described earlier. Differences in the hormonal repertoire between men and women might account for variations in the susceptibility to reinforcers like food. Ovarian hormones in particular, which affect mesolimbic DA system (i.e., reward processing; Sofuoglu et al., 1999; Kaasinen et al., 2001; Evans et al., 2002; Lynch et al., 2002; Carroll et al., 2004) but also HPA functioning (i.e., stress response; Burgess and Handa, 1992; Handa et al., 1994; Patchev et al., 1995; Young, 1995), might be responsible for such differences, making women generally more vulnerable to the reinforcing properties of most drugs of abuse (see Fattore et al., 2008, 2009 for review). As addiction and obesity share several properties (see Volkow et al., 2013 for review), there might be also gender differences in the susceptibility to the reinforcing value of food. For other personality domains and their association with weight status, the gender interaction has already been shown. In a study by Faith et al. (2001) BMI was positively associated with neuroticism and negatively with extraversion in women. In men, BMI was positively associated with extraversion and psychoticism (Faith et al., 2001). Finally, gender moderates obesity-related differences in brain structure. Specifically for women obesity-related variation were observed in regions involved in habitual and goal-directed control of behavior such as the dorsal striatum and dorsolateral prefrontal cortex (Horstmann et al., 2011).

Therapeutic approaches to obesity classically target aspects of eating behavior. Behavioral interventions, for example, aim at increasing *cognitive restraint* and decreasing *disinhibition* (e.g., Jubbin and Rajesh, 2012). Yet, as described above, individual body weight status is also related to personality traits. For a more effective treatment of obesity it is therefore necessary to regard personality traits as well. This study aims to establish a comprehensive model relating BMI to eating behavior and the most relevant obesity-related personality traits (self-reported impulsivity and reward/punishment sensitivity). We investigated questionnaire measures of these traits as they can be easily and quickly assessed in the clinical setting. *TFEQ* scales *cognitive restraint*, *disinhibition*, and *hunger* (Stunkard and Messick, 1985) served as measures of eating behavior. The *BIS/BAS* scales (Carver and White, 1994) were considered as measures of sensitivity to punishment (*BIS*) and sensitivity to reward (*BAS*). Further, self-reported impulsivity, assessed by the *BIS-11* (Patton et al., 1995), was incorporated into the model. The overall goal of our approach was to quantify the individual and joint contribution of these scales to BMI variance explanation.

Based on previous findings, different models were developed to test the following hypotheses:

(1) A significant proportion of BMI variance is explained by *disinhibition*, *hunger*, and *cognitive restraint*. According to previous findings, we assumed positive linear associations of both *disinhibition* and *hunger* with BMI (e.g., Bond et al., 2001; Boschi et al., 2001; Bellisle et al., 2004; Bryant et al., 2008; Lesdéma et al., 2012). As *cognitive restraint* and BMI are positively associated in normal weight individuals and negatively in overweight and obese individuals (e.g., Foster et al., 1998; Lluch et al., 2000; Bellisle et al., 2004; Provencher et al., 2004; de Lauzon-Guillain et al., 2006; Cappelleri et al., 2009), we expected an inverted U-shaped relationship between these variables.

(2) A portion of BMI variance is explained by the interaction of *disinhibition* and *cognitive restraint*, indicated by previous studies (Stunkard and Messick, 1985; Westenhoefer et al., 1990; Williamson et al., 1995; Hays et al., 2002; Dykes et al., 2004).

(3) Additional BMI variance is explained by the level of *BIS* (as a measure of punishment responsiveness) and *BAS* (as a measure of reward responsiveness). Based on previous research, we expected positive linear associations for both variables with BMI in women (Davis et al., 2004, 2007; Franken and Muris, 2005; Harrison et al., 2010, 2011). Despite the lack of previous data for these relationships in men, we expect the positive relationships between *BIS/BAS* and BMI to be specific for women, which is based on gender-dependent differences in the hormonal repertoire influencing the vulnerability to reinforcers (e.g., Sofuoglu et al., 1999; Kaasinen et al., 2001; Evans et al., 2002; Lynch et al., 2002; Carroll et al., 2004).

(4) Further, BMI variance is explained by the level of self-reported impulsivity (*BIS-11*). According to previous findings, we expected a positive linear association with BMI (e.g., Chalmers et al., 1990; Rydén et al., 2003; Mobbs et al., 2010). Considering opposing findings with respect to gender (Pignatti et al., 2006; Brogan et al., 2011; Horstmann et al., 2011; Koritzky et al., 2012), we tested for gender interactions, although they were not expected.

Besides the study's main purpose of modeling BMI, we had two secondary objectives:

(5) *Cognitive restraint*, *disinhibition*, and body weight status mutually influence each other (e.g., Karlsson et al., 1994; Pekkarinen et al., 1996; Foster et al., 1998; Westerterp-Plantenga et al., 1998; Dalle Grave et al., 2009; Savage et al., 2009; Tucker and Bates, 2009). Therefore, we hypothesized the quadratic relationship between BMI and *cognitive restraint* to be moderated by *disinhibition*. Depending on the level of *disinhibition*, we expected the association of BMI and *cognitive restraint* to be as follows: Normal body weight and low *disinhibition* is associated with low *cognitive restraint*. Normal body weight and high *disinhibition* is associated with high *cognitive restraint*. Overweight is associated with high *cognitive restraint* regardless of the level of *disinhibition*. Obesity is associated with low *cognitive restraint* regardless of the level of *disinhibition*.

(6) Davis and Fox (2008) demonstrated an inverted U-shaped relationship between sensitivity to reward and BMI. We aimed to corroborate these findings by testing for a quadratic relationship between *BAS* and BMI. We hypothesized an inverted U-shaped relationship between these measures.

As the focus of this investigation was on self-report questionnaires, i.e., explicit, mentally represented data, this study did not consider implicit or automatic processes (i.e., eating habits) that influence behavior and potentially body weight independently of explicit experience (e.g., Berridge and Robinson, 2003; Finlayson et al., 2008; Papies et al., 2009; Goldstein et al., 2014).

MATERIALS AND METHODS

SUBJECTS

Data were collected by the joint obesity work group of the Max Planck Institute for Human Cognitive and Brain Sciences and the IFB Adiposity Diseases in Leipzig between 2009 and 2013. Healthy adult subjects were invited to participate in different behavioral and neurocognitive experiments in the context of obesity research and were reimbursed for their participation. As part of these experiments, subjects completed various questionnaires this cross-sectional study is based on. Exclusion criteria were age under 18 or over 50 years, BMI under 18 kg/m^2, hypertension, dyslipidemia, metabolic syndrome, depression (Beck's Depression Inventory, cut-off value 18), a history of neuropsychiatric diseases, smoking, diabetes mellitus, vegetarianism, and pregnancy. Although there were no restrictions for ethnicity, only Caucasian subjects volunteered. Age in years and BMI were assessed at the time of the experiment. Height and weight for BMI calculations were measured by scientific staff at the Max Planck Institute in Leipzig. As not all questionnaires were assessed for all participants, we decided to investigate two cohorts (called *TFEQ-only* and *TFEQ-plus* cohort). The total cohort consisted of 326 healthy subjects (*TFEQ-only* cohort; 145 women, 181 men). Besides BMI, age, and gender, the *TFEQ* scores of *CR*, *DIS*, and *HUN* were assessed in these subjects. In a subgroup of 192 participants, *BIS*, *BAS*, and *BIS-11* were additionally assessed (*TFEQ-plus* cohort; 92 women, 110 men). **Table 1** depicts descriptive statistics of the two cohorts. The study was carried out in accordance with the Declaration of Helsinki and approved by the local ethics committee of the University of Leipzig. All subjects gave written informed consent before participation.

Table 1 | Descriptive statistics.

Variable	*n*	Mean (SD)	Range	Mean women (SD)	Mean men (SD)
BMI	326	26.6 (6.1)	18.1–46.5	26.4 (6.6)	26.7 (5.6)
	192	26.7 (6.2)	18.1–46.5	26.6 (6.5)	26.8 (6.0)
Age	326	26.7 (4.8)	18–46	26.3 (4.8)	27.0 (4.9)
	192	26.6 (4.7)	18–46	25.7 (4.1)	27.2 (5.0)
CR	326	6.5 (4.6)	0–19	7.3 (5.0)	5.8 (4.1)
	192	6.7 (4.7)	0–19	7.4 (5.0)	6.2 (4.4)
DIS	326	6.1 (3.2)	0–15	6.8 (3.5)	5.6 (2.8)
	192	6.1 (3.0)	1–14	6.8 (3.3)	5.6 (2.6)
HUN	326	5.5 (3.3)	0–14	5.6 (3.3)	5.5 (3.3)
	192	5.6 (3.3)	0–14	5.9 (3.4)	5.4 (3.3)
BAS	192	30.9 (8.8)	13–51	29.7 (8.5)	31.8 (9.0)
BIS	192	17.0 (3.9)	5–26	16.5 (4.3)	17.4 (3.4)
BIS-11	192	32.2 (8.7)	9–58	32.0 (8.8)	32.3 (8.6)

Descriptive statistics of variables assessed in the TFEQ-only cohort (n = 326, 145 women, 181 men) and the TFEQ-plus cohort (subgroup of TFEQ-only cohort (grey), n = 192, 82 women, 110 men). CR, TFEQ cognitive restraint score; DIS, TFEQ disinhibition score; HUN, TFEQ hunger score; BIS-11, Barratt Impulsiveness Scale 11 total score; BAS, Behavioral Activation System total score; BIS, Behavioral Inhibition System total score; TFEQ, Three-Factor Eating Questionnaire.

QUESTIONNAIRES

Three-Factor Eating Questionnaire (Stunkard and Messick, 1985; German version: Pudel and Westenhoefer, 1989)

The TFEQ is a 51-item self-report assessment of eating behavior. The questionnaire contains three subscales. The 21-item *cognitive restraint* scale (*CR*, scale range: 0–21, Cronbachs Alpha of German version = 0.84) measures intent to control food intake. The 16-item *disinhibition* scale (*DIS*, scale range: 0–16, Cronbachs Alpha of German version = 0.75) quantifies overeating tendencies. The 14-item susceptibility to *hunger* scale (*HUN*, scale range: 0–14, Cronbachs Alpha of German version = 0.76) is a measure for food intake in response to feelings of hunger.

The Behavioral Inhibition System/Behavioral Activation System Scales (Carver and White, 1994; German version: Strobel et al., 2001)

This self-report questionnaire consists of 20 items designed to assess the responsiveness of Gray's (1982, 1987) BAS and BIS as

personality characteristics. The 7-item *BIS* scale measures reactivity of the aversive motivational system (scale range: 7–28, Cronbachs Alpha of German version = 0.78), whereas the 13-item *BAS* scale measures reactivity of the appetitive motivational system (scale range: 13–52, Cronbachs Alpha of German version = 0.81). The *BAS* scale can be divided into three subscales: Drive, Fun-Seeking, and Reward. In this study we applied the *BAS* sum score, as the subscales were not confirmed in the German version.

Barratt Impulsiveness Scale-11 (Patton et al., 1995; German version: Preuss et al., 2008)

The *BIS-11* is a 30-item self-report questionnaire developed to measure impulsivity. Along a four-point scale subjects rate whether statements describing impulsivity pertain to themselves (scale range: 0–90, Cronbachs Alpha of German version = 0.69). For the original English version, six factors were identified. This originally suggested factor structure was not confirmed for the German equivalent. We therefore applied the total score of the *BIS-11*, as it shows adequate internal consistency for German-speaking regions.

STATISTICAL ANALYSES

Statistical analyses were performed using SPSS (IBM Corporation Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corporation) and the SPSS toolbox PROCESS (Hayes, 2013). Associations between BMI and self-reported behavioral data were explored by means of multiple regression analyses. All variables except gender were treated as continuous variables. We separately tested for the association between the three *TFEQ* scales and BMI in the *TFEQ-only* cohort (see Association of the TFEQ Scales with BMI). Age and gender were included as covariates. Significant terms were subsequently used to build a regression model for BMI to assess the proportion of variance solely explained by variables of eating behavior (see BMI Modeling Based on the TFEQ Scales Cognitive Restraint and Disinhibition). Next, we tested *BIS-11*, *BIS*, and *BAS* seperately for their association with BMI in the *TFEQ-plus* cohort (see Association of the Barratt Impulsiveness Scale-11, Behavioral Activation System, and Behavioral Inhibition System Scales with BMI). Additionally, gender interactions for the relationships of the latter three scores with BMI were tested. Age and gender were included as covariates. Again, all significant terms were used to build a comprehensive regression model for BMI including eating behavior and personality traits (see BMI Modeling Based on Cognitive Restraint, Disinhibition, the Behavioral Activation System, and Behavioral Inhibition System Score).

Based on findings of previous studies, quadratic relationships between BMI and *CR* (moderated by *DIS*, see Interactions between Cognitive Restraint, Disinhibition, and BMI) and between BMI and *BAS* (see Quadratic Relationship between BMI and the Behavioral Activation System Score) were tested (Foster et al., 1998; Lluch et al., 2000; Bellisle et al., 2004; Provencher et al., 2004; de Lauzon-Guillain et al., 2006; Davis and Fox, 2008; Cappelleri et al., 2009). BMI was treated as regressor for these analyses.

Table 2 | Regression models and corresponding variables.

Association with regressand	Variables in model	Tested gender interaction
Linear	A, g, a	A*g
Quadratic (e.g., CR^2)	A, A^2, g, a	A^2*g
2-way interaction (DIS*CR)	A, B, A*B, g, a	–
Quadratic 2-way interaction (BMI^2*DIS)	A, B, A^2, A*B, A^2*B, g, a	–

Different regression models were computed to test our individual hypotheses. Corresponding variables of all the investigated models are listed. Partial correlations of the underlined terms were tested against 0. A, B: tested variables, e.g., Three-Factor Eating Questionnaire cognitive restraint (CR) or disinhibition score (DIS); g, gender; a, age.

Table 2 lists the regression models which were used to test all abovementioned associations. As measures of effect size we used partial correlations and squared partial correlations. The latter can be interpreted as the regressand's (e.g., BMI) proportion of variance which can be explained by a single regressor (e.g., *DIS*) when all other variables are held constant. For reasons of consistency, not to indicate causality, BMI was depicted at the x-axis of every graph. We added a table of Pearson Correlations of the assessed variables at the end of the results section (see Pearson Correlations of All Variables of Interest).

RESULTS

TFEQ-ONLY COHORT ($n = 326$)

Association of the Three-Factor Eating Questionnaire scales with BMI

In the total cohort of 326 subjects, a gender difference in *CR* ($p = 0.004$) and in *DIS* ($p = 0.001$) was observed, with women having higher scores in both cases. BMI significantly correlated with *DIS*, CR^2 (hypothesis 1), and the interaction term of *CR* and *DIS* (hypothesis 2; **Figure 1**; partial correlations, all $p < 0.0005$; see **Table 3**). We observed no significant association of *HUN* with BMI.

BMI modeling based on the TFEQ scales cognitive restraint and disinhibition

To obtain a model for BMI regressed on the *TFEQ* scales, a multiple regression analysis using all former significant terms (i.e., *CR*, *DIS*, CR^2, and *CR*DIS*; additional covariates age and gender) was conducted. The underlying adjusted R^2 of this model was 0.232 (women: 0.247, men: 0.208). *CR*DIS* as well as CR^2 separately explained part of BMI variance, as their partial correlations differed from 0 (both $p < 0.0005$). Hence, the *TFEQ* scales *CR* and *DIS* (in addition to age and gender) explained about 23% of the overall variance of BMI in the population of this cohort.

Interactions between cognitive restraint, disinhibition, and BMI

We hypothesized a quadratic relationship between *CR* and BMI (hypothesis 5). The regression of *CR* on BMI^2 confirmed this hypothesis (squared partial correlation: 0.029, $p = 0.002$, age and gender as covariates). Furthermore, this inverted U-shaped

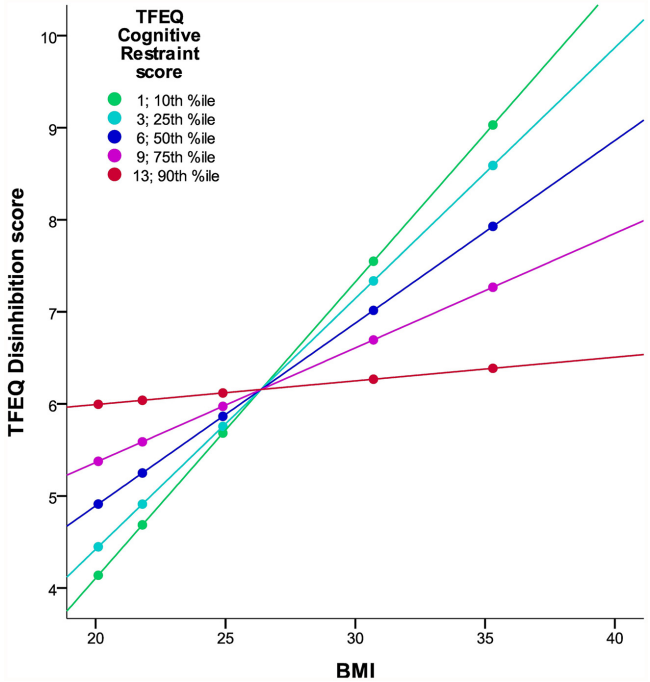


FIGURE 1 | Interaction of DIS and CR on BMI in the TFEQ-only cohort ($n = 326$). The figure illustrates the linear relationship between BMI and DIS moderated by the level of CR with age and gender as covariates. Partial correlation of BMI*CR is −0.203 ($p < 0.0005$; adjusted R^2 change of 0.163 through BMI, CR, and BMI*CR). Dots indicate 10th, 25th, 50th, 75th, and 90th percentiles of BMI (20.1, 21.8, 24.9, 30.7, and 35.3 kg/m^2). Colors indicate 10th, 25th, 50th, 75th, and 90th percentiles of CR (1, 3, 6, 9, 13). CR, cognitive restraint score; DIS, disinhibition score; TFEQ, Three-Factor Eating Questionnaire.

relationship was moderated by *DIS* ($p = 0.001$). In other words, the relationship between BMI and *CR* differed with respect to the *DIS* score (**Figure 2**): For low *DIS* scores the quadratic association between *CR* and BMI was well pronounced, whereas no strong quadratic relationship for high *DIS* scores was observed.

TFEQ-PLUS COHORT ($n = 192$)

Association of the Barratt Impulsiveness Scale-11, Behavioral Activation System, and Behavioral Inhibition System Scales with BMI

With respect to eating behavior (based on the *TFEQ*), results in the subgroup of 192 participants (*TFEQ-plus* cohort) were comparable with the whole sample (*TFEQ-only* cohort, $n = 326$).

BAS and *BIS* scores did not correlate with BMI, but showed a significant interaction with gender (hypothesis 3; all $p = 0.001$). In women, there was a significant positive correlation of *BIS* and BMI (partial correlation = 0.281; $p = 0.011$) as well as a strong tendency for the correlation of *BAS* and BMI (partial correlation = 0.214; $p = 0.055$). In men, we found a significant negative correlation of *BIS* and BMI (partial correlation = −0.208; $p = 0.03$) as well as *BAS* and BMI (partial correlation = −0.295; $p = 0.002$). The relationship of BMI and *BAS*, moderated by gender, is shown in **Figure 3** (results for the association of *BIS* and BMI are comparable). Concerning the association of self-reported impulsivity and BMI, neither a correlation between BMI and *BIS-11* (total score) nor a gender interaction was found (hypothesis 4).

Table 3 | Squared partial correlations (SPC) with BMI.

Variable	Squared partial correlation (η_p^2)	Direction of correlation	*p*-value
CR	(0.009)	(+)	0.083
DIS	0.138	+	<0.0005
HUN	(0.003)	(+)	0.596
CR^2	0.054	−	<0.0005
CR*DIS	0.054	−	<0.0005

Squared partial correlations with BMI in the TFEQ-only cohort ($n = 326$) in a regression model with age and gender as covariates. SPC can be interpreted as the proportion of BMI variance explained only by the corresponding variable, not by covariables. CR, TFEQ cognitive restraint score; DIS, TFEQ disinhibition score; HUN, TFEQ hunger score; TFEQ, Three-Factor Eating Questionnaire.

BMI modeling based on cognitive restraint, disinhibition, the Behavioral Activation System, and Behavioral Inhibition System score

The final model comprised the relevant variables of self-reported eating behavior (see BMI Modeling based on the TFEQ Scales Cognitive Restraint and Disinhibition, *TFEQ-only* model) as well as *BIS*, *BAS*, gender, *BIS**gender, *BAS**gender and age as regressors. The resulting adjusted R^2 was 0.271 (women: 0.324, men: 0.252). R^2 for women and men did not differ significantly ($p = 0.474$, two-tailed Fisher's Z). Independent of eating behavior, *BIS* and *BAS* significantly contributed to variance explanation of BMI (R^2 change of *TFEQ-only* model and *TFEQ-plus* model in the sample of $n = 192$, $p < 0.0005$). Hence, self-reported behavioral measures of *CR*, *DIS*, *BIS*, and *BAS* in addition to age and gender explained about 27% of the overall variance of BMI in the population of this sample. See **Figure 4** for variance proportions of the variables for each gender.

Quadratic relationship between BMI and the Behavioral Activation System score

As Davis and Fox (2008) reported an inverted U-shaped association between sensitivity to reward and BMI, we tested for the quadratic association of *BAS* with BMI (hypothesis 6). We corroborated their finding: BMI showed a quadratic relationship with *BAS* ($p = 0.018$, age and gender as covariates, adjusted R^2 changed by 0.03 after adding BMI and BMI^2). There was only a trend for a gender interaction of this effect ($p = 0.091$, stronger effect in women). Concerning the model, a BMI of around 30 kg/m^2 was associated with the highest *BAS* scores, whereas a higher and lower BMI was associated with lower *BAS* scores (**Figure 5**).

PEARSON CORRELATIONS OF ALL VARIABLES OF INTEREST

For an overview of the assessed variables and how they are interrelated, see **Table 4**. As the correlation of *BIS* and *BAS* was not

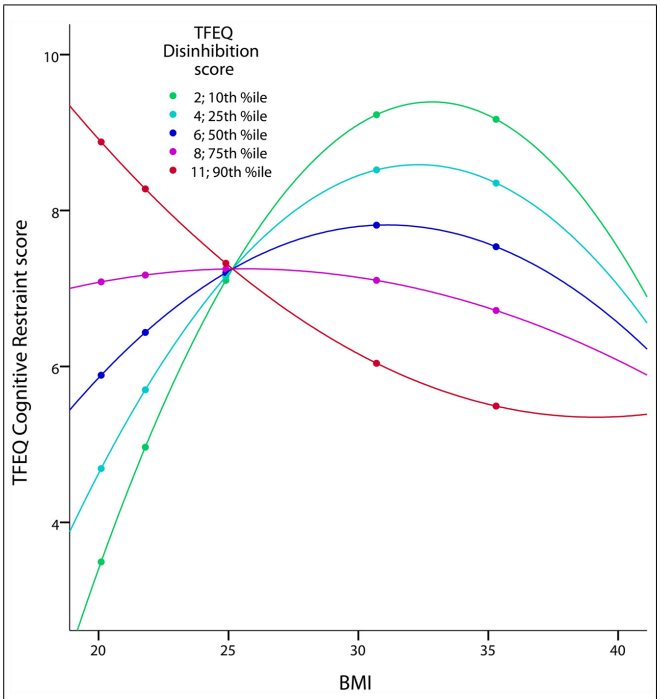


FIGURE 2 | Quadratic interaction of BMI and DIS on CR in the TFEQ-only cohort ($n = 326$). The figure illustrates the quadratic relationship between BMI and CR moderated by the level of DIS with age and gender as covariates. Partial correlation of BMI^2*DIS is 0.185 ($p < 0.001$; adjusted R^2 change of 0.083 through BMI, DIS, BMI^2, BMI*DIS and BMI^2*DIS). Dots indicate 10th, 25th, 50th, 75th, and 90th percentile of BMI (20.1, 21.8, 24.9, 30.7, and 35.3 kg/m^2). Colors indicate10th, 25th, 50th, 75th, and 90th percentiles of CR (2, 4, 6, 8, 10). CR, cognitive restraint score; DIS, disinhibition score; TFEQ, Three-Factor Eating Questionnaire.

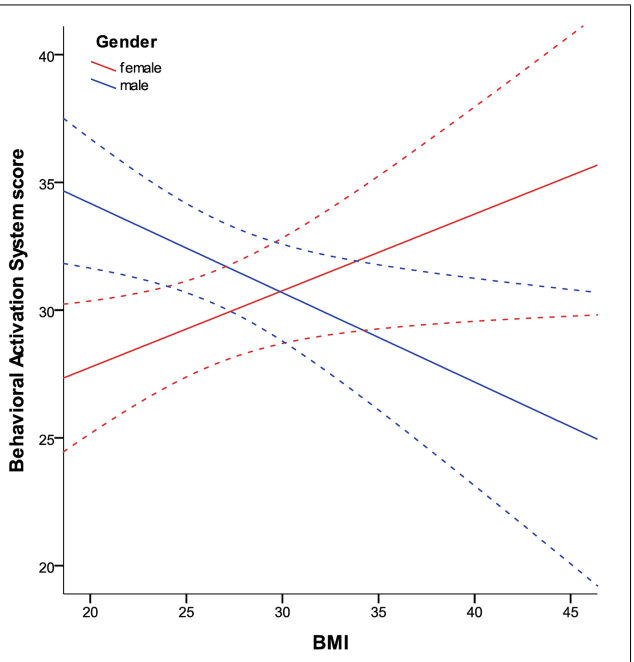


FIGURE 3 | Relationship between BMI and BAS in women and men in the TFEQ-plus cohort ($n = 192$). As the relationship of BAS and BMI is moderated by gender, it is shown separately. Partial correlation of BMI*gender is −0.255 ($p < 0.0005$, age as covariate). Partial correlation of BMI (age as covariate) with BAS is 0.214 in women ($n = 82$) and −0.295 in men ($n = 110$). Dashed lines indicate confidence interval of 95% for the fit lines. BAS, Behavioral Activation System total score.

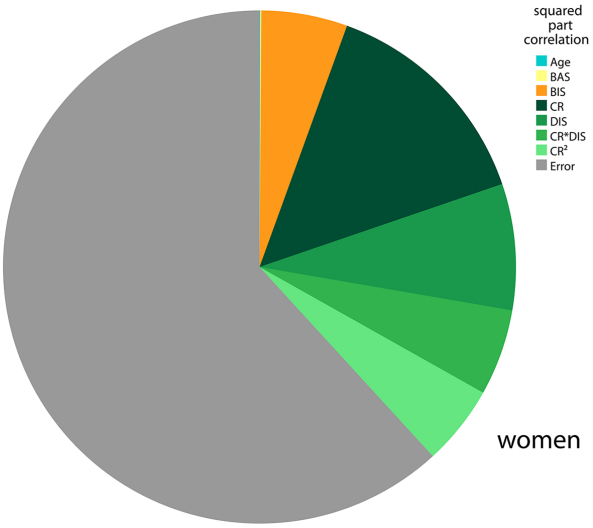
described thus far, this association was further investigated. One reason for this relationship might be the high proportion of obese subjects in our sample. Therefore we tested for an interaction of BMI with *BIS* or *BAS*. Also gender interactions of this assumed effects were tested. We found a 3-way-interaction between BMI, gender and *BIS* ($p = 0.007$ for *BIS**BMI*gender with *BAS* as regressand; age as covariate). Probing this 3-way-interaction revealed that women with a high BMI had a stronger association of *BIS* with *BAS*.

DISCUSSION

RELATIONSHIP BETWEEN EATING BEHAVIOR AND BMI

Interestingly, only two measures of eating behavior, *disinhibition* and *cognitive restraint*, accounted for much of BMI variance (∼23%). In other words, the individual level of overeating tendencies in interaction with the level of conscious efforts to restrict food intake explained a large amount of variance in individual body weight status. *Susceptibility to hunger* did not contribute to variance explanation of BMI. However, an association of *hunger* with *disinhibition* and *cognitive restraint* was shown in our sample, which is in line with previous studies (Bellisle et al., 2004; Lesdéma et al., 2012).

Besides modeling of BMI, we aimed to investigate the apparent non-linear relationship between *cognitive restraint* and BMI. We found an inverted U-shaped association of BMI with *cognitive restraint*. Our model demonstrates low levels of *cognitive restraint* at the outer edges of the BMI range and a high level around the overweight range. Interestingly, this relationship was moderated by the level of *disinhibition*. For low levels of *disinhibition* (low overeating tendencies) the curvilinear relationship between BMI and *cognitive restraint* was well pronounced. Accordingly, we conclude that restrained eating is low in normal weight individuals as food restriction is presumably not necessary. With higher BMI, food restriction becomes necessary, as losing weight or avoiding further weight gain are supposedly more frequent with higher BMI (maximum in the overweight/moderate obese range of the BMI). In the obese BMI range, the positive relationship between BMI and *cognitive restraint* is shifted, resulting in relatively low levels of restrained eating among morbidly obese individuals. Although restrained eating seems desirable in this BMI range, morbidly obese individuals might not be able to raise sufficient self-control resources to restrict food intake. This notion is supported by neuroimaging studies that report structural as well as functional obesity-related alterations in brain structures associated with self-control (Le et al., 2006, 2007; Horstmann et al., 2011). With higher levels of *disinhibition* there was no strong curvilinear relationship between BMI and *cognitive restraint*. This effect indicates that in response to heightened overeating tendencies, normal weight individuals increase conscious efforts to restrict food intake in order to maintain weight/stay slim. Overweight and moderately obese



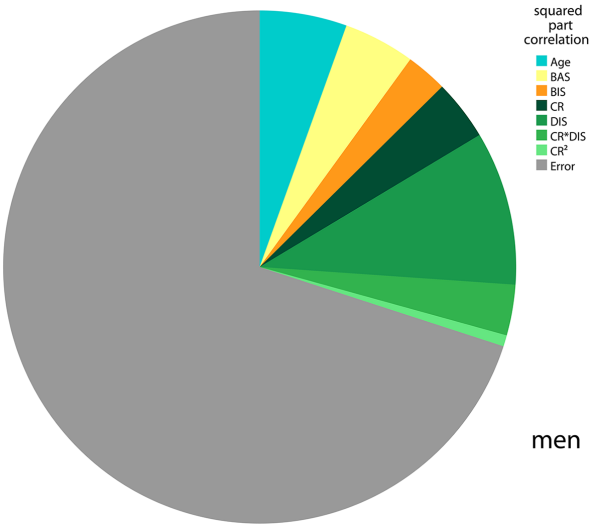


FIGURE 4 | BMI variance explained by final regression model in men and women. The pie charts show the squared part correlations of all variables of the final BMI model in the TFEQ-plus cohort ($n = 192$). All variables with significant correlation to BMI were included. As the directions of the effect of BAS and BIS differed between men and women, separate models comprising the same variables were computed. R^2 for women ($n = 82$) = 0.382. R^2 for men ($n = 110$) = 0.300. CR, TFEQ cognitive restraint score; DIS, TFEQ disinhibition score; BAS, Behavioral Activation System total score; BIS, Behavioral Inhibition System total score; TFEQ, Three-Factor Eating Questionnaire.

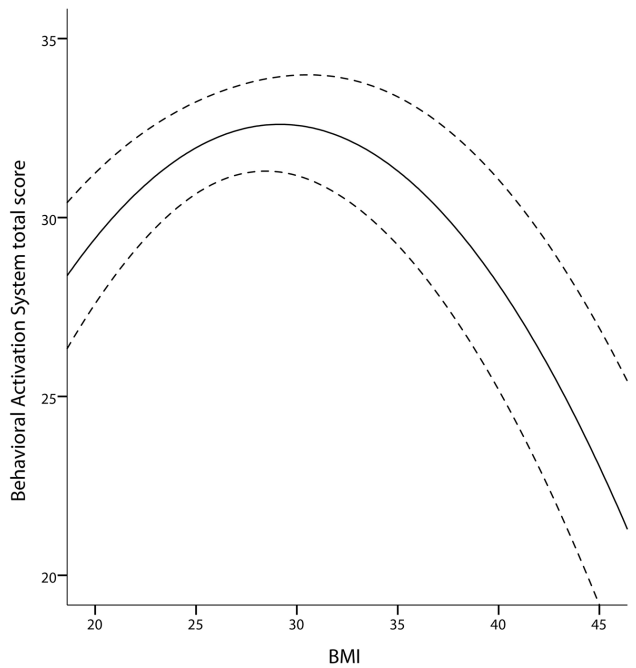


FIGURE 5 | Quadratic association between BAS and BMI in the TFEQ-plus cohort ($n = 192$). Partial correlation of BMI^2 is −0.92 ($p = 0.008$, adjusted R^2 change of 0.039 through BMI and BMI^2, age and gender as covariates). Dashed lines indicate the 95% confidence interval of the quadratic fit line. BAS, Behavioral Activation System total score.

individuals presumably do not adequately adapt their dietary restraint. On the contrary, the model indicates that attempts to restrict food intake decrease (reflected in lower levels of *cognitive restraint*) with stronger disinhibited eating. Eating behavior seems to be more and more dominated by an uncontrolled eating style, driven by, for example, external eating signals or habitual food intake.

GENDER-SPECIFIC RELATIONSHIPS BETWEEN *BIS/BAS* AND BMI

The aforementioned model for BMI based on eating behavior was extended to incorporate personality factors not inherently related to food context but potentially influencing body weight. Both *BIS* and *BAS* explained part of BMI variance independently of eating behavior (∼6%), whereby they inversely accounted for BMI variance in men and women. Both scales were positively associated with BMI in women, but negatively in men.

BAS RESPONSIVENESS AND BMI

Studies already showed that reward responsiveness is positively related to body weight status and eating habits contributing to weight gain in women (Davis and Woodside, 2002; Davis et al., 2004; Franken and Muris, 2005; Loxton and Dawe, 2006). Women report more food cravings than men, indicating heightened motivation for hedonic eating (Lafay et al., 2001; Cepeda-Benito et al., 2003; Meule et al., 2012). Moreover, several studies have shown that women are highly susceptible to the sociocultural pressure resulting from the "lean ideal" portrayed by the media, leading to attempts to lose weight and be slim (Polivy and Herman, 2004; Dittmar, 2005; Mask and Blanchard, 2011; Yean et al., 2013). As a consequence food restriction and avoidance behavior might boost initial vulnerability to and incentive saliency of highly palatable "forbidden" food. In males, drive for a lean body has been shown to be lower (e.g., Cohane and Pope, 2001; Grogan and Richards, 2002; Yean et al., 2013). Their individual motivational value of food might thus be less environmentally influenced.

Table 4 | Pearson correlations.

		BIS-11	BAS	BIS	HUN	DIS
CR	*r*	−0.196**	0.180*	0.018	**−0.227*** **	0.148*
	p	0.006	0.013	0.801	**0.002**	0.041
DIS	*r*	0.046	0.135	−0.022	**0.494*** **	
	p	0.525	0.061	0.764	**<0.0005**	
HUN	*r*	0.195**	−0.050	−0.062		
	p	0.007	0.487	0.391		
BIS	*r*	−0.002	**0.324*** **			
	p	0.981	**<0.0005**			
BAS	*r*	−0.132				
	p	0.068				

**p < 0.05, **p < 0.01, ***p < 0.0033.*

*Pearson correlations between all assessed questionnaire scores in the TFEQ-plus cohort (n = 192). p-values < 0.0033 (***, bold) are considered as significant after Bonferroni correction for multiple comparison. Noticeable are the associations of CR with HUN (negative), DIS with HUN (positive), and BAS with BIS (positive) as well as the trend toward the correlation of CR and BIS-11 (negative). CR, TFEQ cognitive restraint score; DIS, TFEQ disinhibition score; HUN, TFEQ hunger score; BIS-11, Barratt Impulsiveness Scale 11 total score; BAS, Behavioral Activation System total score; BIS, Behavioral Inhibition System total score; TFEQ, Three-Factor Eating Questionnaire.*

For men, reward associated with novelty and excitement might be particularly reinforcing. Studies reported a higher risk for excitement-related addiction like pathological gambling (see van den Bos et al., 2013a for review), alcohol and cannabis (Wagner and Anthony, 2007; NSDUH, 2012; EMCDAA, 2013) or exercise dependence (Crossman et al., 1987; Pierce et al., 1997; Weik and Hale, 2009) in men.

BIS RESPONSIVENESS AND BMI

Emotional eating, which is related to punishment sensitivity (Gray, 1970, 1982, 1987), serves as a way to compensate perceived punishment/negative affect in women (van Strien et al., 1986, 2013; Geliebter and Aversa, 2003; Nolan, 2012). Therefore obesity in women with high *BIS* responsiveness might be related to compensational eating. Men generally show a lower sensitivity to punishment (Cross et al., 2011) as well as stronger emotional and cognitive control over immediate emotional events (especially punishments; van den Bos et al., 2013b), presumably reducing their need for compensation of negative emotionality. Further, there is no clear-cut link between negative emotional eating and BMI in men (Macht et al., 2002; Geliebter and Aversa, 2003; Nolan, 2012), and, in contrast to women, food craving has been associated with positive mood states (Lafay et al., 2001). In contrast to women *BIS* responsiveness in men might reflect differences in risk taking behavior. Koritzky et al. (2012) showed that particularly overweight and obese in comparison to lean men decided more often for high immediate reward despite long-term losses. Accordingly, they might more easily ignore long-term consequences of overeating, such as weight gain, because of low sensitivity to related punishment.

Although the *BIS* and *BAS* scales are assumed to be orthogonal (Gray, 1982, 1987), we found a correlation between the two measures. As BMI moderated the relationship between *BIS* and *BAS* in women, we assume that differences in body weight status accounted for this effect in our sample.

INVERTED U-SHAPED RELATIONSHIP BETWEEN BMI AND *BAS*

We corroborated the inverted U-shaped relationship between sensitivity to reward and BMI demonstrated by Davis and Fox (2008) using the *BAS* scale. Following Davis and Fox (2008), subjects with a high BMI in the non-obese range are supposed to face stronger food cravings and appetitive drive, resulting in enhanced hedonic eating, weight gain, and possibly overweight. Davis and Fox (2008) assumed that these individuals detect rewarding stimuli like palatable food more easily and more likely approach them. The inverse relationship between BMI and *BAS* in the obese range of the BMI is supposed to reflect reward deficiency resulting from hypo-DA functioning in obese individuals (Wang et al., 2001; Volkow et al., 2008; de Weijer et al., 2011). Compensatory hedonic eating probably compensate for this deficiency.

RELATIONSHIP BETWEEN SELF-REPORTED IMPULSIVITY AND BMI

The contribution of self-reported impulsivity on body weight remains vague. Impulsivity did not explain BMI variance in our dataset. Contradictory results regarding the relationship with BMI have been reported previously (Nolan, 2012; van Koningsbruggen et al., 2013). In general, none of the subscales seem to be consistently related to overeating or BMI (Meule, 2013). However, we observed a trend for a negative correlation between *BIS-11* and *cognitive restraint.* This indicates an indirect influence of impulsivity on body weight status via eating behavior, which is in line with previous findings (Leitch et al., 2013).

STUDY LIMITATIONS AND FUTURE DIRECTIONS

This study is based on analyses of self-reported measures, i.e., mentally represented, explicitly accessible information. We have not considered automatic processes (i.e., eating habits) like implicit food attitudes (e.g., Papies et al., 2009; Goldstein et al., 2014) or implicit liking/wanting (e.g., Berridge and Robinson, 2003; Finlayson et al., 2008), which should be regarded in future studies.

Furthermore, impulsivity is a multifaceted construct (e.g., Patton et al., 1995; Whiteside and Lynam, 2001). According to insufficient validity of the factor structure of the *BIS-11* in German (Preuss et al., 2008) we restricted our analysis to the *BIS-11* total score. Another impulsivity scale, the *UPPS Impulsive Behavior Scale* (Whiteside and Lynam, 2001), is recommended as an additional self-report measure of impulsivity. This scale is associated with obesity (Mobbs et al., 2010), but probably measures aspects of impulsivity that are not covered by *BIS-11* (Meule, 2013).

Moreover, *cognitive restraint* has been proposed to be subdivided into a rigid and flexible component (Westenhoefer, 1991; Westenhoefer et al., 1999). For reasons of construct validity, the *cognitive restraint* scale has been expanded by several further items (Westenhoefer et al., 1999). We recommend assessment of these items, because subscaling allows a more detailed analysis of *cognitive restraint's* influence on body weight.

Finally, BMI, although a common way to assess obesity, is a rather course measure. It relates body weight to body height without taking actual body composition into account. As it does not measure body fat directly, erroneous evaluation of body weight status with respect to obesity can occur (Rothman, 2008). Addressing this limitation, we recommend consideration of additional measures like waist/hip ratio or concentration of adipokines like leptin (Badman and Flier, 2005).

SUMMARY

This study demonstrates that responsiveness to the behavioral activation and behavioral inhibition system explains differences in BMI independently of eating behavior. Interestingly the relationships of BMI to *BIS* and *BAS* depend on gender, with opposing directions in men and women. Therefore, specified for men and women, *BIS/BAS* responsiveness should be considered in the treatment of obesity. Further, our study contributes to a better understanding of the complex relationships between eating behavior and body weight status. We showed that *cognitive restraint* and BMI are non-linearly associated (inverted U-shaped relationship). Importantly, this relationship is moderated by the level of *disinhibition.*

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Controversies about a common etiology for eating and mood disorders

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Obesity and depression represent a growing health concern worldwide. For many years, basic science and medicine have considered obesity as a metabolic illness, while depression was classified a psychiatric disorder. Despite accumulating evidence suggesting that obesity and depression may share commonalities, the causal link between eating and mood disorders remains to be fully understood. This etiology is highly complex, consisting of multiple environmental and genetic risk factors that interact with each other. In this review, we sought to summarize the preclinical and clinical evidence supporting a common etiology for eating and mood disorders, with a particular emphasis on signaling pathways involved in the maintenance of energy balance and mood stability, among which orexigenic and anorexigenic neuropeptides, metabolic factors, stress responsive hormones, cytokines, and neurotrophic factors.

Keywords: depression, obesity, reward, overweight, palatable food

INTRODUCTION

Most of antidepressant medications have long been known to induce weight gain, although the obesogenic mechanisms of these treatments remain largely unknown. Most interestingly, a large body of evidence supports the idea that non medicated depressed patients with mood disorders have increased risks of developing obesity compared to the general population, while specific interventions aiming at reducing body weight (including bariatric surgery and increased physical activity) have been found to improve mood. Hence, empirical observations have long suggested a common etiology between obesity and depression: (1) unhealthy diets favoring energy dense foods promote the development of both pathologies; (2) reduced physical activity and sedentary lifestyle are commonly observed in obese and depressed patients; (3) impaired sleep and/or circadian cycles deteriorate mood and increase body weight; (4) recurrent psychological stress and early life trauma have been shown to contribute to a late-onset obesity and depression. Considering the increased prevalence of obesity and depression worldwide, a better comprehension of their common biological bases has become crucial for identifying novel therapeutic targets.

Indeed, obesity-related diseases, including type II diabetes, high blood pressure, and cancer, have become one of the leading causes of preventable death worldwide, reducing life expectancy by 10 years or so. With depression, obesity represents a serious public health concern that contributes to significantly deteriorate the quality of life in people of most countries. Although researches concerning obesity and depression have been conducted for decades separately, recent evidence has shown that these two pathologies might share some neurobiological underpinnings, and possibly a common etiology. Here, we propose to review biological adaptations within several signaling pathways potentially underlying the increased risks of developing both diseases.

Obesity is a pathological condition characterized by excessive body fat accumulation resulting from an imbalance between caloric intake and expenditure. The World Health Organization (WHO) has regularly monitored the worldwide prevalence of overweight and obesity, and a recent estimation suggests that 11% of the world population (more than half a billion people) is obese, and 35% overweighed. Some monogenic forms of obesity have been described (e.g., mutations of leptin and leptin receptor, pro-opiomelanocortin and melanocortin-4-receptor genes) but they are very rare and not sufficient to explain the worldwide distribution of this disorder (Andreasen and Andersen, 2009). As a matter of fact, recent clinical findings rather suggest that obesity is most likely related to common genetic variants and/or single-nucleotide polymorphisms, representing a genetic risk factor of vulnerability (Frayling et al., 2007; Andreasen et al., 2008; Haupt et al., 2008; Hunt et al., 2008).

Depression is the commonest psychiatric disorder, characterized by severe negative mood and inability to experience pleasure from usually pleasurable activities (anhedonia). Depressed mood is associated with fatigue, incapacity to concentrate, altered appetite, sleep disorders, and metabolic complications (Ustun et al., 2004; Kessler and Bromet, 2013). Depressive symptoms often occur at younger age and, in the most serious cases, lead to suicide attempts. The most recent survey conducted by WHO in 2012 estimates that depression affects more than 350 million people worldwide, hence constituting a major threat to public health (Kessler and Bromet, 2013). Major depression is a heterogeneous disease comprising several subtypes, but the atypical depression is one of the most diagnosed forms. Symptoms like psychomotor retardation, insomnia, increased appetite, and body weight gain differentiate atypical depression from the other forms

(Gold and Chrousos, 2002). This subtype of depression is often accompanied by visceral adiposity and shares with obesity certain metabolic, endocrine and behavioral alterations (Vogelzangs et al., 2007; Lasserre et al., 2014). Interestingly, fat accumulation, commonly measured by the body mass index (BMI; weight in kilograms divided by height in squared meters, BMI), has long been used to monitor weight gain. Increasing evidence rather suggests that abdominal obesity [as referred as the waist-to-hip ratio (WHR)], presents a stronger predictive value than total body fat for predicting obesity-depression co-morbidity (Zhao et al., 2011; Wiltink et al., 2013).

Like obesity, the underlying biological causes of depression remain largely unknown, but genetic predisposition is considered to account for ∼40% of cases (Uhl and Grow, 2004). The genetic approach suggests that depression is a polygenic disease, determined by environmental influences in genetically predisposed individuals (Charney and Manji, 2004). Early life stress has been considered as one of the most critical factor triggering depression (Nemeroff and Vale, 2005). Many longitudinal studies have shown that depression is a consistent predictor of metabolic syndrome since its progression is often associated with cardiovascular diseases, diabetes, obesity, and chronic inflammation (Katon and Ciechanowski, 2002). One possible explanation for this observation would take into account the fact that depressed patients often adopt poor health behaviors, including smoking and drinking habits (Davis et al., 2008), reduced physical activity and unhealthy diets (Cizza, 2011).

The aim of this review is to summarize the current knowledge about the obesity-depression relationship and discuss plausible biological mechanisms underlying this association; first by reviewing the clinical findings supporting the intermingled interaction between obesity and depression, second by discussing the possibility that obesity may be a cause of depression and *vice versa*. In particular, we will be focusing our attention on signaling pathways involved in the maintenance of energy balance and mood stability, among which orexigenic and anorexigenic neuropeptides, metabolic factors, stress responsive hormones, cytokines, and neurotrophic factors.

THE OBESITY-DEPRESSION ASSOCIATION AND MOST RELEVANT MEDIATORS

Since obesity has long been considered a metabolic illness, while depression was classified a psychiatric disorder, both basic science and medicine have long investigated these two pathologies separately. Most of the epidemiological and clinical investigations conducted before the end of the 20th century provided only confounding outcomes, unable to prove a direct association between obesity and depression. Nevertheless, a seminal observation proposed that "socio-demographic, psychosocial and genetic factors may render certain obese individuals more prone to depression and *vice versa*" (Faith et al., 2002). The overlap between mood disorders and obesity was later supported by an extensive study reviewing clinical studies from 1966 to 2003 (McElroy et al., 2004). The authors claimed that obesity and mood disorders were likely related disorders with a distinct, but overlapping, pathophysiology. As a consequence, only some forms of obesity and mood disorders that share pathogenic factors would be related. Further supporting this assumption, obesity was found to be strongly associated with a range of common mood and anxiety disorders, suggesting that social and cultural factors may represent possible mediators and/or modulators of the obesity-depression relationship (Simon et al., 2006). More recent publications, including prospective cohort studies and cross-sectional researches, also reported relevant observations supporting a bidirectional causal link between obesity and depression, by which each disease may contribute to the other (Markowitz et al., 2008; Luppino et al., 2010; Faith et al., 2011). In a recent systematic review of the literature, undertaken to identify biopsychological variables associated with the relationship between obesity and depression (Preiss et al., 2013), physical health, decreased social activity, family depression history, childhood abuse, body image distorsion, severity of obesity and binge eating were listed consistent risk factors for developing comorbid obesity and depression. Collectively, these reports suggest that specific risk factors may confer to subgroups of obese individuals higher probability to develop depression, and to subgroups of depressed patients greater vulnerability to become obese. However, it is important to note that the conclusions drawn from of Lupino's, Faith's, and Preiss's works may be limited by quite a strong heterogeneity, in the methodology, the patient identification and the measures of depression and body weight. In particular, the evidence for a mutual influence of depression and obesity relied on reported weight and not on objective body weight measures (Faith et al., 2011), and the negative impact of obesity on depression depended on the evaluation of clinical depression instead of depressive symptoms (Luppino et al., 2010).

Hence, despite limitations, current data suggest a mutual influence between obesity and depression (Atlantis and Baker, 2008), even if a few studies on adolescents would argue that depression represent a clearer risk factor for obesity (Korczak et al., 2013), whereas obesity may not confer any higher risk for later developing depressive symptoms (Roberts and Duong, 2013). A reasonable conclusion would imply that obesity might represent a risk factor for depression only under particular conditions, which are binge eating behavior or abdominal fat deposition (Weber-Hamann et al., 2002; Araujo et al., 2010; Zhao et al., 2011; van Reedt Dortland et al., 2013a,b).

To summarize, the obesity-depression association seems to be strongly dependent on several risk factors that have been clearly established. Reduced physical activity and sedentary lifestyle have been frequently observed in depressed (Azevedo Da Silva et al., 2012; Song et al., 2012) and obese individuals (Bailey et al., 2007; Tucker and Tucker, 2011). Conversely, when depressed patients were encouraged to do physical exercise, improvement of their mood has been repeatedly reported (Conn, 2010; Carek et al., 2011; Rimer et al., 2012). Moreover, the efficacy of exercise in reducing risk of depression has also been observed in overweight and obese adults (Vallance et al., 2011). Sleep disorders and circadian cycle alterations have been shown to be associated with mood changes (Costa e Silva, 2006; Turek, 2007; Kronfeld-Schor and Einat, 2012; Avila Moraes et al., 2013) and weight gain (Bray and Young, 2007; Shi et al., 2013). Gender has also been considered an important factor since a stronger vulnerability in women has been well documented for both diseases (Peveler et al.,

2002). Unhealthy diets characterized by excessive consumption of energy dense foods have been associated with an increased risk of depression (Jeffery et al., 2009; Sanchez-Villegas et al., 2012; Sanchez-Villegas and Martinez-Gonzalez, 2013) as well as obesity (Fulton, 2010; Mozaffarian et al., 2011). In contrast, adherence to Mediterranean diet or to others diets comprising high amounts of vegetables and fruits have been shown to reduce risks of depression (Sanchez-Villegas et al., 2009; Jacka et al., 2010). Finally, stress and early life trauma have been shown to significantly contribute to both late-onset obesity (Gustafson and Sarwer, 2004; Gunstad et al., 2006; D'Argenio et al., 2009) and depression (Cirulli et al., 2009).

In the next part of this article, we will be essentially focusing on two increasing risk factors: stress and diet.

OBESITY AS A CAUSE OF DEPRESSION

The decision to eat is not only ultimately influenced by the internal state of the caloric equation but also by non-homeostatic factors, including food palatability and environmental cues known to trigger conditioned responses (Lutter and Nestler, 2009; Williams and Elmquist, 2012). Hence, a current consensus acknowledges that feeding behaviors are not only influenced by caloric needs, but also by other layers of regulation that involves the processing of reward, notably through dopamine signaling and its ability to pair food consumption to the context predicting its availability (see for review, Volkow et al., 2013a). In an evolutionary perspective, this property of palatable foods used to be critical for surviving in environments where food sources were scarce. Large amounts of food were eaten when available, enabling energy to be stored in the body (as fat) for future use (Spiegelman and Flier, 2001). However, food habits have profoundly changed over the past decades. Energy-dense foods, especially high fat diets, have a reduced satiety capacity and a higher hedonic value compared to that of meals richer in proteins and/or complex carbohydrates, which may explain their excessive consumption and their role in promoting overweight and obesity (Lawton et al., 1993; Blundell and Macdiarmid, 1997). Moreover, in modern societies, food has become plentiful and ubiquitous. As a consequence, the evolutionary adaptation inducing energy storing has become a dangerous liability (Erlanson-Albertsson, 2005a,b) promoting disinhibited and uncontrolled food seeking habits. Energy dense foods are potentially harmful for human health not only for their unbalance contents but also for their capacity to promote overeating behaviors (Berthoud et al., 2011; Egecioglu et al., 2011).

Recent evidence has established that disruption of energy homeostasis can affect the reward circuitry and that overconsumption of palatable food can lead to changes in the reward circuitry that result in compulsive food intake (Bassareo and Di Chiara, 1999; Kenny, 2011). The peripheral signals include peptides and hormones like leptin, insulin, cholecystokinin (CCK), tumor necrosis factor- α (TNF-α) but also nutrients (sugars and lipids), that are transported via the vagus nerve to the nucleus solitary tract and directly through receptors located not only in the hypothalamus, but also in autonomic and limbic brain regions. These multiple signaling pathways ensure that food is consumed when needed. However, with repeated access to highly palatable food, some individuals may eventually override the inhibitory processes that signal satiety and begin to compulsively consume large amounts of food despite nutrition overload (Zheng and Berthoud, 2007; Johnson, 2013). This loss of control and compulsive pattern of food intake is reminiscent of the drug intake patterns seen in addiction and has led to the description of some types of eating disorders inducing obesity as a form of "food addiction" (Volkow et al., 2013b).

Smith and Robbins proposed that overconsumption of palatable foods would lead to habit-driven responses by initiating a devolution from goal-directed to habitual behavior. According to this hypothesis, a process of signaling transfer would devolve impulsively driven and hedonically motivated actions from ventral to dorsal striatal control (Smith and Robbins, 2013). As a consequence, the consumption of high-fat/high-sugar foods would become less pleasurable and instead would turn into a compulsive response triggered by cues such as advertisements, mood, and context. The prefrontal cortex is considered to be critical for self-control, inhibition, and goal representation, and reduced activity in this region is associated with higher levels of impulsivity and compulsivity. Since executive function difficulties have been reported in overweight and obese individuals, and a decrease in orbito-frontal cortex volume correlated with disinhibited eating in obese adolescents, the shift from ventral to dorsal striatal control described above may also be associated with impairments in executive functions such as cognitive control, flexibility, decision-making, and working memory. Ultimately, individuals who experience a lack of control in the face of food persistently overuse their preferred food despite severe health, social, legal, and financial problems; and are unsuccessful at attempting to cut back or reduce their consumption. These behaviors are typically accompanied by feelings of guilt, remorse, sometimes triggering excessive food restriction and distress that in turn, promote palatable food intake to alleviate these dysphoric signs, further accentuating the spiraling distress (Fulton, 2010).

In other words, negative mood, provoked by palatable food withdrawal or by the incapacity to lose weight, often results in the adoption of an abnormal eating behavior characterized by recurrent cycles of dieting and overeating (Polivy and Herman, 1985; Petroni et al., 2007; Stice et al., 2008). Consequently, overeating episodes may turn into an overt binge-eating disorder (BED) that is frequently associated with a feeling of distress. Consistent with this, obese patients suffering from BED often present severe depressive symptoms (Heatherton and Baumeister, 1991; Stice et al., 2000; Spoor et al., 2006; Araujo et al., 2010; Blumenthal and Gold, 2010; Faulconbridge and Bechtel, 2014). These clinical findings are largely supported by experiments in which compulsive overeating has been induced in laboratory animals. For example, the capacity of palatable foods, when taken in a discontinuous pattern, to promote binge eating behavior has been repeatedly demonstrated in rodents (Colantuoni et al., 2002; Bello et al., 2003; Cottone et al., 2008; Corwin et al., 2011). Interestingly, several of these works have also proved that long-term intermittent access to palatable food is accompanied by depressive-like phenotypes and metabolic alterations (Cottone et al., 2009b; Rossetti et al., 2013).

Traumatic stress experiences, especially in early life, are known to affect mood and feeding behavior and therefore, they constitute

a strong element conferring higher vulnerability for developing obesity and depression. Compelling evidence suggests that stress increases palatable food preference and consumption both in humans (Gluck et al., 2004; Gluck, 2006; Adam and Epel, 2007; O'Connor et al., 2008; Tryon et al., 2013a,b) and in rodents (Foster et al., 2006; Moles et al., 2006; Machado et al., 2013; Patterson and Abizaid, 2013). A likely explanation for such stress-induced preference is that palatable food acting as a "comfort food" would be able to alleviate discomfort (Jeffery et al., 2009). In agreement with this hypothesis, a large body of preclinical studies has demonstrated that, not only chronic stress promotes palatable food intake (Dallman et al., 2003; Pecoraro et al., 2004) but its withdrawal increases stress sensitivity and depressive-like behaviors (Avena et al., 2008; Teegarden and Bale, 2008; Iemolo et al., 2012).

Industrial foods that progressively replace healthier fresh cooked meals worldwide are harmful for human health not only for their strong rewarding properties but also for their low content of poly-unsatured fatty acids (PUFAs). Accordingly, higher intake of saturated fatty acids has been found to promote visceral fat deposition and mood alterations (Schulze et al., 2006; Akbaraly et al., 2009; Molenaar et al., 2009; Jacka et al., 2010). Diets containing low levels of PUFA also increase the risk of depression (Appleton et al., 2010), while lower levels of omega-3 PUFA have been recently reported in the blood of depressed patients (Ross et al., 2007; Lin et al., 2010). Similar conclusions about the role of PUFA in depression have been indirectly drawn from people adhering to a Mediterranean diet. This diet, privileging vegetables, fruits and fish and containing high levels of PUFA, has been shown to limit excessive weight gain and obesity (Schroder et al., 2004, 2007; Romaguera et al., 2009) as well as depression (Sanchez-Villegas et al., 2009). Beside these human studies, the relevance of PUFA in the prevention of depressions has also been reported in rodents (Fedorova and Salem, 2006; Huang et al., 2008) and non human primates (Chilton et al., 2011).

In sum, protracted consumption of unhealthy diets and recurrent stress could interfere with the homeostatic control of the energetic balance and modify the reactivity of the rewarding system leading ultimately to a loss of control over food intake. The resulting compulsive overeating might trigger a BED and eventually lead to abdominal fat deposition, two factors tightly associated with depression (Weber-Hamann et al., 2002; Rivenes et al., 2009; Zhao et al., 2011; van Reedt Dortland et al., 2013a,b).

LEPTIN SIGNALING

Leptin is a peptide hormone belonging to the adipokines family that is secreted primarily from adipocytes of the white adipose tissue. Leptin serum levels change depending on the feeding/fasting state and correlate with body mass (Seeley and Woods, 2003). This peptide is coded by the obese gene *ob* and exerts its physiological function mainly in the brain. After release in the bloodstream, leptin pass across the blood–brain barrier (BBB) using a receptor-mediated transport (Banks et al., 1996). LepRb is the long form of the leptin receptor and is critical for leptin signaling cascade (Banks et al., 2000). LepRb was first identified in the hypothalamus and its activation was related to the anorectic effect of leptin, that is its ability to reduce food intake and increase energy expenditure (Chehab, 2000). Later, the presence of this receptor in other brain structures, namely the hippocampus, prefrontal cortex, ventral tegmental area (VTA) and amygdala has suggested additional roles of leptin in other physiological functions, among which learning and memory (Farr et al., 2006; Harvey et al., 2006), motivation for reward (Farooqi et al., 2007; Grosshans et al., 2012), and mood regulation (Guo et al., 2013; Milaneschi et al., 2014).

The role of leptin in the orchestration of food intake and energy expenditure in the hypothalamus has been largely described in the literature (Cone, 2005; Coll et al., 2007). Briefly, leptin is released by the adipose tissue, crosses the BBB and reaches the arcuate nucleus (ARC) of the hypothalamus where interacts with its receptors localized on two neuron populations: the orexigenic neuropeptide Y (NPY)/agouti related peptide (AgRP) neurons and the anorexigenic proopiomelanocortin (POMC)/cocaine and amphetamine regulated transcript (CART) neurons. Leptin inhibits the former and activates the latter, leading to a satiety signal (Spiegelman and Flier, 2001). It has long been known that impairment in leptin signaling causes obesity, increased food intake and fat deposition (Pelleymounter et al., 1995a; Speakman et al., 2007). These observations gave rise to the hypothesis that leptin could be used as anti-obesity treatment, but clinical investigations have shown that obese patients have chronically high levels of circulating leptin (Lu et al., 2006). This apparent contradiction suggests the emergence of a leptin resistance syndrome, a phenomenon also observed with insulin in diabetic patients. The precise mechanism responsible for leptin resistance is not yet known but may be linked to down-regulation of leptin receptors, reduced transport of leptin across the BBB or altered intracellular transduction of leptin signaling (Jung and Kim, 2013).

Abnormalities in leptin functioning are also believed to enhance the motivation for palatable food and promote its overconsumption. This assumption comes from the discovery that leptin receptors are also localized in brain reward structures (Figlewicz and Benoit, 2009). In particularly, leptin receptors have been detected in dopaminergic neurons of the VTA and electrophysiological studies in rodents have established that leptin decreases the firing rate of these neurons reducing dopamine release and food intake (Hommel et al., 2006). There is also evidence that leptin is able to regulate the incentive salience of reward because food restriction in rodents (that corresponds to a reduction of leptin signal) increases the preference for sucrose and other drugs in a conditioned place-preference paradigm (Figlewicz and Benoit, 2009). In other words, leptin impairment may result in a higher stimulation of dopaminergic neurons that, in turn, may increase the incentive for palatable food and ultimately its consumption. In support of this hypothesis, some animal studies demonstrated that injection of leptin in the VTA reduced food intake whereas a viral-mediated knockdown of leptin receptor in the same brain structure had opposite effect (Hommel et al., 2006).

In the last years, the scientific interest for leptin has also been extended to psychiatric disorders, such as anxiety and depression. Mutant mice that lack leptin signaling have been found to develop depressive symptoms (Collin et al., 2000; Sharma et al., 2010; Yamada et al., 2011) whereas systemic and central administration

of leptin in wild-type mice reduced anxiety and depressive-like behaviors (Asakawa et al., 2003; Lu et al., 2006; Finger et al., 2010; Liu et al., 2010; Yamada et al., 2011; Guo et al., 2013). In addition, other preclinical studies have shown that circulating leptin levels are modulated by stress since chronic unpredictable stress or chronic social defeat, but not acute stress, decreased basal levels of leptin in rats (Lu et al., 2006; Ge et al., 2013).

Although these findings seem to support a reduction of leptin levels in animal models of depression, the current knowledge on the role of leptin signaling in human depression remains unclear. Leptin levels in depressed patients have been reported to be lower, higher or equal to those observed in control patients (Deuschle et al., 1996; Antonijevic et al., 1998; Rubin et al., 2002; Esel et al., 2005; Zeman et al., 2009). However, studies with larger size samples reported a negative correlation between plasma leptin levels and major depression, hence supporting the idea that decreased leptin signaling may be a shared biological alteration in both obesity and depression (Kraus et al., 2001; Atmaca et al., 2002; Westling et al., 2004; Jow et al., 2006; Lawson et al., 2012). In conclusion, there is a current consensus suggesting a reduced leptin signaling in human depression, even though, this association might be stronger in certain subtype of depression or might be influenced by different factors including age, sex, body mass, and co-morbidity with other disorders (Gecici et al., 2005).

Nevertheless, the involvement of the leptin hormone in the emergence of depressive symptoms remains unclear. In this regards, some findings suggest that leptin may exert an antidepressant effect by modulating the hypothalamic-pituitary-adrenal (HPA) axis function. An early *in vitro* study on primary cultures of bovine adrenocortical cells demonstrated the capacity of leptin to reduce the transcription of cortisol (Bornstein and Chrousos, 1999). Mutant mice with altered leptin signaling exhibit hypercorticosteronemia, whereas leptin replacement reduces corticosterone levels in these mice (Chen et al., 1996; Chua et al., 1996; Arvaniti et al., 2001). Similarly, an inverse correlation between basal levels of leptin and release of glucocorticoids (GCs) has been found in humans (Licinio, 1998; Komorowski et al., 2000). Beside a direct effect on GCs, it has also been speculated that leptin may limit the activity of the HPA axis by suppressing the hypothalamic corticotropin-release factor (CRF) release (Ahima et al., 1996; Huang et al., 1998; Arvaniti et al., 2001).

A second hypothesis in favor of the antidepressant role of leptin is linked to the neurotrophic hypothesis of depression, since this adipokine may facilitate neurogenesis. In particular, mutant mice exhibiting an altered leptin signaling present reduced brain volume and abnormal expression of neuronal and glial proteins. These morphological changes have been attributed to the lack of leptin, since leptin administration was shown to normalize the brain morphology (Vannucci et al., 1997; Ahima et al., 1999; Steppan and Swick, 1999). Consistent with a neurotrophic role of leptin, it has been shown that this hormone facilitated the formation of specific neuronal projection pathways inside the hippocampus (Bouret et al., 2004). It also has been shown that leptin increased the motility and density of dendritic filopodia, and enhanced the number of hippocampal synapses (O'Malley et al., 2007). Further, convergent evidence demonstrated the importance in the hippocampus: first, mice with a selectively ablation of Lep-Rb in glutamatergic hippocampal neurons showed long-term potentiation (LTP) impairment, anhedonia-like phenotype, behavioral despair and enhanced social avoidance, and second, specific reduction of leptin receptors in the dentate gyrus, where neurogenesis is considered critical, was shown to induce marked depressive-like behavior (Guo et al., 2013).

In summary, accumulating findings established multiple and complex implications of leptin signaling in physiological and cognitive functions that extend energy balance regulation, and a compromised leptin signaling represents a serious candidate contributing to the development of pathological adaptations underlying the overlap between obesity and depression.

INFLAMMATION AND CYTOKINES RELEASE

Obesity is associated with a low-grade chronic systemic inflammation and in particularly interleukin-6 (IL-6), TNF-α and C-reactive protein (CRP) are present at high levels in the serum of obese people (Shelton and Miller, 2010; Gregor and Hotamisligil, 2011). Very recent epidemiological studies reported that increased levels of CRP correlated with depressive symptoms and abdominal obesity (Alvarez et al., 2013; Daly, 2013; Wium-Andersen et al., 2013) and other clinical works have found CRP to be the most consistent marker of the obesity-depression association (van Reedt Dortland et al., 2013a,b).

Two factors are responsible for chronic increase of circulating cytokines in obese patients. The first is fat deposition. Fat is stored in the adipocytes of the white adipose tissue and an increased adiposity, especially around the abdomen, stimulate these cells to release inflammatory factors, including adipokines, cytokines, and chemokines (Shelton and Miller, 2010; Gregor and Hotamisligil, 2011). Macrophages attracted into the adipose tissue by chemokines massively produce inflammatory factors, leading to the systemic inflammation observed in human obesity (Clement et al., 1997; Wellen and Hotamisligil, 2003). The second factor is diet quality. In a study conducted on a sample of healthy Greek population, the consumption of several food items was scored during a year. Higher scores, reflecting a stronger adherence to the Mediterranean diet, were inversely associated with biomarkers of systemic inflammation (Chrysohoou et al., 2004). The protective effect of the Mediterranean diet was more recently confirmed by other studies revealing that lower plasma concentration of CRP, IL-6, TNF-α were closely related to this type of diet (Camargo et al., 2012; Rimer et al., 2012; Urpi-Sarda et al., 2012). To date, the identification of the Mediterranean diet nutrients supposed to alleviate signs of chronic inflammation remain unclear. A recent study reviewed 26 randomized clinical trials and established that ω-3 PUFA was of particular interest to lower inflammatory markers (Bloch and Hannestad, 2012; Kiecolt-Glaser et al., 2012; Calder, 2013).

Cytokines are mostly produced in peripheral tissues and due to their large molecular weight cannot freely pass cellular membranes, but they can enter the brain through the leaky regions of the BBB or via cytokine-specific transporters. Cytokine signal also arrives inside the brain by afferent nerve fibers (the vagus nerve for example) or infiltration in the brain parenchyma of peripherally activated monocytes (Plotkin et al., 1996; Rivest et al., 2000;

Quan and Banks, 2007; D'Mello et al., 2009). Evidence for elevated levels of neuroinflammatory cytokines has been found in the hypothalamus of rodents after 4 weeks of high-fat feeding, and in *post mortem* brain samples of obese patients as well (Thaler et al., 2012). Noteworthy, whether it is diet-induced or genetically programmed, animal models of obesity present severe inflammation of hypothalamus (Velloso et al., 2008; Wisse and Schwartz, 2009). As a consequence, hypothalamic neurons are injured and the activity of some neurotransmitters, NPY and POMC, compromised (Thaler and Schwartz, 2010; Thaler et al., 2010). Hence, it is likely that excessive amounts of cytokines may exaggerate hypothalamic neuroinflammation and consequently, alter food intake regulation and energy expenditure, worsening signs of obesity in a spiraling down mechanism.

Concomitantly, the neuroinflammation response may exacerbate signs of depression as well, since chronic increase in pro-inflammatory and inflammatory markers has been repetitively observed in depressed patients. It is important to note that inflammation is a protective mechanism for the body to fight microbial and viral attacks. The short-term release of pro-inflammatory cytokines in the blood, including interleukin-1α (IL-1α), interleukin-1β (IL-1β), TNF-α, and IL-6, triggers fatigue, psychomotor retardation, sleep alteration and anhedonia. The "sickness behavior" is supposed to save energy, minimize risks and promote recovery. However, similitudes with the depressive symptoms are quite striking and suggest that depression could be associated with a long-term activity of cytokines (Maes et al., 1995; Charlton, 2000).

In other words, the "cytokine hypothesis of depression" considers depression as the result of a maladaptive response that occurs after a sustained and persistent cytokine release (Dantzer et al., 2008). In favor of this theory, many recent clinical data have shown elevated concentrations of inflammatory markers (including TNF-α, IL-6, IL1-β, and CRP) in the blood and in the cerebral spinal fluid of patient with major depression (Kim et al., 2007; Simon et al., 2008; Howren et al., 2009; Dowlati et al., 2010).

It is also important to note that psychiatric disorders, among which depression, have frequently been described in patient afflicted by pathologies associated with chronic inflammation; it is particularly true for rheumatoid arthritis and autoimmune disorders. Systemic lupus erythmatosus for instance is frequently associated with episodes of major depression. And further, elevated IL-6 spinal fluid levels found in these patients often correlate with the severity of their neuropsychiatric symptoms (Fragoso-Loyo et al., 2007).

The assumption that inflammatory processes may take part in the etiology of depression is also supported by the observation of patients receiving cytokine treatments (e.g., interferon-α) as an anti-cancer or anti-viral therapy. Despite the clinical efficacy of such therapy, more than 45% of treated subjects developed a major depression (Capuron and Miller, 2004; Raison et al., 2006). Moreover, with regards to antidepressant therapies, it has also been noted that their efficacy was related to a reduction of plasmatic levels of inflammatory markers (Lanquillon et al., 2000; O'Brien et al., 2007; Hannestad et al., 2011).

Meanwhile, preclinical findings have demonstrated that mice with targeted deletions of the gene coding for IL-6 or for TNF-α receptor show depressive-resistant phenotypes (Chourbaji et al., 2006; Simen et al., 2006). In other genetically and pharmacologically induced inflammation models, the presence of depressive-like behaviors has been demonstrated in several paradigms including the tail suspension and forced swim tests, as well as in protocols based on social interaction monitoring (Anisman et al., 2005; Moreau et al., 2008; Sukoff Rizzo et al., 2012). Collectively, these findings suggest that depression may represent a maladaptive response to a sustained cytokines release, which may occur in predisposed subjects when the activation of the immune system is heightened in intensity or duration.

While the plasmatic increase of cytokines in depression is confirmed by several studies, little is known about how these modulators of the immune response may affect mood regulation. Recently, it has been proposed that chronic inflammation can affect central neurotransmission, in particular, those of the serotonergic and dopaminergic systems. Particular interest has been accorded to the interaction between cytokines and serotonin because of the serotonergic hypothesis of depression (Coppen and Wood, 1978; Blokland et al., 2002; Wichers and Maes, 2004).

Briefly, cytokines seem to interfere with the serotonin system at different levels: synthesis, release and synaptic reuptake (Dunn and Wang, 1995; Dunn et al., 1999; Anisman et al., 2005). The amount of serotonin in the brain is highly dependent on the availability of the amino acid tryptophan and its transformation in 5-hydroxytryptophan by the enzyme tryptophan hydroxylase (TH; Delgado et al., 1990). Tryptophan levels also depend on an alternative metabolic pathway via its conversion in kynurenine upon the activation of the enzyme indoleamine 2,3-dioxygenase (IDO) localized in multiple cell types including macrophages, astrocytes, and microglia. Hyperactivation of this pathway leads to a depletion of tryptophan and ultimately, decreases the brain amounts of serotonin (Schwarcz and Pellicciari, 2002; Schrocksnadel et al., 2006). Intriguingly, several cytokines (e.g., TNF-α, interferon-α and interferon-γ) and their signaling pathways have been found to activate IDO, hence limiting tryptophan availability (Takikawa et al., 1999; Popov et al., 2006). Consistent with these *in vitro* observations, high plasmatic levels of pro-inflammatory cytokines have been associated with IDO induction in mice displaying depressive-like phenotypes (O'Connor et al., 2009a,b). In line with this interpretation, it also has been shown that: (1) IL-1, IL-6, and TNF-α are able to reduce brain levels of serotonin by accelerating its catabolism (Clement et al., 1997; Dunn, 2006) and (2) cytokines may facilitate serotonin synaptic reuptake (Blakely and Berson, 1992; Bull et al., 2009; Lotrich et al., 2009) hence reducing serotonin neurotransmission.

In parallel, chronic inflammation was shown to alter dopamine neurotransmission as well. Symptoms of depression have been shown to correlate with a reduced prefrontal and striatal dopamine activity (Dunlop and Nemeroff, 2007) and repeated interferon-α administration has been shown to decrease dopaminergic neural activity in the mouse brain (Shuto et al., 1997). Cytokines may also interfere with the dopamine transporter, or affect dopamine synthesis by inhibiting tetrahydrobiopterin (BH4), an important co-factor for tyrosine hydroxylase enzyme that converts tyrosine into L-DOPA (Kitagami et al., 2003). Finally, cytokines also affect

the HPA axis functioning. Hyperactivity of the HPA axis is a hallmark of depression (see next section) and several observations have demonstrated that cytokines and cytokine-inducers can potently activate the stress cascade. Administered acutely, cytokines have been shown to increase the expression and the release of CRF, adrenocorticotropic hormone (ACTH), and cortisol (Besedovsky and del Rey, 1996; Pariante and Miller, 2001; Pace and Miller, 2009).

Even though these findings linking cytokines to depression have to be confirmed, the idea that neuroinflammation might be a leading cause of depression, represents a fascinating prospective connecting mood to eating disorders.

DEPRESSION AS A CAUSE OF OBESITY

The hyperactivation of the HPA axis and reduced neurogenesis and/or brain plasticity are two current hypotheses trying to reconcile the past and recent assumptions about the pathogenesis of depression. Interestingly, these biological processes have been demonstrated to participate to the etiology of obesity as well, and thus may represent a thread union between these two pathologies (Sinha and Jastreboff, 2013; Sominsky and Spencer, 2014).

HYPERACTIVATION OF THE HPA AXIS

A large body of clinical and preclinical evidence has led to the general consensus that hyperactivation of the HPA axis following a chronic stress experience is a leading cause of depression. Animal and human survival depends on the capacity to recognize and face harmful stimuli (Chrousos, 2009). In this context, stress response allows to react to potentially harmful threat and to maintain body homeostasis through transient physiological and behavioral adaptations (Chrousos and Gold, 1992; McEwen, 1998). This temporary adaptive stress response is directed to increase energy availability in those organs of the body involved to counteract the stressor. As a consequence, cardiac output and respiration are accelerated, catabolism is increased and blood flow is potentiated in the brain and muscles (Gilbey and Spyer, 1993).

These adaptive responses rely on the activation of the autonomic nervous system and the HPA axis, a complex neuroendocrine system composed of multiple brain structures and peripheral organs (Tsigos and Chrousos, 2002). Emotional and stressful stimuli, processed in the amygdala, activate the paraventricular nucleus of the hypothalamus (PVN) and trigger a cascade of physiological adaptations through the release of corticotropin-releasing hormone/factor (CRH/CRF; de Kloet, 2000; Charmandari et al., 2005). CRF target neurons are located in the anterior pituitary gland and release the ACTH in the bloodstream. This hormone stimulates the cortex of the adrenal gland to secrete GCs, cortisol in humans, and corticosterone in rodents. GCs receptors are widely distributed in the body and their binding with GCs leads to the activation or repression of a plethora of genes (Bamberger et al., 1996; Dostert and Heinzel, 2004), among which those coding for enzymes involved in promoting the hepatic synthesis of glucose from non-glucidic substrates (i.e., lactate, pyruvate, and amino acids). GCs also contribute to increase blood glucose levels, and antagonize the anabolic activities of insulin, growth, and thyroid hormones (Rizza et al., 1982).

When the exposure to stressful stimuli is limited, the stress response has short duration because GCs exert a feedback inhibition on the HPA axis. Specifically, they act on the pituitary and hypothalamus limiting the release of CRF and ACTH, which reduces their own activity. GCs also stimulate GC receptors in the hippocampus, where inhibitory GABAergic projections to PVN neurons block the CRF release (Boudaba et al., 1996; Herman et al., 2002). Higher cortical structures, including the dorsomedial prefrontal cortex and the prelimbic cortex, also control the stress response (Diorio et al., 1993; Radley et al., 2008).

During the past three decades, several groups reported compelling evidence showing that prolonged stress response (in particular sustained CRF and GCs release) may represent a biological mechanism triggering depression (Carroll, 1982; Holsboer, 2000; Pariante, 2003; Strohle and Holsboer, 2003) and obesity (Bjorntorp and Rosmond, 2000; Pasquali and Vicennati, 2000), even though psychosocial stress would exert only a modest effect on weight gain (Wardle et al., 2011). CRF has been particularly studied and its role in the emergence of depressive symptoms has been well documented (Grigoriadis, 2005). The activity of CRF depends on its binding on two types of receptors, both widely express in the brain and body, called CRF-R1 and CRF-R2. Three other endogenous ligands with different affinity for CRF receptors have been revealed and named urocortin1 (UCN1), UCN2, and UCN3 (Nakayama et al., 2011). Consistent with the CRF hypothesis of depression, some studies have shown that depressive patients exhibit high levels of CRF in the cerebrospinal fluid (Nemeroff et al., 1984), increased number of CRF expressing neurons in the PVN, elevated expression of CRF mRNA in the same neurons and reduced CRF receptor density (Banki et al., 1987; Raadsheer et al., 1994; Merali et al., 2004). Early observations in rodents also found that intracerebroventricular (ICV) administration of CRF induced anxiety and depression-like behaviors, whereas the injection of CRF antagonists produced the opposite effect, confirming the potential antidepressive properties of CRF ligands (Deak et al., 1999; Zobel et al., 2000; Seymour et al., 2003). Beside pharmacological data, genetic manipulations in rodents confirmed these observations: overexpression of the CRF gene in mice led to increased anxiety-like behaviors and impaired stress (Stenzel-Poore et al., 1994). Interestingly, these behavioral changes were accompanied by enhanced food intake, weight gain and insulin (Coste et al., 2001). However, whereas transgenic mice lacking CRF-R1 showed a reduced stress response and blunted anxiety-like behaviors (Smith et al., 1998; Timpl et al., 1998), those lacking CRF-R2 exhibited pronounced anxiety-like behaviors and stress hypersensitivity (Bale et al., 2000; Coste et al., 2000).

Compared to depression, less is known about a direct role of CRF signaling on obesity. The involvement of the CRF family peptides (CRF, UCN1, UCN2, and UCN3) in energy balance and food intake regulation has been documented (Chalew et al., 1995; Richard et al., 2002), but most of the data are rather contradictory (Levine et al., 1983; Negri et al., 1985; Asakawa et al., 1999; Ushikai et al., 2011). Similarly to what is observed with anxio-depressive like behaviors, the impact of the CRF system on energy balance largely depends on CRF ligands, the type of receptor and the brain structure targeted. Indeed, recent observations reported

that blocking CRF-R1 signaling in the central nucleus of the amygdala prevented palatable food intake and anxiety-like behaviors in the rat (Cottone et al., 2009a; Iemolo et al., 2013). Meanwhile, CRF-R2 activation with UCN2 was shown to reduce high-fat food consumption when injected ICV in the rat, confirming a former observation in mice lacking CRF-R2, which consume larger meals compared to wild type mice (Bale and Vale, 2003; Tabarin et al., 2007).

Further confirming a role of the HPA axis dysfunction in the emergence of excessive food intake and obesity, it has been recently demonstrated in humans that the systemic administration of a low dose of CRF stimulated food intake, likely through GCs release (George et al., 2010). The Cushing's syndrome, a disease resulting from pituitary/adrenal tumors or chronic treatment with corticosteroids, also represents a coincident model of depression and obesity (Sonino et al., 1998). Clinical observations of these patients revealed that cortisol hypersecretion is accompanied by visceral adiposity, metabolic syndrome and mood related disorders. In order to explain this, early and more recent researches have pointed out that the excessive release of GCs impairs the ability of insulin to promote glucose uptake, induces metabolic syndrome and promotes body fat deposition (Brindley and Rolland, 1989; Black, 2006). Likewise, chronically elevated GCs contribute to visceral fat accumulation in primates (Shively, 1998; Shively et al., 2009) and humans (Marin et al., 1992; Rosmond et al., 1998; Epel et al., 2000; Spencer and Tilbrook, 2011). Finally, the diurnal variations of circulating cortisol levels positively correlated with the hip-to-waist ratio in obese patients (Weaver et al., 1993; Lasikiewicz et al., 2008). Beside insulin and leptin resistance (Zakrzewska et al., 1997, 1999; Jéquier, 2002a,b), GCs may alter the energetic balance and stimulate food intake acting on different brain structures. GCs receptors are widely distributed in hypothalamic nuclei implicated in energy homeostasis such as ARC, LH, and PVN (Morimoto et al., 1996) and long-lasting stimulation of GC receptors in these brain regions is known to potentiate orexigenic signals modulating the expression of genes involved in energy balance. In particular, the expression of one gene contributing to satiety signaling, the POMC gene, has been found to be reduced following GC receptor stimulation and increased after adrenalectomy (Cavagnini et al., 2000; Savontaus et al., 2002).

While clinical data strongly support that chronically elevated GCs receptors affect the hedonic regulation of food intake and increase the preference for palatable food, which most likely contributes to excessive fat deposition (Dallman et al., 2003, 2006; Pecoraro et al., 2004; Germano et al., 2007), enhanced levels of corticosterone have been found in different animal models of obesity including fa/fa rats, db/db, and ob/ob mice (Guillaume-Gentil et al., 1990). Conversely, the absence of GCs, due to adrenalectomy, provoked body weight loss and food intake reduction in rodents, effects that could be reversed by corticosterone administration (Saito and Bray, 1984; Pralong et al., 1993; Makimura et al., 2000). Accumulating evidence suggests that diet composition may also play a role in modulating the HPA axis functions. Although, only a few studies have covered this topic, some of them indicate that unbalanced fat diets may perturb lipid metabolism and, as a consequence, increase circulating levels of GCs. Consistent with this hypothesis, a recent study on woman health has reported a positive correlation between consumption of saturated fatty acids and diurnal variation of cortisol (Garcia-Prieto et al., 2007). In addition, converging evidence has found that patients who received diets supplemented with PUFA or fish oil had reduced ACTH and cortisol rise after acute stress (Delarue et al., 2003; Michaeli et al., 2007).

Collectively, these observations emphasizes that stress alters the HPA axis homeostasis and most likely triggers the onset and worsening of depression. Since the hypothalamus is the main brain orchestrator regulating energy balance and food behavior, it is not surprising that alterations in the HPA axis may lead to overeating and obesity.

NEUROGENESIS AND THE BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)

The monoaminergic hypothesis of depression has long proposed that depression originates from decreased monoamine neurotransmission. Despite the impressive number of pre-clinical and clinical studies that have been conducted over the last 40 years, the validity of this theory remains debatable (Mulinari, 2012; Blier and El Mansari, 2013). In particularly, the discrepancy between the rapid increase of monoamine signaling occurring upon antidepressant treatment and their delayed therapeutic effect remains unclear. Moreover, inconsistent clinical findings have failed to demonstrate a decrease in monoamine basal levels in depressed patients compared with health people. Thus, the altered monoamine neurotransmission classically reported in depression is now considered to reflect the consequences of neuronal damage. Historically neurons have been presumed to not regenerate. However, recent evidence demonstrated that the mammalian central nervous system retains the capacity to produce new neurons that can integrate neural network (Ming and Song, 2011). Two brain regions have been extensively studied: the subventricular zone of the lateral ventricles and the subgranular region of the dentate gyrus in the hippocampus (Duman et al., 2001; Duman, 2002; Kempermann and Kronenberg, 2003). Hippocampal neurogenesis, which is pivotal for cognitive functions and mood control, is strongly affected by chronic stress which causes shortening and pruning of neuron dendrites and reduction of the final number of newborn neurons (McEwen, 1999; Duman, 2004a,b). Accordingly, morphologic and morphometric studies of brains of depressed patients have found a reduction of hippocampal volume due, at least in part, to an impaired neurogenesis (Sheline, 1996; Bremner et al., 2000; Sheline et al., 2003; Stockmeier et al., 2004; Lucassen et al., 2010). Interestingly, antidepressant treatments have shown to reverse neural atrophy, cell loss and stimulate neurogenesis (Duman and Monteggia, 2006). Similar observations have been reported with preclinical investigations, confirming that unpredictable chronic stress protocols suppressed hippocampal neurogenesis in rodents (Watanabe et al., 1992; Malberg and Duman, 2003; Pham et al., 2003; Mineur et al., 2007).

Neurotrophins constitute an important class of signaling molecules in the brain, playing a pivotal role in brain development, neuron survival and synaptic plasticity. The brain-derived neurotrophic factor (BDNF) has received particular attention,

since it is considered a relevant biomarker of depression and suicidal behavior, and a possible downstream target of a variety of antidepressant drugs (Dwivedi, 2009; Lee and Kim, 2010). Hippocampal neurogenesis is an important process that appears to be involved in maintaining balanced mood. A large body of evidence identifies BDNF, and its TrkB receptor, as two key elements in the orchestration of different phases of neurogenesis such as cell proliferation, migration, differentiation and death. The rationale for the involvement of BDNF in depression comes from the observation that stress can down-regulate the expression of this neurotrophic factor in brain structures known to control emotions (Dwivedi, 2009; Lee and Kim, 2010). Stress-related disorders, among which major depression, is considered to induce morphologic damages in the hippocampus, which is intimately connected to the HPA axis (Bremner et al., 2000). Accordingly, the expression of BDNF, BDNF regulated genes and TrkB receptors are decreased in *post mortem* hippocampal tissues harvested from depressed human brains (Tripp et al., 2012). Similar findings have also been reported for the prefrontal cortex, another brain region essential for mood regulation. Moreover, BDNF protein levels are reduced in the serum of depressed patients (Castren et al., 2007; Castren and Rantamaki, 2010; Thompson Ray et al., 2011). It is important to note that, *post mortem* hippocampal BDNF levels and serum concentrations were normalized in depressed patients successfully treated with antidepressants, suggesting that the therapeutic effect of these compounds is related to BDNF activity (Duman and Monteggia, 2006).

However, human studies are only correlative and a clear causal association between impairment of BDNF brain activity and depression has not been yet demonstrated in human patients. Thus, preclinical studies have attempted to validate this theory. Converging studies have shown that stress (both physical and psychological) is able to lower BDNF expression levels in rat hippocampus (Duman and Monteggia, 2006) and that direct BDNF infusion in this area reduces stress-induced depressive-like behaviors (Hoshaw et al., 2005; Hu and Russek, 2008). Viral-mediated deletion of BDNF or TrkB genes in the VTA resulted in a significant antidepressant-like response as well. However, results discrepancy made complicated the interpretation. For instance, mice exposed to a chronic social defeat exhibited increased depressive-like behaviors and increased BDNF protein levels in the nucleus accumbens and amygdala (Berton and Nestler, 2006; Yu and Chen, 2011). Therefore, the current hypothesis, suggesting a strong link between low BDNF brain levels and depression, appears to be too simplistic and has to be reconsidered. One possible explication is that BDNF gene expression may be down regulated in some brain structures (like hippocampus and prefrontal cortex) and up regulated in others.

Interestingly, compelling evidence demonstrated that BDNF has a direct role in the regulation of homeostatic and hedonic eating as well (Lyons et al., 1999; Kernie et al., 2000; Rios et al., 2001; Xu et al., 2003). With regards to the homeostatic regulation, early studies showed that ICV injection of BDNF in rats led to a reduction of body weight, suggesting that BDNF could take part in the central control of feeding behavior (Lapchak and Hefti, 1992; Pelleymounter et al., 1995b). More recently, BDNF deficiency has been associated with increased weight in mice (Noble et al., 2011; Schwartz and Mobbs, 2012), while leptin injected in the ventromedian hypothalamus (VMH) would exert an anorexigenic effect activating the expression of BDNF (Komori et al., 2006). The relevance of BDNF in energy balance was also confirmed in mutant mice. Heterozygous BDNF +/- mice show hyperphagia, body weight gain, insulin resistance, dyslipidemia, and hyperglycemia. In addition, these mice were more sensitive to negative effects of a high fat diet (Lyons et al., 1999; Kernie et al., 2000). Similarly, mice expressing only about 25% of TrkB receptors display excessive feeding (Xu et al., 2003). Inversely, BDNF infusion in the VMH of adult wild-type mice resulted in decreased food intake and body weight (Wang et al., 2007). Consistent with a role in homeostatic mechanisms, levels of expression of BDNF and trkB in hypothalamus and hindbrain regions are influenced by the energy status (Xu et al., 2003; Bariohay et al., 2005; Tran et al., 2006; Unger et al., 2007). Preclinical studies also support a role for BDNF in regulating hedonic feeding by modulating the mesolimbic dopamine (Seroogy et al., 1994; Numan and Seroogy, 1999; Cordeira et al., 2010). Meanwhile, only a limited number of human studies managed to correlate BDNF expression to obesity. Human BDNF haploinsufficiency was linked to elevated food intake and obesity (Gray et al., 2006; Han et al., 2008). Recent evidence also associated a functional polymorphism of the Bdnf gene, Bdnf Val66met which impedes a correct secretion and signaling of BDNF, with obesity predisposition (Beckers et al., 2008; Skledar et al., 2012), and the missense mutation in the TrkB gene, which prevents TrkB function, has been identified in patients exhibiting overweight and severe obesity (Yeo et al., 2004).

Interestingly, adhering to a balanced diet was shown to influence neurogenic factors, and potentially alleviates signs of depression and obesity. Depressed patients following a Mediterranean diet exhibited signs of remission concomitant to increased plasma BDNF concentrations (Sanchez-Villegas et al., 2011; Sanchez-Villegas and Martinez-Gonzalez, 2013). Meanwhile, overweight and obese patients exposed to a 3-months calorie restricted diet displayed increased levels of serum BDNF (Araya et al., 2008). On the other hand, high fat meals were shown to decrease plasma BDNF of almost 30% in healthy patients (Karczewska-Kupczewska et al., 2012). These observations have been confirmed in preclinical studies since BDNF level decreased in rats maintained on a high carbohydrate diet (Maioli et al., 2012), and high fat diet (Yamada-Goto et al., 2012), whereas caloric restriction increases BDNF expression (Lee et al., 2002; Duan et al., 2003).

Further investigation has revealed that ω-3 PUFA may play a role in neurogenesis. In animals, ω-3 PUFA supplementation provided protection against reduced plasticity and normalized BDNF after traumatic brain injury (Wu et al., 2004), whereas diets deficient in ω-3 PUFA lowered BDNF brain levels (Rao et al., 2007; Bhatia et al., 2011).

Taken together these data suggest that BDNF pathway may be a relevant biological substrate underlying the pathogenesis of both mood and eating disorders. However, considering the emerging data on complexity of BDNF pathway, further findings are needed to better understand whether BDNF is a real causal factor for the depression-obesity association.

CONCLUDING REMARKS

Obesity and depression represent a global health burden, and the diagnostic is even more severe for those individuals suffering from both diseases. The increasing prevalence of depression-obesity co-morbidity strongly suggests that these disorders may share a common pathogenesis.

Earlier and current findings clearly demonstrated a link between the two pathologies but the nature of their association is still to be fully understood. The complexity comes from the fact that both obesity and depression are heterogenic diseases, influenced by multiple environmental and genetic factors. Clinical works have identified environmental factors that seem to facilitate the development of obesity as well as that of depression. In particular, factors like stress and diet quality have a strong impact on both pathologies and may constitute key mediators for the obesity-depression association. Other risk factors, reduced physical activity, sleep impairments and altered circadian rhythms, are also considered relevant but most likely as factors worsening the acquired pathologies rather that factors influencing the emergence of the diseases.

Collectively, clinical studies reviewed in this article suggest that obesity and depression are closely related but may not be globally interconnected. Indeed, only subgroups of obese patients are at higher risk for developing depression, and *vice versa*. Accumulating evidence emphasizes that mainly patients with BEDs and those with abdominal fat deposition and metabolic syndrome are at higher risk for depression. The identification of these two subgroups of obese individuals is promising for discovering the biological mechanisms underlying the obesity-depression association.

In the last years, clinical and preclinical studies have also found that leptin may represent a biological substrate underlying the pathogenesis of both obesity and depression. Circulating leptin increases proportionally with body mass and is significantly elevated in obese patient in comparison with non-obese individuals. However, persistent high levels of the hormone alter leptin signaling in the brain, most likely due to leptin resistance compensatory mechanisms. This deficit is believed to trigger a maladaptive functioning of the brain homeostatic system, ultimately leading to obesity. Since converging evidence suggests that impaired leptin signaling may affect mood regulation and food reward perception in humans, impaired leptin signaling cascades may represent a biological mechanism binding obesity and depression, in particular when obesity is paired with compulsive overeating. This assumption opens novel perspectives for the development of therapeutic medications able to correct clinical signs of obesity and depression.

Substantial fat deposition is known to stimulate inflammatory processes, which in turn, promote peripheral inflammatory cytokines release. These molecules enter the brain, not only affecting the hypothalamic control of food intake but also impacting other brain functions that are pivotal for mood regulation. Consistent with this hypothesis, some findings demonstrated that high levels of inflammatory cytokines might interfere with serotonin and dopamine neurotransmission, and with HPA axis as well. Although they need to be confirmed, these findings suggest that the inflammatory hypothesis of eating and mood disorders should get a larger attention in the near future.

The stress response is known to alter the HPA axis functioning and to trigger depressive symptoms and eating disorders. Deciphering the contribution of CRF signaling in these two pathologies remains quite difficult though. Indeed, CRF has opposite effects on depressive symptoms depending on which brain receptors are activated, but CRF binding to CRF receptor 1 seems to exacerbate signs of depression while increasing food intake. The development of CRF receptor 1 antagonists may represent another relevant strategy for improving mood and eating disorders. Inhibitors of GCs are currently used for treating the Cushing's syndrome and some cancers of the adrenal gland, but no medications are available to date for treating depression or obesity.

The neurothrophic hypothesis of depression has received particular attention, but the current knowledge remains elusive and recent evidence, rather than establishing a clear-cut role for BDNF to causally link depression to obesity, mainly claim for further studies before delineating any mechanism underlying the association obesity-depression.

In conclusion, compelling evidence shows that obesity and depression are two overlapping pathologies. However, the causal link between eating and mood disorders needs to be clarified. Most likely, different mechanisms may contribute to the worsening of each disease, and a global cure does not sound realistic. Instead, a better understanding of the molecular and cellular adaptations occurring in subgroups of obese (or depressed) patients identified as highly vulnerable to develop comorbidities should be a clinical priority. Meanwhile, improving animal models of eating and mood disorders is critical for unraveling the underpinnings of the obesity-depression association.

However, given the deleterious impact of stress and junk food consumption on the onset and progression of these two pathologies, the most effective prevention program remains public campaign defending the adherence to healthy balanced diets and promoting effective programs of stress management.

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The endocannabinoid system: directing eating behavior and macronutrient metabolism

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For many years, the brain has been the primary focus for research on eating behavior. More recently, the discovery of the endocannabinoids (EC) and the endocannabinoid system (ECS), as well as the characterization of its actions on appetite and metabolism, has provided greater insight on the brain and food intake. The purpose of this review is to explain the actions of EC in the brain and other organs as well as their precursor polyunsaturated fatty acids (PUFA) that are converted to these endogenous ligands. The binding of the EC to the cannabinoid receptors in the brain stimulates food intake, and the ECS participates in systemic macronutrient metabolism where the gastrointestinal system, liver, muscle, and adipose are involved. The EC are biosynthesized from two distinct families of dietary PUFA, namely the n-6 and n-3. Based on their biochemistry, these PUFA are well known to exert considerable physiological and health-promoting actions. However, little is known about how these different families of PUFA compete as precursor ligands of cannabinoid receptors to stimulate appetite or perhaps down-regulate the ECS to amend food intake and prevent or control obesity. The goal of this review is to assess the current available research on ECS and food intake, suggest research that may improve the complications associated with obesity and diabetes by dietary PUFA intervention, and further reveal mechanisms to elucidate the relationships between substrate for EC synthesis, ligand actions on receptors, and the physiological consequences of the ECS. Dietary PUFA are lifestyle factors that could potentially curb eating behavior, which may translate to changes in macronutrient metabolism, systemically and in muscle, benefiting health overall.

Keywords: food intake behavior, endocannabinoids, polyunsaturated fatty acids, appetite, cannabinoid receptors, brain and neuronal function

INTRODUCTION

Decades ago, it was recognized that macronutrient expenditure and maintenance of dietary intake are controlled by complex physiological processes involving endocrine, neurological, and behavioral factors (Mayer and Thomas, 1967). More recently, the ECS was found to be integral in the control of food intake and a target to mitigate obesity (Cota, 2007). The cannabinoid receptors of this system are present in large numbers in the brain, and their activation by endogenous agonists, called EC, has revealed yet another aspect of the neuroendocrine system's participation in caloric intake (Mackie, 2008; Viveros et al., 2008). Furthermore, studies using pharmacological antagonists of the cannabis receptors in the brain have demonstrated marked improvements in weight loss and appetite control in humans but with undesirable side effects that include heart disease and depression (Di Marzo and Despres, 2009). The first discovered EC were found to be biosynthesized from PUFA of the n-6 family, however, those derived from the n-3 PUFA family have shown the potential of a dietary approach to modulate activation of the cannabinoid receptors to diminish food intake and downstream events that reduce macronutrient metabolism directed toward fat accumulation (Naughton et al., 2013). Indirect evidence to support this premise comes from studies in n-3 PUFA deficient-mice which abolished EC mediated neuronal functions (Lafourcade et al., 2011) and where feeding n-3 PUFA decreased the levels of the n-6 PUFA derived EC (Watanabe et al., 2003; Alvheim et al., 2012). The n-3 PUFA may lower activation of cannabinoid receptors, thereby attenuating food intake (Watkins et al., 2010; Kim et al., 2013).

The ECS includes both synaptic and peripheral signaling functions, and the cannabinoid receptors are found in many organs and tissues besides the nervous system (Mackie, 2008). Cannabinoid receptors are G protein-coupled receptors that upon activation, lead to multiple complex signaling pathways (Lafourcade et al., 2011). AEA and 2-AG, both derived from (20:4n-6 an n-6 PUFA), are the two most abundant endogenous EC for the cannabinoid receptors CB1 and CB2 (Bisogno et al., 1999; Bosier et al., 2010). However, other EC have been identified which include those biosynthesized from n-3 PUFA, such as eicosapentaenoyl ethanolamide from eicosapentaenoic acid (EPA or 20:5n-3) and docosahexaenoyl ethanolamide from docosahexaenoic acid (DHA

Abbreviations: AA, arachidonic acid; AEA, arachidonylethanolamide; 2-AG, 2-arachidonoylglycerol; DHA, docosahexaenoic acid; EC, endocannabinoids; ECS, endocannabinoid system; EPA, eicosapentaenoic acid; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAE, *N*-acylethanolamine; NAPE-PLD, p *N*-acyl phosphatidylethanolamine selective phospholipase D; PUFA, polyunsaturated fatty acids.

or 22:6n-3; Rossmeisl et al., 2012; Kim et al., 2014a). Binding affinities differ between the various EC ligands for the cannabinoid receptors, and as such, result in varying degrees of activation of the receptor and downstream effects (Mackie, 2008), which, in the case of the central nervous system (CNS), includes blocking of neurotransmitter release from the presynaptic neuron (Okamoto et al., 2004). The binding affinities of the EC derived from either EPA or DHA toward cannabinoid receptors are shown to be comparatively weaker than the AA-derived EC (Brown et al., 2010). In cells and tissues, a dynamic competition exists in the biochemistry of the PUFA families, and recently, their actions on ECS gene expression in both humans and rodents have varied (Watkins et al., 2010; Kim et al., 2013). Examples of how PUFA and EC change the expression of ECS-related genes have been reported in C2C12 myoblast cultures (Kim et al., 2014a) and in mice (Hutchins-Wiese et al., 2012). In studies with rodents, the consumption of dietary lipids varying in the amounts of n-6 and n-3 PUFA as well as monounsaturated fatty acids is now believed to be one approach to control appetite and obesity by changing endogenous levels of EC and subsequent receptor activation (Naughton et al., 2013).

During development, measurable increases in the expression of cannabinoid receptors (mRNA and protein) and circulating levels of EC have been reported, along with receptor binding in the fetal and early post-natal brains of rodents (Berrendero et al., 1999). Along with aging, it is suggested that a decrease in ECS activity is associated with a decline in neuroprotective regulation in the brain of rodents (Bilkei-Gorzo, 2012). Evidence supporting the neuroprotective benefits conferred by ECS intervention is scarce but growing (Goncalves et al., 2008; Marchalant et al., 2008, 2009). While the means of protection are a result of pharmacological intervention on the cannabinoid receptors, aging and environmental factors, such as dietary n-6 PUFA, may provide a protective mechanism to maintain circulating levels of EC that would offset the decline of biosynthetic enzyme levels for EC synthesis. However, at the same time, the n-3 PUFA may help to minimize overstimulation of the ECS during obesity to reduce the n-6 PUFA derived EC (Kim et al., 2014b). Thus, dietary PUFA studies are of great research interest to better understand eating behavior and excess caloric intake in the adolescent and young adult populations, as well as suppressed appetites in older adults. The aim of this research is to determine how specific families of dietary PUFA alter EC levels, receptor excitability, and signaling to improve health outcomes throughout the life cycle.

Herein, we describe the ECS and how it is integrated in the biological control of food intake; maintenance of the ECS; actions of PUFA on genes of the ECS; relationships between muscle, adipose, and liver; and aging. Throughout this review, we will suggest research to further elucidate the interactions between PUFA and the ECS and recommend approaches to advance the understanding of how EC and receptors function in the body.

OVERVIEW OF THE ENDOCANNABINOID SYSTEM IN THE BRAIN AND PERIPHERAL ORGANS

The functional components of the ECS are the cannabinoid receptors, biosynthesis and degradation enzymes of the EC ligands, and their signaling pathways (Murray et al., 2007). Although the brain and CNS exhibit all of these attributes of the ECS (Mackie, 2008), the components of this system have also been shown to be present and active in several organs, tissues, and cells (Vettor et al., 2008; De Petrocellis and Di Marzo, 2009). In the late 1980s the central receptor for the psychotropic component of cannabis, tetrahydrocannabinol, was identified as cannabinoid receptor 1 (CB1; Devane et al., 1988), and it was later cloned in 1990 by Matsuda et al. (1990). Endogenous agonists for CB1 and the predominantly localized peripheral cannabinoid receptor, CB2, were also identified in the early and mid-1990s (Mechoulam et al., 1995). AEA was first discovered as a cannabinoid ligand, followed by 2-AG; these fatty acid derived compounds are made on demand and act locally in a paracrine or autocrine fashion. CB1 has since been identified to be localized on numerous peripheral tissues such as adipose, liver, and muscle (Kim et al., 2013). CB2 was first identified in immunocompetent cells and has since been identified in various peripheral organs (i.e., bone, muscle, heart) and the CNS, albeit to a lesser degree than CB1 expression. The cannabinoid receptors are Gi/o protein-coupled receptors and share 44% overall identity (Howlett, 2005). Upon activation, a signaling cascade occurs and results in the inhibition of adenylyl cyclase, cAMP, and protein kinase A. The signaling pathways of the ECS are recognized as a target for therapeutic applications (Bosier et al., 2010), but the complex relationships between the endogenous ligands, receptors, and signaling are poorly understood.

The ECS signaling pathways are multifaceted because the many endogenous ligands, including those derived from the n-6 and n-3 PUFA families, result in varying degrees of receptor activation and involve different intracellular transduction pathways to influence numerous physiological functions (Bosier et al., 2010). The modification of receptor activation by the different endogenous and exogenous ligands selectively directs the downstream actions of the ECS *in vivo*. At present, the actions of the multitude of ligands on the ECS are not well described in the literature. Potentially, modification of the ECS by endogenous ligands derived from dietary PUFA may be a means to blunt food intake and alter physiological processes to reduce obesity and improve skeletal muscle response to glucose and insulin sensitivity (Kim et al., 2013, 2014a). These relationships are now under investigation in rodents and humans (Viveros et al., 2008); however, there are few clinical published studies (McPartland et al., 2014). Adequate amounts of dietary n-3 PUFA are necessary for proper cannabinoid signaling, and the long chain n-3 PUFA, both DHA and EPA, moderate the cellular levels of AEA and 2-AG, thus acting as a means to alter ECS signaling to reduce over stimulation of signaling and its negative consequences on health, such as obesity (Kim et al., 2013; McPartland et al., 2014).

Additional downstream signaling pathways resulting from the ECS include extracellular receptor kinase, mitogen activated protein kinase, c-Jun *N*-terminal kinases, and c-fos (Wartmann et al., 1995; Howlett, 2005). CB1 activation in neurons and other cranial tissues is responsible for inhibition of intracellular cAMP, dephosphorylation of potassium and calcium ion channels, and increased intracellular free Ca^{2+} (Howlett, 2005). CB2 activation in immune cells is generally considered anti-inflammatory and

immunomodulatory (Han et al., 2009; Hao et al., 2010). AEA acts as a partial CB1 agonist and a weak CB2 agonist, while 2-AG is a full CB1 and CB2 agonist (Bisogno et al., 2005). Two additional receptors, GPR55 (Ryberg et al., 2007) and transient receptor potential vanilloid type 1 (Ross, 2003) are also activated by AEA; although, their interactions with the ECS are currently under investigation.

The next research area for the ECS must include characterization of the downstream actions and should also include the relationships of related substrates, such as the ligands for the EC receptors and those that can be used by cyclooxygenase (Kim and Watkins, 2014). This research will have implications on brain functions, inflammatory status, and disease pathogenesis. The physiological aspects of this research have implications in obesity and insulin resistance, but with regards to food intake, such research can integrate the role of the ECS in systemic macronutrient metabolism and fat accretion. The ECS has some impact on directing systemic macronutrient metabolism, which involves the intestinal tract, liver, muscle, and adipose (Viveros et al., 2008; Kim et al., 2013). Hence, the interest in the ECS on food intake and energy balance is of vital importance to human health (Di Marzo and Matias, 2005; Tibirica, 2010).

ENDOCANNABINOID BIOSYNTHESIS, DEGRADATION AND THE BRAIN

Arachidonylethanolamide and 2-AG are biosynthesized from AA in the phospholipid membrane and are the two most studied endogenous ligands for the cannabinoid receptors. AEA is synthesized from AA in the sn-1 position on phospholipids and is found at pmol/g concentrations in tissues, whereas 2-AG is formed from AA in the sn-2 position. AA is more abundant in the sn-2 position of the phospholipids in the cell membrane, leading to higher tissue concentrations of 2-AG (ng/g range) than AEA (Bab et al., 2009). Currently, there are three known routes of AEA formation from AA. The two step synthesis by NAPE-PLD is the major pathway, where AA is first cleaved as a phosphatidylethanolamine by an acyltransferase to form NAPE and then the NAE is released from NAPE by a selective PLD (Okamoto et al., 2004). When AA is the starting substrate, the released NAE is AEA. NAE has been previously found to function as a signaling molecule in various tissues, primarily in the CNS (Schmid, 2000). Another route of synthesis is via hydrolysis of NAPE from phospholipase C, forming an *N*-arachidonoylethanolamine phosphate which can then be hydrolyzed by a phosphatase to form AEA. Synthesis of AEA can also be achieved by a condensation pathway where a FAAH works in reverse, starting with AA and ethanolamine. As for the synthesis of 2-AG, various pathways have been characterized (Sugiura et al., 1995). For example, phosphatidylinositol (PI) can be hydrolyzed by phospholipase A, forming a lysophosphatidylinositol which is then hydrolyzed by phospholipase C to form 2-AG. Additionally, 2-AG can also be produced in response to a stimulus, such as ionomycin, in which PI is hydrolyzed by phospholipase C forming triacylglycerol (DAG) and hydrolyzed once more by DAG lipase (DAGL). A third mechanism in forming 2-AG is via conversion of 2-arachidonoyl lysophosphatidic acid to 2-AG, which is accomplished by monoacylglycerol kinase. Degradation of AEA, and to a lesser degree 2-AG, occurs by FAAH to produce AA and an ethanolamide. The degradation of 2-AG occurs by MAGL to form AA and monoglycerol (Sugiura et al., 2002).

Previously, NAPE-PLD was shown to be expressed by particular populations of neurons in the brain, specifically targeting axon and axon terminals to mediate anterograde signaling at synapses (Egertova et al., 2008). This is evident in the observations where AEA acts as a retrograde messenger to bind to the axonal terminal of presynaptic neurons in the hippocampus (Wilson and Nicoll, 2001; Alger, 2002).

Agonists and inverse agonist actions on the cannabinoid receptors result in high to low responses, compared to basal cellular levels (Mackie, 2008). The *in vitro* response is dependent upon the binding affinity of the ligand to the cannabinoid receptor, the activation of the receptor, and the resulting downstream actions through the signaling pathways, which include gene expression; however, these are not well characterized *in vivo* (Bosier et al., 2010). What is clear is that the ECS in the brain controls food intake via the hypothalamus and limbic systems, where activation of cannabinoid receptors induces fat accumulation (Viveros et al., 2008). In addition, activation of receptors may divert macronutrient metabolism toward lipid synthesis in adipose (Osei-Hyiaman et al., 2005; Bluher et al., 2006). Moreover, dietary PUFA directly affect the concentration of specific EC in blood and tissues *in vivo* (Artmann et al., 2008; Wood et al., 2010; Kim et al., 2014b). The effects of dietary n-6 PUFA on the enzymes of synthesis and degradation of the EC is worthy of investigation *in vivo* and in cell cultures (Kim et al., 2014a). Conversely, sufficient evidence supports the need to conduct human studies that examine the full extent in which n-3 PUFA derived EC alter the ECS in health and in controlling obesity and insulin resistance (McPartland et al., 2014).

ENDOCANNABINOIDS, THE GASTROINTESTINAL TRACT, AND CONTROL OF FOOD INTAKE

The ECS is well known to be involved in the regulation of appetite, food intake, and energy metabolism (Tibirica, 2010; Kim et al., 2011). Initially, it was thought that the effects of EC were localized in the CNS. The EC can act as neurotransmitters between neurons in various regions of the brain (Bermudez-Silva et al., 2012). They behave as retrograde messengers on CB1 at presynaptic glutamatergic terminals in the hypothalamus, resulting in the inhibition of the release of the excitatory neurotransmitter glutamate and leading to an overall suppressive effect on neuroendocrine function (Di et al., 2003). One of the major consequences of ECS action on the hypothalamus is affecting neuroendocrine functioning (Harmon and Aliapoulios, 1972). From an evolutionarily perspective, the primary physiological function of the ECS appears to shift energy balance toward energy storage (Piazza et al., 2007), and thus, can lead to fat accumulation. While the necessity for the ECS was more obvious for our hunter and gatherer ancestors when food supply was not guaranteed, the technological advances of today provide greater stability in regards to food availability. However, today this is more of a disadvantage with regard to being overweight and obese. An excessive food supply and an overactivation of CB1 can lead to overeating and a susceptibility of metabolism to favor energy storage and obesity (Piazza et al., 2007).

Stimulation in the CNS of CB1 by AEA and 2-AG increases hyperphagia, and the response is a higher food intake (Williams and Kirkham, 1999; Kirkham et al., 2002). The ECS controls food intake in two ways: (1) reinforces the motivation to find and consume food with high incentive value, and (2) induces appetite by regulating levels and actions of orexigenic and anorectic mediators (Di Marzo and Matias, 2005; Monteleone et al., 2005). The hyperphagic properties of EC were reported in Williams and Kirkham (1999) when AEA was injected peripherally to stimulate feeding behavior and overeating in satiated rats, an effect that was attenuated by selectively blocking CB1 with the antagonist SR141716. AEA caused a modest hyperphagic response that appeared over a longer time course, as compared to Δ^9-THC (Williams and Kirkham, 1999). Injection of 2-AG into the nucleus accumbens shell, an area of the basal forebrain associated with appetite stimulation, induced eating in rats (Kirkham et al., 2002). This increase in food intake from 2-AG treatment was prevented by pre-treatment with the antagonist SR141716, while the CB2 antagonist SR144258 had no effect; thus, it was demonstrated that the hyperphagic properties of AEA and 2-AG are specifically mediated by central CB1 (Williams and Kirkham, 1999; Kirkham et al., 2002). Besides reinforcing the action of SR14176 on suppressing food intake in the rat, behavioral aspects related to motivational processes in both the appetitive and consummatory phases of feeding behavior are involved (Thornton-Jones et al., 2005). Subsequently, others reported that the CB1 receptor antagonists/inverse agonists (e.g., Rimonabant, analog AM 251) actions on reducing food intake appears to be linked to or mediated by behavioral aspects in the rat (Tallett et al., 2007). Adiponectin mRNA and plasma adiponectin were elevated in vehicle-treated chow-fed animals compared to obese controls, and did not differ between rimonabant-treated and pair-fed animals. The similarities between rimonabant-treated and pair-fed animals in body weight loss and the absence of differences in measures of adiponectin activity between drug-treated and pair-fed animals suggest that the outcomes of this experiment were solely mediated by the drug-induced reduction in food intake (Thornton-Jones et al., 2006). Others have reported a reduced but transient food intake in rats treated with a neutral CB1 receptor antagonist AM4113 (Cluny et al., 2011). Weight control resulting in a lower food intake with AM4113 was also observed in the rats that were subjected to pair-feeding. With regard to sex differences and diet, Foltin and Haney (2007) found that baboon males ate more food pellets than females, few other sex differences were observed in this study.

Signals to the brain from the small intestines and other organs of the gastrointestinal system play a role in regulating energy balance, and the ECS appears to have a role in these pathways (Di Marzo and Matias, 2005). The interaction between the gastrointestinal tract and the ECS is carried out by both endocrine and neural pathways. CB1 is present in neurons of the enteric nervous system and in sensory terminals of vagal and spinal neurons in the gastrointestinal tract (Massa et al., 2005). Activation of CB1 is shown to modulate nutrient processing, such as gastric secretion, gastric emptying, and intestinal motility. Intestinal derived hormones such as CCK and the adipocyte derived hormone, leptin, decrease food intake while ghrelin has the opposite effect by increasing appetite (Pagotto et al., 2006). These hormones function as satiety and hunger signals by triggering nerve impulses in sensory nerves that travel to the hindbrain and hypothalamus via blood. Food intake and hunger may be under the influence of the ECS by regulating the expression and action of orexigenic and anorectic mediators that originated from the hypothalamus. Leptin has also been found to lower 2-AG and AEA levels in the hypothalamic region in rats (Di Marzo et al., 2001). The higher levels of EC in ob/ob and db/db mice support this observation. Remarkably, it has been reported that inactivation of CB1 results in a decrease of plasma insulin and leptin levels (Ravinet Trillou et al., 2004). CB1 is shown to co-localize with the food intake inhibiting neuropeptide, corticotrophin-releasing hormone, in the paraventricular nucleus of the hypothalamus, and with the two orexigenic peptides, melanin-concentrating hormone in the lateral hypothalamus and with pre-pro-orexin in the ventromedial hypothalamus (Inui, 1999; Horvath, 2003). CB1 knockout (KO) mice showed higher levels of CRH mRNA, suggesting that hypothalamic EC receptors are involved in energy balance and may be able to mediate food intake (Cota et al., 2003).

The gastrointestinal tract is a site for EC production. It has been reported that feeding influences levels of EC, such as AEA. Gomez et al. (2002) found that after a 24-h fast, AEA levels in the small intestine were seven times higher than that of a littermate that had not fasted. When the ECS was blocked via antagonist (SR141716), food intake was reduced in fasted and partially satiated rats. Thus this work suggests that EC levels are responsive to nutrient status. By increasing n-3 PUFA intake, the dietary ratio of n-6/n-3 PUFA would be lowered and lead to a decrease in the synthesis of AEA and 2-AG from AA. This would mimic the hormonal and behavioral alterations that are observed in animals with treatment of a CB1 antagonist. This association between PUFA and EC supports the premise that a lower dietary ratio of n-6/n-3 PUFA would lead to a reduction in food intake by dietary manipulation of tissue AA levels.

Another route where the ECS has been found to regulate food intake is through the vagus nerve, which connects the medulla and brainstem nuclei associated with satiety with the gastrointestinal tract to monitor the status of digestive processes. After consuming food, CCK is secreted from the duodenum and then binds to CCK receptors that are located on afferent terminals of the vagus nerve. The signal is taken up the vagal axon and to the hypothalamus to signal the decrease of food intake. Leptin receptors have also been found on these same nerve terminals. Reports have indicated decreased CB1 receptor mRNA in rats that had been fed after previously fasting or receiving CCK. In addition, in a study looking at the effect of leptin, the investigators reported that acute leptin treatments reduced AEA in the hypothalamus (Di Marzo et al., 2001). The hypothalamus plays a critical role in receiving signals from peripheral organs to inform the brain of the state of energy status (Kirkham et al., 2002). Upon eating, wild-type rats were observed to have reduced hypothalamic 2-AG levels compared to when they were previously fasted.

An increase in food intake was observed when ghrelin was infused into the paraventricular nucleus of the hypothalamus in

rats (Tucci et al., 2004). However, when a CB1 receptor antagonist was added to the ghrelin treated animal, the food intake returned to baseline. Elevated levels of the endogenous ligands, AEA and 2-AG, have also been found in obese individuals (Engeli et al., 2005; Osei-Hyiaman et al., 2005) and correlates with intra-abdominal adiposity (Cote et al., 2007). Additionally, diet-induced obese mice demonstrated higher levels of AEA and 2-AG in hippocampal regions and displayed an increase in DAGL, which is one of the enzymes responsible for the synthesis of 2-AG (Massa et al., 2010). In the same study, ECS-mediated synaptic plasticity was observed to have changed in the CA1 region, as depolarization-induced suppression of inhibition and long-term depression of inhibitory synapses were enhanced. This finding demonstrated the potential for the ECS to remodel and influence aspects of cognition. In another study, AEA injection into the ventromedial hypothalamus of satiated rats induced significant appetite stimulation through CB1 receptor activation (Jamshidi and Taylor, 2001).

Although feeding a mixture of different n-3 PUFA was found to increase plasma leptin in blood of insulin-resistant rats, food intake did not change (Peyron-Caso et al., 2002). While leptin functions to reduce appetite and increase energy expenditure, resistance to leptin's effects is well documented in metabolic complications, such as obesity (Myers et al., 2010, 2012). Recently, treatment with a CB1 inverse agonist was shown to reverse leptin resistance and reduce obesity in diet induced obese mice (Tam et al., 2012). In another study, both plasma insulin and leptin levels were lower in CB1 KO mice, compared to wild-type mice (Ravinet Trillou et al., 2004). Both exogenous cannabinoids and AA-derived EC increase food intake and promote weight gain via CB1 receptor activation (Jamshidi and Taylor, 2001; Kirkham et al., 2002). From these studies, one can surmise that an overactivation of the ECS is indicative of the increased adiposity observed with obesity. In addition, leptin status appears to be influenced by the levels of endogenous cannabinoids, thus validating the idea that the ECS plays a major role in energy homeostasis. Unfortunately, the precise actions of the n-6 and n-3 families of dietary PUFA on activation of cannabinoid receptors and their signaling downstream is not known. For this reason, it is necessary to investigate how dietary PUFA influence the ECS in order to understand food intake and macronutrient metabolism and their effects on fat accretion and insulin sensitivity.

DIETARY PUFA AND THE ENDOCANNABINOIDS

It is well recognized that dietary PUFA can alter the fatty acid composition of glycerolipids of cells, tissues, and organs in the body with some significant physiological outcomes (Watkins et al., 2006; Li et al., 2010; Hutchins-Wiese et al., 2012). In addition, the remodeling of plasma membrane PUFA composition is well documented in humans. Moreover, families of n-3 PUFA change membrane phospholipid composition in most organs, including the brain. Feeding the long chain n-3 PUFA, such as EPA and DHA, will increase their concentrations *in vivo*, and to some extent will lower the concentrations of n-6 PUFA, specifically AA. Thus, remodeling the phospholipid composition of cell membranes and organelles by dietary PUFA is a means to change substrate for and the biosynthesis of prostanoids (Watkins et al., 2000), and more recently for the biosynthesis of EC (Watkins et al., 2010; Kim and Watkins, 2014; Kim et al., 2014a).

As suggested, tissue EC levels are responsive to substrate PUFA availability and can be modulated by dietary levels of n-6 and n-3 PUFA. Rodents or piglets fed diets rich in the EC substrate AA showed greater 2-AG and AEA levels in the brain (Berger et al., 2001), small intestine, and liver (Artmann et al., 2008). Diets enriched with long chain n-3 PUFA (EPA and DHA) decreased AA levels and resulted in lower EC levels in the brain (Berger et al., 2001; Watanabe et al., 2003; Artmann et al., 2008; Wood et al., 2010), small intestine, liver (Artmann et al., 2008), visceral adipose tissue (Batetta et al., 2009), and plasma (Wood et al., 2010). These results demonstrate the link between dietary intake of PUFA, tissue PUFA concentrations, and EC levels.

As described, EC are products of dietary lipids. Modification of dietary fat intake can modulate the EC levels, both EPA and DHA can displace AA in cell membranes and then the derived EC, consequently reducing AEA and 2-AG production (Naughton et al., 2013). Similarly, oleoyl ethanolamide, a product of oleic acid, induces satiety, decreases circulating fatty acid concentrations, increases the capacity for β-oxidation, and inhibits the action of AEA and 2-AG in adipose tissue. The dietary lipids typically higher in n-6 PUFA drive the formation of AEA and 2-AG and likely support excessive energy intake and weight gain. Thus, understanding how dietary fats alter ECS activity is a pertinent area of research due to public health messages promoting a shift toward terrestrial plant and vegetable oils.

While dietary n-3 PUFA deficiencies have been linked to neuropsychiatric diseases (Parker et al., 2006), a potential underlying cause may be due to the desensitization and uncoupling of CB1 in the prelimbic prefrontal cortex and accumbens (Lafourcade et al., 2011). Recently, consumption of n-3 PUFA was shown to abolish negative consequences of non-functional CB1 activation regarding mood and behavior. In a study using 80 Male C57/blk6 mice (21-days-old), fed a modified AIN-93G diet (containing 11.04% fat) and assigned to either the control diet containing safflower oil or a DHA enriched diet (both diets were isocaloric and isonitrogenous), there were significant changes in the concentrations of n-3 and n-6 PUFA in tissues (serum, anterior tibialis, epididymal fat pads, and liver), including the brain (Kim et al., 2014b). After 62 days, mice fed the DHA diet had higher levels of 14:0, 16:1n7, 18:1n9, 18:2n6, 20:3n6, 22:5n3, and 22:6n3 in the brain, compared to the mice fed the control diet (**Table 1**; Kim et al., 2014b). Not surprisingly, the ratio of n-6/n-3 PUFA in the brain was significantly reduced in the DHA diet group, as compared to the control group (0.71 vs. 1.26). More specifically, the ratio of AA to DHA was reduced in the DHA diet fed mice (0.48 vs. 0.77). As shown in **Table 2**, after 118 days, brains from the mice given the DHA diet were observed to have higher levels of 14:0, 18:1n9, 18:2n6, 20:3n6, 20:5n3, 22:5n3, and 22:6n3, compared to the control diet fed mice (Kim et al., 2014b). Once again, the ratio of n-6/n-3 PUFA in the brain was significantly reduced in the mice fed the DHA diet, as compared to the mice fed the control diet (0.71 vs. 1.50). The ratio of AA to DHA was also reduced (0.48 vs. 0.86). The change in PUFA levels of the brain of mice given

Table 1 | Mouse brain fatty acid composition after 62 days of feeding a semi-purified diet.

FA	Control		DHA		*t*-test *p* value
	Mean	SD	Mean	SD	
12:0	ND		ND		
14:0	0.13	0.00	0.14	0.005	0.012
14:1n5	ND		ND		
15:0	ND		ND		
16:0	19.38	0.13	19.13	0.22	0.0076
16:1t	0.15	0.004	0.13	0.002	<0.0001
16:1n7	0.44	0.02	0.47	0.01	0.0017
17:0	0.14	0.01	0.14	0.01	0.77
18:0	19.57	0.13	19.51	0.10	0.31
18:1n9	15.14	0.12	16.06	0.21	<0.0001
18:1n7	3.79	0.07	3.46	0.05	<0.0001
18:2n6	0.79	0.08	1.20	0.11	<0.0001
18:3n6	ND		ND		
18:3n3	ND		ND		
20:0	0.35	0.01	0.34	0.02	0.067
20:1n9	1.88	0.08	1.88	0.12	0.98
20:2n6	0.23	0.03	0.23	0.02	0.94
20:3n6	0.36	0.01	0.78	0.02	<0.0001
20:4n6	9.92	0.10	8.00	0.11	<0.0001
20:5n3	ND		0.02	0.04	0.17
22:0	0.21	0.01	0.22	0.01	0.35
22:1n9	0.19	0.01	0.18	0.01	0.55
22:4n6	2.99	0.06	1.89	0.04	<0.0001
22:5n6	2.06	0.09	ND		<0.0001
22:5n3	ND		0.19	0.01	<0.0001
22:6n3	12.96	0.17	16.71	0.25	<0.0001
24:0	0.25	0.02	0.26	0.02	0.16
24:1n9	0.31	0.06	0.33	0.05	0.60
TOTS	40.04	0.15	39.74	0.23	0.0047
TOTM	21.76	0.26	22.38	0.38	0.0009
PUFA	29.32	0.27	29.01	0.29	0.036
TN6	16.35	0.18	12.09	0.15	<0.0001
TN3	12.96	0.17	16.92	0.25	<0.0001
AA/DHA	0.77	0.01	0.48	0.01	<0.0001
n-6/n-3	1.26	0.02	0.71	0.01	<0.0001
Area%	91.27	0.13	91.27	0.11	0.95
Total area	4644	483	4397	397	0.25

Values are weight percentages determined by gas chromatography (Watkins et al., 2010). N = 9 control group, and n = 9 for the DHA group. ND, not detected at the integration condition applied on these data.

Table 2 | Mouse brain fatty acid composition after 118 days of feeding a semi-purified diet.

FA	Control		DHA		*t*-test *p* value
	Mean	SD	Mean	SD	
12:0	ND		ND		
14:0	0.12	0.003	0.13	0.003	0.017
14:1n5	ND		ND		
15:0	ND		ND		
16:0	18.87	0.28	18.90	0.19	0.76
16:1t	0.15	0.005	0.13	0.01	<0.0001
16:1n7	0.47	0.02	0.48	0.01	0.093
17:0	0.13	0.01	0.13	0.003	0.73
18:0	20.09	0.14	19.93	0.07	0.0072
18:1n9	15.51	0.21	16.52	0.17	<0.0001
18:1n7	3.83	0.13	3.45	0.02	<0.0001
18:2n6	0.75	0.08	0.95	0.07	<0.0001
18:3n6	ND		ND		
18:3n3	ND		ND		
20:0	0.34	0.03	0.31	0.01	0.024
20:1n9	2.06	0.16	2.06	0.10	0.91
20:2n6	0.19	0.01	0.20	0.02	0.74
20:3n6	0.31	0.02	0.75	0.03	<0.0001
20:4n6	9.86	0.15	7.87	0.22	<0.0001
20:5n3	ND		0.09	0.03	<0.0001
22:0	0.21	0.02	0.20	0.01	0.18
22:1n9	0.19	0.01	0.18	0.01	0.032
22:4n6	3.25	0.09	1.99	0.04	<0.0001
22:5n6	2.90	0.19	ND		<0.0001
22:5n3	ND		0.20	0.01	<0.0001
22:6n3	11.51	0.24	16.29	0.24	<0.0001
24:0	0.24	0.03	0.23	0.02	0.44
24:1n9	0.40	0.05	0.36	0.06	0.20
TOTS	40.00	0.24	39.82	0.16	0.084
TOTM	22.46	0.39	23.05	0.29	0.0020
PUFA	28.77	0.39	28.33	0.32	0.017
TN6	17.26	0.26	11.75	0.18	<0.0001
TN3	11.51	0.24	16.58	0.26	<0.0001
AA/DHA	0.86	0.02	0.48	0.01	<0.0001
n-6/n-3	1.50	0.04	0.71	0.01	<0.0001
Area%	91.38	0.25	91.33	0.09	0.60
Total area	4646	256	4472	225	0.14

Values are weight percentages determined by gas chromatography (Watkins et al., 2010). N = 9 control group, and n = 9 for the DHA group. ND, not detected at the integration condition applied on these data.

the DHA enriched diet were nearly identical to the levels found after 62 days. These data support the premise that dietary PUFA alter the fatty acid composition of the brain and the substrate for the biosynthesis of EC (Wood et al., 2010; McPartland et al., 2014). Dietary supplementation with n-3 PUFA predictably increased the concentration of EPA and/or DHA in tissues, cells, and plasma, and it decreased the relative concentration of AA. However, the data showing the effects of feeding n-3 PUFA to mice are more complex than a simple decrease in AA and increase in n-3 PUFA, as indicated by other aspects of their biochemistry.

MUSCLE, LIVER, ADIPOSE AND THE ENDOCANNABINOID SYSTEM

The primary organs of macronutrient metabolism and energy expenditure that support growth and impact obesity and diabetes include the gastrointestinal tract, muscle, liver, adipose, and the endocrine system. The gastrointestinal tract is a site for the production of EC, and along with its associated organs, it is influenced by the ECS during the digestion and absorption of macronutrients. Muscle and liver are sites for glucose storage, as glycogen and adipose are for fat deposition. Furthermore, biochemical pathways of glucose, amino acids, and lipid metabolism link the muscle, liver, and adipose to cooperatively sustain the supply of intermediates that maintain energy balance in the fed and fasting state, as well as for growth and maintenance. It is clear that the ECS is a key player in macronutrient metabolism between the organs involved in food intake and systemic energy balance (Di Marzo and Matias, 2005; Viveros et al., 2008; Tibirica, 2010; Kim et al., 2013). At the gene level, sensitivity of muscle to insulin and glucose uptake appears to be influenced by the ECS (Kim et al., 2014a). Aspects of the involvement of the ECS in obesity, with specific studies in adipocyte cell cultures and adipose tissues, are described in the literature (Naughton et al., 2013). In many studies, the levels of EC and the activities of enzymes of EC synthesis and degradation suggest that a diet emphasizing n-6 PUFA, specifically AA, is a factor of concern.

We reported that feeding DHA to mice compared to feeding a semi-purified control diet resulted in an increase in the gene expression of cannabinoid receptors and enzymes for the synthesis/degradation of EC and for glucose metabolism (Kim et al., 2014b). The change in expression of ECS genes also indicated differences between muscle and adipose in mice. Furthermore, epididymal fat mass was lower in mice fed the DHA containing semi-purified diet, as compared to mice fed the control diet. If the action of DHA is as the EC, docosahexaenoyl ethanolamide, it may alter cannabinoid downstream signaling to improve glucose uptake in myoblasts (Kim et al., 2014a) and *in vivo*, the potential for n-3 PUFA to reduce diabetes is worthy of investigation. In support of these findings and the link to macronutrient metabolism, these initial studies in mice show that DHA, most likely through the ECS, alters metabolite profiles to favor fatty acid oxidation and reduce fat accretion (Kim et al., 2014b). Although these are early findings, they support the premise that dietary PUFA have roles in the ECS and eating behavior. At the least, mechanistic research in rodent models and clinical investigations on dietary PUFA in glucose metabolism and fat accretion are warranted to understand the relationships of fat intake and the ECS.

High circulating levels of AA-derived EC and excessive endocannabinoid production by adipocytes are associated with human obesity and fat accretion in rodents (Engeli et al., 2005; Cote et al., 2007). The ECS works through many anorexigenic and orexigenic pathways where ghrelin, leptin, adiponectin, endogenous opioids, and corticotropin-releasing hormones are involved (Viveros et al., 2008). Taken together, with the emerging role played by the ECS in obesity and with over production of the AA-derived EC prolonging stimulation of CB1 that leads to dysregulation (Matias et al., 2006, 2008), there is convincing evidence to focus future research on dietary PUFA and the ECS in metabolic syndrome.

Systemic macronutrient metabolism of carbohydrates, amino acids, and fatty acids now places the ECS as a target to redirect the fate of energy metabolism in the gastrointestinal tract, liver, muscle, and adipose. The ECS is an established player in CNS control of food intake. Emerging evidence of the phylogenic and developmental aspects of the ECS can be useful in understanding this complex system (Viveros et al., 2008). This research suggests that the genes for endocannabinoid enzymes, especially DAGL-α and NAPE-PLD, may contain alleles that express disease-related phenotypes, and therefore, place a greater emphasis on fully exploring the nature of how dietary PUFA influence activation of the cannabinoid receptors and downstream signals.

CONCLUSIONS: ENDOCANNABINOIDS, EATING BEHAVIOR, AND AGING

As described in this review, the ECS plays an important role in eating, and specifically, when activated, CB1 leads to stimulation of food intake, which includes the behavioral aspects observed in fasted mice (Soria-Gomez et al., 2014). The mechanisms and actions of the ECS in the full array of the drive to eat are not well understood. Mechoulam and Parker (2013) recently reviewed several behavioral aspects of the ECS in the brain, suggesting that the brain contains numerous EC-like compounds that activate the cannabinoid receptors to influence mood, depression, cognition, and learning; however, further study is needed to advance the knowledge of the CNS and brain functions. Interestingly, the distribution of the CB1 receptors, which is a primary receptor in the brain (Wilson and Nicoll, 2002) and CNS, differs in neonatal and adult brains. Moreover, since cannabinoid receptor distribution and expression are influenced by aging, research should determine how the ECS functions during aging and especially with the loss of appetite in older adults. These findings underscore the complex nature of the ECS, which is a comprehensive component that ultimately impacts all aspects of eating behavior.

It is well known that the ECS and the endogenously produced EC are crucial components for inducing food intake and controlling macronutrient metabolism (Di Marzo and Matias, 2005). The flux through metabolic pathways for glucose, fatty acids, and amino acids is largely dependent on rate regulating enzymes of metabolic pathways in major organs, such as muscle, liver, and adipose, which are all impacted by the ECS. Hence, understanding these intermediary pathways, endocrine factors, and gene expression will encourage important areas of research to determine the role of the ECS on eating behavior (Richard et al., 2009). As previously stated, one research approach should be to characterize how the different PUFA families integrate in the processes of metabolic flux of macronutrient pathways in major organs where the ECS is of consequence.

In this review, we described the current research on the ECS and food intake, macronutrient metabolism, and the participating dietary PUFA that serve as substrate for the biosynthesis of EC. The evidence clearly indicates that the activation of the cannabinoid receptors in the brain stimulates food intake and overstimulation

of the ECS contributes to overeating and obesity. The newly discovered feature of the ECS is that diet, specifically PUFA, influences genes of the ECS in cells and tissues. In this regard, the ECS participates in macronutrient metabolism and energy status in adipose and muscle that appears to be highly dependent on the type of EC derived from dietary PUFA. Future studies should focus on the underlying aspects of how the n-6 and n-3 dietary PUFA families affect the ECS, alter the types of EC synthesized, change gene expression of the ECS, and direct the signaling pathways downstream of receptor activation. These investigations will potentially impact obesity risk and metabolic syndrome.

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Ketosis, ketogenic diet and food intake control: a complex relationship

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Though the hunger-reduction phenomenon reported during ketogenic diets is well-known, the underlying molecular and cellular mechanisms remain uncertain. Ketosis has been demonstrated to exert an anorexigenic effect via cholecystokinin (CCK) release while reducing orexigenic signals e.g., via ghrelin. However, ketone bodies (KB) seem to be able to increase food intake through AMP-activated protein kinase (AMPK) phosphorylation, gamma-aminobutyric acid (GABA) and the release and production of adiponectin. The aim of this review is to provide a summary of our current knowledge of the effects of ketogenic diet (KD) on food control in an effort to unify the apparently contradictory data into a coherent picture.

Keywords: ketones, ketogenic diet, hunger, brain, hypothalamus, appetite

INTRODUCTION

Hunger and satiety are two important mechanisms involved in body weight regulation. Even though humans can regulate food intake by will, there are systems within the central nervous system (CNS) that regulate food intake and energy expenditure. This complex network, whose control center is spread over different brain areas, receives information from adipose tissue, the gastrointestinal tract (GIT), and from blood and peripheral sensory receptors. The actions of the brain's hunger/satiety centers are influenced by nutrients, hormones and other signaling molecules. Ketone bodies are the major source of energy in the periods of fasting and/or carbohydrate shortage and might play a role in food intake control.

HYPOTHALAMIC CONTROL OF FEEDING/APPETITE/HUNGER

ROLE OF NUTRIENTS IN FOOD INTAKE CONTROL

The hypothalamus is the brain's main center responsible for hunger/satiety (H/S) control. In the theory that Mayer proposed more than 60 years ago, he assigned a central role to glucose levels in the H/S control: the so-called "glucostatic theory" (Mayer, 1955). Mayer suggested that depletion of carbohydrate availability leads to hunger, and the hypothalamic centers with receptors sensitive to glucose levels might be involved in the short-term regulation of energy intake (Mayer, 1955). The "feeding center" in the lateral hypothalamic area (LHA), according to the glucostatic theory, reacts to the between-meal fall of blood glucose and stimulates food intake. The LHA contains glucose-inhibited neurons that are stimulated by hypoglycemia, a process crucial to mediating the hyperphagia normally induced by hypoglycemia. The subsequent post-prandial hyperglycemia activates the "satiety center" in the ventromedial hypothalamus (VMH), which contains glucose-excited neurons and inhibits both "feeding center" and food intake.

In 1953, Kennedy proposed the lipostatic hypothesis suggesting that lipid metabolites could also be involved in food regulation (Kennedy, 1953), and in 1956, Mellinkoff studied the effects of protein metabolism suggesting an aminostatic hypothesis (Mellinkoff et al., 1956).

Glucose-sensitive neurons have been identified in a number of CNS regions including the metabolic control centers of the hypothalamus. Medeiros et. al. have used patch-clamp electrophysiology to examine whether neurons in a specific specialized region known as the subfornical organ (SFO), an area where the blood-brain barrier is not present, are also glucose sensitive or not. These experiments demonstrated that SFO neurons are glucose-responsive and that SFO is an important sensor and integrative center of circulating signals of energy status (Medeiros et al., 2012).

But comprehensive transcriptional profiling of glucose-sensing neurons is challenging, as glucokinase (Gck) and other key proteins that transduce glucose signals are expressed at low levels. Glucose also exerts a hormonal-like action on neurons; electrophysiological recordings demonstrated, for example, that hypoglycemia activates growth hormone-releasing hormone (GHRH) neurons, suggesting a mechanistic link between low blood glucose levels and growth hormone release (Stanley et al., 2013).

Nutrient-sensitive neurons reacting to glucose but also to fatty acids (FAs) concentrations are present at many sites throughout the brain and may play a key role in the neural control of energy and glucose homoeostasis. Central administration of oleate, for example, inhibits food intake and glucose production in rats.

This suggests that daily variations in plasma FA concentrations could be detected by the CNS as a signal that contributes to the regulation of energy balance (Moulle et al., 2014).

Even though intracellular metabolism and activation of the ATP-sensitive K^+ channels appear to be necessary for some signaling effects of FAs, a great amount of the FA responses in the ventromedial hypothalamic neurons are mediated by interactions with fatty acid translocase (FAT)/CD36. Translocase is a FA transporter/receptor that activates downstream signaling even in the absence of intracellular metabolism (Moulle et al., 2014).

The classical unified model is based on the role of the three metabolic substrates: lipids, glucose and protein/amino acids in maintaining the nutritional status throughout the corresponding loci in the CNS, but there are many other signals and brain targets (Williams et al., 2001).

ROLE OF THE NEUROENDOCRINE SYSTEM IN FOOD INTAKE CONTROL

More recently, other hypothalamic appetite control regions have been identified, including those in the arcuate nucleus (ARC), the periventricular nucleus (PVN) and the dorsomedial hypothalamic nucleus (DMH) (Valassi et al., 2008). These are sites of convergence and integration of many central and peripheral signals, not just macronutrients, that are involved in food intake and energy expenditure mechanisms, e.g., a group of neurons in the ARC stimulating food intake via neuropeptide Y (NPY) and agouti gene-related protein (AGRP). These neurons interact with those producing the anorexigenic pro-opiomelanocortin (POMC) and the cocaine/amphetamine-regulated transcript (CART) (Williams et al., 2001). Thus, a more comprehensive, unified model should include macronutrients as well as many single amino acids and other signaling molecules.

There are two distinguished types of food intake regulation: a) the short-term (satiety signals, SS) occurring at the beginning and end of a single meal; it also includes the length between meals and b) the long-term regulation (adiposity signal, AS) that is influenced by such factors as body fat deposition.

The SS providing information to the brain mainly send information to the nucleus of the solitary tract (NTS). These signals are generated in the GIT and abdominal viscera, as well as in the oral cavity and provide information about mechanical and chemical properties of food. The information is transmitted via vagal and spinal nerve to the NTS. The ASs arrive to the median eminence through ARC or through the blood-brain barrier (BBB). All these afferents are integrated in a complex and not fully understood network.

Hormones like leptin and insulin, both secreted into the blood, reflect the stored body fat. These hormones can pass the BBB and stimulate specific receptors. Hypothalamic areas are richly supplied by axons from ARC, which has greater concentrations of leptin and insulin receptors than any other hypothalamic site (Valassi et al., 2008).

The ARC exerts opposing actions on food intake responding not only to leptin and insulin, but also to gut hormones (the most studied are ghrelin and, recently, PYY). The neurophysiological pathways suggest that feeding is regulated by a feedback loop, where the hypothalamus provides the long-term regulatory input to the NTS, which acts as a setpoint (Williams et al., 2001).

It has recently been proposed that the ARC is required for the coordination of homeostatic circadian systems including temperature and activity. Authors tested this hypothesis by injecting saporin toxin conjugated to leptin into the ARC of rats. Wiater et al. showed that the leptin-sensitive network is required for entrainment of activity by photic cues and entrainment of temperature by food but is not required for entrainment of activity by food or temperature by photic cues (Wiater et al., 2013).

ANOTHER PLAYER: THE GASTROINTESTINAL TRACT AND GUT MICROBIOTA

The gastrointestinal tract (GIT) plays a central role in the control of energy balance. Many molecules produced by the GIT exert hunger or satiety effects on the brain. Ghrelin is a peptide produced mainly by the stomach's oxyntic cells that stimulates ghrelin secretion in the hypophysis and has some neuroendocrine activities. However, its orexigenic properties are the most relevant to us and ghrelin is the only known peripheral orexigenic hormone (Date, 2012). Cholecystokinin (CCK) is a peptide produced mainly in the duodenum and jejunum that acts on the vagus nerve and directly on the hypothalamic nuclei. CCK is an anorexigenic factor and it reduces food intake, meal size and duration (Murphy et al., 2006). Three other related hormones are pancreatic polypeptide (PP), amylin, and peptide YY (PYY). PP is a peptide produced by the endocrine pancreas in relation to the caloric content of meals, and it reduces food intake both in rodents and humans. Amylin is a peptide co-secreted with insulin; its main effect on food control is a reduction of meal sizes and food intake (Murphy et al., 2006). Peptide YY (PYY) is produced in the gut and is similar to PP. PYY is stored in intestinal cells and released into the circulation as PYY_{3-36}, a truncated form of PYY. The release of PYY_{3-36} is dependent on a meal's caloric and fat content (Veldhorst et al., 2008). The glucagon-like peptide 1 (GLP-1) is produced by the cleavage of pro-glucagon gene in the intestine. It acts as incretin at a pancreatic level, promoting insulin secretion and as neuro hormone on hypothalamic nuclei, inducing satiety (Valassi et al., 2008).

The gut-brain link is important not only for the hormones produced by the gut, but also for the long-term body weight regulation. Studies in mice indicate that the gut microbiome influences both sides of the energy balance by contributing to nutrient absorption and regulating host genes that affect adiposity [however there are conflicting reports (Parks et al., 2013; Schele et al., 2013)]. However, it remains uncertain just how important gut microbiota are for nutrient absorption in humans. A cohort study has demonstrated that the nutrient load is a key variable that can influence the gut/fecal bacterial content over short time frames. Furthermore, the observed associations between gut microbes and nutrient absorption indicates a possible role of the human gut microbiota in the regulation of the nutrient intake and utilization (Jumpertz et al., 2011).

Moreover, according to recent evidence, meal onset appears to be biochemically induced only in the case of serious energy deprivation, while usually it is controlled by social, cultural and environmental factors strictly related to the lifestyle (Karatsoreos et al., 2013).

SYSTEMIC KETOSIS IN KD THERAPY

Ketogenic diets have become popular in recent decades for their demonstrated positive effects on weight loss (Bueno et al., 2013), though the precise mechanism of action is not fully understood (Paoli, 2014). In fact there is contradictory data about KD in mice and rats. In fact, there are contradictory data about KD in mice and rats. For example whilst a huge amount of data confirm that KD in humans is effective in weight reduction, improving lipidemia and glucose tolerance (Bueno et al., 2013), it has been recently demonstrated that a long-term KD (22 weeks) caused dyslipidemia, a pro-inflammatory state, hepatic steatosis, glucose intolerance and a reduction in beta and alpha cell mass, all without weight loss in mice (Ellenbroek et al., 2014). Two considerations should be made: (1) the induction of ketosis and the response to ketosis in humans and mice are quite different and (2) mice and humans have different life spans, and results obtained in mice after several weeks on the diet can correspond to months on the diet in humans (Demetrius, 2005, 2006).

Regardless of its efficacy for weight loss, the medium-long diet duration (Paoli et al., 2013) is over-cautiously received by the physicians, perhaps due to the lack of attention to the topic in specialized medical education courses. As a result, most physicians associate the term "ketosis" only in the context of diabetic ketoacidosis.

Meanwhile, the KD induces a ketosis that is not a pathological but physiological condition occurring on a daily basis. Hans Krebs was the first to use the term "physiological ketosis" despite the common view of it as oxymoron (Krebs, 1966); this physiological condition, i.e., ketosis, can be reached through fasting or through a drastically reduced carbohydrate diet (below 20 g per day). In these conditions, glucose reserves become insufficient both for normal fat oxidation via the supply of oxaloacetate in the Krebs cycle and for the supply of glucose to the central nervous system (CNS) (Felig et al., 1969; Owen et al., 1969) (**Figure 1**). It is well-known that the CNS cannot use FAs as an energy source because free FAs cannot cross the blood-brain barrier (BBB). This is why the brain normally uses only glucose. After 3–4 days without carbohydrate intake (KD or fasting) the CNS must find alternative energy sources as demonstrated by Cahill et al. (Owen et al., 1967, 1969; Felig et al., 1969; Cahill, 2006). These alternative energy sources are the ketones bodies (KBs): acetoacetate (AcAc), β-hydroxybutyric acid (BHB) and acetone and the process of their formation occurring principally in the mitochondrial matrix in the liver is called ketogenesis (Fukao et al., 2004). Usually the concentration of KB is very low (<0.3 mmol/L) compared to glucose (≅4 mmol) (Veech, 2004; Paoli et al., 2010). Since glucose and KB have a similar KM for glucose transport to the brain the KB begin to be utilized as an energy source by the CNS when they reach a concentration of about 4 mmol/L (Veech, 2004), which is close to the KM for the monocarboxylate transporter (Leino et al., 2001).

KBs can cross the BBB but not in a homogenous manner. For example, past experiments have demonstrated that BHB utilization is different in various brain areas (Hawkins and Biebuyck, 1979). Areas without BBB, hypothalamic regions and the lower cortical layers have a higher BHB metabolism compared to the lower one of the basal ganglia (Hawkins and Biebuyck, 1979).

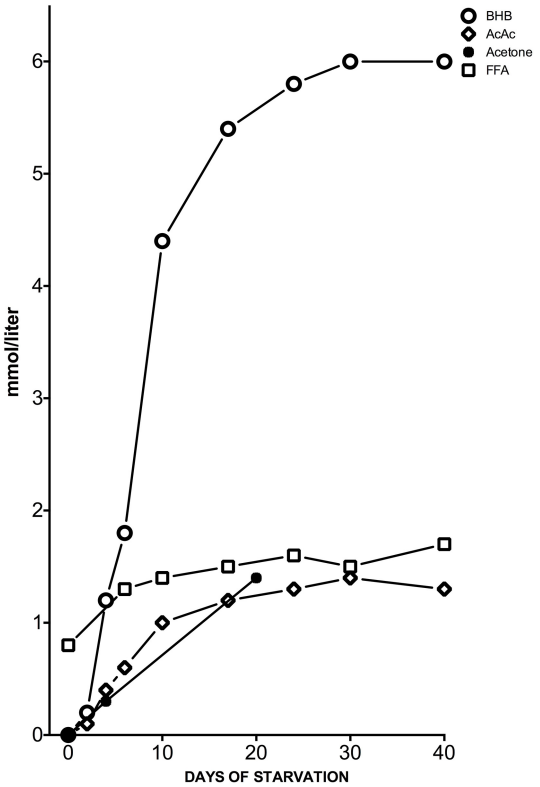

FIGURE 1 | Concentrations of KB: acetone, BHB and acetoacetic acid (AcAc), and plasma free FAs (FFA) from the post-absorptive state to 40 days of starvation in human subjects. Y axis was expanded to better describe the great change in BHB concentration. Modified from Fukao et al. (2004), Owen (2005).

Also the metabolic meaning of the three KBs is different: while the main KB produced in the liver is AcAc, the primary circulating ketone is BHB. The third one, acetone, is produced by spontaneous decarboxylation of AcAc, and it is the cause of the classic "fruity breath." Acetone does not have any metabolic functions, but it can be used as a clinical diagnostic marker. BHB acid is not, strictly speaking, a KB because the ketone moiety has been reduced to a hydroxyl group. Under normal conditions the production of free AcAc is negligible and this compound, transported via the blood stream, is easily metabolized by various tissues including skeletal muscles and the heart. In conditions of overproduction, AcAc accumulates above normal levels and a part is converted to the other two KBs. The presence of KBs in the blood and their elimination via urine causes ketonemia and ketonuria. Apart from being the fundamental energy supply for CNS, glucose is necessary for the replenishment of the quota of oxaloacetate, since this intermediate of the tricarboxylic acid cycle (TCA) is labile at body temperature and cannot be accumulated in the mitochondrial matrix. Hence it is necessary to refurnish the TCA with oxaloacetate via the anaplerotic cycle that derives it from glucose through ATP dependent carboxylation of pyruvic acid by pyruvate carboxylase (Jitrapakdee et al., 2006). This pathway is the only way to create oxaloacetate in mammals. Once produced by the liver, KBs are used by tissues as a source

of energy (Fukao et al., 2004; Veech, 2004; McCue, 2010): initially BHB is converted back to AcAc that is subsequently transformed into Acetoacetyl-CoA that undergoes a reaction producing two molecules of Acetyl-CoA to be used in the Krebs cycle (**Figure 2**).

It is interesting to note that the KB are capable of producing more energy than glucose due to the changes in mitochondrial ATP production induced by KB (Kashiwaya et al., 1994; Sato et al., 1995; Veech, 2004). During fasting or KD glycaemia, though reduced, remains within physiological levels (Seyfried and Mukherjee, 2005; Paoli et al., 2011). This euglycemic response to extreme conditions comes from two main sources: glucogenic amino acids and glycerol liberated via lysis from triglycerides (Vazquez and Kazi, 1994; Veldhorst et al., 2009). Glucogenic amino acids (neoglucogenesis from amino acids) are more important during the earlier phases of KD, while the glycerol becomes fundamental as the days go by. Thus, the glucose derived from glycerol (released from triglyceride hydrolysis) rises from 16% during a KD to 60% after a few days of complete fasting (Vazquez and Kazi, 1994). According to Bortz (1972) 38% of the new glucose formed from protein and glycerol is derived from glycerol in the lean while 79% in the obese (Bortz et al., 1972). It is important to note that during physiological ketosis (fast or very low calorie ketogenic diets) ketonemia reaches maximum levels of 7–8 mmol/L with no change in blood pH, while in uncontrolled diabetic ketoacidosis blood concentration of KBs can exceed 20 mmol/L with a consequent lowering of blood pH (Robinson and Williamson, 1980; Cahill, 2006) (**Table 1**).

We can say that no species, including humans, could have survived for millions of years without the ability to withstand brief periods of hunger or starvation (Amen-Ra, 2006). These periods of fasting are themselves ketogenic (McCue, 2010) during which the concentrations of insulin and glucose decrease while that of glucagon increases in the attempt to maintain normal blood glucose levels. When the body passes from a condition of food abundance to one of deprivation (or else via VLCKD simulated deprivation), there is, with a slight delay, an increase in the concentration of free FAs as well as KB in the blood. Thus, from this point of view KD could be compared to caloric restriction for fasting. These manipulations of nutrients, both in quantity and quality, seem to not only act on blood glucose/KB level but also to promote changes in metabolic pathways and cellular signaling. How this kind of metabolic condition (ketosis) can affect satiety and hunger mechanisms is still a matter of debate.

EFFECTS OF KETOSIS ON HUNGER AND SATIETY

Although convincing, the bulk of evidence in relation to the inhibitory effects of ketosis on appetite is still anecdotal. Preliminary scientific reports seem to support this phenomenon, and the evidence shows that KD is more effective, at least in the short/medium-term, on fat loss (Paoli, 2014). It was demonstrated that diet-induced weight loss leads to changes in energy

Table 1 | Blood levels during a normal diet, ketogenic diet, and diabetic ketoacidosis (Paoli et al., 2012).

Blood levels	Normal diet	Ketogenic diet	Diabetic ketoacidosis
Glucose (mg/dL)	80–120	65–80	>300
Insulin (μU/L)	6–23	6.6–9.4	≅ 0
KB conc (mmol/L)	0.1	7–8	>25
pH	7.4	7.4	<7.3

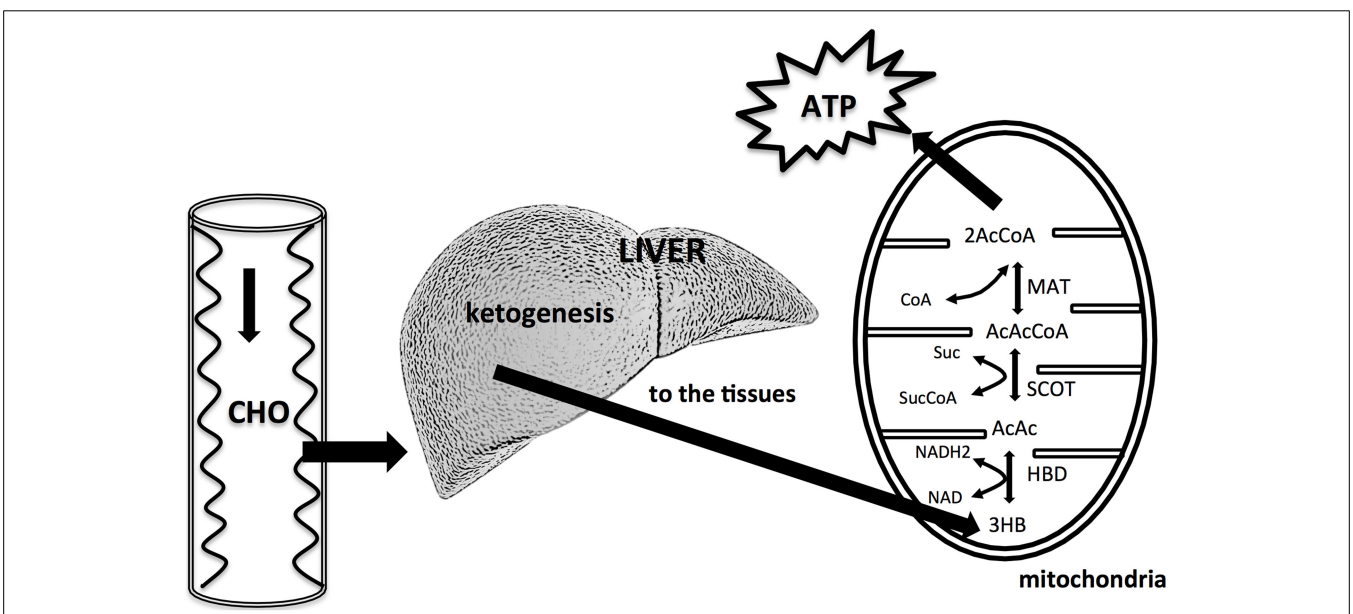

FIGURE 2 | A reduced availability of dietary carbohydrates leads to an increased liver production of KBs. The liver cannot utilize KBs because it lacks the mitochondrial enzyme succinyl-CoA: 3-ketoacid (oxoacid) CoA transferase (SCOT) necessary for activation of acetoacetate to acetoacetyl CoA. KBs are utilized by tissues, in particularly by brain. KBs enter the citric acid cycle after being converted to acetyl CoA by hydroxybutyrate dehydrogenase (HBD), succinyl-CoA: 3–CoA transferase (SCOT), and methylacetoacetyl CoA thiolase (MAT). Modified from Owen (2005), Paoli et al. (2014).

expenditure and in appetite-regulating hormones that facilitate weight regain and the return to initial energy homeostasis (Sumithran et al., 2011). This response to alteration of energy balance nullifies the success of many dietary approaches. It is well-known that the long-term success of a nutritional approach is defined by the amount of weight regain and is the main problem regarding the so-called weight cycling or "yo-yo" effect (Jeffery, 1996). A recent study by our group has demonstrated that a brief ketogenic period, if followed by a longer period of correct Mediterranean diet could avoid this yo-yo effect (Paoli et al., 2013). During the ketogenic period subjects reported less hunger, confirming previous studies (Nickols-Richardson et al., 2005; Johnston et al., 2006; Johnstone et al., 2008) on hunger-suppression effect of ketogenic diet. Despite these clinical findings, the mechanisms of action of ketosis on appetite reduction are still not completely understood. Clinical results are suggestive of both direct and indirect (via modifications of hunger-related hormones concentration) actions of KBs on appetite (Sumithran et al., 2013).

ROLE OF KETOSIS IN NUTRIENT-SPECIFIC CONTROL OF HUNGERS AND SATIETY

The findings of a stable (Chearskul et al., 2008) or slightly increased response (Sumithran et al., 2013) of post-prandial FFA after KD can be viewed in the nutrient-static context. Elevated circulating FFA may actually reduce food intake and glucose production through actions on specific hypothalamic neurons (Obici et al., 2003). It has been suggested that this effect could be mediated by the increase of cellular concentration of long-chain FAs-CoA in the arcuate nuclei of the hypothalamus (Obici et al., 2003).

Brain glucose and KB uptake was investigated in rats subjected to mild experimental ketonemia induced by 2 weeks on the KD or by 48 h fasting. To test this, researchers developed a carbon-11 labeled AcAc (11)C-AcAc for PET use. They found in rats that after 10 days of KD (11)C-AcAc brain uptake increased up to 8-fold, an increase comparable to those measured after 48 h of fasting (Pifferi et al., 2008).

The BBB, largely formed by the brain capillary endothelial cells, provides a protective barrier between the systemic blood and the extracellular environment of the CNS. Passage of FAs from the blood to the brain may occur either by diffusion or by proteins that facilitate their transport. Studies indicate that FATP-1 and FATP-4 are the predominant FA transport proteins expressed in the BBB based on human and mouse expression studies (Mitchell et al., 2011).

As a matter of fact, in animal models intracerebroventricular injections of long-chain FA reduced hypothalamic expression of NPY. NPY is an important orexogenic neuropeptide that is a downstream target of leptin and insulin in the hypothalamus. In some forms of hyperphagic obesity, characterized by elevated plasma leptin and insulin levels, the lack of action of insulin on NPY expression could explain the pathological condition. Central administration of oleic acid, fatty-acid synthase, or CPT-1 inhibitors prevents the rise in hypothalamic NPY mRNA induced by fasting (Obici et al., 2003). But glucose level is also involved in KD's food control mechanisms. According to glucostatic theory (Mayer, 1955) data indicates that ketosis did not influence FA glucose but instead stimulated the elevation of post-prandial glucose (Sumithran and Proietto, 2013) in non-diabetic subjects, while in diabetics there was a reduction of fasting glucose (Westman et al., 2008). It is important to note that carbohydrate availability may increase cellular levels of long-chain FA-CoA through an increase of malonyl-CoA, which inhibits oxidation of FAs.

Another product of elevated levels of free FA is polyunsaturated FA (PUFA). The potential ability of PUFA to block seizure activity in the brain is speculated to be associated with KD. Some mechanisms are thought to be a direct inhibition of voltage-gated sodium and calcium channels, modulation of a lipid-sensitive potassium channel, the activity of the sodium pump to limit neuronal excitability, or the induction of expression and activity of proteins in the mitochondria, thereby inducing a neuroprotective effect by partially inhibiting the production of reactive oxygen species (ROS) (Bough and Rho, 2007; Paoli et al., 2014).

ROLE OF KETOSIS ON NEUROENDOCRINE CONTROL OF HUNGERS AND SATIETY

The discovery of many appetite-related hormones provided molecular basis for appetite control, decreasing the relevance of the metabolites hypothesis (Karatsoreos et al., 2013). Recently, Sumithran et al. demonstrated that there is a long-term persistence of changes in some peripheral hormones involved in food control (Sumithran et al., 2011). In this study, they found a significant difference in mean levels of many food intake-related hormones 1 year after the cessation of weight loss via the hypocaloric diet. There was a long lasting decrease of anorexigenic compounds: leptin, PYY, cholecystokinin, insulin, and pancreatic peptide and an increase of the orexigenic molecule ghrelin. Moreover, they found that hunger remained elevated 1 year after diet cessation. In a successive study the same group investigated hunger-related hormones after 8 weeks of KD, demonstrating that during ketosis the increase of ghrelin (a strong stimulator of appetite) was suppressed (Sumithran et al., 2013). These results are consistent with those of Ratliff et al (Ratliff et al., 2009), who found no significant change in fasting plasma ghrelin after 12 weeks of VLCD.

Moreover, in the above study of Sumithran et al. (2013), ketosis maintains post-prandial secretion of CCK as previously demonstrated by other researchers (Chearskul et al., 2008). Note that the orexigenic effect of BHB is blocked by transection of the common hepatic branch of the vagus nerve (Langhans et al., 1985). The hepatic branch contains fibers from the proximal small intestine, stomach and pancreas, and is sensitive to CCK (Horn and Friedman, 2004); ghrelin signals to brain are also transmitted via vagus nerve (Habara et al., 2014). Thus, the effects of ketosis on these two appetite-related hormones could be one of the many factors related to the effects of such nutritional regimen on food control.

Many questions about the role of such an important intermediate of lipid metabolism remains unanswered, e.g., the role of BHB in food control. For example, whether or not BHB could act as a satiety signal in the brain, considering its role in energy supply to CNS. We have to consider that the effects of KBs on

hunger reduction can only be seen after many days following fasting or KD initiation (Paoli et al., 2010); this is consistent with the abovementioned threshold of brain utilization of KB as an energy source, i.e., 4 mmol/L (Veech, 2004), which is close to the Km for the monocarboxylate transporter (Leino et al., 2001). During the first days of fasting or KD there is a rise of BHB and adiponectin concentrations (Halberg et al., 2005). One of the putative causes of hunger in starved humans may be due—together with other causes—to adiponectin. When adiponectin binds to its receptor AdipoR1, AMP-activated protein kinase (AMPK) is phosphorylated in the ARC of the hypothalamus (Valassi et al., 2008). The increase of AMPK activity in the hypothalamus may increase food intake and hepatic glucose output in mice while the decrease seems to reduce food intake (Zhang et al., 2009). KDs can also act similarly to a caloric restriction on AMPK (Newman and Verdin, 2014). Interestingly, AMPK seems to have opposing actions on the liver, muscle tissues and the brain: in liver and muscle AMPK activation increases FA oxidation by decreasing malonyl-CoA concentrations (Malonyl-CoA is the first intermediate in the lipogenic pathway and is also an inhibitor of carnitine palmitoyltransferase-1 (CPT-1). CPT-1 activity can be limiting for FA oxidation), through the inactivation of the acetyl-CoA carboxylase 1 (ACC1). AMPK can also increase the activity of malonyl-CoA decarboxylase (MCD), which enhances the decrease of malonyl-CoA levels.

On the contrary, in the brain, as mentioned above, the increase of AMPK activity leads to higher food intakes. But the effect of AMPK in the brain is more complicated; mice lacking AMPKa2 in pro-opiomelanocortin neurons develop obesity, while the deficiency of AMPKa2 in agouti-related protein neurons results in an age-dependent phenotype. Thus, the conclusion is that even while AMPK is a regulator of hypothalamic functions, it does not act as a signal for energy deficit or excess (Claret et al., 2007). However, the picture is more complex than this (**Figure 3**); BHB induces AgRP expression while increasing ATP and inhibiting AMPK phosphorylation (Cheng et al., 2008). Moreover, Laeger and colleagues have recently demonstrated that under physiological conditions BHB decreases AMPK phosphorylation and AgRP mRNA expression in GT1-7 hypothalamic cells (Laeger et al., 2012).

Another mechanism that could be involved in food-regulation during KD is the gamma aminobutyric acid (GABA) and glutamate regulation. Wu et al. demonstrated that GABAergic signaling from the NPY/AgRP neurons to the parabrachial nucleus (located in the dorsolateral part of the pons) is involved in many regulatory sensory stimuli including taste and gastric distension, regulate feeding behavior. GABA signaling seems to prevent animals from anorexia when AgRP neurons were destroyed (Wu et al., 2009). These findings are yet another contradictory aspect of KDs and food behavior; ketosis should increase the availability of glutamate (via diminution of transamination of glutamate to aspartate) and therefore increase GABA and glutamine levels; moreover, in ketosis, the brain imports a huge amount of acetate and converts it through glia into glutamine (an important precursor of GABA) (Yudkoff et al., 2008). The result of these mechanisms, together with the increased mitochondrial metabolism and flux through the TCA cycle, is an increased synthesis of glutamine and a "buffering" of glutamate. These results are not consistent with the well-documented anorexigenic effect of KDs, and therefore the GABA hypothesis cannot be taken into account despite the mild euphoria often reported during a KD that is probably due to the action of BHB (Brown, 2007) and can help to reduce appetite.

OTHER POSSIBLE MECHANISMS

GUT MICROBIOTA

It is known that different dietary components exert some effects on gut microbiome composition, mainly in relation to obesity and inflammatory states. In general, a Mediterranean diet has a positive effect while a high-protein diet seems to have detrimental effects due to putrefaction phenomena (Lopez-Legarrea et al., 2014; Flint et al., 2015). Few data are available at this time about the effects of KD on gut microbiota. For example, a study by Crawford et al. (2009) investigated the regulation of myocardial ketone body metabolism by the gut microbiota and demonstrated that, during fasting, the presence of gut microbiota improved the supply of ketone bodies to the heart where KBs were oxidized. In the absence of a microbiota, low levels of KB was associated with a related increase in glucose utilization, but heart weight was still significantly reduced. The myocardial-mass reduction was completely reversed in germ-free mice feeded with a ketogenic diet. Regarding food control we can hypothesize that the particular metabolic state of ketosis could provide some benefit to weight and food control via synergic actions between butyrate production by gut bacteria and circulating high blood ketones (Sanz et al., 2015).

REACTIVE OXYGEN SPECIES

As is in the case of GABA, the intracellular reactive oxygen species (ROS) hypothesis works against the hunger-suppressive role of KD: it has been demonstrated that the hypothalamic ROS increase through NADPH oxidase is required for the eating-inhibitory effect of insulin (Jaillard et al., 2009); moreover it

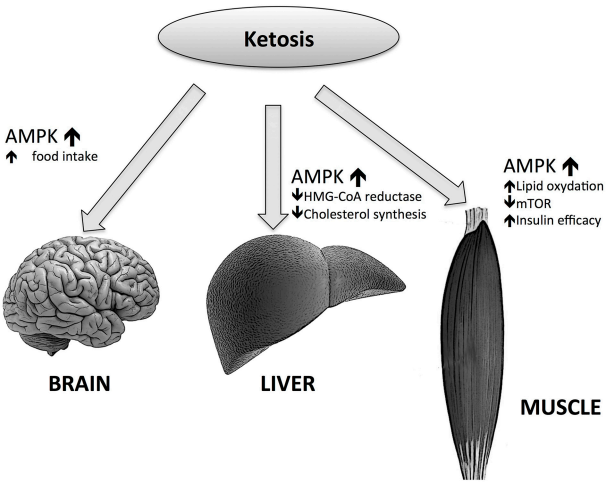

FIGURE 3 | Effects of ketone bodies on AMP-activated protein kinase (AMPK) actions in different tissues.

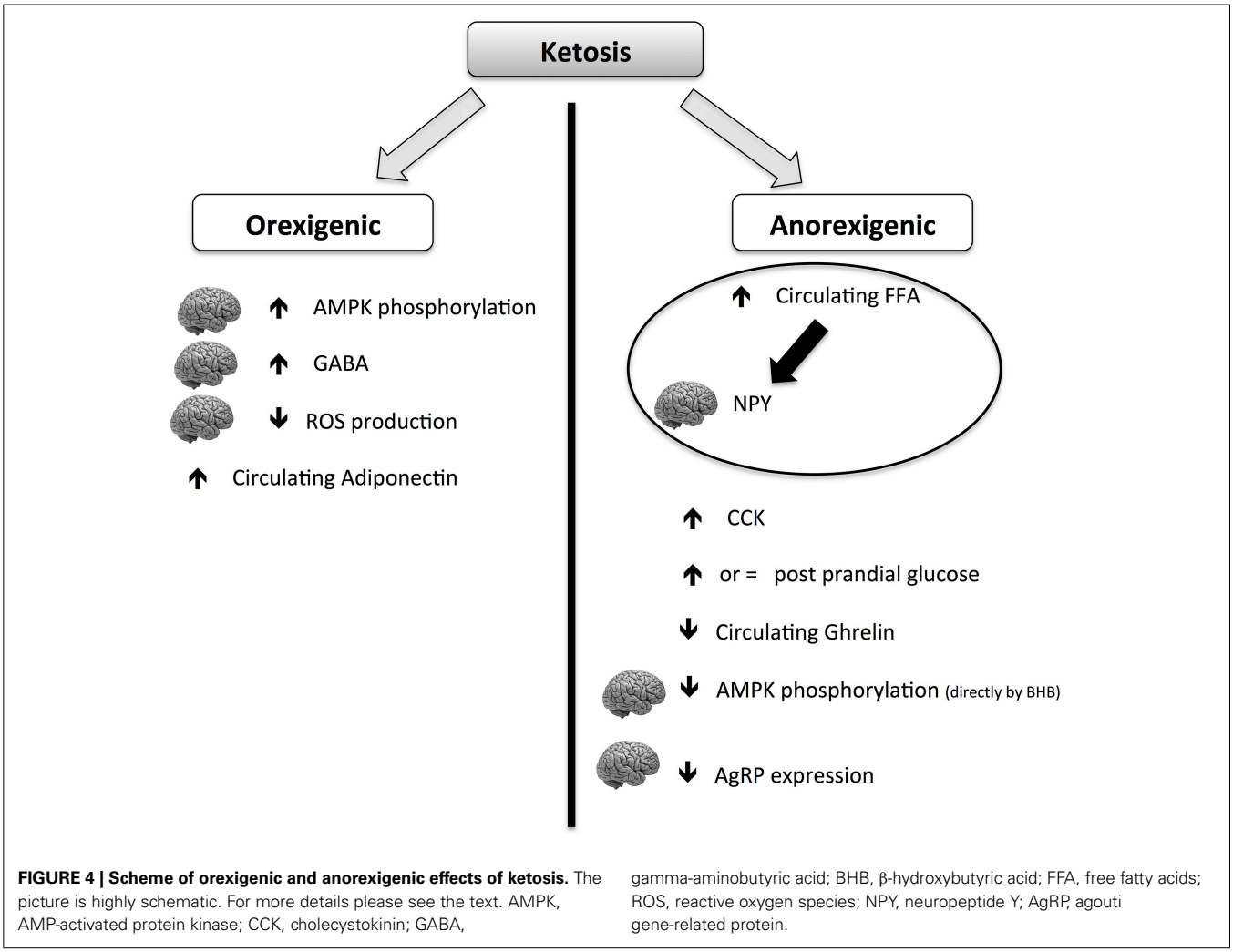

FIGURE 4 | Scheme of orexigenic and anorexigenic effects of ketosis. The picture is highly schematic. For more details please see the text. AMPK, AMP-activated protein kinase; CCK, cholecystokinin; GABA, gamma-aminobutyric acid; BHB, β-hydroxybutyric acid; FFA, free fatty acids; ROS, reactive oxygen species; NPY, neuropeptide Y; AgRP, agouti gene-related protein.

has been demonstrated that there is a ROS-dependent signaling pathway within the hypothalamus that regulates the energy homeostasis, and that activation of ROS-sensitive mechanisms could be sufficient to promote satiety (Benani et al., 2007). On the other side, KBs decreases mitochondrial production of ROS by increasing NADH oxidation in the mitochondrial respiratory chain (Maalouf et al., 2007).

CONCLUSIONS

Although the hunger-reducing effect of KD is well-documented, its main mechanisms of action are still elusive. The global picture is complicated by the contradictory role of ketosis on anorexigenic and orexigenic signals (summarized in **Figure 4**). Ketones (mainly BHB) can act both orexigenically or anorexigenically. In the orexigenic mechanism, it increases the circulating level of adiponectin, increasing brain GABA and AMPK phosphorylation and decreasing brain ROS production. The anorexigenic mechanism triggers a main normal glucose meal response, increasing circulating post-meal FFA (thus reducing cerebral NPY), maintaining CCK meal response and decreasing circulating ghrelin. It can be postulated that the net balance of the contrasting stimuli results in a general reduction of perceived hunger and food intake. More studies are needed to explore the mechanism of potential beneficial effects of KD on food control.

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