CURRENT VIEWS OF HYPOTHALAMIC CONTRIBUTIONS TO THE CONTROL OF MOTIVATED BEHAVIORS

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PUBLISHED IN: Frontiers in Systems Neuroscience

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-199-5
DOI 10.3389/978-2-88963-199-5

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CURRENT VIEWS OF HYPOTHALAMIC CONTRIBUTIONS TO THE CONTROL OF MOTIVATED BEHAVIORS

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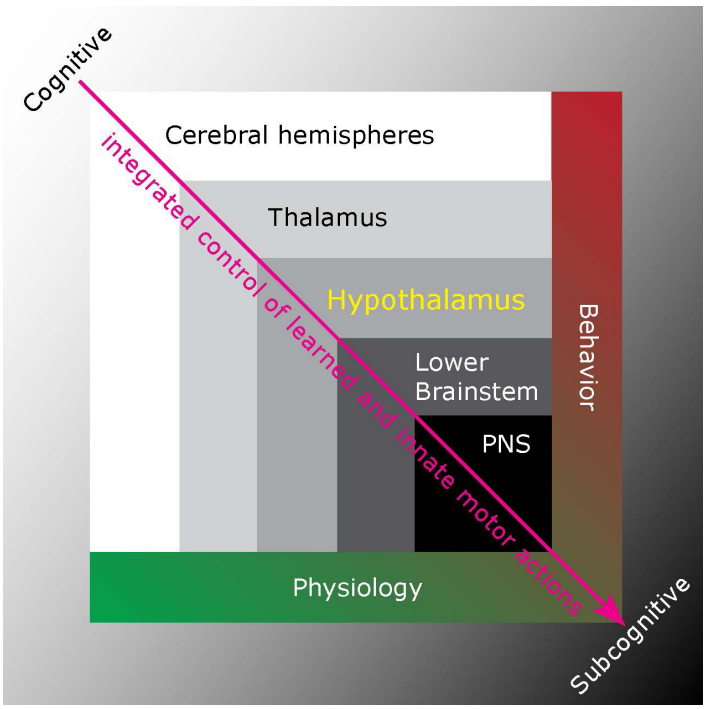

A composite schematic of the major divisions of the nervous system in relation to the function of the nervous system to control physiology and behavior. The cognitive (voluntary) control of complex learned behaviors requires the cerebral hemispheres; whereas the control of simpler innate (instinctive) behaviors and physiology prominently involves the hypothalamus. The direct influence of cognitive control arising in the cerebral hemispheres generally decreases as the influence of subcognitive control increases. The centrally located hypothalamus has direct neuronal connections with all divisions of the brain and is well placed to integrate cognitive and subcognitive information that support its essential role in the control of fundamental motivated behaviors and physiology that are critical for survival. Abbreviations: PNS, peripheral nervous system.

Image: Joel D. Hahn.

The increasing availability of technologies for interrogating genetically targeted neurons is driving a resurgence of empirical research aimed at determining the structure and function of the neural systems that control motivated behaviors. This

has refocused attention on the hypothalamus, whose central role in behavioral control was identified about a century ago. As a result, new insights into hypothalamic contributions to the control of motivated behaviors are emerging, driven not only by the application of new technologies, but also by the application in parallel of iteratively refined established techniques, and increasingly by informatics approaches applied to maturing neuroscience databases. With this renewed interest in decrypting hypothalamic contributions to the control of motivated behaviors, it is timely to provide an updated overview that bridges current insights and historical foundations.

Citation: Hahn, J. D., Fink, G., Kruk, M. R., Stanley, B. G., eds. (2019). Current Views of Hypothalamic Contributions to the Control of Motivated Behaviors. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-199-5

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frontiers in Systems Neuroscience

EDITORIAL
published: 13 August 2019
doi: 10.3389/fnsys.2019.00032

Editorial: Current Views of Hypothalamic Contributions to the Control of Motivated Behaviors

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Keywords: hypothalamus, brain, motivated behavior, innate behavior, sensory - motor coordination

Editorial on the Research Topic

Current Views of Hypothalamic Contributions to the Control of Motivated Behaviors

WHAT ARE MOTIVATED BEHAVIORS?

The goal of this Research Topic was to assemble a diverse collection of current views of the hypothalamus relating to its role in the control of motivated behaviors. This editorial highlights the included articles directly and also indirectly via two perspectives (from George Fink and Menno Kruk) that frame the topic in a historical context. However, before these, it is apt to reconsider briefly what is meant by the term "motivated behaviors."

According to the Oxford English Dictionary, the noun "motivation" (from adjective "motive") stems from the Latin *movēre*, meaning "to move[1]," and the noun "behavior" (from the verb "behave") stems from a combination of "be-" (as a prefix) and "have," conveying "to have or bear oneself (in a specified way)," that is to conduct oneself intentionally[2]. Motivated behaviors may then be thought of literally as the expression of intentional (or purposeful) movements. This understanding is reflected in their common description of being oriented, directed, or driven by a goal.

From a neuroscientific standpoint, the terms goal-oriented, goal-directed, and goal-driven, all convey essentially the same basic idea that orientation, direction, or drive toward a goal (that which motivates) occurs when a change in the internal (body) or external environment that is detected by the sensory division of the nervous system achieves a level of input stimulation that is sufficient to activate a behavioral output response from the body via the motor division of the nervous system. A goal is attained when the behavioral response counteracts the originating stimulus to a level at which it no longer stimulates the behavioral response (**Figure 1**). Examples include the drive to regulate body temperature, fluid balance, and energy status in response to sensed changes in these, in order to maintain homeostasis (Watts and Swanson, 2002).

Through a process of natural selection, animals have evolved motivated behaviors that support the life goals of survival and reproduction, and the motivated behaviors that fundamentally support these goals include those for which the hypothalamus plays a central role: ingestive (eating

[1]The Oxford English Dictionary Dictionary (OED). *"motive, adj."*. Oxford University Press.
[2]The Oxford English Dictionary Dictionary (OED). *"behave, v."*. Oxford University Press.

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Received: *29 March 2019*
Accepted: *08 July 2019*
Published: *13 August 2019*

Citation:
Hahn JD, Fink G, Kruk MR and Stanley BG (2019) Editorial: Current Views of Hypothalamic Contributions to the Control of Motivated Behaviors. Front. Syst. Neurosci. 13:32. doi: 10.3389/fnsys.2019.00032

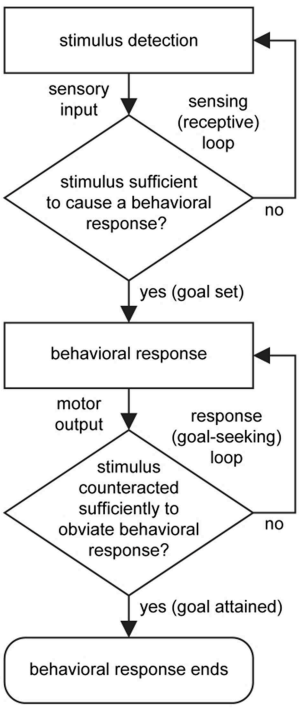

FIGURE 1 | Basic flow diagram for the control of behavior. A stimulus is detected and transmitted (sensory input). If the stimulus is sufficient to cause a behavioral response, then a motor output will ensue. If the response sufficiently counteracts the originating stimulus then the behavioral response ends. Two stages of processing are looped: The first is a sensing (or receptive) loop that confers vigilance, and the second is a response (or goal-seeking) loop that enables error correction. Fundamentally, the setting, seeking, and attaining of a goal is a determined by interaction between the sensory and motor divisions of the nervous system. Note that "selection" of a behavioral response occurs when a stimulus reaches the response threshold, and that temporally, multiple sensing loops, responsive to different sensory stimuli, operate in parallel and concurrently with behavioral output.

and drinking), agonistic (defensive and aggressive), sexual, and allied to these the control of behavioral state (the level of intrinsic behavioral arousal) (Swanson, 1987; Simerly, 2015).

In seeking to understand motivated behaviors, it is noteworthy that the distinction between movement *per se* and purposeful movement that is considered motivated behavior, is neither obvious nor absolute. For example, reflexes such as the patellar stretch reflex (knee-jerk) are not typically thought of as motivated behaviors, but they do involve movement that is ostensibly purposeful (postural retention in the case of the patellar reflex). Nevertheless, behaviors can to some extent be classified according to the parts of the nervous system that are necessary and sufficient for their expression.

Voluntary (cognitive) control of behavior requires the cerebral cortex; whereas control of innate (instinctive) behaviors is classically associated with the hypothalamus. At the lowest hierarchical level are reflex behaviors, such as the patellar reflex that involves a monosynaptic reflex arc between sensory and motor neurons in the spinal cord. Classic lesion experiments have shown that innate behaviors can be performed to some extent without the cerebral cortex, and spinal reflexes without the forebrain and much of the brainstem. However, it is also clear that hypothalamic (and lower) level behavioral control is to varying degrees subject to cerebral cortical control, and that all behavior occurs in concert with the activity of the body as a whole (Mogenson et al., 1980; Swanson and Mogenson, 1981; Swanson, 2000; Canteras, 2018).

RESEARCH TOPIC CONTRIBUTIONS

Four of the included articles focus specifically on the spatially-extensive lateral hypothalamic area (LHA) that has received renewed attention in recent years, as successive inroads into its structural organization (Goto et al., 2005; Hahn, 2010; Hahn and Swanson, 2010, 2012, 2015; Canteras et al., 2011) have encouraged further forays into its functional roles (Leinninger, 2011; Li et al., 2011; Petrovich et al., 2012; Betley et al., 2013; Hsu et al., 2015). The first article, by Rangel et al., elucidates a novel role for an LHA region juxtaposed to the dorsomedial hypothalamic nucleus (the LHAjd), in relation to socially-relevant defensive behaviors; the second article, by Tyree and de Lecea, focuses on the relevance of LHA and ventral tegmental area (VTA) connections to the motor-output that is necessary for behavioral goal-seeking; the third article, by Petrovich, reviews recent evidence on the control of feeding behavior to support a view of the LHA as an interface between cognitive and sub-cognitive control; the fourth article, by Haller, delves into LHA involvement in aggression, and relates physiology to behavior, arguing the case that the LHA has a central role in deviant forms of aggressive behavior that are promoted by chronic glucocorticoid deficiency. In addition to these four LHA-related articles, a fifth, by Diniz and Bittencourt, relates broadly to them all as it provides a comprehensive and nicely illustrated review of the role of largely LHA-located melanin-concentrating hormone (MCH) neurons in relation to their participation in control of motivated behaviors.

Of the three remaining topic articles, one, by Hashikawa et al., also focuses on aggressive behavior: its neuroanatomical focus is the ventromedial hypothalamic nucleus (VMH), and a specific locus is the ventrolateral subdivision (VMHvl). Evidence to support a role for the VMHvl in generation of aggression is reviewed in relation to VMHvl neuronal connections. Hypothalamic connections are also the subject of an article by Micevych and Meisel, who focus their attention on circuit integration in relation to the control of female sexual behavior. Lastly, an article by Khan et al. demonstrates implementation of a novel computer-assisted method to facilitate interoperability between different brain atlases. To illustrate the approach (that has broad potential application), the authors use their hypothalamic datasets relating to behavioral control.

HISTORICAL PERSPECTIVES

To round out this editorial are two illustrated perspectives (edited by JDH). The first, by George Fink, is broadly relevant to the

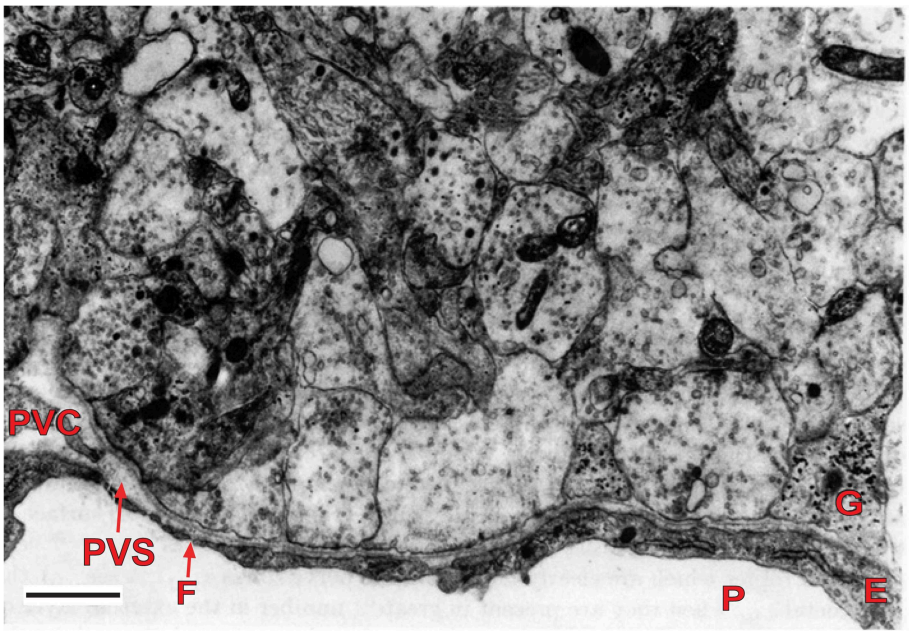

FIGURE 2 | Electron micrograph of the external layer of the median eminence of a rat at the first postnatal day. Note the high density of nerve terminals, containing several agranular and granular vesicles, around part of a primary portal capillary vessel (P), which is fenestrated (F). The vesicles contain packaged neurohormone or neurotransmitter that undergo quantal release upon nerve depolarization resulting from action potentials. The neurohormones are released into the perivascular space (PVS), and from there they move rapidly into portal vessel blood for transport to the pituitary gland. This arrangement is typical of the neurohemal junctions found in the several circumventricular organs of the brain. Scale bar = 1 μm. E, endothelial cell; G, glial process; P, portal vessel; PVC, perivascular cell (reproduced with permission from Fink and Smith, 1971).

topic, and the second, by Menno Kruk, relates more closely to some of the included articles. Both are historically-informed vignettes that serve to frame the included articles and the topic, and are also offered to inspire future research into hypothalamic structure and function.

External Layer of the Median Eminence a Neurovascular Synapse

The external layer of the median eminence (MEex) is comprised of hypothalamic neuron axons that terminate on the primary plexus of hypophysial portal vessels, where they form neurovascular synapses (**Figure 2**). This organization has been exploited experimentally as a model system for investigating central neurotransmission (Fink and Smith, 1971), and to investigate interactions between multiple different neurotransmitters expressed by different types of hypothalamic neurons whose axons converge in the MEex. This is exemplified by physiological and pharmacological studies on the release into hypophysial portal blood of several neurohormones, most of which are neuropeptides, such as gonadotropin-releasing hormone (GnRH) and corticotropin releasing factor (CRF) (Fink, 2012). However, non-peptide neurotransmitters such as dopamine, which inhibits prolactin release, are also released into hypophysial portal blood. The hypophysial portal vessels (**Figure 3**) convey these neurohormones to the anterior pituitary gland where they stimulate or inhibit the release of pituitary hormones (Fink, 2012).

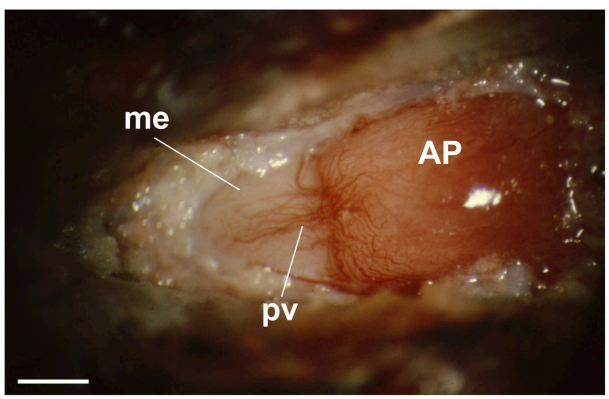

FIGURE 3 | View through a dissecting microscope of the hypophysial portal vessels on the anterior surface of the pituitary stalk (left) of an anesthetized rat. The portal vessels (pv) (veins) arise from the primary capillary bed on the median eminence (me) (pink area to the left) and fan out over the anterior pituitary gland (AP) (right) at the me-AP junction. The tuberoinfundibular artery, a branch of the superior hypophysial artery, can be seen arching across the top of the me-AP junction, where it enters the AP. This artery passes through the anterior pituitary gland to supply arterial blood to the neurohypophysis. (reproduced with permission from Fink, 2012). Scale bar = ∼500 μm.

It is possible to collect hypophysial portal vessel blood experimentally and thereby determine directly the characteristics of neurohormone/transmitter release under experimental conditions. The interaction of neurohormones is exemplified by

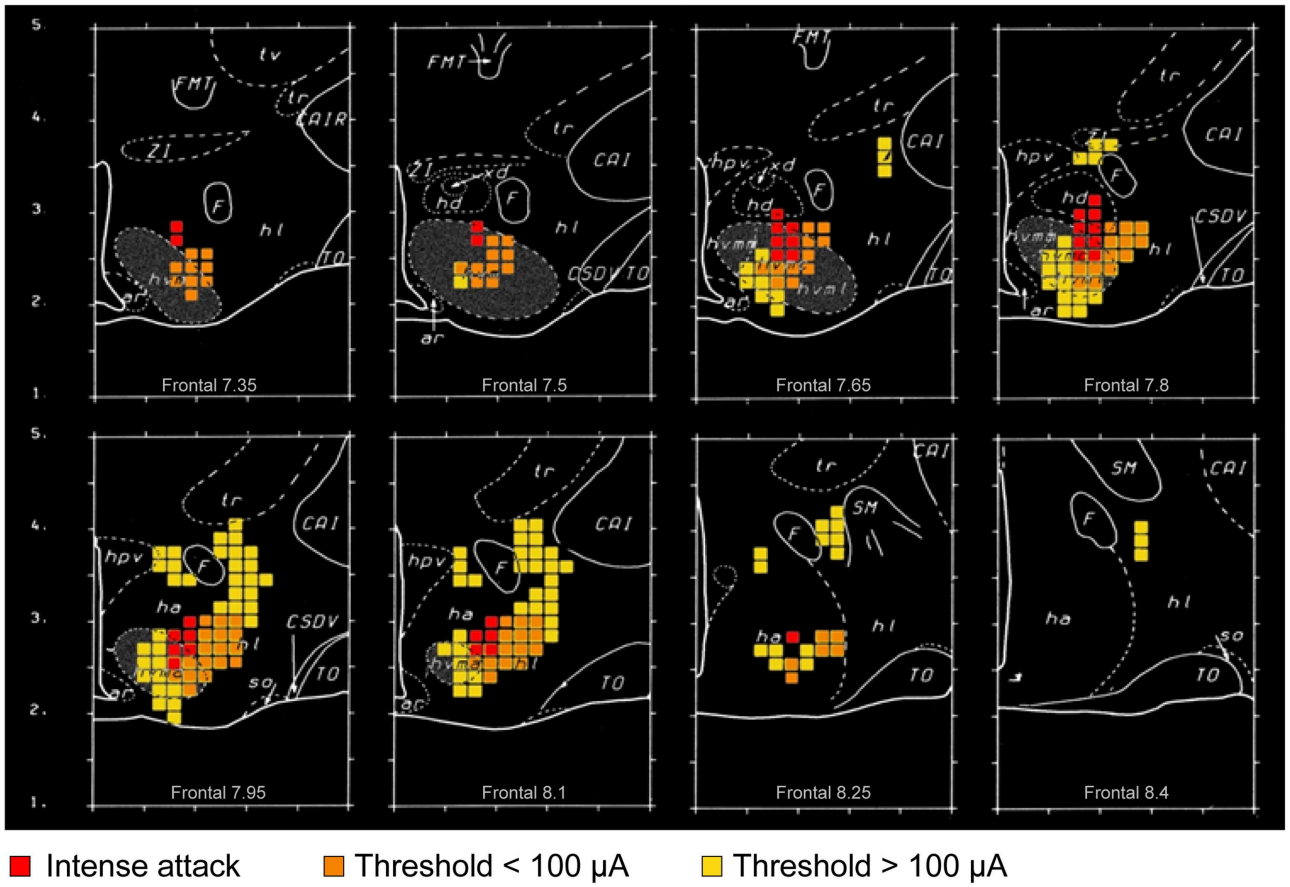

FIGURE 4 | Graphic summary of mathematical analyses of the distribution of attack-eliciting electrodes in the rat hypothalamus (adapted and reproduced with permission from ref. Kruk et al., 1983). Colored square dimensions (as voxels) are 150 μm. Fiercest attacks (attack jumps) at the lowest current intensity are evoked from sites within and closely adjacent to the ventromedial hypothalamic nucleus (VMH) (red squares); whereas, milder attacks at low current intensity are evoked from a wider range of sites within and close to the VMH (orange squares). Yellow squares represent sites where attacks were elicited reliably at higher current intensities. The extensions (orange and yellow squares) of the "attack area" beyond the VMH somewhat overlap direct and indirect projections from parts of the amygdala, and cerebral cortex to the VMH and lateral hypothalamic area. It is noteworthy that the excitability of amygdala, hippocampal, and prefrontal cortical region neurons is subject to slow and rapid, as well as genomic and non-genomic, effects of corticosteroids (Joels et al., 2018). Impairing the adrenocortical stress response impairs elicited attacks, especially at sites indicated by the orange and yellow squares. Collectively, these findings suggest that corticosteroid effects on "fight-or-flight" responses in social conflict may be transmitted by amygdalar, hippocampal or prefrontal cerebral cortical connections to the VMH "core" and "shell." Distances shown are mm ("Frontal" distances are relative to an interaural zero point). Abbreviations (for additional information see Kruk et al., 1983): ar, arcuate hypothalamic nucleus; CAI, internal capsule (R, rostral); CSOV, hypothalamic supraoptic decussations; F, fornix; FMT, mammillothalamic tract; ha, anterior hypothalamus (general region of); hd, dorsal hypothalamus (general region of); hl, lateral hypothalamus (general region of); hpv, hypothalamic paraventricular nucleus; hvmm, ventromedial hypothalamic nucleus, dorsomedial part; hvmc, ventromedial hypothalamic nucleus central part; hvml, ventromedial hypothalamic nucleus ventrolateral part; so, supraoptic nucleus; TO, optic tract; tr, reticular thalamic nucleus; tv, ventral thalamus; xd, dorsal region (of hd); ZI, zona incerta.

the potentiation of CRF anterior pituitary signaling by arginine vasopressin (AVP) (Gillies et al., 1982; Sheward and Fink, 1991). Portal vessel blood measurements may also provide information on the processing of neuropeptide precursors and identify potentially novel signaling molecules (Antoni et al., 1992; Fink et al., 1992; Caraty et al., 2010; Clarke et al., 2012).

Direct measurements of GnRH in hypophysial portal blood confirmed the existence of the estrogen-induced ovulatory surge in spontaneously ovulating mammals (Sarkar et al., 1976; Sherwood et al., 1980; Caraty et al., 2010; Clarke et al., 2012), and demonstrated the way that estrogen feedback moderates pulsatile GnRH release (Sarkar and Fink, 1980; Clarke and Cummins, 1982; Fink, 2018). The latter explains why pulsatile gonadotropin release occurs in ovariectomized, but not intact, rhesus monkeys (Dierschke et al., 1970), and the differences in gonadotropin pulse frequency in post-menopausal compared with pre-menopausal women (Yen et al., 1972). Similarly, glucocorticoid negative feedback inhibition of adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary gland, depending on its duration, is mediated by central moderation of CRF and AVP release as well as well as blockade of the pituitary response to CRF (Plotsky et al., 1986; Fink et al., 1988; Sheward and Fink, 1991).

The post-synaptic consequences of MEex neurovascular synaptic signaling can readily be determined by studying pituitary hormone release, which has elucidated novel mechanisms such as the self-priming effect of GnRH, by which the decapeptide can increase by several fold its effect on gonadotropin release, can enable small pulses of GnRH to induce

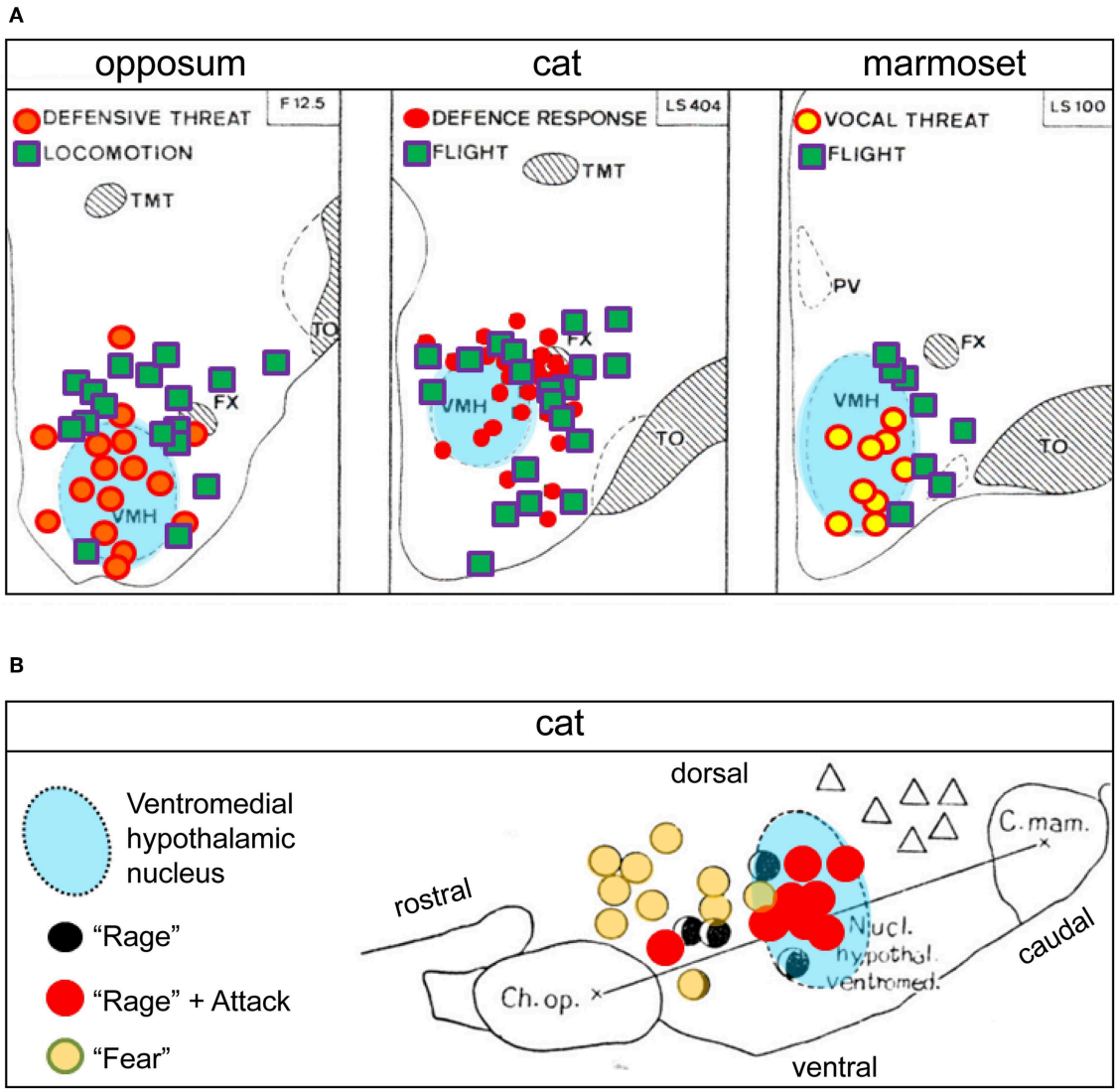

FIGURE 5 | (A) Hypothalamic sites at which electrical stimulation elicited social conflict responses in three mammalian species: opossum, cat, and marmoset (adapted and reproduced with permission from Lipp and Hunsperger, 1978). The similar distribution of response sites for the three species suggests evolutionary conservation of "fight-or-flight" neuronal circuits at the level of the ventromedial hypothalamic nucleus (VMH). The different types of "social conflict" motor responses indicated reflect different aspects of "fight-or-flight" behaviors. Similar response site distribution patterns in the vicinity of the VMH have also been reported in rat and mouse (Lammers et al., 1988; Wong et al., 2016). **(B)** Comparative distribution from an earlier study of social conflict responsive sites (low-threshold) in the cat, shown in sagittal section (adapted and reproduced with permission from Yasukochi, 1960). Attacks and "rage" are elicited mostly within the VMH, while the response site for "rage" alone is shifted rostrally, and that for "fear" alone still further rostral in the hypothalamus, suggesting a VMH-centric circuit organization to control "fight-or-flight" behaviors in the cat (for additional perspective see Hinde, 1970). Ch. Op, optic chiasm; C. mam, mammillary body; FX, fornix; PV, paraventricular hypothalamic nucleus; TMT, thalamic mammillothalamic tract; TO, optic tract. Triangles in **(B)** = "yearning".

an ovulatory gonadotropin surge, and has been used extensively in artificial insemination, animal husbandry, and fish farming (Fink, 1995, 2015).

The Hypothalamic Ventromedial Nucleus: A Crucial Node in the Fight-Flight Balance?

Establishing that estrogen receptor-α (ESR1)-expressing neurons within the VMH ventrolateral part (VMHvl) are necessary and sufficient for aggressive behavior (Lin et al., 2011; Falkner et al., 2014; Kennedy et al., 2014) transformed the neuroscience of aggression, as it provided a specific locus from which to explore the "aggressive network" (Anderson, 2012; Yang et al., 2013, 2017; Hashikawa et al., 2016, 2017, 2018; Remedios et al., 2017; Hashikawa et al.). Other studies have identified cell groups in the amygdala and lateral septum that modify VMH activity (Choi et al., 2005; Wong et al., 2016). Moreover, activation of inhibitory (GABAergic) neurons in the medial amygdala can also elicit aggressive behavior (Hong et al., 2014). How the activity of these

different cell groups is integrated is not fully understood, but a recent physiological experiment suggests a possible mechanism. Differential innervation of the "core" and "shell" of the VMH, directly from the basomedial amygdala, and indirectly from the anterior bed nucleus of the stria terminalis, produces "…a net inhibition or disinhibition of core neurons…depending on the firing rate of shell neurons," imparting "…flexibility to this regulator of defensive and social behavior" (Yamamoto et al., 2018). Such flexibility might explain the episodic nature and context-sensitivity of fighting and underlie dynamic selection of appropriate behavioral responses in general (Brown et al., 1969b; Lammers et al., 1989; Haller et al., 1998a; Anderson, 2012; Yang et al., 2013, 2017; Hong et al., 2014; Kennedy et al., 2014; Hashikawa et al., 2016, 2018; Remedios et al., 2017; Todd et al., 2018; Todd and Machado, 2019).

Stimulation of the VMH and its surround is reported to evoke aggressive and defensive responses in several mammalian species (Yasukochi, 1960; Roberts et al., 1967; Brown et al., 1969a; Lipp and Hunsperger, 1978; Lammers et al., 1988; Kruk et al., 1983) (**Figures 4**, **5**). However, predominant VHMvl association with overtly aggressive responses (Lin et al., 2011; Falkner et al., 2014, 2016; Kennedy et al., 2014) contrasts with VMH dorsolateral and central part association with defensive responses (Wang et al., 2015). This suggests the existence of a VMH-centric circuit for controlling opposing agonistic responses, echoing earlier ethological concepts of a mechanism for controlling "fight or flight" balance (Hinde, 1970).

In a manner similar to feedback (and feed-forward) control of the pituitary gland by circulating hormones (mentioned in the first perspective), the adrenocortical stress response (ACSR) (Joels et al., 2018) controls spontaneous and hypothalamus-elicited agonistic responses in experienced and inexperienced animals in different ways (Haller et al., 1998b, 2000a,b; Kruk et al., 1998, 2004, 2013; Mikics et al., 2007). An impaired ACSR tilts the balance toward "flight or freeze" in rats naïve to conflict but produces "pathological" attacks on opponents in bouts of spontaneous aggression (Haller et al., 2001, 2004). The behavioral changes correlate to altered hypothalamic excitability and enhanced amygdalar activity (Halasz et al., 2002; Kruk, 2014; Haller, 2018; Haller). A dynamic ACSR is clearly required for an adaptive response to social conflict. Interestingly, the absence of a well-timed ACSR in humans results in misguided aggression and poor conflict handling (Haller), possibly reflecting dysfunctional hypothalamic control.

CONCLUDING REMARKS

The ability to perform motivated behaviors (purposeful movements) is a defining characteristic of animals. In this ability, with respect to the control of fundamental behaviors in mammals and other vertebrates, the hypothalamus takes center stage. The works of twentieth century ethologists, exemplified in those of Tinbergen (1951), paved a path that has led inexorably into the hypothalamus, and they continue to inspire neuroscientists interested in the study of behavior.

The current Research Topic, and the articles that comprise it, reflect ongoing and growing interest in the hypothalamus, driven partly by the increasing availability of investigative tools borne of molecular biology and computer science. However, with regard to those tools, Tinbergen's advocacy for observations of nature, rather than availability of technique, to direct one's research, seems prescient. More generally, current interest is also driven by a renewed recognition that a better understanding of hypothalamus structure and function has potential relevance for numerous diseases that impact the vital and varied physiological and behavioral functions in which the hypothalamus plays a central role (Hahn et al., 2019).

AUTHOR CONTRIBUTIONS

JH wrote the editorial. GF and MK provided illustrated perspectives (edited by JH). All authors reviewed the editorial and provided editorial guidance.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Systems Neuroscience

REVIEW
published: 08 June 2018
doi: 10.3389/fnsys.2018.00026

The Role of the Lateral Hypothalamus in Violent Intraspecific Aggression—The Glucocorticoid Deficit Hypothesis

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This review argues for a central role of the lateral hypothalamus in those deviant forms of aggression, which result from chronic glucocorticoid deficiency. Currently, this nucleus is considered a key region of the mechanisms that control predatory aggression. However, recent findings demonstrate that it is strongly activated by aggression in subjects with a chronically downregulated hypothalamus-pituitary-adrenocortical (HPA) axis; moreover, this activation is causally involved in the emergence of violent aggression. The review has two parts. In the first part, we review human findings demonstrating that under certain conditions, strong stressors downregulate the HPA-axis on the long run, and that the resulting glucocorticoid deficiency is associated with violent aggression including aggressive delinquency and aggression-related psychopathologies. The second part addresses neural mechanisms in animals. We show that the experimental downregulation of HPA-axis function elicits violent aggression in rodents, and the activation of the brain circuitry that originally subserves predatory aggression accompanies this change. The lateral hypothalamus is not only an integral part of this circuitry, but can elicit deviant and violent forms of aggression. Finally, we formulate a hypothesis on the pathway that connects unfavorable social conditions to violent aggression via the neural circuitry that includes the lateral hypothalamus.

Keywords: violence, aggression, hypothalamus, humans, rodents

OPEN ACCESS

Edited by:
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Leiden University, Netherlands

Reviewed by:
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Received: *05 January 2018*
Accepted: *16 May 2018*
Published: *08 June 2018*

Citation:
Haller J (2018) The Role of the Lateral Hypothalamus in Violent Intraspecific Aggression—The Glucocorticoid Deficit Hypothesis. Front. Syst. Neurosci. 12:26. doi: 10.3389/fnsys.2018.00026

INTRODUCTION

Early studies by Hess (1928) attributed the hypothalamus a central role in aggression control, by showing that the electrical stimulation of particular hypothalamic sites rapidly induces biting attacks. This methodology contributed to aggression research by delimitating aggression-related hypothalamic regions, and by elucidating the major components of aggression-related neural networks (for reviews see Kruk, 1991; Siegel et al., 1999). Initially, hypothalamic regions involved in attack behavior were divided into two separate mechanisms. Mediobasal aspects of the hypothalamus (the mediobasal hypothalamus in cats, the hypothalamic attack area in rats, and the ventrolateral part of the ventromedial hypothalamus in mice) were shown to control intraspecific aggression. Albeit disparate early studies suggested that the electric stimulation of the lateral hypothalamus might also promote intraspecific aggression (Woodworth, 1971; Koolhaas, 1978), the effect remained somewhat elusive and occurred at rather large current intensities. Consequently,

the consensus is that the lateral hypothalamus (in both cats and rats) controls predatory aggression (Smith et al., 1970; Kruk et al., 1979; Shaikh et al., 1987; Lin et al., 2011). In all the species studied so far (including humans) hypothalamic areas located medioventrally to the fornix were associated with intraspecific aggression, whereas hypothalamic areas located laterally to the fornix were shown to control interspecific (predatory) aggression (Haller, 2013).

Our recent studies, however, indicated that the lateral hypothalamus is involved also in the control of intraspecific aggression, particularly in its violent forms. We found that the lateral hypothalamus was strongly activated during resident-intruder conflicts, but only if subjects were submitted to the glucocorticoid hypofunction model of abnormal aggression (Tulogdi et al., 2010, 2015). In this model of abnormal aggression, rats attack vulnerable body parts of opponents (head, throat band belly) without appropriately signaling attack intentions by social signals. We also showed that a subpopulation of prefrontal neurons specifically project to, and influence violent aggression controlled by the lateral hypothalamus (Biro et al., 2017, 2018). We recently proposed that the control mechanisms of violent forms of aggression are combinations of the mechanisms that subserve intraspecific (rivalry) and interspecific (predatory) aggressions (Haller, 2017; **Figure 1**).

The present review argues in favor of the role of the lateral hypothalamus in violent aggression, specifically in aggression associated with glucocorticoid deficits. The first part of the review is devoted to the role of glucocorticoid deficits in human aggression. The ultimate aim of this section is to clarify the translational value of the mechanisms discussed in the second part of the review. This latter section will in fact provide explanations for, and arguments supporting the mechanisms schematically represented in **Figure 1**. We suggest that glucocorticoid deficits are important contributors of deviant aggression in humans, and that animal findings with translational value suggest that the lateral hypothalamus has an important role in mediating the violence-related roles of glucocorticoid deficits.

GLUCOCORTICOID DEFICITS AND AGGRESSION IN HUMANS

Virkunnen reported in 1985 that cortisol secretion by habitually violent offenders with antisocial personality disorder was considerably lower than that of all three antisocial personality disordered subjects without habitual aggression, recidivist arsonists, and male clinic personnel. This report was published at a time when current thought associated aggression with increased rather than decreased stress responses (Posner and Conway, 1981; Public Health Report, 1983; Mason and Blankenship, 1987; Susman et al., 1988). Not surprisingly, reference to the now classical work by Virkkunen (1985) had a slow start. The first independent citation (a review) was published 4 years later (Zuckerman, 1989), and it required about a decade till the first confirmatory studies were published (Vanyukov et al., 1993; van Goozen et al., 1998; McBurnett et al., 2000; Dolan et al., 2001; Pajer et al., 2001; Kariyawasam et al., 2002). More than a decade elapsed until the first laboratory model of the condition was developed (Haller et al., 2001, 2004), albeit associations between low glucocorticoid levels and aggression were observed earlier (Poole and Brain, 1974).

Taken together, these findings suggested that certain types of human aggression and aggression-related psychopathologies are associated with downregulated hypothalamus- pituitary-adrenocortical (HPA) axis function, and that mimicking this condition in rodents leads to the development of abnormal forms of aggression (Haller, 2014). Several authors questioned the assumption that low glucocorticoid levels are associated with aggression problems (Klimes-Dougan et al., 2001; von der Pahlen, 2005; Marsman et al., 2008), and indeed, findings are often contradictory, especially in children diagnosed with various aggression-related psychopathologies (**Table 1**). Nevertheless, the findings presented in **Table 1** clearly demonstrate that such psychopathologies are associated with downregulated HPA-axis function in certain study populations at least, which raises the question of how such conditions develop.

Conditions Leading to Glucocorticoid Deficits in Humans

Yehuda et al. (1990) were the first to suggest that on the long-run stressors may decrease HPA-axis function. They observed that cortisol secretion was significantly lower in post-traumatic stress disorder (PTSD) patients than in controls; moreover, cortisol levels correlated with PTSD symptomatology. Importantly, a recent paper from the same lab suggested that glucocorticoid treatments can ameliorate the symptoms of PTSD, suggesting causal relationships between the hormonal response and psychiatric symptoms (Yehuda et al., 2015). Despite these early findings in PTSD, more than a decade later Gunnar and Vazquez (2001) still felt the need to encourage those who observed similar phenomena. They wrote "Lower than expected cortisol values should not necessarily be relegated to the file drawer because they contradict the central dogma that stress must be associated with elevations in cortisol" (Gunnar and Vazquez, 2001). Scientific opinion has changed considerably currently; an increasing number of stressors that lower HPA-axis activity have been reported since.

A Brief Account of the Findings

Detrimental conditions acquired via the mother such as exposure to various illicit drugs, caffeine, nicotine, alcohol, as well as synthetic glucocorticoids (administered as medications) were all shown to inhibit HPA-axis function in adulthood (Kapoor et al., 2008; Vázquez et al., 2012; Zhang et al., 2014; Buckingham-Howes et al., 2016).

Deficient parental care also inhibits HPA-axis function on the long run (Van der Vegt et al., 2009; Koss et al., 2014; Martin et al., 2014; McLaughlin et al., 2015); moreover, this effect becomes irreversible if "treatment" by adoption comes too late e.g., after the age of 2–3 years (McLaughlin et al., 2015). Family tradition of aggressiveness (Saxbe et al., 2012; Arbel et al., 2016) as well as parental psychopathology (antisocial personality disorder, and post-traumatic stress disorder) also decrease HPA-axis function on the long run (Vanyukov et al., 1993; Cordero et al., 2017).

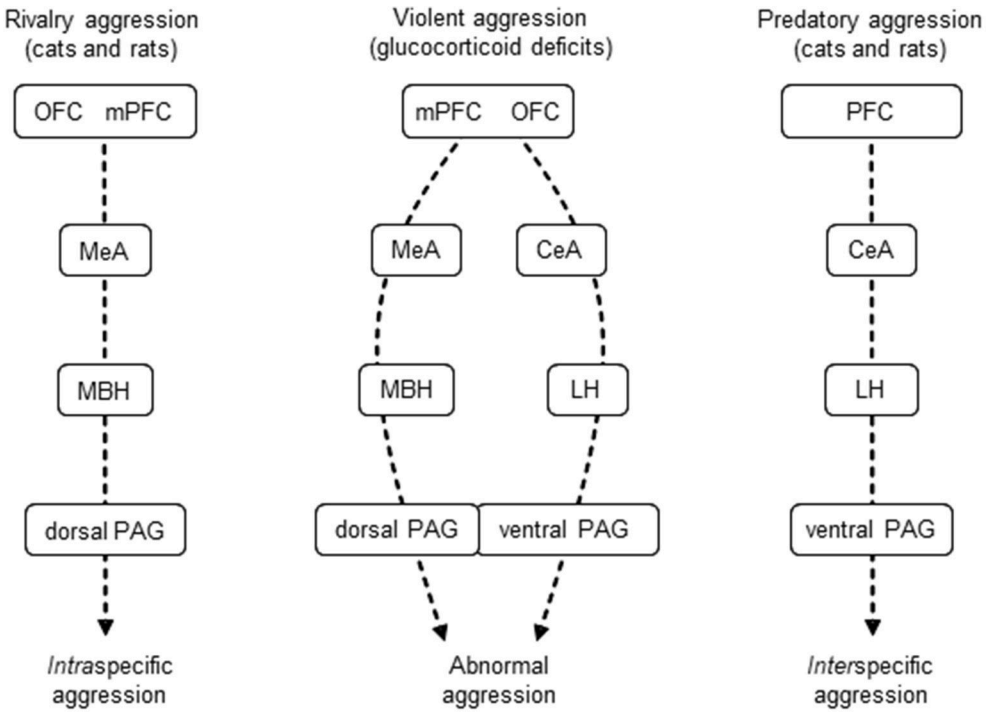

FIGURE 1 | Mechanisms of glucocorticoid deficit-induced aggression are combinations of those subserving intraspecific and predatory aggressions. For explanations, see section Introduction. Dashed arrows indicate hypothetical information flow. CeA, central amygdala; LH, lateral hypothalamus; MBH, mediobasal hypothalamus (hypothalamic attack area); MeA, medial amygdala; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; PAG, periaqueductal gray. Dashed arrows, hypothetical flow of information.

Various forms of early maltreatment e.g., neglect, and all forms of abuse (emotional, physical, sexual abuse) as well as traumatic stressors have a similar effect (Weissbecker et al., 2006; Elzinga et al., 2008; Carpenter et al., 2009; Bosch et al., 2012; Lovallo et al., 2012; Doom et al., 2014; Trickett et al., 2014; Peckins et al., 2015; Puetz et al., 2016).

Extra-familial conditions, e.g., violent neighborhoods and community violence in general, bullying in schools, the proximity of pubs were all negatively associated with indices of HPA-axis function in the adulthood of subjects (*blunted stress responses*: Busso et al., 2017; *blunted cortisol responses*: Ouellet-Morin et al., 2011; Janusek et al., 2017; *steeper diurnal decline in cortisol levels during the day*: Theall et al., 2017; *low basal cortisol levels*: Vaillancourt et al., 2008).

Finally, adversities suffered in adulthood also decrease HPA-axis function on the long run (Carsia and McIlroy, 1998; Dayan et al., 2016; Pinto et al., 2016; Ewing-Cobbs et al., 2017). Such stressors include malnutrition, intimate partner violence, traumatic brain injury, and traumatic stress. HPA-axis function also showed chronic deficits in certain psychopathologies e.g., antisocial personality disorder, atypical depression, chronic fatigue syndrome, cocaine addiction, post-traumatic stress disorder, and schizophrenia (Buydens-Branchey et al., 1997; Yehuda, 1999; Strous et al., 2004; Bührsch et al., 2009; Flory et al., 2009; Nijhof et al., 2014; Berger et al., 2016; Juruena et al., 2017; Pruessner et al., 2017; Das et al., 2018). While the association was likely expected with certain disorders (e.g., antisocial personality and post-traumatic stress disorders), others may need some clarification. Depression overall is clearly associated with chronic stress; yet depressed patients who suffered strong stressful events in their early life showed decreased awakening cortisol in adulthood (Strous et al., 2004). Noteworthy, the awakening-induced increase in cortisol production is believed to reflect best the overall functional status of the HPA-axis as it is free of confounds from daily events (Kudielka and Wüst, 2010). Decreased HPA-axis function was also observed in atypical depression, a condition that may be associated with aggressiveness (Hasler et al., 2004; Juruena et al., 2017). Although stressors were earlier associated with the *development* of schizophrenia, it was recently shown that *the disorder per se* is associated with low cortisol plasma levels throughout the day–possibly as a response to early stress exposure (Strous et al., 2004; Berger et al., 2016; Pruessner et al., 2017; Das et al., 2018). Moreover, decreased cortisol levels were shown to contribute to aggression in psychosis. Finally, cocaine addiction *per se* was not associated with decreased plasma levels of cortisol, but aggressive addicts had a blunted cortisol response to meta-chlorophenylpiperazine challenge (Buydens-Branchey et al., 1997).

Overall Evaluation of Findings

HPA-axis deficits evidenced by the studies briefly reviewed above may take many forms, from decreased morning cortisol and

TABLE 1 | Cross sectional studies in children: associations between aggression-related psychological conditions and cortisol plasma levels.

Condition		Cortisol measurement						Association	References
Primary	**Secondary**	**Single**	**Multiple**	**Diurnal**	**Total**	**Awakening**	**Stress response**		
CD		X						↑	van Bokhoven et al., 2005
DIS		X						→	Scerbo and Kolko, 1994
ADHD	ODD	X						↓	Kariyawasam et al., 2002
CD		X						↓	Vanyukov et al., 1993
CD	CU	X						↓	Loney et al., 2006
CD	ODD, AGG	X						↓	Oosterlaan et al., 2005
CD			X					↓	Pajer et al., 2001
CD			X					↓	Pajer et al., 2006
DIS	ANX		X				X	↓**	Schoorl et al., 2016
CD				X				→	Fairchild et al., 2008
UNR	AGG			X				→	Van den Bergh et al., 2008
UNR	AGG			X				↓	Oberle et al., 2017
UNR	EXT			X				↓	Martin et al., 2014
DIS					X			→	Kruesi et al., 1989
UNR	EXT				X			↓	Puetz et al., 2017
UNR	EXT					X		↑	Marsman et al., 2008
UNR	EXT					X		→	Klimes-Dougan et al., 2001
CD	CU					X		↓	von Polier et al., 2013
UNR	EXT					X		↓	Cicchetti and Rogosch, 2001
DIS	AGG					X		↓	Van de Wiel et al., 2004
UNR	EXT					X		↓	Puetz et al., 2016
DIS							X	↑‡	McBurnett et al., 2005
ADHD							X	↓ ‡	Pesonen et al., 2011
ADHD	CD						X	↓*	Northover et al., 2016
CD							X	↓†	Yang et al., 2007
DIS							X	↓	van Goozen et al., 1998
DIS							X	↓‡	van Goozen et al., 2000
ODD							X	↓$	Snoek et al., 2002
ADHD	CU						X	↓€	Stadler et al., 2011

Psychiatric/psychological conditions. *ADHD, attention deficit hyperactivity disorder; AGG, aggression; ANX, anxiety; CD, conduct disorder; CU, callous emotional traits, DIS, disruptive behavior; EXT, externalizing behavior; ODD, oppositional-defiant disorder; UNR, unreferred.* ***Cortisol measurements***. *X, type of measurement performed; single, condition associated with a single cortisol measurement; multiple, condition associated with averaged multiple cortisol measurements; diurnal, condition associated with altered diurnal secretion rhythm; total, 24 h urinary cortisol or equivalent; awakening, diurnal increase in cortisol measured in the morning; stress response, stress response independent from aggression.* ***Association with cortisol***. *↓, condition associated with downregulated HPA-axis function; → , condition not associated with changes in HPA-axis function; ↑, condition associated with upregulated HPA-axis function; *association with CD traits; **complex interaction with anxiety; † anticipation of stress (e.g., public speaking); ‡ psychological challenge; $ pharmacological challenge; € psychological stress, association with CU traits.*

blunted awakening response, trough decreased levels over the day to blunted cortisol responses to various types of challenges. Although the relative importance of such individual events is unclear at present, it should be noted that one and same stressful event elicited the full range of changes albeit in different studies. For example, early adversity decreased cortisol stress responses in some studies (Elzinga et al., 2008; Trickett et al., 2014), decreased basal levels or flattened the diurnal rhythm of cortisol secretion in others (Sánchez et al., 2005; Bosch et al., 2012; Peckins et al., 2015), and decreased morning cortisol in yet other studies (Weissbecker et al., 2006; Puetz et al., 2017). As such, effects may cover a variety of indices of HPA-axis deficits, even if one particular aspect was addressed in a particular study. Taken together, the findings demonstrate that various stressors suffered from prenatal to adult ages can inhibit the function of the HPA-axis on the long run.

Albeit the studies reviewed above were primarily endocrinological in their scope, some of them did investigate the behavior of subjects. In all such studies, low HPA-axis function was associated with indices of aggression (*bullying*: Ouellet-Morin et al., 2011; *cocaine addicts with low stress responses*: Buydens-Branchey et al., 1997; *early life stress*: Puetz et al., 2016; *poor parental monitoring*: Martin et al., 2014; *prenatal drug exposure*: Buckingham-Howes et al., 2016; *prenatal synthetic glucocorticoids*: Kapoor et al., 2008; *schizophrenia*: Strous et al., 2004; Das et al., 2018). In these studies, aggression was conceptualized as problem behavior, social and behavioral problems, externalizing behavior, high scores on aggression

inventories, the display of aggression-related psychopathologies, and aggression histories. Particular behavioral descriptions were usually not provided. Thus, long-term decreases in HPA-axis function was associated with various indices of aggressiveness.

Comparison of HPA-Axis Down- and Upregulating Factors

Stressful events that led to a downregulated HPA-axis on the long run (see above) are surprisingly similar to those, which augmented HPA-axis function in other studies (see the reviews by Kuhlman et al., 2017; Raymond et al., 2017). This leads to the surprising conclusion that one and same stressor may down—or upregulate the stress system on the long run, depending on an unknown array of circumstances. While such circumstances cannot be identified at present, the available data provide some hints toward the solution of the contrasting findings.

The first aspect to be considered is related to the temporal evolution of events. It has been repeatedly shown that stressors that upregulate the HPA-axis may ultimately lead to its downregulation. For instance, caffeine, nicotine or alcohol consumed by the mother enhanced cortisol secretion in both the mother and the infant; blunted stress responses were observed only when subjects reached adulthood (Zhang et al., 2014). In the study by Bosch et al. (2012), pre- and post-natal stressors also elicited a cortisol response acutely. Subsequently, the adversity was not associated with cortisol outcomes up to the age of 5, the upregulation of the HPA-axis was observed up to the age of 11, whereas a few years thereafter low cortisol levels were observed in subjects. Although not detailed to a similar degree, the trajectory of HPA-axis changes followed the same pattern in a number of cases. Acute and (sub)chronic stress responses were followed over time by a downregulation of the HPA-axis (Gunnar and Vazquez, 2001; Fries et al., 2005; Sánchez et al., 2005; Doom et al., 2014; Zhang, 2017). These findings show that the glucocorticoid deficit develops over time, and subjects frequently reach this phase after a period of HPA-axis upregulation. It also occurs that the process may take years.

Another factor that should be taken into account is the variability in HPA-axis responses. Peckins et al. (2015) categorized their subjects with respect to stress responses rather than calculating overall averages. They identified three cortisol profiles emerging after stress exposure (blunted, moderate, and elevated stress responses), and found that maltreated youth were more likely to show a blunted cortisol profile on the long run, but some of them fell into the other two categories. Van der Vegt et al. (2009) found that the magnitude of HPA-axis dysregulation depended largely on the severity of early maltreatment. Individual variability in HPA-axis responses to stress was observed in other studies as well (Weissbecker et al., 2006; Flory et al., 2009). Thus, the direction of HPA-axis changes (up- or down-regulation) and the magnitude of the effect strongly depend on the individual features of subjects, and/or to life events subsequent to the initial stressor. Naturally, other factors e.g., genetic susceptibility, and the type of stress are also important (see Daskalakis et al., 2013 for a review).

Conclusions

The long-term decrease in HPA-axis function that develops in response to strong stressors may be best conceptualized as an "allostatic crash," a term coined by Van Houdenhove et al. (2009). This concept suggests that severe stressors, when associated with low individual resilience lead to an allostatic load that overpasses the adaptive capacities of the HPA-axis. This—metaphorically saying—results in the "fatigue" or "burnout" of the system. Such a "burnout" may be facilitated by the transient phase of upregulation (see above) or by subsequent stressors. Collectively, the excessive allostatic load leads to a failure of the adrenals to produce adequate quantities of glucocorticoids under basal conditions, and/or to failures of the system to adequately respond to acute stressors.

The likely factors that differentiate conditions that lead to the chronic upregulation or the chronic downregulation of the HPA-axis are the severity and duration of the triggering event (the initial stressor), individual susceptibility to stress effects, and life events that follow the initial stressor. Their relationship may place the HPA-axis on opposing trajectories and may lead either to the up- or to the downregulation of the HPA-axis.

Glucocorticoid Deficits and Human Aggression

The study by Virkkunen (1985) implicitly suggests that glucocorticoid deficiency is associated with severe aggression; moreover, superimposed deficits e.g., antisocial personality disorder and habitual offending. Some recent findings also imply that HPA-axis hypoactivity characterizes a particularly severe subgroup of subjects who show both chronic antisocial behavior and callous-unemotional traits (Hawes et al., 2009). Other evidence, however, suggests that glucocorticoid deficits can be associated with less severe forms of aggression.

A Brief Account on Findings

By a thorough Medline search using various keywords, we identified 76 studies on the interaction between HPA-axis function and aggressiveness. We summarized findings in **Tables 1–4**. Individual tables refer to studies in particular subjects; **Table 1** for instance shows cross-sectional studies performed in children who showed various aggression-related conditions. Findings were arranged according to the method of cortisol measurements to visualize possible confound resulting from this variable. As argued elsewhere (Haller et al., 1998, 2008; Haller, 2014), types of measurement are not equivalent. Single measurements appear to be the least reliable due to accidental variations in plasma cortisol levels. Averaged multiple measurements eliminate such confounds, whereas awakening cortisol as well as indices of total cortisol production (e.g., 24 h urinary cortisol) can be considered general indices of HPA-axis function. Diurnal variations provide detailed information, which may reveal alterations specific to particular phases of the day. Finally, the magnitude of the stress response may vary independently from basal levels, by this providing a yet different measure of HPA-axis function.

Out of the 29 cross-sectional studies performed in children, 21 (72.4%) revealed a negative association between HPA-axis

TABLE 2 | Longitudinal studies in children: associations between cortisol plasma levels and long-term changes in aggression-related conditions.

Condition		Cortisol measurement						Association		References
Concurrent	Longitudinal	Single	Multiple	Diurnal rhythm	Daily production	Awakening response	Stress response	Concurrent	Longitudinal	
UNR	DIS	X						→	↓	Sondeijker et al., 2008
UNR	DIS		X					→	↓	McBurnett et al., 2000
UNR	DIS		X					→	↓	Alink et al., 2012
UNR	EXT		X					→	↓	Shirtcliff and Essex, 2008
UNR	EXT		X					→	↓	Shirtcliff et al., 2005
UNR	AGG		X					→	↓	Shoal et al., 2003
INT	INT		X					→	↓*	Ruttle et al., 2011
UNR	AGG			X				→	↓	Salis et al., 2016
AGG	AGG					X		↓	↓**	Platje et al., 2013

Temporal relationships. *Concurrent, first time point of the study; longitudinal, subsequent time-points of the study (usually years after "current").* ***Psychological conditions***. *AGG, aggression; DIS, disruptive behavior; EXT, externalizing behavior; INT, internalizing behavior; UNR, unreferred.* ***Cortisol measurements.*** *X, type of measurement performed; single, condition associated with a single cortisol measurement; multiple, condition associated with averaged multiple cortisol measurements; diurnal, condition associated with altered diurnal secretion rhythm; total, 24 h urinary cortisol or equivalent; awakening, diurnal increase in cortisol measured in the morning; stress response, stress response independent from aggression.* ***Association with cortisol***. *→ , condition not associated with changes in HPA-axis function concurrently (i.e., during the first time-point of the study); ↓, the condition associated with downregulated HPA-axis function over time (cortisol levels at the first time point were compared with psychiatric conditions observed in subsequent time-points); *increase in internalizing behavior predicted the decrease in cortisol plasma levels over time; **study subjects were adolescents (16–19 years old).*

function and conditions associated with aggressiveness; 5 (17.3%) found no association, whereas the opposite relationship was reported in the remaining 3 studies (10.3%) (**Table 1**). The method of glucocorticoid measurement does not seem to have a role in discrepancies, because all the 6 ways of HPA-axis evaluation was found in all the three categories of interactions. Out of the primary conditions (diagnoses) investigated, attention deficit hyperactivity disorder (ADHD) showed the negative association consistently, whereas findings in disruptive behavior were the least consistent. Findings in unreferred subjects evaluated for externalizing behavior were rather inconsistent as well. Out of the secondary conditions, callous-unemotional traits showed the negative association consistently whereas findings in externalizing behavior were the least consistent. Studies lacking a secondary moderating factor also provided inconsistent findings.

Taken together, the findings summarized in **Table 1** show that the association between aggression-related conditions and HPA-axis function are conflicting in cross-sectional studies performed in children. Albeit the overwhelming majority of studies reported a negative association, a significant proportion of studies are at variance with this conclusion. No contradictions were found with certain conditions e.g., ADHD and callous unemotional traits, but the number of relevant studies is too small to draw definite conclusions regarding these conditions.

In sharp contrast to cross-sectional studies, longitudinal ones performed in children appear highly consistent (**Table 2**). In the majority of these studies, unreferred subjects were studied at time-point 1, when there was no interaction between cortisol levels and behavior. Note that no problem behavior was identified at this time-point. Low levels of cortisol at time-point 1, however, predicted the development of problem behaviors at the 2nd time-point i.e., more than 2 years later (up to 6 years in some studies). There were only two exceptions, but neither produced conflicting findings. In one study, childhood internalizing behavior predicted downregulated HPA-axis function in adolescence; no similar association was found for externalizing behavior (Ruttle et al., 2011). In the second study, the negative association was observed at the first time-point already, and this was maintained over time (Salis et al., 2016). Noteworthy, however, the subjects of this study were adolescents at the first time-point. One can hypothesize that the prediction of problem behavior by low cortisol was valid for these subjects as well, but they were over the critical age. Again, the method of cortisol measurement did not affect the relationship between cortisol and behavior.

Out of the 19 studies performed in adolescents and adults, low HPA-axis functioning was associated with various indices of aggressiveness in 18 studies (**Table 3**). The only exception was a study performed in antisocial personality-disordered subjects, where psychopathic traits did not correlate with the diurnal patterns of cortisol secretion (Loomans et al., 2016). The lack of interaction cannot be attributed to the way of cortisol measurement, as two other studies employed the same approach; moreover, one of these found associations with psychopathic traits (Vaillancourt and Sunderani, 2011; Das et al., 2018). This discrepancy is difficult to explain. However, the rest of the studies are remarkably consistent as it regards the association between aggressiveness and cortisol, despite the large variation in subject types (from unreferred through psychotic and drug addiction to antisocial personality disorder) and the various ways of HPA-axis evaluations.

Finally, findings obtained in delinquents also appear rather consistent (**Table 4**). Out of the 18 studies, delinquents had downregulated HPA-axes in 15 (83%). Interestingly, the association did not depend on the type of delinquency, as low HPA-axis function was observed in both violent and nonviolent offender populations. In two studies, no association was observed (Feilhauer et al., 2013; Gostisha et al., 2014). However, the impact

TABLE 3 | Studies in adolescents and adults: associations between plasma cortisol and aggression-related psychiatric conditions.

Condition		Cortisol measurement						Interaction	References
Primary	**Secondary**	**Single**	**Multiple**	**Diurnal rhythm**	**Daily production***	**Awakening**	**Stress responses**		
UNR	AGG	X						↓	Yu and Shi, 2009
ALC	VIOL	X						↓	Bergman and Brismar, 1994
UNR	PP	X						↓*	Glenn et al., 2011
SCH	AGG	X						↓	Strous et al., 2004
UNR	CU		X					↓**	Fanti and Kimonis, 2017
UNR	AGG		X					↓	Victoroff et al., 2011
UNR	PP1			X				↓†	Vaillancourt and Sunderani, 2011
PSYCH	VIOL			X		X		↓	Das et al., 2018
APD	PP			X				→	Loomans et al., 2016
UNR	AGG				X			↓*	Grotzinger et al., 2018
UNR	AGG						X	↓‡	Böhnke et al., 2010
UNR	AGG						X	↓$	Gordis et al., 2006
HERadd (ABST)	AGG						X	↓‡	Gerra et al., 2004
HERadd (METH)	AGG						X	↓‡	Gerra et al., 2001
APD	-						X	↓€	Almeida et al., 2010
UNR	EXT						X	↓*	Portnoy et al., 2015
UNR	PP						X	↓	O'Leary et al., 2007
COCadd	AGG						X	↓€	Buydens-Branchey et al., 1997
PDE	AGG, EXT						X	↓	Buckingham-Howes et al., 2016

Psychiatric/psychological conditions*. ABST, abstinent at the time of the study; AGG, aggression; ALC, alcoholic; APD, antisocial personality disorder; CU, callous emotional traits, EXT, externalizing behavior; HERadd, heroin addiction; METH, on methadone at the time of the study; PDE, prenatal drug exposure; PP, psychopathic traits; PP1, psychopathy type 1; PSYCH, psychosis; SCH, schizophrenia; UNR, unreferred; VIOL, violence.* ***Cortisol measurements.*** *X, type of measurement performed; single, condition associated with a single cortisol measurement; multiple, condition associated with averaged multiple cortisol measurements; diurnal, condition associated with altered diurnal secretion rhythm; total, 24 h urinary cortisol or equivalent; awakening, diurnal increase in cortisol measured in the morning; stress response, stress response independent from aggression.* ***Association with cortisol****. ↓, condition associated with downregulated HPA-axis function; → , condition not associated with changes in HPA-axis function; ↑, condition associated with upregulated HPA-axis function; *association with testosterone/cortisol ratio; **association with externalizing and internalizing behaviors; †females only; ‡experimental provocation unrelated to aggressiveness; $interaction dependent on alpha amylase activity;€pharmacological challenge.*

of antisocial and psychopathic traits was investigated in these studies. If HPA-axis deficits were associated with delinquency *per se* (as the rest of the studies suggest), the lack of an additional impact by such traits becomes explainable. The only finding that was discrepant indeed reported that violent delinquents show enhanced stress responses (Soderstrom et al., 2004). The discrepancy cannot be due to the way of HPA-axis evaluation as four other studies found decreased stress responses in delinquent populations; these were done either in violent or non-violent offenders (Moss et al., 1995; Popma et al., 2006; Couture et al., 2008; Johnson et al., 2015).

Taken together, conditions associated with aggressiveness or violence show a more reliable association with downregulated HPA-axis than generally believed (Klimes-Dougan et al., 2001; von der Pahlen, 2005; Marsman et al., 2008). The findings briefly reviewed above reveal that the negative association is neither restricted to extreme violence nor to callus-unemotional traits as previously suggested (Virkkunen, 1985; Hawes et al., 2009). Moreover, it may be valid for delinquents in general, irrespective to the violent nature of offenses committed. At the same time, however, there are a series of discrepant findings as well. Although these represent a rather small share of the studies, there are no obvious reasons to neglect them.

Discrepant Findings—Possible Explanations

One possible explanation to the discrepant findings may reside in the putative long-term association of glucocorticoid deficits and aggressiveness. Such findings were summarized in **Table 2.** One can hypothesize that the negative association between glucocorticoid production and aggressiveness may not be obvious in some cases because the behavioral change is subsequent to the decrease in glucocorticoid production, and the temporal difference between the two can be measured in years. This may at least partly explain discrepant findings in children. However, discrepant findings were reported at older ages too. Another explanation may reside in the type of aggression performed by subjects, which is considered rather rarely.

Aggression is often divided into two types: instrumental (proactive) and emotional (reactive). The former is non-impulsive and gain oriented, whereas the latter is a response to threat or provocation, is impulsive, and is not performed for gain (Feshbach, 1971; Blair, 2001; Kempes et al., 2005; Raine et al., 2006; van Honk et al., 2010). The two forms of aggression appear to have a differential association with cortisol. Instrumental (proactive) aggression appears associated with blunted, whereas emotional (reactive) aggression seems to be associated with upregulated HPA-axis function (McBurnett et al., 2003, 2005;

TABLE 4 | Studies in delinquent populations: associations between plasma cortisol and delinquency.

Condition	Cortisol measurement						Interaction	References
Delinquent type	Single	Multiple	Diurnal rhythm	Daily production*	Awakening response	Stress responses		
M/nV	X						↓*	Popma et al., 2007b
M/nV	X						↓**	Poustka et al., 2010
M/nV	X						↓	Dolan et al., 2001
M/nV	X						↓†	Horn et al., 2014
M/nV	X					X	↓‡	Couture et al., 2018
PP		X					↓*	Dabbs et al., 1991
PP		X					→	Feilhauer et al., 2013
ANTS			X				→ ⁂	Gostisha et al., 2014
M/nV			X				↓	Popma et al., 2007a
VIOL			X				↓	Brewer-Smyth et al., 2004
PP			X				↓	Cima et al., 2008
VIOL				X			↓	Virkkunen, 1985
VIOL					X		↓$	Holi et al., 2006
M/nV						X	↓€	Popma et al., 2006
VIOL						X	↓€	Moss et al., 1995
VIOL						X	↑€	Soderstrom et al. (2004)
M/nV						X	↓¥	Johnson et al., 2015
M/nV						X	↓‡	Couture et al., 2008

Delinquent type. *ANTS, delinquents with antisocial traits; M / nV, mixed delinquent population, committing mostly non-violent crime; PP, delinquents with psychopathic traits; VIOL, violent crime.* ***Cortisol measurements****. X, type of measurement performed; single, condition associated with a single cortisol measurement; multiple, condition associated with averaged multiple cortisol measurements; diurnal, condition associated with altered diurnal secretion rhythm; total, 24 h urinary cortisol or equivalent; awakening, diurnal increase in cortisol measured in the morning; stress response, stress response independent from aggression.* ***Association with cortisol****. ↓, delinquency associated with downregulated HPA-axis function; ↑, delinquency associated with upregulated HPA-axis function; *interaction with testosterone/cortisol ratio; **males only; † interactions mediated by personality and substance use disorders; ‡ interaction mediated by risk taking; ⁂, complex interactions with stress exposure; $ interaction with psychopathic features; € anticipation of stress (e.g., public speaking); ¥ interaction with the number of incarcerations.*

Kempes et al., 2005; van Bokhoven et al., 2005; Lopez-Duran et al., 2009; O'Neal et al., 2010; Poustka et al., 2010; van Honk et al., 2010; Geniole et al., 2011; Stoppelbein et al., 2014). Noteworthy, a similar dichotomy was found in animal models (Haller, 2016).

Evaluating the relationship between aggression and aggression-induced changes in cortisol production, as well as the relationship between reactive aggression and enhanced glucocorticoid production is outside the scope of this review, which aims at evaluating the role of the lateral hypothalamus in aggression performed under conditions of downregulated HPA-axis. However, studies where reactive and proactive forms of aggression were directly compared as it regards their glucocorticoid background strongly suggest that the two types of aggression substantially differ in this respect, and may explain discrepancies in the studies reviewed above.

Overall Evaluation of Human Findings

The studies briefly reviewed above show that on the long run, a large number of stressors elicits HPA-axis deficits in humans. These stressors coincide largely with those, which on the long run may also elicit a chronic enhancement of HPA-axis function. There are no explanations for such contrasting long-term trajectories of HPA-axis responses to stress, but disparate findings suggest that the severity of the "initial" stressor, as well as subsequent life events, together with individual vulnerabilities may have an important role. The long-term decrease in HPA-axis function may result from an "allostatic crash" i.e., from an allostatic load that overpasses the adaptive capacities of the HPA-axis. HPA-axis deficits develop slowly over time, and subjects often go through a phase of HPA-axis hyperfunction before the "final" glucocorticoid deficit develops, albeit this assumption requires further evidence.

Although initially suspected to be related to extreme aggression, a series of studies suggest that glucocorticoid deficiency is associated with a wide variety of aggressive behaviors. Overall, the findings suggest that the "prime suspects" are proactive (instrumental) forms of aggression. Psychopathic e.g., callous-unemotional traits strengthen the association. Glucocorticoid deficiency was repeatedly associated violent crime as well.

The available findings suggest that glucocorticoid deficiency precedes behavioral changes. The vast amount of evidence on the association of glucocorticoid deficiency with aggression as well as the precedence of hormonal over behavioral changes suggest a causal relationship. However, this assumption is based on indirect evidence only, and the nature of the phenomenon precludes the obtaining of direct evidence in humans. The issue requires animal experimentation.

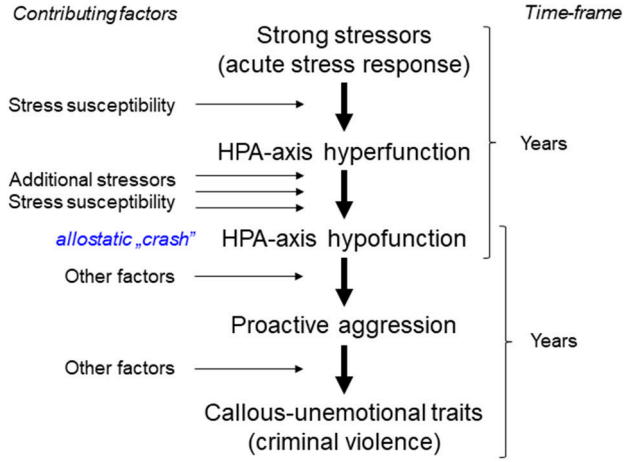

FIGURE 2 | Hypothetical pathway from strong stressors to violent aggression through HPA-axis hypofunction. The latter is attributed to an "allostatic crash" (see section Glucocorticoid Deficits and Aggression in Humans). "Other factors" were included to indicate that hormonal conditions are likely embedded in a wider array of biological, psychological, and social factors. The time frame is based on a number of longitudinal studies performed in late childhood—early adolescence.

THE ROLE OF THE LATERAL HYPOTHALAMUS

Findings in humans were tentatively summarized in **Figure 2**. In addition to factors that were based on the findings reviewed above, we added "other factors" to indicate that hormonal conditions are likely embedded in a wider array of biological, psychological, and social factors (Dodge et al., 1990; Loeber and Hay, 1997; van Honk et al., 2010).

Despite the clear involvement of the hypothalamus in the control of aggression, little effort was invested in understanding its role in humans. The available studies either focus on the "triangle of Sano" (a hypothalamic region, the destruction of which strongly inhibits aggressive behavior; Sano et al., 1970) or the technologies employed were unable to differentiate hypothalamic nuclei, and consequently considered it as a unit (Rosa et al., 2012; Van den Stock et al., 2015; Mutic et al., 2017). As the triangle of Sano includes parts of the lateral hypothalamus, but is not restricted to it, and the term "hypothalamus" is not sufficiently precise for the purposes of this study, we have to turn to animal models.

The Concept of Abnormal Aggression in Animals

Over the last decades, rodent abnormal aggression models have increasingly been used to mimic symptoms of aggression-related psychopathologies. Such models (1) mimic in rodents etiological factors of human aggression problems; (2) study the resulting aggressiveness by focusing on the form of aggression and its behavioral contexts, and (3) strive at investigating aggression-independent features (e.g., autonomic responses, anxiety, and social behaviors beyond aggressiveness) which are important concomitants of aggression-related psychopathologies (for reviews see Haller et al., 2004, 2014; Miczek et al., 2013).

The overall conclusion deriving from such studies is that the etiological factors of human aggression problems result in abnormal forms of aggression in rodents. Human etiological factors modeled so far to study their effects on animal aggression include but are not restricted to alcohol, early social isolation, frustration by the omission of scheduled reward, glucocorticoid deficiency, peripubertal social or physical stressors, repeated maternal separation, repeated treatment with cocaine, amphetamine, or anabolic steroids during adolescence, and selection for extremes in aggression and anxiety to mimic the genetic component of human aggression (Benus et al., 1991; Miczek et al., 1992; Melloni and Ferris, 1996; Delville et al., 1998; Haller et al., 2001; de Almeida and Miczek, 2002; Ricci et al., 2005; Veenema et al., 2006; Tóth et al., 2008; Gobrogge et al., 2009; Neumann et al., 2010; Márquez et al., 2013). All these etiological factors elicit abnormal manifestations of aggression in rodents, which are perceived as models for the symptoms of aggression-related psychopathologies. As an example, the glucocorticoid deficiency model changed the behavior and physiology of subjects in the following ways. (1) Attack targeting shifted from less vulnerable body parts (back and flanks) to more vulnerable ones (head, throat, belly, and occasionally paws and testicles); (2) The propensity of signaling attack intentions by social signals (threats) decreased; (3) Attacks were performed under conditions of low autonomic arousal; (4) The social behavior of subjects showed important deficits (beyond those related to aggression). This array of features mimics important aspects of human proactive (antisocial) aggressiveness (Haller et al., 2004; Haller, 2017).

Aggression Models Associated With Glucocorticoid Deficits

The glucocorticoid deficit model of abnormal aggression was purposefully developed to mimic the human condition discussed in this review (see above). In addition to this model, there are several other abnormal aggression models, where the induction of glucocorticoid deficits was not an aim, but deficient HPA-axis function was observed later on. This was observed in the early subjugation model of aggression (Ferris, 2003), in mouse lines selected for aggressiveness, particularly the short attack latency mice (Veenema et al., 2004; Natarajan and Caramaschi, 2010), and rats selected for extremes in anxiety (Neumann et al., 2010). In the pubertal stress model, corticosterone secretion was not decreased overall, but was decreased relative to testosterone secretion (Márquez et al., 2013).

Maternal aggression is also associated with low glucocorticoid stress responses (Neumann, 2001; Neumann et al., 2001); moreover, acute stressors suffered before the aggressive encounter and increased stress responsiveness (as an individual feature) decrease maternal aggression (Gammie et al., 2005; Gammie and Stevenson, 2006). Albeit maternal aggression cannot be considered abnormal, it shares several features with the glucocorticoid deficit model of abnormal aggression. When protecting their pups, dams attack vulnerable body part of their

opponents, and fail to signal their attack intentions by social signals (Parmigiani et al., 1988). Finally, predatory aggression is also performed under low arousal, and consist of lethal attacks on vulnerable body parts (Siegel et al., 1999).

The studies briefly reviewed above suggest that in certain abnormal aggression models as well as in two models of natural aggressiveness (maternal and predatory aggressions) violent forms of aggression are associated with decreased HPA-axis function.

Violent Aggression and the Lateral Hypothalamus

Where the lateral hypothalamus was investigated, glucocorticoid deficit-associated violent aggression was also associated with a marked activation of this hypothalamic region (*glucocortioid deficit model*: Tulogdi et al., 2010; *mouse lines selected for aggressiveness*: Haller et al., 2006; *rats selected for extremes in anxiety*: Beiderbeck et al., 2012; *predatory aggression*: Tulogdi et al., 2015; *maternal aggression*: Hasen and Gammie, 2006). In these models, the mediobasal hypothalamus was also activated, with the exception of the predatory aggression model. A detailed analysis of activation patterns observed in aggression models associated with glucocorticoid deficit and those associated with normal or enhanced glucocorticoid responses revealed three different brain activation patterns (Haller, 2014, 2017). The overall conclusion of these studies was that: (1) regularly performed resident-intruder test activate the medial amygdala-mediobasal hypothalamus-dorsal periaqueductal gray pathway. (2) In abnormal aggression models associated with *increased* glucocorticoid stress responses, the same pathway was activated, but the medial amygdala and mediobasal hypothalamus together with certain areas of the prefrontal cortex and the basolateral amygdala showed increased activations. (3) Abnormal aggression models associated with *decreased* glucocorticoid stress responses activate the same pathway but in addition they also activate the central amygdala-lateral hypothalamus-ventral periaqueductal gray pathway. Finally (4) predatory aggression activates exclusively the central amygdala-lateral hypothalamus-ventral periaqueductal gray pathway (**Figure 1**).

Naturally, correlations between behavioral, endocrine and brain activation patterns are indicative of, but not proofs of causal relationships. Recent studies, however, do suggest such causal relationships. These studies were prompted by the finding that hypothalamic areas involved in aggression control receive direct inputs from the prefrontal cortex, a brain area tightly involved in aggression control (Sesack et al., 1989; Siegel et al., 2007; Toth et al., 2010). Blair (2001) attributed a large role of such direct prefrontal cortex–hypothalamus connections in his model of human reactive aggression, albeit subsequent studies by the same author suggest that the prefrontal cortex–hypothalamus link is not direct but is mediated by the amygdala (Blair, 2012, 2016). Recent rodent findings, however, appear to re-establish the notion that the prefrontal cortex controls aggression by direct effects on the hypothalamus, a mechanism that may complements the more established prefrontal cortex–amygdala–hypothalamus pathway (Biro et al., 2018). We established in a recent study that the hypothalamus is directly (monosynaptically) innervated by the prefrontal cortex; moreover, the mediobasal and lateral hypothalamus received inputs from dominantly distinct prefrontal neuron populations. These neurons were located in the prelimbic and infralimbic region of the medial prefrontal cortex. Prefrontal projecting neurons were dispersed in the layers III–V of the infralimbic and prelimbic cortices, and their dense axon terminals at hypothalamic sites exclusively contained vesicular glutamate transporter 1, i.e., these neurons were glutamatergic. The optogenetic stimulation of mPFC terminals in the MBH distinctively increased bite counts in resident/intruder conflicts, whereas stimulation of similar terminals in LH specifically resulted in violent bites. These were aimed at vulnerable targets of opponents (head, throat and belly, occasionally paws), and/or were not preceded by social signals (threats). No other behavior was affected, suggesting that dedicated prefrontal neurons control particular aspects of aggressiveness via the hypothalamus in a highly selective manner. Besides shedding light on the way in which the prefrontal cortex controls aggressive behavior (e.g., by highly dedicated subcortical efferents), these findings are to our knowledge the first proof of a causal involvement of the lateral hypothalamus in intraspecific aggression. These findings are in agreement with the role of this brain area in violent aggression, which was assumed earlier based on brain activation patterns, but surprisingly show that subpopulations of prefrontal neurons may increase aggressiveness; moreover, may make it more violent. In principle, this finding is at variance with the prefrontal deficit theory of aggression (Blair, 2001; Siegel et al., 2007). The role of the prefrontal cortex in aggression control is outside the scope of this review, therefore this aspect of the findings will not be discussed here in detail. We showed earlier, however, that aggressiveness is associated with both chronic prefrontal deficits and increased acute aggression-induced prefrontal activation in abnormal aggression models (Biro et al., 2017), and repeatedly argued elsewhere, that the involvement of the prefrontal cortex in aggression control should be revised (Haller, 2014, 2017). Albeit chronic prefrontal deficits increase the likelihood and severity of aggressive behavior, this brain area is activated by aggressive behavior acutely; moreover, activation is stronger in models of abnormal aggression and in subjects with aggression-related psychopathologies. Based on these findings, one can hypothesize that HPA-axis hypofunction affects the aggression circuitry at least partly at the level of the prefrontal cortex.

THE IMPACT OF GLUCOCORTICOID DEFICITS ON AGGRESSION: PUTATIVE MECHANISMS

The basic question raised by the findings reviewed above is how the mechanisms controlling aggression are shifted from the ones that control rivalry aggression to the ones that control predatory aggression under normal circumstances. Low HPA-axis activity after allostatic overload or experimental manipulations is not likely to cause behavioral maladaptation by itself. Glucocorticoids affect the properties of neurons rather than elicit membrane

depolarizations or activate brain circuitries. Consequently, the basic question is how the absence of well-timed, adaptive adrenocortical response in the face of a social challenge or emergency alters the properties of aggression-controlling networks.

Some time ago we speculated that the dynamics of the endocrine responses accompanying social challenges and aggression,—most notably the dynamics of the HPA-axis—, would rapidly affect the way animals cope with actual and future conflicts. We also suggested that studying the underlying mechanisms at the level of the hypothalamus and its interactions with frontal systems could possibly contribute to our understanding of inadequate, maladaptive conflict behavior in humans (Kruk et al., 1998). We also suggested that the hypothalamus serves as a crucial link between frontal areas involved in memory, the appraisal of the environments the recognition of conspecifics and the execution of specific aggressive responses. Such frontal areas, as we suggested at that time could either facilitate or inhibit hypothalamic aggression in rats (Kruk et al., 1998). Since then animal studies have provided strong support for these ideas reviewed e.g., in Kruk (2014). The dynamics of the adrenocortical response facilitates hypothalamic,- as well as territorial aggression within the time frame of one single conflict (Kruk et al., 2004; Mikics et al., 2004) by an initially non-genomic rapid mechanism. In the absence of such a response inadequate conflict handling takes over. Rapid adrenocortical signaling via the mineralocorticoid receptor also enables the first emergence of an adaptive aggressive response in a novel environment (Kruk et al., 2013). More recent studies clearly suggested the involvement of structures "up-stream" from the hypothalamus, such as the amygdala and the frontal cortex, in such behavioral effects of the adrenocortical response (Biro et al., 2017, 2018; Mikics et al., 2018).

These experimental studies suggest that the precise timing of the adrenocortical response with respect to the timing of a social challenge determines the adaptive nature of the aggressive response. Therefore, it stands to reason to predict that the absence of a well-timed adrenocortical response as a consequence of repeated severe stressors and the ensuing allostatic crash, could also be a major factor in inadequate conflict handling in humans. Recent results from animal experiments summarized here, suggest that the behavioral changes accompanying an allostatic crash in humans could be due to a reorganization of hypothalamic connections with "upstream," modulating circuits, resulting in a change from affective social conflict handling toward a predatory like responding in conflicts.

Albeit acute deficits in the regulatory roles of glucocorticoids may have effects *per se* via the so-called non-genomic effects (Haller et al., 2008), the consequences of HPA-axis downregulation for aggression appear to develop slowly. This renders slow glucocorticoid actions better candidates for mediating the behavioral effects of glucocorticoid deficits, even if such slow alterations interact with rapid effects (Joëls et al., 2013).

A typical example of slow glucocorticoid effects are the ones that are mediated by epigenetic mechanisms. For example, early adversity was shown to durably alter the epigenetic regulation of glucocorticoid signaling genes and to induce DNA methylation in various brain areas (Lutz and Turecki, 2014; Tyrka et al., 2016). Importantly, glucocorticoids control gene expression in the prefrontal cortex, the brain site that shapes behavioral strategies during social conflict (Costin et al., 2013; Biro et al., 2018). The assumption that epigenetic changes in the prefrontal cortex elicit abnormal aggression is supported by recent findings showing that such behaviors largely depend on prefrontal neuronal plasticity, particularly plasticity in the prelimbic and infralimbic cortex (Mikics et al., 2018).

In line with these findings, it was recently shown that there is a complex interplay between early stressors, plasma glucocorticoid levels, and decision-making at the level of the prefrontal cortex (Fan et al., 2014; Quevedo et al., 2017; Joëls et al., 2018). A key component of this interaction is HPA-axis functioning in adulthood, that depends to a large extent on early adversities (Raymond et al., 2017; Kaiser et al., 2018). Albeit the issue clearly needs further studies, findings obtained so far suggest that the link between early adversity, adult glucocorticoid deficits and abnormal aggression is established by epigenetic changes, and one of the major loci of these processes is the prefrontal cortex.

CONCLUSIONS AND HYPOTHESIS

Ample human evidence suggests that strong stressors downregulate the HPA-axis on the long run. Stressors that have this outcome largely coincide with those that have an opposite effect i.e., which upregulate the HPA-axis on the long run. The factors that underlie these opposite trajectories of HPA-axis responses are largely unknown at present, but disparate findings suggest that individual differences in stress responsiveness, and exposure to subsequent stressors may play an important role. The stress-induced decrease in glucocorticoid secretion capacities develop slowly. Low HPA-axis function on its turn was consistently associated with increases in aggressiveness and aggressive delinquency in humans. Findings suggest that the association is valid primarily for proactive, antisocial aggressiveness. Longitudinal studies indicate that the decrease in glucocorticoid secretion precedes the increase in aggressiveness by years, raising the possibility of a causal relationship. These human findings demonstrate the relevance of the relationship between glucocorticoid hypofunction and aggressiveness.

Hypothalamic mechanisms possibly underlying this relationship are difficult to study in humans, for which such mechanisms need to be investigated in animal models.

In rodents, the deliberate limitation of glucocorticoid secretion as well as conditions that on the long run decrease HPA-axis function result in increased aggression. This was observed in models of abnormal aggression, i.e., models that mimic the etiological factors of pathologic human aggression and where aggressive behavior strongly deviates from species-typical patterns. Recent findings suggest that an alternate neural route of aggression control mediates the effects of glucocorticoid deficits on aggression. Particularly, neural mechanisms of predatory aggression are activated in conditions associated with glucocorticoid deficits. Within this alternate mechanism, the

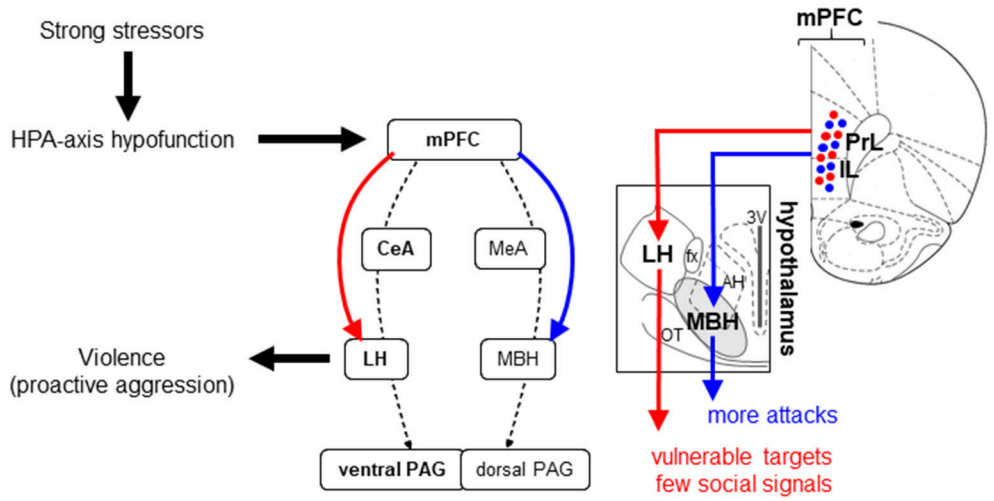

FIGURE 3 | Brain mechanisms that mediate the effects of glucocorticoid deficits on aggression. Dashed arrows indicate hypothetical information flow based on earlier studies. Continuous arrows indicate the pathways discovered by Biro et al. (2018). The left hand panel is a simplified version of **Figure 2**. The arrows to the right and then to the left indicate the likely neural mechanism that link the hormonal to the behavioral event. The middle panel shows the "mixed" mechanism of glucocorticoid hypofunction-induced aggression complemented with two pathways that directly connect the medial prefrontal cortex to hypothalamic centers of aggression. The right hand panel shows on Paxinos and Watson slides (Paxinos and Watson, 1998) two subpopulations of medial prefrontal neurons, which project either to the lateral or mediobasal hypothalamus. The latter is an electrophysiologically defined area of the hypothalamus that covers several hypothalamic nuclei (Kruk, 1991). The optogenetic stimulation of axon terminals in the lateral hypothalamus increases the share of attacks on vulnerable targets that are poorly signaled socially. When stimulations are aimed at the mediobasal hypothalamus, the number of bites increases. Both effects are highly selective behaviorally (Biro et al., 2018). 3V, the third ventricle; AH, anterior hypothalamic nucleus; fx, fornix; IL, infralimbic cortex; OT, optic tract; PrL, prelimbic cortex. For other abbreviations, see **Figure 1**.

lateral hypothalamus, especially the prefrontal cortex-lateral hypothalamic pathway seems to play an important role.

By corroborating human and animal findings, we hypothesize the following (**Figure 3**):

(1) Strong stressors associated with subsequent stress exposure and high individual stress sensitivity decrease the glucocorticoid secretion capacity of the HPA-axis by a slowly developing process ("allostatic crash.")
(2) HPA-axis hypofunction alters the function of the aggression circuitry. One of the primary targets of this change is the prefrontal cortex. This process also develops slowly.
(3) The ultimate consequence of brain alterations is that social challenges elicit the activation of brain mechanisms that originally control predatory aggression. This results in violent, harmful aggression.
(4) The lateral hypothalamus plays a key role in eliciting violent forms of aggression. When the aggression circuitry is altered by glucocorticoid hypofunction, this brain region is activated by the prefrontal cortex and possibly by the central amygdala upon social challenge, and its activation leads to aggression patterns that are substantially more damaging than species-typical ones.

Ultimately, this hypothetical sequence of events explains how unfavorable (stressful) social conditions lead to endocrine and later to neural alterations that result in proneness to violence, which on its turn may restart the cycle of violence by creating social tensions that elicit hyporesponsive HPA-axes in others.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

FUNDING

This study was supported by NKFI grant No. 112907, and the KÖFOP-2.1.2-VEKOP-15-2016-00001 grant.

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Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Systems Neuroscience

METHODS
published: 01 May 2018
doi: 10.3389/fnsys.2018.00007

Computer Vision Evidence Supporting Craniometric Alignment of Rat Brain Atlases to Streamline Expert-Guided, First-Order Migration of Hypothalamic Spatial Datasets Related to Behavioral Control

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The rat has arguably the most widely studied brain among all animals, with numerous reference atlases for rat brain having been published since 1946. For example, many neuroscientists have used the atlases of Paxinos and Watson (*PW*, first published in 1982) or Swanson (*S*, first published in 1992) as guides to probe or map specific rat brain structures and their connections. Despite nearly three decades of contemporaneous publication, no independent attempt has been made to establish a basic framework that allows data mapped in *PW* to be placed in register with *S*, or vice versa. Such data migration would allow scientists to accurately contextualize neuroanatomical data mapped exclusively in only one atlas with data mapped in the other. Here, we provide a tool that allows levels from any of the seven published editions of atlases comprising three distinct *PW* reference spaces to be aligned to atlas levels from any of the four published editions representing *S* reference space. This alignment is based on registration of the anteroposterior stereotaxic coordinate (*z*) measured from the skull landmark, Bregma (β). Atlas level alignments performed along the *z* axis using one-dimensional Cleveland dot plots were in general agreement with alignments obtained independently using a custom-made computer vision application that utilized the scale-invariant feature transform (SIFT) and Random Sample Consensus (RANSAC) operation to compare regions of interest in photomicrographs of Nissl-stained tissue sections from the *PW* and *S* reference spaces. We show that *z*-aligned point source data (unpublished hypothalamic microinjection sites) can be migrated from *PW* to *S* space to a first-order approximation in the mediolateral and dorsoventral dimensions using anisotropic scaling of the vector-formatted atlas templates, together with expert-guided relocation of obvious outliers in the migrated datasets. The migrated data can be contextualized with other datasets mapped in *S* space, including neuronal cell bodies, axons, and chemoarchitecture; to

OPEN ACCESS

Edited by:
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Reviewed by:
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Received: 08 December 2017
Accepted: 07 March 2018
Published: 01 May 2018

Citation:
Khan AM, Perez JG, Wells CE and Fuentes O (2018) Computer Vision Evidence Supporting Craniometric Alignment of Rat Brain Atlases to Streamline Expert-Guided, First-Order Migration of Hypothalamic Spatial Datasets Related to Behavioral Control. Front. Syst. Neurosci. 12:7. doi: 10.3389/fnsys.2018.00007

generate data-constrained hypotheses difficult to formulate otherwise. The alignment strategies provided in this study constitute a basic starting point for first-order, user-guided data migration between *PW* and *S* reference spaces along three dimensions that is potentially extensible to other spatial reference systems for the rat brain.

Keywords: stereotaxic, stereotactic, atlas, data migration, registration, computer vision, subject matter expert, behavioral control

INTRODUCTION

Following the 1930s, when the design for the original Horsley-Clarke stereotaxic instrument (Horsley and Clarke, 1908) underwent modifications (Ranson and Ingram, 1931; Harrison, 1938) and was later diversified for performing intracranial surgery in the laboratory rat (Clark, 1939; Beattie, 1952; Stellar and Krause, 1954; Greer et al., 1955; Andreas and Legler, 1969; Krieg, 1975; also see Hillarp, 1947 for an alternate technology), several investigators published various stereotaxic coordinate systems to aid in the precise manipulation of small brain structures in this animal model, beginning with Krieg's atlas of 1946 (Krieg, 1946) (see Table 4 in Khan, 2013). Such manipulations have included ablation or stimulation of brain structures (Sheer, 1961; Myers, 1974; Thompson, 1978), tissue microdissection for biochemical analyses (Palkovits and Brownstein, 1988), chemical sampling of brain extracellular space via microdialysis or electrochemistry (Parada et al., 1998; also see Carter and Shieh, 2015), delineation of neural circuits using tracers (Heimer and Robards, 1981; Zaborszky and Heimer, 1989; Zaborszky et al., 2006), or molecular neurobiological techniques involving antisense, RNA interference, or viral-based vector delivery of various constructs to activate or silence activity in a cell-specific manner (Khan, 2013). More recently, such manipulations have also included optogenetic studies in rats (e.g., Gradinaru et al., 2009; Witten et al., 2011), including studies involving *in vivo* stimulation of hypothalamic cell bodies, their axonal projections, or their axonal inputs (Larson et al., 2015; Gigante et al., 2016), a structure that we also focus on in this study. Stereotaxic-based methods to manipulate brain structures to control behavior in the rat have contributed richly to our collective understanding of structure-function relations in the brain.

However, an inevitable outcome from these efforts—which collectively now span over seven decades of research using rat brain stereotaxic atlases—has been that anatomical data have been mapped within several different stereotaxic coordinate systems, hampering our abilities to interrelate formally the hard-earned and valuable results published in numerous studies. For example, the locations of injection sites published by a laboratory using a particular stereotaxic rat brain atlas may be difficult to place in register with corresponding locations, *within the same physical space*, of neuronal populations that might lie underneath such injections, but which have been mapped by another laboratory using a different stereotaxic atlas. This is because of several variables that will differ between such atlas reference spaces: plane of section, intervals between sections, originations of various "zero" points for Cartesian coordinates calibrated to landmarks on the skull surface, and strains and body weights of the animals used to create the atlases (Kruger et al., 1995; Khan, 2013). Indeed, the idea of "interoperability" between different software and hardware systems in computer science is now being extended to describe similar needs for anatomical reference frameworks of the brain (Zaslavsky et al., 2010; Hawrylycz et al., 2011), which have also been represented digitally in three-dimensional space (Toga et al., 1989, 1995; Timsari et al., 2001; Hjornevik et al., 2007).

The problem of poor interoperability is compounded further by the progression of time. Older editions of brain atlases fall out of fashion, go out of print, or are supplanted by more popular coordinate systems of other atlases, or by newer editions of the same atlas. Take, for instance, a laboratory that published critical data about a neural system two decades ago, using what were then state-of-the-art techniques to map their anatomical data to what was then a current edition of a specific rat brain stereotaxic atlas. Today, data from that study may no longer be so useful to laboratories that routinely use a different atlas reference space and entirely different coordinates based on a radically different plane of section. Thus, the high quality data from this 20 year-old study are now "trapped" within an old reference space, effectively sealed by coded locks that no longer have appropriately registered keys. The consequence of this is that if no other laboratory has taken up the same problem, those trapped data continue to represent all that is known about that particular structure-function relation in the brain, but our abilities to interpret that information continue to decrease with time. A related consequence is that current investigators may have to repeat the same experiment because they cannot contextualize such data with their own observations. These issues are similar to those envisioned over 75 years ago (Asimov, 1942), and also discussed in relation to the "Digital Dark Age(s)," in which older information may not be obsolete, but simply locked or uninterpretable, similar to software or hardware that no longer is accessible due to modernization of digital standards (Sanders, 1997; Rosenzweig, 2003; Lima, 2011; also see Lepore, 2015). The locked data may still be useful and relevant if there was a living key. Also, even if neuroanatomical data from a study are not yet "trapped," migrating or registering them to additional anatomical reference spaces ensures their continued widespread use, lasting preservation, and broader contextualization with other (both older and newer) datasets [see, for example, the GitHub methods package release (https://github.com/RittmanResearch/maybrain) from Whitaker et al. (2016) to contextualize human brain MRI data with human brain gene expression data collected by the Allen Institute for Brain Sciences]. If supported by a durable and upgradable infrastructure, an extant anatomical reference space

can serve as a stable repository and unified model for all spatial information concerning the brain of a particular species.

A viable solution to ensure that neuroanatomical datasets remain within living reference spaces is to make them as widely available as possible, a task that can be achieved in part by migrating the data to more than one living reference space. This strategy also affords scientists the ability to contextualize the migrated spatial dataset with unique resident datasets already mapped within the host atlas. Such migration could serve as a powerful means for investigators to formulate new hypotheses about diverse spatial datasets that they discover for the first time to be co-registered to the same region of the brain, a discovery process akin to the classic, albeit now mythologized, discoveries of the spatial relationships among cholera deaths, sewer access points and city water pumps by Edmund Cooper and John Snow using co-registered spatial datasets (Brody et al., 2000). As one of us has argued before (see section 4.6.2.2ff of Khan, 2013), co-registering datasets from experimental neuroscience studies, for example, to the same reference space allows investigators to formulate new ideas concerning the relationships between an experimental manipulation in the brain, the underlying neural substrates being manipulated, and the behavioral or physiological outcomes of such manipulation. Additionally, since the prevalence of numerous reference atlases stems partly from the need for atlas-makers to furnish their own interpretations about how brain structures are organized and parcellated, migration of a spatial dataset into a new host atlas allows investigators to place their results within the unique universe of discourse (Boole, 1854) of the host atlas creator, which could lead to new theoretical and/or empirical determinations of how the dataset contributes to our understanding of brain structure and function.

In this study, portions of which have been presented in preliminary form (Khan, 2013; Wells and Khan, 2013; Hernandez and Khan, 2016; Perez et al., 2017; Wells, 2017), we sought to fulfill three objectives: (1) to establish a basic alignment of 11 rat brain atlases based on their shared set of anteroposterior (AP) stereotaxic coordinates derived from the Bregma landmark on the skull surface; (2) to develop and implement a novel computer vision algorithm to independently provide evidence—from internal landmarks in the brain—about the usefulness of the basic AP stereotaxic alignment; (3) to migrate unpublished spatial datasets related to behavioral control experiments involving the hypothalamus, and in the process, determine whether expert-guided mapping would be required to migrate data from one atlas space to another in the mediolateral and dorsoventral dimensions.

MATERIALS AND METHODS

Creation of an Anteroposterior Alignment Tool

Data Entry and Sorting

Coordinates based on the distance from the skull landmark, Bregma (β), in mm (hereafter designated as "β coordinate," or z) listed for each of the 312 unique atlas levels from all editions of *PW* and *S* were entered manually into a spreadsheet (Microsoft Excel for Mac 2011, version 14.2.3; Microsoft Corp., Redman, WA). The numerical sequences of atlas levels for *PW* atlas editions fell within three separate groups: (1) a "1982/86/97" group (*PW1*) that is derived from the same tissue set and has identical atlas level assignments (Paxinos and Watson, 1982, 1986, 1997); (2) a "1998" group (*PW2*; Paxinos and Watson, 1998) that is derived from the same tissue set as *PW1*, but is assigned as a separate group because it includes from that tissue set two previously unpublished tissue sections and associated atlas drawings; thereby altering the numerical sequence of the atlas levels; and (3) a "2005/2007/2014" group (*PW3*) that is based on a tissue set with drawings and atlas levels completely distinct from *PW1* and *PW2* (Paxinos and Watson, 2005, 2007, 2014). These three *PW* groups were organized into separate columns, alongside a column containing *S* atlas levels (these are identical for all four editions and based on the same brain: Swanson, 1992, 1998, 2004, 2018), and a column of z values pooled from all 11 atlases (*PW*'82,'86,'97,'98,'05,'07,'14; *S*'92,'98,'04,'18). All atlas levels sharing the same coordinates were assigned to a common row within the spreadsheet.

Construction of Dot Plots

All atlas levels were to be calibrated to the same scale (z), and required separate plotting along this scale by atlas group. Thus, these levels needed to be plotted within one-dimensional rather than two- or three-dimensional (Cartesian) space (i.e., there are no x- or y-axis values for this dataset). For this purpose, two sets of resources were very helpful. First, the guidelines offered by Wilkinson (2005) and Carr and Pickle (2010) for the representation of one-dimensional data, along with the seminal papers of Cleveland (1984) and Cleveland and McGill (1984) on graphical perception theory, prompted the decision to represent the data as a set of Cleveland dot plots. Second, the online guidelines provided by O'Day for plotting climate change data (see O'Day, 2011) were very helpful to transform the raw data in Excel to such plots. Specifically, a dummy y-axis was created to numerically rank order distinct atlas level groups (see above) and then plot all atlas levels from these groups along a z-axis scale of β coordinate values. The arbitrary y-axis rankings were assigned specific atlas group labels to sort them, and the resulting graph of dot plots was re-drawn for publication using Adobe Illustrator Creative Suite 4 (Adobe Systems, Inc., San Jose, CA). Individual dots falling along the z scale in *PW* reference space (z_{PW}) were color-coded on the basis of their proximity to dots in *S* space along the same scale (z_S). Specifically, those levels where $z_{PW} = z_S$ (and technically, where $z_S = z_{PW}$) were coded as "*Fully in Register*"; those where $z_{PW} - z_S \leq 50$ μm (or $z_S - z_{PW} \leq 50$ μm) were coded as "*Narrowly in Register*"; and those where $z_{PW} - z_S > 50$ μm (or $z_S - z_{PW} > 50$ μm) were coded as "*Not in Register.*"

Creation and Implementation of a Computer Vision Algorithm to Compare Atlas Levels

To begin efforts toward automating the process of pairing *PW* and *S* atlas levels, we developed an algorithm to compare images of the Nissl-stained tissue accompanying the atlas levels and rank matches based on a similarity metric. For each image under analysis, the algorithm builds a descriptor by finding a set of

local features that are invariant to changes in scale, illumination, and orientation, and partially invariant to geometric distortion. Given two images, their similarity is estimated by determining the number of local features that they have in common, subject to geometric constraints. Image descriptors are computed using the Scale Invariant Feature Transform (SIFT) (Lowe, 1999, 2004) while feature matching under geometric constraints is attained by applying the Random Sample Consensus (RANSAC) algorithm (Fischler and Bolles, 1981).

SIFT Algorithm

After selecting a region of interest (ROI) from a given image, its features are computed and encoded using SIFT. The SIFT algorithm includes both a detector—which selects points of interest by finding high-contrast points that are maxima or minima of the difference of Gaussians in scale space for the ROI—and a descriptor, which encodes the selected points as a 128-dimensional feature vector describing the frequency distribution of the gradient orientations in a circular region surrounding the point of interest. Rotation invariance is attained by measuring all gradients with respect to the region's dominant orientation.

Matching

For every feature vector u in the descriptor of the ROI, we find the two most similar feature vectors v and w in the descriptor of the target image, according to their Euclidean distance $|u - v|$. If $|u - v|$ is smaller than a predefined threshold, and the ratio $|u - v|/|u - w|$ is less than 0.8, u and v are considered a match.

RANSAC

Once a set of matches between the ROI and an image is obtained, we find the largest subset of matches that are geometrically consistent. A set of matches $M = \{(p_1, q_1), (p_2, q_2),...,(p_n, q_n)\}$, where $p_1,..., p_n$ and $q_1,...,q_n$ are points of interest in the ROI and the target image, respectively, is geometrically consistent if there is an affine transformation or homography H such that $H(p_i) = q_i$, for $1 \leq i \leq$ n. To find the largest set of matches we use the RANSAC algorithm. RANSAC is a randomized iterative procedure that consists of the following steps: first, we randomly select from the set of matches the minimum number of matches required to compute a homography, which is four in this case. Then we compute the corresponding homography H and, for each match (p_i, q_i) in the set, we measure the reprojection error $|H(p_i) - q_i|$. If the error is less than 10 pixels, we consider the match correct and the point is labeled as an inlier, otherwise it is labeled as an outlier. This process is repeated for 2,000 iterations; at the end the homography with the largest number of inliers is retained and its corresponding number of inliers or geometrically consistent matches is considered the metric of similarity between the ROI and the candidate image.

The whole process of feature extraction, matching, and homography search is repeated for every candidate image and the output is a list of images sorted by similarity to the ROI. The pseudocode in **Figure 1** illustrates the complete process. The program was written in Python using OpenCV3 (Open Source Computer Vision Library; opencv.org), PyQt5, Scikit-Learn, SciPy, and NumPy; and is available for download at http://www.github.com/DeveloperJose/Vision-Rat-Brain.

Experiments to Test Algorithm

To test the computer vision algorithm, three experiments were conducted. *Experiment 1a*, which was essentially a proof of concept, was designed to task the algorithm to determine the Nissl image of origin within the Swanson atlas from where a test region of interest (ROI) was extracted. To implement this, a region of interest was extracted from Level 34 of *S* space, rotated 155 degrees, and distorted slightly using random point warping. It was then used to test the algorithm's ability to identify it as being part of Level 34's Nissl plate. Additionally, comparisons were performed to test the overall matching output before and after the RANSAC module of the algorithm was applied. In *Experiment 1b*, a comparison test was performed to determine the algorithm's ability to recognize the source of an undistorted test ROI from L34 as originating from the Nissl photomicrograph of L34 as opposed to a photomicrograph from a different level of the *S* atlas. In *Experiment 2*, the ability of the algorithm to recognize the appropriately matching plate from *S* space, which corresponds to a test ROI extracted from a Nissl image from *PW* space, was evaluated. For *Experiments 1b and 2*, the number of SIFT matches and RANSAC inliers was computed by the algorithm and the results tabulated in rank order with the highest ranking match being the solution associated with the highest number of SIFT matches and RANSAC inliers.

Data Transformation

Transformation of Unpublished Experimental Data

Since the injection site locations for experiments from a published behavioral study for the hypothalamus (Khan et al., 2004) had not been included in that publication, we decided to re-visit this dataset and use it to test our data transformation and migration methods and, in the process, place some of these sites in the published record within an atlas reference space. The goal of this exercise was to illustrate how an unpublished dataset could be migrated into an atlas reference space years after it had been generated. Below, each step is described in detail to aid readers in their own attempts to update and unlock older datasets. For the process described here, the data to be migrated were originally mapped into a reference space for which digital formats did not exist, requiring first a series of transformations to migrate them to vector-formatted space.

Mapping injection sites in PW space (histological to graphical transformation)

In the behavioral experiments reported by Khan et al. (2004), adult male Sprague-Dawley rats (350–500 g BW) received stereotaxic implantations of chronic indwelling stainless steel guide cannulas targeting the LHA. The stereotaxic coordinates were: +6.1–6.4 mm anterior to the interaural line, +1.8 mm lateral to the midsagittal sinus, and –8.2 mm ventral to the skull surface; with the incisor bar set at −3.3 mm (Paxinos and Watson, 1986). The injection sites were originally preserved in tissue and mapped as follows. Tissue was prepared by

Algorithm 1 Image Matching Using SIFT and RANSAC

```
function FindMatchingPlate(R, A)        ▷ Where R is a region and A = {P_1, ...P_n} is an Atlas
    Compute SIFT descriptors S(P_1), ..., S(P_n)        ▷ They can be precomputed before matching
    Compute SIFT descriptor S(R)
    max_A = 0
    for i = 1 to n do                                   ▷ Find matches in plate P_i
        for every point p ∈ S(R) find matching point q ∈ S(P_i) if it exists
        Let M = {⟨p_1, q_1⟩, ..., ⟨p_m, q_m⟩} be the set of matches
        max_p = 0
        for j = 1 to r do                               ▷ where r is the number of RANSAC iterations
            Let {⟨p_m1, q_m1⟩, ..., ⟨p_m4, q_m4⟩} be 4 randomly selected from M
            Compute H such that Hp_mi = q_mi for i ∈ {1, ..., 4}
            m = 0
            for k = 1 to |M| do
                if |Hp_i − q_i| < ε then                ▷ Counting matches for iteration j
                    m = m + 1
                end if
            end for
            if m > max_p then
                max_p = m
                H_p = H                                 ▷ Where H_p is the best homography for plate P_i
            end if
        end for
        if max_A > max_p then
            max_A = max_p
            H_A = H_p                                   ▷ Where H_A is the best homography for the atlas
            match = i
        end if
    end for
    return match, H_A        ▷ Returns index of best matching plate and corresponding homography
end function
```

FIGURE 1 | Pseudocode delineating the operations of the custom-made algorithm developed for this study, based on SIFT and RANSAC operations.

transcardially perfusing each subject with 10% formalin. After removal from the skull, brains were stored in 10% formalin at room temperature until sectioned, at which time they were blocked and frozen in powdered dry ice. The portion of the hypothalamus containing the injection site was cut into 100 μm-thick sections on the freezing stage of a Reichert sliding microtome. Sections were collected through the full extent of the injector needle track and injection site. Sections were mounted onto glass slides, air dried, stained with thionin, dehydrated in an ascending series of ethanol concentrations, cleared in xylene, and coverslipped using Permount or DPX. Selected slides containing thionin-stained sections with regions of interest were each mounted onto the stage of a Bausch & Lomb Microprojector™ projection microscope (Bausch & Lomb, Inc., Rochester, NY). The projected image of the tissue section containing the injection site was traced onto a size-adjusted, cropped, paper photocopy of the relevant figure from the second edition of the Paxinos and Watson rat brain atlas (Paxinos and Watson, 1986; *PW86*). The selection of each figure was determined by visual comparison of the Nissl-stained regions in the tissue with those found in the atlas photomicrographic plate accompanying each figure.

Graphical to digital transformation

Twelve to fifteen years after they were originally drawn, a total of 183 of the manually produced tracings on their selected *PW86* maps were bulk-scanned as digital images and imported into a vector graphics editor [Adobe Illustrator (AI) CS5, Adobe Systems Inc., San Jose, CA]. Each digital image was a composite scan that included (a) the traced outline of the injection site, and (b) the underlying map from *PW86*. Composite scans of injection site cases were imported into a common .ai file if the map onto which they were traced was at the identical anteroposterior (AP) stereotaxic coordinate (z). For example, if the composite scan was obtained for an injection site traced onto a photocopy of Figure 25 of *PW86* ($z = -1.80$ mm), it was imported into an .ai file along with other such composite scans for $z = -1.80$ mm. In the file, each scan resided on a separate transparent layer, and each .ai file therefore contained a set of injection sites that had been localized in the AP axis to the same atlas level.

Raster to vector transformation

The next step in the transformation of our data was to separate the injection site tracings from the underlying *PW*

atlas plates onto which they were traced, which required that the separate components within each composite digital scan (tracings, underlying maps) were rendered into vector objects. The first and second editions of the Paxinos and Watson atlas (*PW82*, *PW86*) do not include electronic versions of the atlas plates, but the third edition (*PW98*) does. Since *PW86* and *PW98* reference spaces differ only in slightly revised drawings and the inclusion of two additional plates in the latter space, but are both derived from the same set of animal subjects and hence the same brains, we selected to trace the imported composite scans into a vector format using the *PW98* digital atlas maps as a template. For this purpose, the digital file from the *PW98* atlas that corresponded to the *PW86* map within the composite scan was imported into the .ai file as a separate layer, and aligned with the raster image of the scan. This was done such that registration of structures at and immediately surrounding the lateral hypothalamus ("LH" in *PW98*) was maximized, at the expense of the alignment of more distal structures. The *Pencil Tool* was used to trace over the injection site drawing on an additional transparent layer, thereby producing a vector drawing over the raster outline. A different transparent layer was created for each injection site, so long as all were mapped to the same atlas level within the given .ai file. The result of this effort was a single .ai file containing a *PW98* atlas layer and separate layers of injection site drawings in vector format, each drawn over the *PW98* layer and which could be visualized together or separately with the other injection sites, depending on whether the visibility of each layer was toggled on or off.

The format of the dataset produced from the procedures just described can be summarized as follows. Vector-formatted drawings of each injection site for a common AP level (*z*) of *PW98* were now present as individual transparent layers within a single .ai file. Importantly, the injection site outline was now digitally separated from its original *PW86* photocopied map and on a separate *PW98* layer in the .ai file. The benefit of this arrangement was that each injection site outline existed as a separate 2-D object that could be overlaid onto a separate data layer representing *PW98* space. The dataset was therefore now amenable for data migration (described next), since this would entail importing the comparable Swanson (*S*) atlas map into this stack of layers, permitting alignment of injection sites to *PW* space and also to *S* space.

Migration of Experimental Data

Data alignment

The alignment tool shown in **Figure 2** was used to identify the levels of the Swanson (2004) atlas (*S* space) corresponding to the *PW98* levels, and the electronic version of the appropriate *S* atlas level was imported as a separate layer into each file and centered in the horizontal axis. The agreement of the *S* and *PW* maps was inspected visually and additional *S* maps anterior or posterior to the first map were also imported and centered as appropriate to correct for dorsoventral plane of section differences between the atlases. Mediolateral plane of section differences were less than one level (although this determination is complicated by the fact that only one hemisphere of the brain is mapped in the *S* atlas). The imported *S* atlas maps were cropped and combined to create a single *S* atlas "composite map" for each file.

Anisotropic scaling of atlas plates

For the levels of *PW98* used for this exercise (Figures 26, 31, and 33 of Paxinos and Watson, 1998), the *S* map was scaled anisotropically such that the digitally represented stereotaxic coordinate grid in the AI environment exactly matched that of the *PW* map. (This can also be achieved, in our experience, by using the scaling factors provided by (Swanson, 1992): 139% in the horizontal axis and 161% in the vertical axis; and then normalizing the proportions using a scaling factor of 139.5% in both axes on *PW*; the difference between these two methods of scaling the *S* map was 0.4% in the horizontal axis and 0.1% in the vertical axis).

Final transformation and migration of experimental data

After various warping methods (Wells, 2017) yielded limited success, it was determined that 2-D drawings needed to be represented as point-source data to enable accurate migration of the locations of the injection sites. Each injection site was approximated as a point-source datum by placing a circle upon its ventral margin in Adobe Illustrator, as near to the site's midline as possible, which corresponded to the ventral tip of each injection site. Toggling the visibility of the relevant layers in the .ai file permitted the point-source sites to be displayed on the *S* map in the file. Two steps were then conducted sequentially to migrate the data. First, the locations of the point source data in *PW* space were migrated to their directly corresponding stereotaxic locations in *S* space. Because this approach yielded several outliers that did not migrate to lawful locations in *S* space on the basis of stereotaxic coordinates alone, a second step was employed. Specifically, each site was shifted—in a subject expert-guided fashion—to the appropriate location in *S* space so that the original relationship between the site and nearby fiducials on the *PW* plate was recapitulated as closely as possible on the *S* map. A total of 24 injection sites were migrated in this study (**Table 7**), 20 of which were from experiments described in detail in Khan et al. (2004).

Quantitative analysis of data migration procedures

Converting the destination atlas reference space to a Cartesian workspace. In order to compute the errors in migrating point-source data prior to the step where expert-guided corrections were implemented, the mapped sectors covering the locations in *S* space where the data were migrated were treated as quadrants of a 2 × 2 mm Cartesian plane, with the ordinate defined as the dorsoventral axis, the abscissa as the mediolateral axis, and their intersection as origin *O* at (0,0) mm. A separate Cartesian plane was constructed in Adobe Illustrator (AI) for each point-source dataset in *PW*98 that was migrated to a unique *S* level. Thus, three separate Cartesian planes were constructed in AI, for $PW98_{26} \rightarrow S_{26}$, $PW98_{31} \rightarrow S_{29}$, and $PW98_{33} \rightarrow S_{30}$ injection site migrations, respectively.

Error calculations. Within the AI environment, the Cartesian plane, the migrated data points prior to expert-guided correction, and the data points relocated after expert-guided correction; were all placed on separate layers so they could be toggled visible or invisible as needed for the analysis. Millimeter units were assigned in the *Preferences*, rulers were toggled to visible, and the

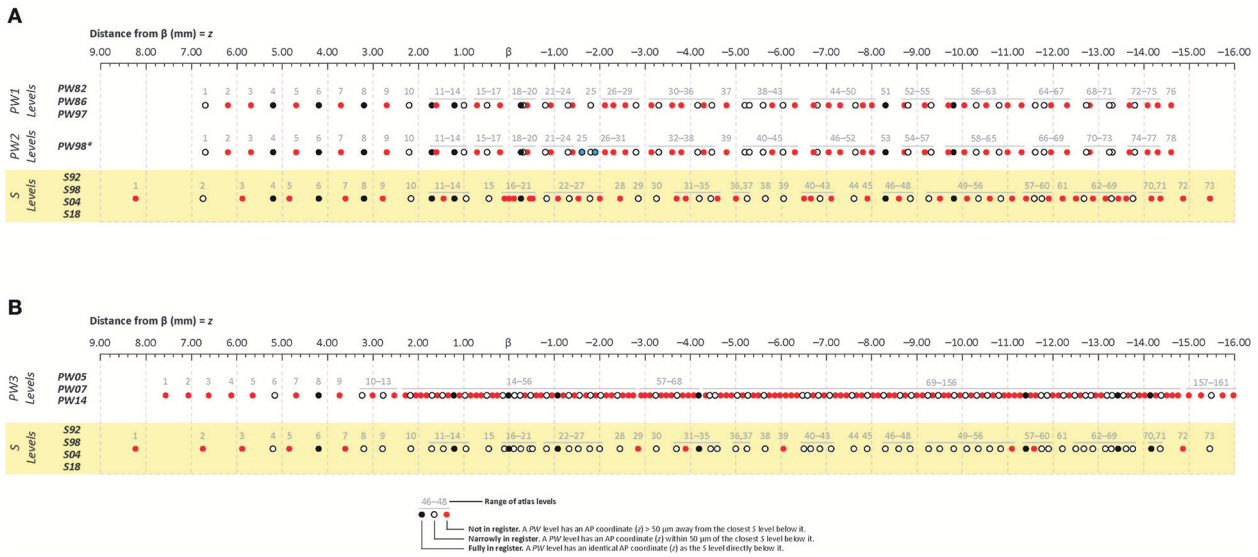

FIGURE 2 | Cleveland dot plot charts illustrating craniometric alignments of sequential levels for Paxinos & Watson (*PW*) reference atlases with the Swanson (*S*) reference atlas. The charts are calibrated to a millimeter scale (found at the top of **A,B**) denoting anteroposterior (AP) distance from the cranial suture-based landmark, Bregma (β). The legend at the bottom of the figure defines each symbol, with filled black dots, filled red dots, and open circles in *PW* spaces denoting levels that are fully in register, not in register, or narrowly in register; respectively, with the corresponding dots directly below them in *S* space. **(A)** Cleveland plots aligning the atlas levels of two *PW* reference spaces ("*PW1 Levels*" and "*PW2 Levels*") to *S* reference space ("*S Levels*"). *"PW1 Levels"* denote atlas levels from the first three editions of *The Rat Brain in Stereotaxic Coordinates* by Paxinos and Watson (1982, 1986, 1997) and are designated "*PW82*," "*PW86*," and "*PW97*," respectively. *"PW2 Levels"* denote atlas levels from the fourth edition of *The Rat Brain in Stereotaxic Coordinates* by Paxinos and Watson (1998), which is designated "*PW98*." Editions 2–4 contain refinements of the atlas drawings in the first edition, but the actual tissue sections on which the drawings are based are the same as those used in the original edition. *The only exception to this rule is that "*PW98*." differs from "*PW82*," "*PW86*," and "*PW97*" in the addition of two levels from the original tissue set that had not been published in the earlier editions. These are highlighted as filled blue dots. Because these additions alter the numbering scheme for the "*PW98*" levels from those of previous *PW* editions, they have been displayed separately from those editions. **(B)** Cleveland plots aligning the atlas levels of a third *PW* reference space ("*PW3 Levels*") to *S* reference space ("*S Levels*"). *"PW3 Levels"* denote atlas levels from the fifth, sixth and seventh editions of *The Rat Brain in Stereotaxic Coordinates* by Paxinos and Watson (2005, 2007, 2014) and are designated "*PW05*," "*PW07*," and "*PW14*," respectively. They are in a separate reference space because the tissue used was from a different animal than that used for the earlier editions, which were actually based on tissue sections from several animals. **(A,B)** "*S Levels*" comprise atlas levels from all four editions of *Brain Maps: The Structure of the Rat Brain* by Larry W. Swanson, published in 1992, 1998, 2004, and 2018 (designated "*S92*," "*S98*," "*S04*," and "*S18*"; respectively). They are all within one reference space because the same tissue set has been used for each edition, with the editions differing primarily in the refinement of the drawings and cytoarchitecturally derived mapped sub-regions from this single tissue set.

ruler origin was dragged to align precisely with the origin of the Cartesian plane being analyzed. With this arrangement, the *x* and *y* positions of each point-source datum could be queried by using the *Selection Tool* to select an individual data point and then consulting the *Info* window for specific positional information for the data point, expressed in mm from *O*. The *x* and *y* positions of the original (uncorrected) migrated data points were tabulated in an Excel spreadsheet in relation to the positions of the expert-guided (relocated) data points, and the differences in position calculated by subtracting the value of each uncorrected coordinate from the value of its corresponding relocated point. The mean and SEM for the errors in position along the *x* (ML) and *y* (DV) axes were calculated across migrated levels.

Analysis of expert-guided corrections to the migrated datasets. In order to compute the magnitudes and directions of the corrections performed by the subject expert on the migrated datasets, an additional layer within our Cartesian workspace in AI was created. In this layer, vectors were drawn from each pair of original and relocated data points in order to prepare diagrams showing the nature of the corrections performed by the subject matter expert. To compute the magnitude of each vector (AB), the numerical difference in the position of each original (A_x, A_y) and each relocated (B_x, B_y) data point was used to calculate the positional differences of vector AB along each axis (AB_x and AB_y). The vector AB was computed by taking the square root of $AB_x^2 + AB_y^2$, and the direction of AB computed by calculating the arctangent (in radians) of AB_x and AB_y. To this end, the two-argument variant of the arctangent was used (ATAN2 function in Excel) rather than the one-argument variant (ATAN), in order to have the calculation take into account the signs of both positions when assigning the direction to a specific quadrant. The resulting value was then converted to degrees to obtain φ, the final direction for AB. The mean magnitude and direction for all the vectors was then calculated to obtain the general behavior of the subject expert in correcting the migration of the datasets as a whole.

RESULTS

Anteroposterior Alignments Based on Craniometric Measures Using Cleveland Dot Plots

To facilitate anteroposterior (AP) alignment between *PW* and *S* reference spaces, atlas levels corresponding to values along the

z-axis (expressed as distance in mm from Bregma) were tabulated for all editions of each reference space and compared (**Table 1**). From these values, Cleveland dot plots were generated for the tabulated data as described in the Methods. **Figure 2** presents Cleveland dot plots for all atlas levels of *PW* and *S* editions alongside one another. Comparisons between early *PW* atlas editions ("*PW1 Levels*" and "*PW2 Levels*") and *S* atlas editions ("*S Levels*") are made in **Figure 2A**, whereas later *PW* editions ("*PW3 Levels*"), based on a new set of tissue, are compared against *S* atlas editions (again, "*S Levels*") in **Figure 2B**. The colored symbols in **Figure 2** specify which levels between these reference spaces are *fully in register* ($z_{PW} = z_S$; black dots), *narrowly in register* ($|z_{PW} - z_S| \leq 50$ μm (or $|z_S - z_{PW}| \leq 50$ μm); open circles), or *not in register* ($|z_{PW} - z_S| > 50$ μm (or $|z_S - z_{PW}| > 50$ μm); red dots) with one another along z.

Differences in Atlas Level Registration Based on Craniometric Measures

When calibrated along z, only eight atlas levels were *fully in register* between *PW1 Levels*, and *S* levels and between *PW2 Levels* and *S Levels* (**Figure 2A**: black dots; **Table 2**). Similarly, only eight atlas levels were *fully in register* between *PW3 Levels* and *S Levels* (**Figure 2B**: black dots; **Table 3**). In contrast, there were several more *PW* atlas levels that were *narrowly in register* with corresponding *S* levels; in some cases, the distance separating the levels was as little as 10 μm (**Figures 2A,B**: white circles; **Tables 4, 5**). Since the atlases of the *PW3* group are based on a brain that was sampled at higher spatial resolution than those of the *PW1* and *PW2* groups (at primarily 120 μm intervals instead of 500 μm intervals; see the dense pattern of dots that reflects this fine-grained sampling for *PW3 Levels* in **Figure 2B**), registration of the atlas levels from this group with *S Levels* resulted in much greater numbers of *narrowly in register* pairs of atlas levels between the reference spaces. This is evident at a glance when examining the dot plots for *S* space in **Figure 2**: many more *not in register* levels (*red dots*) exist in *S* space in Panel A than in Panel B, where the red dots have been converted to white dots to denote their updated status as *narrowly in register* with the newer *PW3* reference space.

Creation and Implementation of a Computer Vision Algorithm

In order to provide an independent means to determine whether the external craniometric alignments were a reasonable first-order solution to determine the most similar anteroposterior levels between *PW* and *S* reference spaces, we sought evidence from within the reference tissue sets themselves. This was achieved by developing a computer vision algorithm that enabled feature-based matching of selected regions of interest from within the Nissl-stained tissue used to create each reference space. This tissue is in the form of digital photomicrographs that accompany each atlas edition. For the purposes of algorithm development, test photomicrographs were used from the seventh edition of *PW* (Paxinos and Watson, 2014) and the third edition of *S* (Swanson, 2004).

Basic Operations of the Custom-Made Computer Vision Algorithm

Figure 3 shows the points of interest found in sampled parts of Nissl-stained photomicrographs from Paxinos and Watson (2014) (**Figure 3A**) and from Swanson (2004) (**Figure 3B**). The small lines inside the circles each indicate the dominant orientation of the area, with different region sizes corresponding to different scales.

Results of *Experiments 1a* and *1b*: Feature-Based Matching to Tissue Section of Origin

Figure 4 shows the results of *Experiment 1a*. A region of interest was extracted from the Nissl image corresponding to Level 34 of *S* space, rotated and distorted, and then used as a test ROI for the algorithm to produce the correct *S* level from which the test region originated. The results of SIFT operations before and after the application of RANSAC are shown (**Figures 4A,B**, respectively). **Figure 5** and **Table 6** show representative results of *Experiment 1b*, comparing the feature-based matching of an ROI from Level 34 of *S* to its correct and incorrect tissue photomicrograph of origin from the *S* atlas. As shown in **Figure 5A**, the algorithm successfully matched a number of features of the ROI to the plate of origin. The highest number of SIFT matches and RANSAC inliers was for Level 34, with SIFT matches being nearly three times greater and RANSAC inliers an order of magnitude greater, respectively, than the values of the next highest ranking plate match (**Table 6**). In contrast, images that clearly were from a different *S* plate, such as Level 22 shown in **Figure 5B**, resulted in a predictably low number of matches and inliers.

Results of *Experiment 2*: Feature-Based Matching to Determine Plate Correspondence

In *Experiment 2*, an ROI from the Nissl image associated with a specific plate in *PW* reference space (Plate 70 (L70) of *PW14*) was used as a test for the algorithm's ability to determine the appropriately matched Nissl plate in *S* space. **Figure 6** shows the robust feature-based matching that the algorithm achieved for the top-ranked *S* photomicrographic plate. **Table 6** shows the values for both the SIFT matches and RANSAC inliers for the five highest-ranking matches, which are in the range of *S* Levels 29–36. As can be seen in **Table 1** and **Figure 7**, the levels within this range are all flanking *S* L34, which is assigned as being *narrowly in register* with *PW* L70 on the basis of craniometric alignments (**Figure 2B**). These results support craniometric alignments as being a reliable first-order means to align the two reference spaces along the anteroposterior axis.

Data Transformation

Khan et al. (2004) reported the effects of centrally microinjecting membrane-permeable protein tyrosine kinase inhibitors (PTKIs) on feeding behavior produced by central injections of the glutamate receptor agonist, *N*-methyl-D-aspartate (NMDA). Specifically, the PTKIs, Tyrphostin A48 and PP1, were able to powerfully suppress NMDA-elicited eating when injected into the lateral hypothalamic area. However, in their study, the central microinjection sites were not published. We therefore

TABLE 1 | Alignments of *PW* atlas levels (Groups 1–3) with *S* atlas levels (Group 4) by distance from β.

	PW			*S*		*PW*			*S*		*PW*			*S*		*PW*			*S*		*PW*			*S*
z **(mm)**	**1**	**2**	**3**	**4**	***z*** **(mm)**	**1**	**2**	**3**	**4**	***z*** **(mm)**	**1**	**2**	**3**	**4**	***z*** **(mm)**	**1**	**2**	**3**	**4**	***z*** **(mm)**	**1**	**2**	**3**	**4**
8.24				1	−0.11				18	−3.80	33	35			−7.68			97		−11.64			130	
7.56			1		−0.12			34		−3.84			65		−7.80	49	51	98		−11.75				59
7.08			2		−0.24			35		−3.90				32	−7.90				45	−11.76			131	
6.60			3		−0.26	18	18		19	−3.96			66		−7.92			99		−11.80	65	67		
6.74				2	−0.30	19	19			−4.08			67		−8.00	50	52			−11.88			132	
6.70	1	1			−0.36			36		−4.16	34	36			−8.04			100		−11.90				60
6.20	2	2			−0.40	20	20			−4.20			68	33	−8.16			101		−11.96	66	68		
6.12			4		−0.46				20	−4.30	35	37			−8.28			102		−12.00			133	
5.88				3	−0.48			37		−4.36			69		−8.30	51	53		46	−12.12			134	
5.70	3	3			−0.51				21	−4.44			70		−8.40			103		−12.20				61
5.64			5		−0.60			38		−4.45				34	−8.52			104		−12.24			135	
5.20	4	4		4	−0.72			39		−4.48	36	38			−8.60				47	−12.30	67	69		
5.16			6		−0.80	21	21			−4.56			71		−8.64			105		−12.36			136	
4.85				5	−0.83				22	−4.60				35	−8.72	52	54			−12.48			137	
4.70	5	5			−0.84			40		−4.68			72		−8.76			106		−12.50				62
4.68			7		−0.92	22	22			−4.80	37	39	73		−8.80	53	55			−12.60			138	
4.20	6	6	8	6	−0.96			41		−4.92			74		−8.85				48	−12.68				63
3.72			9		−1.08			42	23	−5.00				36	−8.88			107		−12.72	68	70	139	
3.70	7	7			−1.20			43		−5.04			75		−9.00			108		−12.80	69	71		
3.60				7	−1.30	23	23			−5.16			76		−9.12			109		−12.84			140	
3.24			10		−1.32			44		−5.20	38	40			−9.16	54	56			−12.88				64
3.20	8	8		8	−1.33				24	−5.25				37	−9.24			110		−12.96			141	
3.00			11		−1.40	24	24			−5.28			77		−9.25				49	−13.08			142	
2.80				9	−1.44			45		−5.30	39	41			−9.30	55	57			−13.15				65
2.76			12		−1.53				25	−5.40			78		−9.36			111		−13.20			143	
2.70	9	9			−1.56			46		−5.52			79		−9.48			112		−13.24	70	72		
2.52			13		−1.60		25			−5.60	40	42			−9.50				50	−13.28				66
2.28			14		−1.72			47		−5.64			80		−9.60			113		−13.30	71	73		
2.20	10	10			−1.78				26	−5.65				38	−9.68	56	58			−13.32			144	
2.16			15		−1.80	25	26	48		−5.76			81		−9.72			114		−13.44			145	67
2.15				10	−1.88		27			−5.80	41	43			−9.80	57	59		51	−13.56			146	
2.04			16		−1.92			49		−5.88			82		−9.84			115		−13.60				68
1.92			17		−2.00				27	−6.00			83		−9.96			116		−13.68	72	74	147	
1.80			18		−2.04			50		−6.04	42	44			−10.04	58	60			−13.76				69
1.70	11	11		11	−2.12	26	28			−6.06				39	−10.08			117		−13.80	73	75	148	
1.68			19		−2.16			51		−6.12			84		−10.10				52	−13.92			149	
1.60	12	12			−2.28			52		−6.24			85		−10.20			118		−14.04			150	
1.56			20		−2.30	27	29			−6.30	43	45			−10.30	59	61			−14.08	74	76		
1.45				12	−2.40			53		−6.36			86		−10.32			119		−14.16			151	70
1.44			21		−2.45				28	−6.48			87		−10.35				53	−14.28			152	
1.32			22		−2.52			54		−6.50				40	−10.44			120		−14.30	75	77		
1.20	13	13	23	13	−2.56	28	30			−6.60			88		−10.52	60	62			−14.36				71
1.08			24		−2.64			55		−6.65				41	−10.56			121		−14.40			153	
1.00	14	14			−2.76			56		−6.72	44	46	89		−10.60				54	−14.52			154	
0.96			25		−2.80	29	31			−6.80	45	47			−10.68			122		−14.60	76	78		
0.95				14	−2.85				29	−6.84			90		−10.80	61	63	123		−14.64			155	
0.84			26		−2.92			57		−6.85				42	−10.85				55	−14.76			156	
0.72			27		−3.00			58		−6.96			91		−10.92			124		−14.86				72
0.70	15	15			−3.12			59		−7.04	46	48			−11.00	62	64			−15.00			157	
0.60			28		−3.14	30	32			−7.08			92		−11.04			117		−15.24			158	
0.48	16	16	29		−3.24			60		−7.10				43	−11.10				52	−15.46				73

(Continued)

TABLE 1 | Continued

	PW			*S*		*PW*			*S*		*PW*			*S*		*PW*			*S*		*PW*			*S*
z **(mm)**	**1**	**2**	**3**	**4**	***z*** **(mm)**	**1**	**2**	**3**	**4**	***z*** **(mm)**	**1**	**2**	**3**	**4**	***z*** **(mm)**	**1**	**2**	**3**	**4**	***z*** **(mm)**	**1**	**2**	**3**	**4**
0.45				15	−3.25				30	−7.20			93		−11.16			118		−15.48			159	
0.36			30		−3.30	31	33			−7.30	47	49			−11.28					−15.72			160	
0.24			31		−3.36			61		−7.32			94		−11.30	63	65	119		−15.96			161	
0.20	17	17			−3.48			62		−7.44			95		−11.40				53					
0.12			32		−3.60	32	34	63		−7.56			96		−11.52			129						
0.10				16	−3.70				31	−7.60				44	−11.58				58					
0.00			33	17	−3.72			64		−7.64	48	50			−11.60	64	66							

PW, Paxinos and Watson atlas levels; S, Swanson atlas levels. The numbers 1–3 denote different PW atlas groups, as described in the Methods: Group 1 = PW atlases published in 1982, 1986, and 1997. Group 2 = PW atlas published in 1998. Group 3 = PW atlases published in 2005, 2007, and 2014. All Swanson atlas editions (1992, 1998, 2004, 2018) are within Group 4. These tabulated data are represented using dot plots in ***Figure 2****. Note that the Bregma values were measured directly by Paxinos & Watson for their (PW) atlases using stereotaxic procedures; these values have been used by Swanson to derive Bregma value estimates for his (S) atlases.*

TABLE 2 | *PW1* and *PW2* levels fully in register with *S* levels along the AP axis (*z* mm from Bregma).

z	***PW* Atlas Level**		***S* Atlas Level**
	PW1	***PW2***	***S92, S98, S04, S18***
+5.20	4	4	4
+4.20	6	6	6
+3.20	8	8	8
+1.70	11	11	11
+1.20	13	13	13
−0.26	18	18	19
−8.30	51	53	46
−9.80	57	59	51

TABLE 3 | *PW3* levels fully in register with *S* levels along the AP axis (*z* mm from Bregma).

z	***PW3* Atlas Level**	***S* Atlas Level**
	PW05, PW07, PW14	***S92, S98, S04, S18***
+4.20	8	6
+1.20	23	13
0.00	33	17
+0.00	11	11
−1.08	42	23
−4.20	68	33
−13.44	145	67
−14.16	151	70

TABLE 4 | Distances between *PW1* & *PW2* levels narrowly in register with *S* levels along the AP axis (*z* mm from Bregma).

***PW* Atlas Level**			***S* Atlas Level**		**Distance**
z_{PW}	***PW1***	***PW2***	z_S	***S92, S98, S04, S18***	$\|\Delta z\|$, μm
+6.70	1	1	+6.74	2	40
+2.20	10	10	+2.15	10	50
+1.00	14	14	+0.95	14	50
+0.48	16	16	+0.45	15	30
−0.80	21	21	−0.83	22	30
−1.30	23	23	−1.33	24	30
−1.80	25	26	−1.78	26	20
−2.80	29	31	−2.85	29	50
−3.30	31	33	−3.25	30	50
−4.16	34	36	−4.20	33	40
−4.48	36	38	−4.45	34	30
−5.20	38	40	−5.25	37	50
−5.30	39	41	−5.25	37	50
−5.60	40	42	−5.65	38	50
−6.04	42	44	−6.06	39	20
−6.80	45	47	−6.85	42	50
−7.64	48	50	−7.60	44	40
−8.80	53	55	−8.85	48	50
−10.30	59	61	−10.35	53	50
−10.80	61	63	−10.85	55	50
−11.60	64	66	−11.58	58	20
−11.80	65	67	−11.75	59	50
−12.72	68	70	−12.68	63	40
−13.24	70	72	−13.28	66	40
−13.30	71	73	−13.28	66	20
−13.80	73	75	−13.76	69	40

decided to use these unpublished injection sites as a test for our data migration procedures and to publish their locations in both *PW* and *S* reference spaces. The first step needed to enable data migration for these sites was their transformation from graphical to digital form, and then their conversion from raster to vector format within digital space. **Figure 8** shows a representative example of a central microinjection site (**Figure 8A**) and the steps by which its drawn 2-D representation was transferred into a digital vector format (**Figure 8B**, Panels 1–4).

TABLE 5 | Distances between *PW3* levels narrowly in register with *S* levels along the AP axis (*z* mm from Bregma).

PW Atlas Level		*S* Atlas Level		Distance	*PW* Atlas Level		*S* Atlas Level		Distance
z_{PW}	*PW05, PW09, PW14*	z_S	*S92, S98, S04, S18*	$\|\Delta z\|$, μm	z_{PW}	*PW05, PW09, PW14*	z_S	*S92, S98, S04, S18*	$\|\Delta z\|$, μm
+5.16	6	+5.20	4	40	−6.60	88	−6.65	41	50
+3.24	10	+3.20	8	40	−6.84	90	−6.85	42	10
+2.76	12	+2.80	9	40	−7.08	92	−7.10	43	20
+2.16	15	+2.15	10	10	−7.56	96	−7.60	44	40
+1.68	19	+1.70	11	20	−7.92	99	−7.90	45	20
+1.44	21	+1.45	12	10	−8.28	102	−8.30	46	20
+0.96	25	+0.95	14	10	−8.64	105	−8.60	47	40
+0.48	29	+0.45	15	30	−8.88	107	−8.85	48	30
+0.12	32	+0.10	16	20	−9.24	110	−9.25	49	10
−0.12	34	−0.11	18	10	−9.48	112	−9.50	50	20
−0.24	35	−0.26	19	20	−9.84	115	−9.80	51	40
−0.48	37	−0.46	20	20	−10.08	117	−10.10	52	20
−0.48	37	−0.51	21	30	−10.32	119	−10.35	53	30
−0.84	40	−0.83	22	10	−10.56	121	−10.60	54	40
−1.32	44	−1.33	24	10	−10.80	123	−10.85	55	50
−1.56	46	−1.53	25	30	−11.76	131	−11.75	59	10
−1.80	48	−1.78	26	20	−11.88	132	−11.90	60	20
−2.04	50	−2.00	27	40	−12.24	135	−12.20	61	40
−2.40	53	−2.45	28	50	−12.48	137	−12.50	62	20
−3.24	60	−3.25	30	10	−12.72	139	−12.68	63	40
−3.72	64	−3.70	31	20	−12.84	140	−12.88	64	40
−4.44	70	−4.45	34	10	−13.20	143	−13.15	65	50
−4.56	71	−4.60	35	40	−13.32	144	−13.28	66	40
−5.04	75	−5.00	36	40	−13.56	146	−13.60	68	40
−5.28	77	−5.25	37	30	−13.80	148	−13.76	69	40
−5.64	80	−5.65	38	10	−14.40	153	−14.36	71	40
−6.48	87	−6.50	40	20	−15.48	159	−15.46	73	20

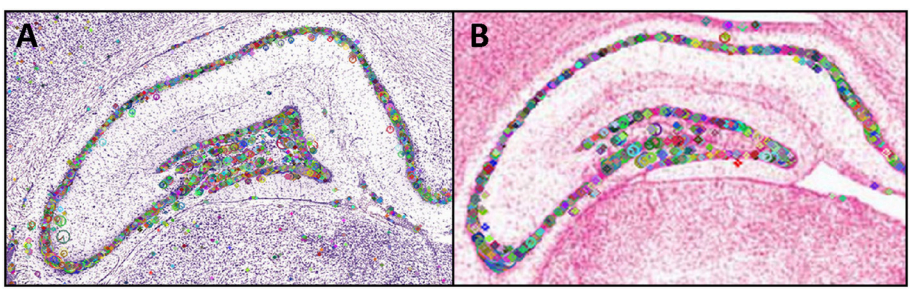

FIGURE 3 | Points of interest found in a region of interest from a plate in the **(A)** Paxinos and Watson (2014) atlas and **(B)** in the Swanson (2004) atlas. The small horizontal lines in each panel inside the circular regions indicate the region's dominant orientation. Different region sizes correspond to different scales. The portion of the *PW14* atlas photomicrograph (Level 70) is reproduced in **(A)** with permission from Elsevier. The photomicrograph in **(B)** is reproduced from Swanson (2004) under the conditions set forth by a Creative Commons BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/legalcode).

Data Migration

Migration

The alignment tool in **Figure 2A** was used to determine the atlas level in *S* space that corresponds most closely to the *PW* level serving as the source of the migrated injection site data. The *PW* and *S* reference spaces were first brought into register with one another in the mediolateral and dorsoventral axes. This was achieved by taking advantage of the stereotaxic grid embedded in the .ai files comprising the digital atlas maps of Swanson (2004), which was provided to users of the atlas as a means to contextualize their data according to the stereotaxic coordinates of Paxinos and Watson (1986). Using this grid and the scaling factors provided by Swanson (1992), three atlas maps from *PW*98 were aligned with the corresponding closest matching maps in *S*04: $PW98_{26} \rightarrow S_{26}$, $PW98_{31} \rightarrow S_{29}$, and $PW98_{33} \rightarrow S_{30}$. A point-source datum representing the ventral tip of each injection site was drawn on the 2-D rendering of the injection site, and each of these data points was then migrated to the appropriate

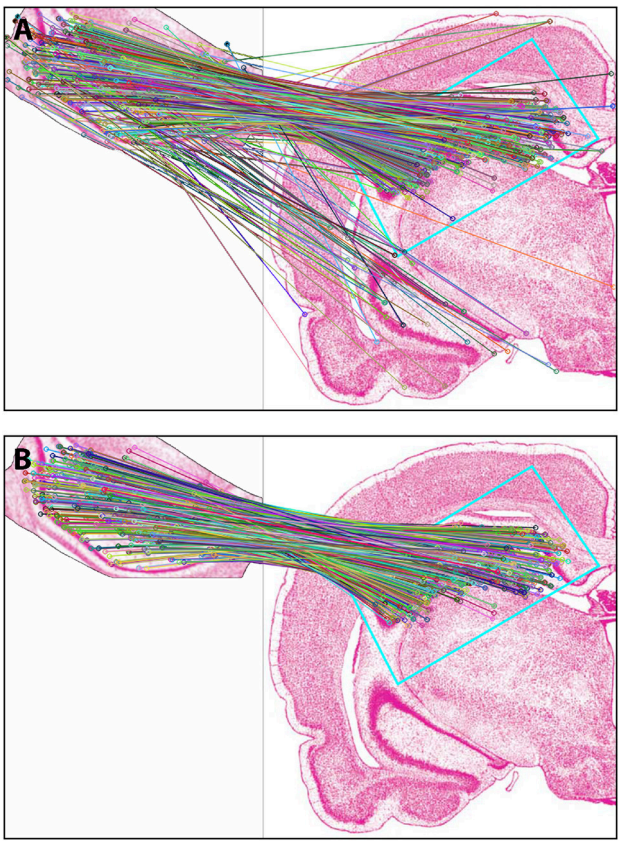

FIGURE 4 | Results of *Experiment 1a*. The algorithm successfully matched the exact *S* atlas plate (Level 34), containing the desired Nissl-stained tissue section, with the ROI extracted digitally from that section, which was rotated 155 degrees and distorted slightly through random point-warping, and used as a test image. SIFT matches are shown for the test image before **(A)** and after **(B)** RANSAC was applied to remove outliers. The photomicrographs are reproduced from Swanson (2004) under the conditions set forth by a Creative Commons BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/legalcode).

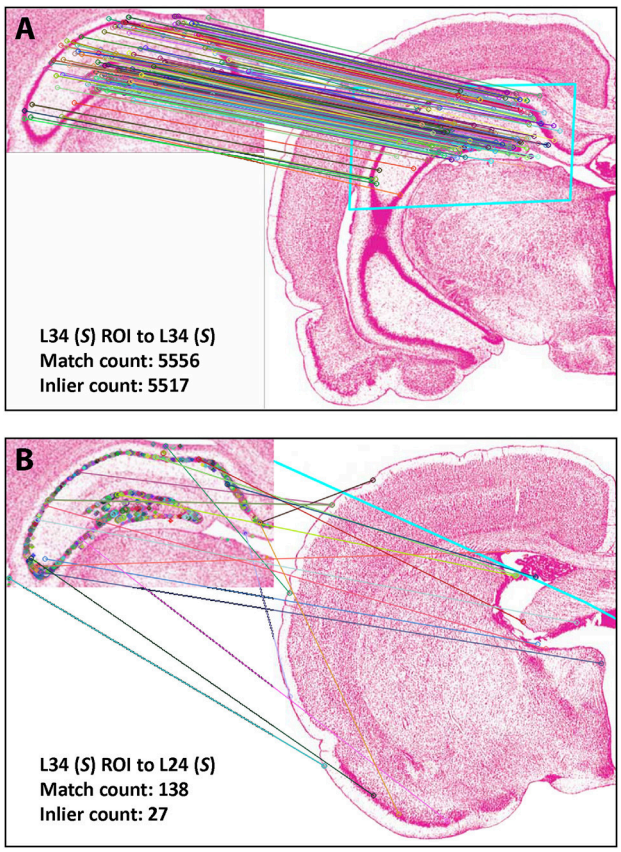

FIGURE 5 | Results of *Experiment 1b*. Examples of a good match between an ROI and an image **(A)**, and a poor one **(B)**. The photomicrographs are reproduced from Swanson (2004) under the conditions set forth by a Creative Commons BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/legalcode).

S level. **Figures 9–11** show the datasets for the $PW98_{26}$➔S_{26}, $PW98_{31}$➔S_{29}, and $PW98_{33}$➔S_{30} migrations, respectively. Each of these figures shows three panels (A–C) that depict the positions of the data points in *PW*98 space (**Figures 9A**, **10A**, **11A**), their transfer to *S* space (**Figures 9B**, **10B**, **11B**), and finally, their adjusted positions in *S* space after expert-guided corrections to the migrated datasets were performed (**Figures 9C**, **10C**, **11C**).

Analysis

In order to ascertain the amount of error in data migration that required correction by a subject matter expert, we quantitatively determined the location of each migrated data point within a Cartesian workspace derived from the original reference space quadrants into which the data were migrated (*S* space from *PW* space) (**Figure 12**). As seen in the *left column* of data points in **Figure 12**, the positions of all data points fell within a 2 × 2 mm Cartesian plane, as did the locations of the relocated points after expert intervention (**Figure 12**, *middle column*). The differences in positions for the original vs. relocated data points were expressed in the form of vectors (**Figure 12**, *right column*).

TABLE 6 | Top matches (Experiments 1b & 2).

S Level	SIFT Matches	RANSAC Inliers
EXPERIMENT 1b		
34	3618	3494
71	1316	822
35	652	278
EXPERIMENT 2		
30	483	212
29	456	197
32	431	173
34	410	144
36	525	137

The magnitude and direction of each vector were calculated as described in the Methods. The results of these calculations are summarized in **Table 7**. The average magnitude of the vectors was 76 μm in the mediolateral dimension and 442 μm in the dorsoventral dimension (**Table 7**), demonstrating that the error in migrating the data was dominated by errors in the latter dimension. This was supported by the arctangent calculations computed for each vector, which produced a mean value of 78.4°

for φ. If φ had been less than 45°, the result would have suggested a more dominant deviation laterally (i.e., along the ML axis), but this was not the case. Thus, both the mean *xy* components of the vector and its mean direction demonstrate that the overall correction performed by the expert user was to shift the migrated points in the dorsolateral direction, with the greater component of this correction occurring along the DV axis.

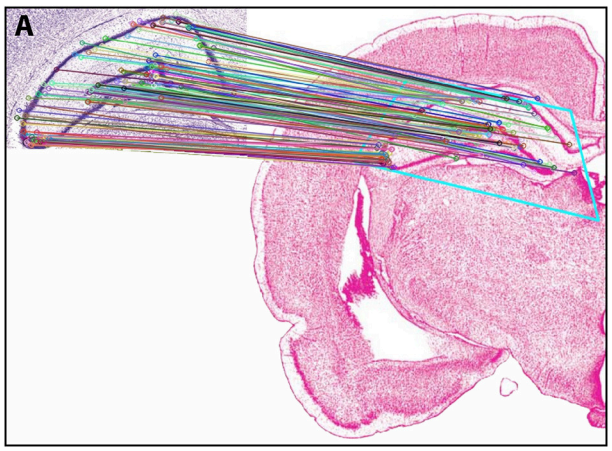

FIGURE 6 | Results of *Experiment 2*. View of the match obtained after *Experiment 2* was implemented, between an ROI extracted from *PW* space and the closest matching *S* atlas photomicrograph. In the inset (labeled A), the portion of the *PW14* atlas photomicrograph (Level 70) is reproduced with permission from Elsevier. The photomicrograph in the main image is reproduced from Swanson (2004) under the conditions set forth by a Creative Commons BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/legalcode).

Data Decoding

Figures 13, **14** show summaries of the migrated data points from **Figures 9–11**, but decoded with respect to the experiments conducted in the original study (Khan et al., 2004). Specifically, three different experiments were performed using protein tyrosine kinase inhibitors (PTKIs). In the first experiment (Exp. 4 in Khan et al., 2004), the PTKI, Tyrphostin A48, was tested at varying doses against NMDA (*TyrA48 dose-response*). In the second experiment (Exp. 5a in Khan et al., 2004), PP1 was tested at varying doses against NMDA (*PP1 dose-response*). Finally, in a third experiment (Exp. 5b in Khan et al., 2004), two doses of PP1 were again tested against the feeding stimulatory effects of NMDA (*PP1* vs. *NMDA*). **Figures 13**, **14** show the injection sites, sorted and then migrated onto Swanson (2018) atlas maps, for each of these three coded experiments, along with a few injection sites from a related experiment involving PP1 that was not reported in Khan et al. (2004) (see **Table 7**). In the host *S18* reference space, the injection sites fell within the following portions of the lateral hypothalamic area: anterior group, anterior region, ventral zone (LHAav; **Figure 14C**); middle group, lateral tier, dorsal region (LHAd; **Figures 13A,B, 14A,D**); middle group, lateral tier, ventral region, magnocellular nucleus (LHAma; **Figures 13A**, **14A,D**); and middle group, lateral tier, ventral region, medial zone (LHAvm; **Figures 13A**, **14A**). Several injection sites were also migrated to a region near but not within the lateral hypothalamic area, middle group, lateral tier, tuberal nucleus, lateral part (TUl; **Figures 13A**, **14A**).

DISCUSSION

In this study, we sought to establish a basic framework for migrating spatial datasets between two sets of canonical

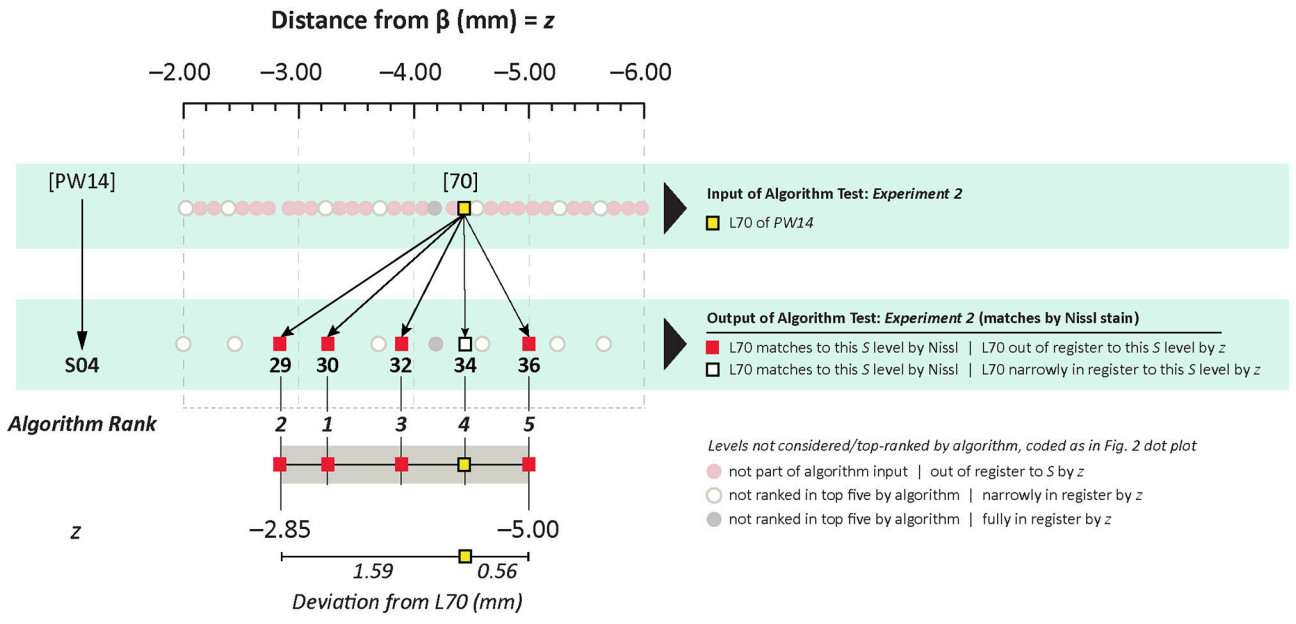

FIGURE 7 | Summary of *Experiment 2*. The algorithm successfully matched its top-ranked *S* atlas plates (most plates within Levels 29–36) with the ROI extracted digitally from Plate 70 of *PW3* space. This range is in close agreement with the general range of levels between the two reference spaces, as indicated by craniometric measures.

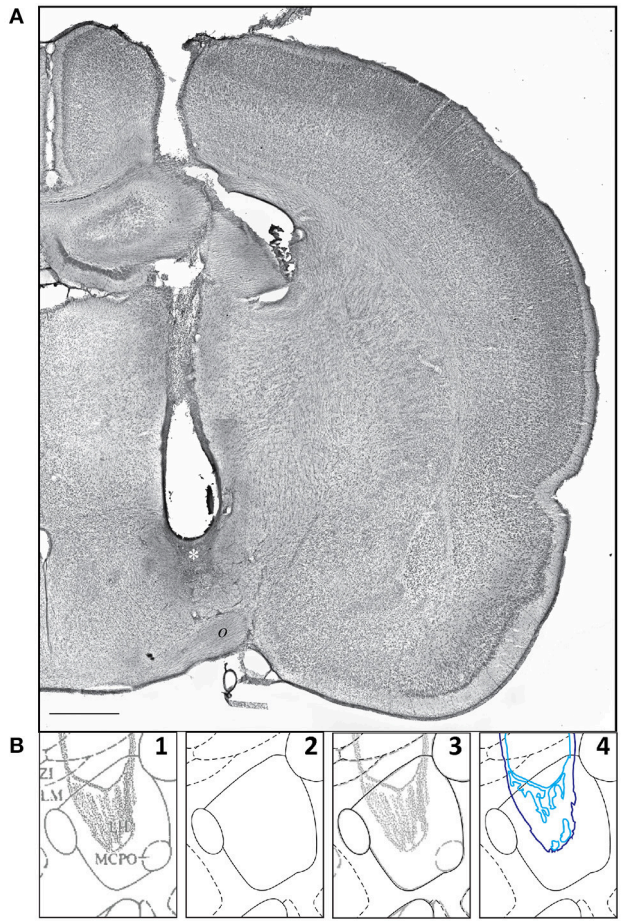

FIGURE 8 | (A) A representative example of a microinjection site, with the injection scar denoted by a white asterisk (*). This photomicrograph shows one half of a transverse section through the rat brain, with a needle track targeting the hypothalamus. *o*, optic tract. Scale bar = 1 mm. **(B)** Sequential steps in the transformation of a graphical injection site drawing to a vector-formatted object: *Step 1*: graphical drawing based on *PW*86; *Step 2*: the *PW*98 digital version of the same atlas drawing; *Step 3*: an overlay of the drawing in 1 and the digital drawing in 2 (graphical drawing over digital boundaries); *Step 4*: vector drawing, in a separate layer, of the injection site. The portion of the *PW* atlas figure (Level 26) is reproduced here with permission from Elsevier.

reference spaces for the rat brain, the Paxinos & Watson (*PW*) and Swanson (*S*) rat brain atlases. The major findings from this effort can be summarized as follows. First, concerning *alignment*, calibrating the *PW* and *S* atlas levels to the Bregma landmark allowed a basic tool to be created to interrelate the reference spaces. Second, concerning *matching*, the novel computer vision algorithm we created to match the image features from atlas photomicrographs of Nissl-stained tissue provided independent support for the utility and general accuracy of the craniometric alignments. Finally, with regard to *migration*, we demonstrate that the transfer of unpublished spatial datasets from a behavioral study of the hypothalamus between rostrocaudally registered *PW* and *S* spaces could be achieved by expert-guided mapping in the mediolateral and dorsoventral dimensions. Each of these outcomes will be discussed below in relation to the value of data migration across atlases for domain experts in behavioral neuroscience and neuroanatomy.

Craniometric Alignment

The approach taken in this study to interrelate the *PW* and *S* reference spaces owes its existence, in part, to the prescient decision made by Swanson (1992) to provide a detailed description of the spatial relationships between his atlas and that published by Paxinos and Watson (1986). The first attempt to interrelate the two reference spaces, therefore, occurs in Swanson (1992); our approach should be considered as simply an independent attempt to do the same. Additionally, from the basic starting point set down by Swanson (1992), we have taken the logical next step of applying and testing his basic system of *PW*/*S* registration across each of the levels that collectively populate the other editions of *PW* and *S* that have been published since 1992.

The rationale for performing such registration is based on certain favorable starting conditions that suggest immediately to discerning users of both series of atlases that registration between the two would appear to be feasible. First, the plane of section for the brain used to create the *S* atlas series, at least through the rostral forebrain and midbrain, is very similar to that provided by Paxinos and Watson (1986), reportedly differing by only four degrees in the mediolateral plane (Swanson, 1992). Second, *S* space derives its stereotaxic coordinates from the *PW*86 atlas, and scaling factors in both mediolateral and dorsoventral dimensions have been provided in Swanson (1992) to facilitate registration. Finally, the vector graphics files provided by Swanson (1992) in Adobe Illustrator format, together with AI's native system of data layers, provide a very useful means to scale the maps from both atlas series using transparent overlays.

The alignment tool we furnish in this study has been created with the needs of the behavioral neuroscientist in mind. We selected 50 μm as the interval that defines levels between *PW* and *S* reference spaces that are *narrowly in register* vs. *not in register* with one another, because this interval is greater in resolution than the resolution of most probes used for manipulations in the rat, which typically range from 100 to 300 μm in diameter (Zhang et al., 2010; Larson et al., 2015; Gigante et al., 2016). In our experience (e.g., Khan et al., 1999, 2004, 2007), greater resolution is not required at this time to achieve the practical goals of performing a successful, reproducible intracranial manipulation of neural substrates in this animal model. Higher resolution may, in fact, impair the clarity required of any useful map for a bench neuroscientist (Wagner, 2011). Of course, as the technology used to manipulate neural substrates becomes smaller in scale (e.g., Kim et al., 2013), the need for further refinements in resolution will arise for maps to remain maximally useful.

Image Feature Detection and Matching

Despite the parameters described in the preceding section that suggest the feasibility of bringing *S* and *PW* spaces into register with one another, we sought an independent means to determine whether such registration was accurate. This is because the use of Bregma coordinates has been evaluated in the context of registration of rat brain representations by others, and has been

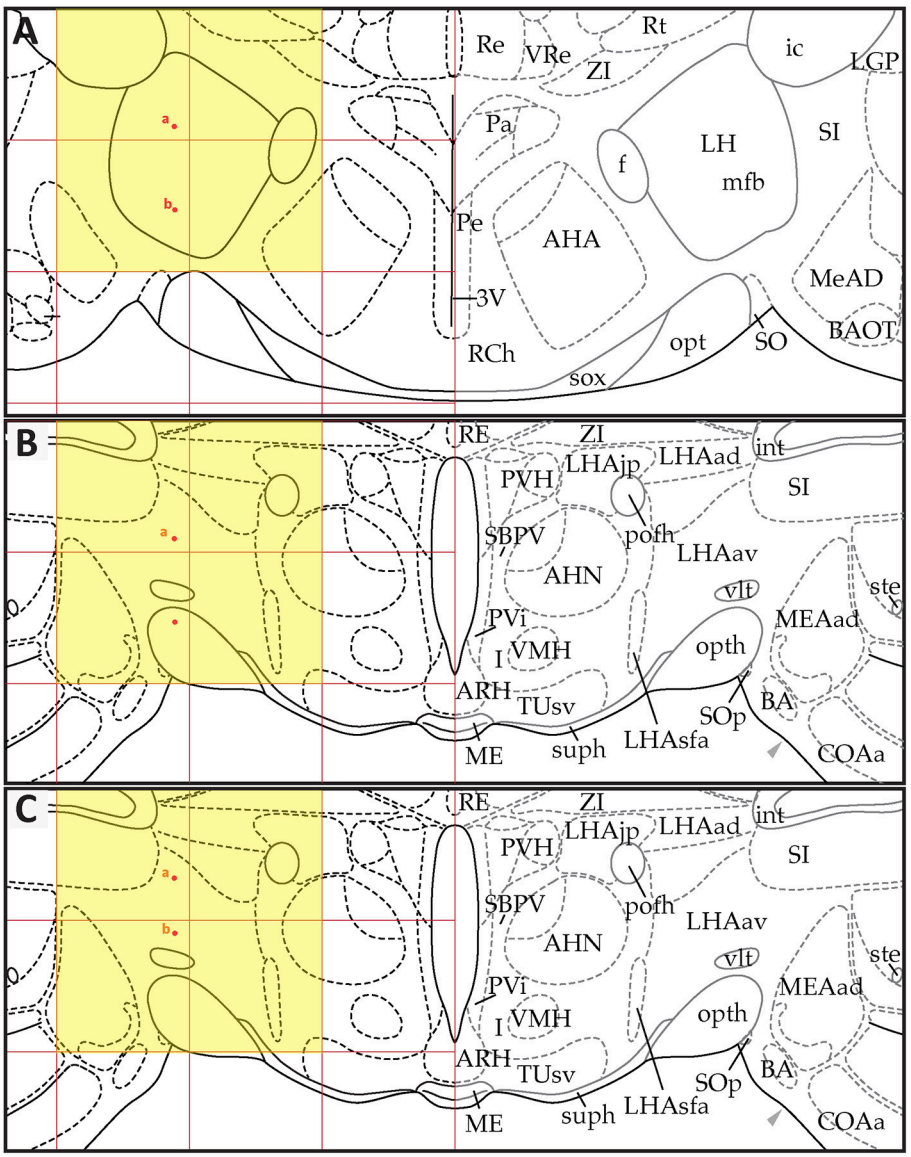

FIGURE 9 | Point-source data migrated from the digital map in Paxinos and Watson (1998), Figure 26, to Swanson (2018), Level 26. **(A)** Paxinos and Watson (1998), Figure 26. Point-source data are shown by red dots, which have been shifted contralaterally. **(B)** Swanson (2018), Level 26, with stereotaxic grid aligned to that of Paxinos and Watson (1998), Figure 26. Point-source data appear at their original stereotaxic coordinates. **(C)** Swanson (2018), Level 26, with stereotaxic grid aligned to that of Paxinos and Watson (1998), Figure 26. Point-source data have been shifted to more closely match their original locations with regard to nearby fiducials. Note that to minimize reader distraction, the general appearance (but not boundaries or nomenclature) of the *S* levels in **(B**,**C)** have been altered to match those of *PW*. Figure 26 from *PW98* is reproduced with permission from Elsevier. Level 26 from *S18* is reproduced from Swanson (2018) under the conditions of a Creative Commons BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/legalcode). For an explanation of the abbreviations in **A**, see List of Abbreviations, Paxinos and Watson Nomenclature. For an explanation of the abbreviations in **B** and **C**, please see List of Abbreviations, Swanson Nomenclature.

reported to be error-prone (Kline and Reid, 1984; Santori and Toga, 1993; Blasiak et al., 2010; Sergejeva et al., 2015; Rangarajan et al., 2016; but see Slotnick and Brown, 1980), although the precise contexts within which such tests have been conducted differ from our own. Moreover, key differences exist between the brains used to create both reference spaces, including strains and body weights of the source subjects, with *PW* reference space based on the brains of several male Wistar rats ranging 290 ± 16 g in body weight and the *S* atlas series based on a single 315 g, male Sprague-Dawley rat. Kruger et al. (1995) have reported that the midbrain and hindbrain of the Wistar rat is longer in the AP axis than these brain divisions in the Sprague-Dawley rat. On the other hand, Whishaw et al. (1977) have determined empirically that body weight in the laboratory rat is correlated linearly with the location of the Bregma coordinate, a finding supported by a detailed statistical analysis by Slotnick and Brown (1980). Moreover, Paxinos et al. (1985) report that stereotaxic coordinates for their atlas could be applied to animals

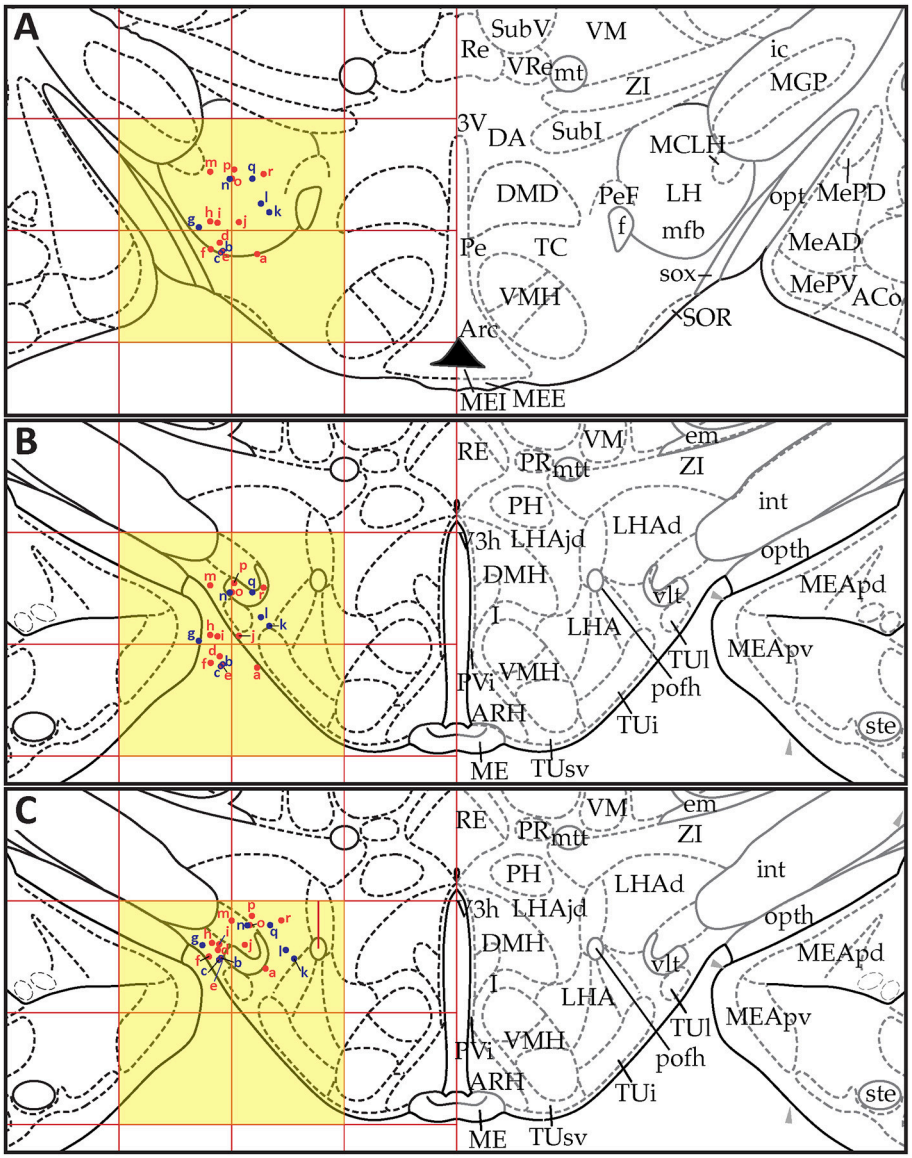

FIGURE 10 | Point-source data migrated from Paxinos and Watson (1998), Figure 31, to Swanson (2018), Level 29. **(A)** Paxinos and Watson (1998), Figure 31. Point-source data are shown by red dots, which have been shifted contralaterally; and blue dots, which have not been shifted. **(B)** Swanson (2018), Level 29, with stereotaxic grid aligned to that of Paxinos and Watson (1998), Figure 31. Point-source data appear at their original stereotaxic coordinates. **(C)** Swanson (2018), Level 29, with stereotaxic grid aligned to that of Paxinos and Watson (1998), Figure 31. Point-source data have been shifted to more closely match their original locations with regard to nearby fiducials. Note that to minimize reader distraction, the general appearance (but not boundaries or nomenclature) of the *S* levels in **(B,C)** have been altered to match those of *PW*. Figure 31 from *PW98* is reproduced with permission from Elsevier. Level 29 from *S18* is reproduced from Swanson (2018) under the conditions of a Creative Commons BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/legalcode). For an explanation of the abbreviations in **A**, see List of Abbreviations, Paxinos and Watson Nomenclature. For an explanation of the abbreviations in **B** and **C**, please see List of Abbreviations, Swanson Nomenclature.

of differing strains and sexes provided that the animals are of similar surgical body weight to the animals used in their atlas. Nevertheless, it remains unclear whether registration remains accurate at the level of individual transverse sections between the atlases.

Accordingly, we decided to create a semi-automated, quantitative method to examine key landmarks (fiducials) within the tissue sections themselves that form the bases of both atlas reference spaces, as we have noted previously (Khan, 2013; Wells and Khan, 2013). The computer vision algorithm that resulted from this effort utilizes a well-established feature detection technique, known as the Scale-Invariant Feature Transform (SIFT), developed by Lowe (1999, 2004), to detect salient local features in a region of interest (ROI) and the image of the Nissl-stained tissue section documented in each atlas photomicrograph. To match ROIs from one reference

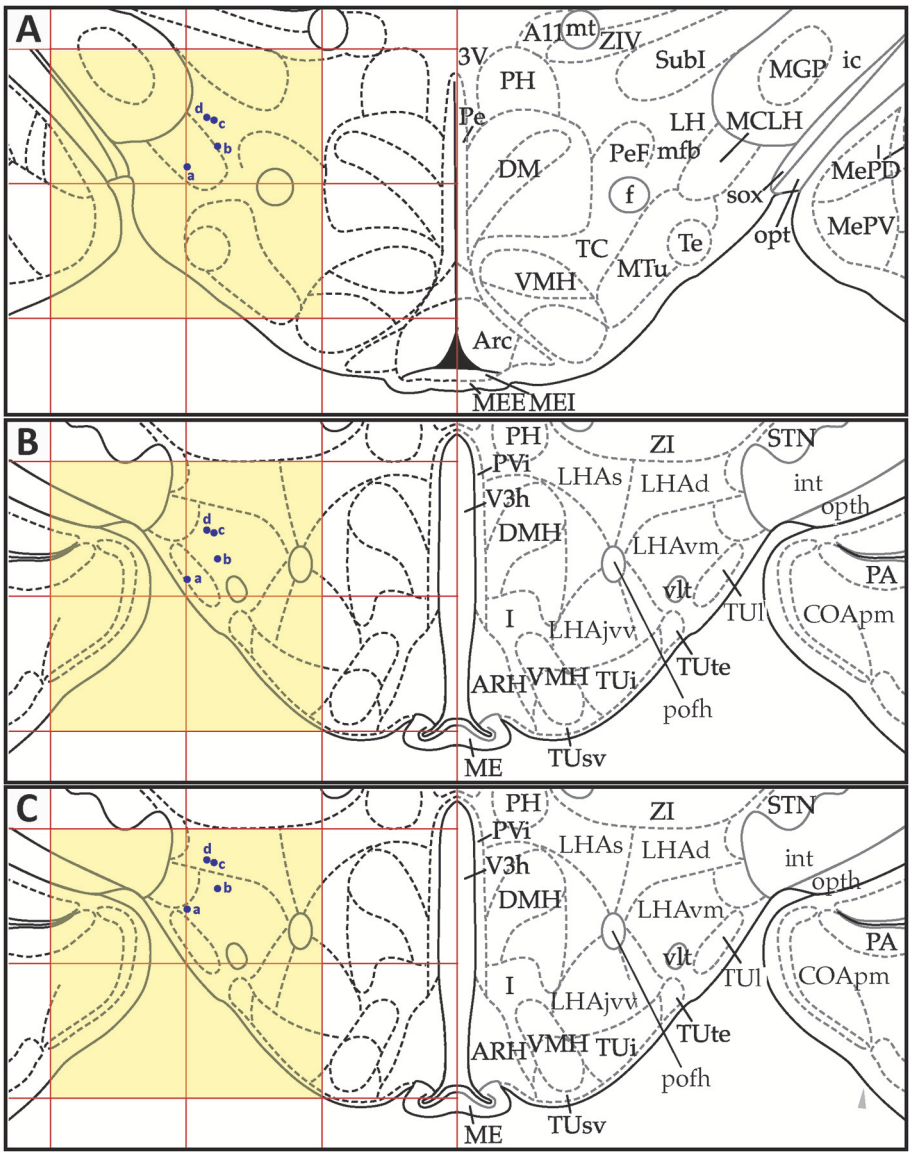

FIGURE 11 | Point-source data migrated from Paxinos and Watson (1998), Figure 33, to Swanson (2018), Level 30. **(A)** Paxinos and Watson (1998), Figure 33. Point-source data are shown by blue dots. **(B)** Swanson (2018), Level 30, with stereotaxic grid aligned to that of Paxinos and Watson (1998), Figure 33. Point source information appears at its original stereotaxic coordinates. **(C)** Swanson (2018), Level 30, with stereotaxic grid aligned to that of Paxinos and Watson (1998), Figure 33. Point source data have been shifted to more closely match their original locations with regard to nearby fiducials. Note that to minimize reader distraction, the general appearance (but not boundaries or nomenclature) of the *S* levels in **(B,C)** have been altered to match those of *PW*. Figure 33 from *PW98* is reproduced with permission from Elsevier. Level 30 from *S18* is reproduced from Swanson (2018) under the conditions of a Creative Commons BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/legalcode). For an explanation of the abbreviations in **A**, see List of Abbreviations, Paxinos and Watson Nomenclature. For an explanation of the abbreviations in **B** and **C**, please see List of Abbreviations, Swanson Nomenclature.

atlas space to the other, we implemented a Random Sample Consensus (RANSAC) operation, first developed by Fischler and Bolles (1981), which uses stochastic search to find the affine transformation that yields the largest number of feature correspondences between the images.

We found a striking consensus between the ranked matches returned by the algorithm and the predicted pairings produced by craniometric alignments (summarized in **Figure 7**). In particular, not only did the algorithm return ranked matches that clustered in the same range as that specified by the craniometric alignment tool, but the differences in the levels between the external (skull-based) and internal (tissue-based) alignments were not more than around 1.6 mm. This distance, if applied as a general "error" value, is probably an overestimate considering that the ROI used to produce this result, the dentate gyrus of the hippocampus, is a relatively large structure that spans many rostrocaudal levels without undergoing drastic variation at the resolution examined here. It is possible that the rostrocaudal difference between the

TABLE 7 | Positions, errors, and corrections for migrated datasets.

Levels	Subject # (Expt #)	Label	Distance of Scaled Data from Cartesian Origin (mm)				Expert-Guided Mapping			
			− Expert guidance		+ Expert guidance		Error (mm)		Correction	
			A_x (ML)	A_y (DV)	B_x (ML)	B_y (DV)	AB_x (ML)	AB_y (DV)	AB	φ°
$PW_{26} \rightarrow S_{26}$	00/115 (5b)	a	−0.12776	−0.11052	−0.12456	−0.34712	0.00320	0.23660	0.236622	89.225
	00/123 (5b)	b	−0.12360	0.53560	−0.12040	0.07792	0.00320	0.45768	0.457691	89.599
$PW_{31} \rightarrow S_{29}$	99/153 (4)	a	0.20612	0.16380	0.29084	−0.41396	0.08472	0.57776	0.583938	81.658
	99/144 (4)	b	−0.10444	0.16428	−0.11252	−0.51160	0.00808	0.67588	0.675928	89.315
	99/152 (4)	c	−0.12844	0.18468	−0.13288	−0.49116	0.00444	0.67584	0.675855	89.624
	99/138 (4)	d	−0.13240	0.09252	−0.14044	−0.58232	0.00804	0.67584	0.675888	89.318
	00/049 (5a)	e	−0.11320	0.17572	−0.12124	−0.50012	0.00804	0.67584	0.675888	89.318
	00/036 (5a)	f	−0.21412	0.15276	−0.22216	−0.52308	0.00804	0.67584	0.675888	89.318
	99/151 (4)	g	−0.32236	−0.04632	−0.28208	−0.62544	0.04028	0.57912	0.580519	86.021
	00/044 (5a)	h	−0.21908	−0.09960	−0.19492	−0.64652	0.02416	0.54692	0.547453	87.471
	00/092 (*)	i	−0.15284	−0.08728	−0.12868	−0.63420	0.02416	0.54692	0.547453	87.471
	00/039 (5a)	j	0.04104	−0.09264	0.10288	−0.62924	0.06184	0.53660	0.540152	83.426
	99/143 (4)	k	0.31640	−0.18180	0.55008	−0.50312	0.23368	0.32132	0.397307	53.973
	99/137 (4)	l	0.24096	−0.26124	0.47464	−0.58256	0.23368	0.32132	0.397307	53.973
	00/057 (*)	m	−0.21908	−0.54960	−0.01672	−0.84860	0.20236	0.29900	0.361041	55.910
	99/147 (4)	n	−0.04248	0.48416	0.12672	−0.80544	0.16920	0.32128	0.363111	62.227
	00/084 (*)	o	−0.02108	−0.48688	0.14808	−0.80820	0.16916	0.32132	0.363128	62.235
	00/050 (5a)	p	−0.00176	−0.56996	0.16744	−0.89124	0.16920	0.32128	0.363111	62.227
	99/135 (4)	q	0.16320	−0.56996	0.33240	−0.80776	0.16920	0.32128	0.363111	62.227
	00/086 (*)	r	0.26500	−0.52916	0.43420	−0.85048	0.16920	0.32132	0.363146	62.230
$PW_{33} \rightarrow S_{30}$	99/153 (4)	a	−0.01084	−0.14860	−0.01832	−0.44964	0.00748	0.30104	0.301133	88.577
	99/144 (4)	b	0.21572	−0.30200	0.20692	−0.60216	0.00880	0.30016	0.300289	88.321
	99/152 (4)	c	0.18920	−0.49628	0.18056	−0.79532	0.00864	0.29904	0.299165	88.345
	99/138 (4)	d	0.13552	−0.51648	0.12720	−0.81544	0.00832	0.29896	0.299076	88.406
	Mean		−0.00668	−0.16578	0.06363	−0.60778	0.07613	0.44201	0.460175	78.351
	SEM		0.03564	0.06012	0.04593	0.04195	0.01679	0.03134	0.028963	2.747

indicates an experiment conducted in association with those published in Khan et al. (2004) but not included in that study. Letter designations in the "Label" column refer to labels in **Figures 9–11 which mark the locations for each injection site.*

craniometric and computer vision-based alignments would be smaller with selection of ROIs that are more restricted along the rostrocaudal axis, a possibility that will be explored in future expansions of this work.

This deviation notwithstanding, the results demonstrate that there appears to be a strong agreement, under the experimental conditions examined in this study, between craniometric measures of atlas levels for the two reference spaces and the histological features of the underlying tissue sets themselves on which these spaces are based. Future efforts to test this relationship further could include embedding a computational framework within our algorithm that focuses on stable fiducials within the brain tissue sets rather than random features of the test ROI. These fiducials could include white matter tracts and medially located, cytoarchitectonically defined brain structures that are less prone to the variability in appearance that can arise between tissue sections due to differences in their planes of section. Our finding that there is a general consensus between craniometric and histological matchings between the reference spaces is also supported by the statistical analysis conducted by Slotnick and Brown (1980), who found robust correlations (0.68–0.92, mean: 0.81, $n = 14$) of Bregma values with five fiducial points examined within the brains of male albino Holtzmann rats: the most anterior and posterior points of the cerebrum, the genu and splenium of the corpus callosum, and the center of the anterior commissure.

Although our results demonstrate that there is a strong case to be made in using craniometric measures as a first-order means to coarsely align the two reference spaces, a few points concerning our approach are worth noting. First, the search space for our algorithm is limited to an affine transformation between the region of interest and the image as defined by the homography matrix. For tissue that is distorted or warped, further work will be required to generate robust matches, although the results from *Experiment 1a* demonstrate that the algorithm is invariant to

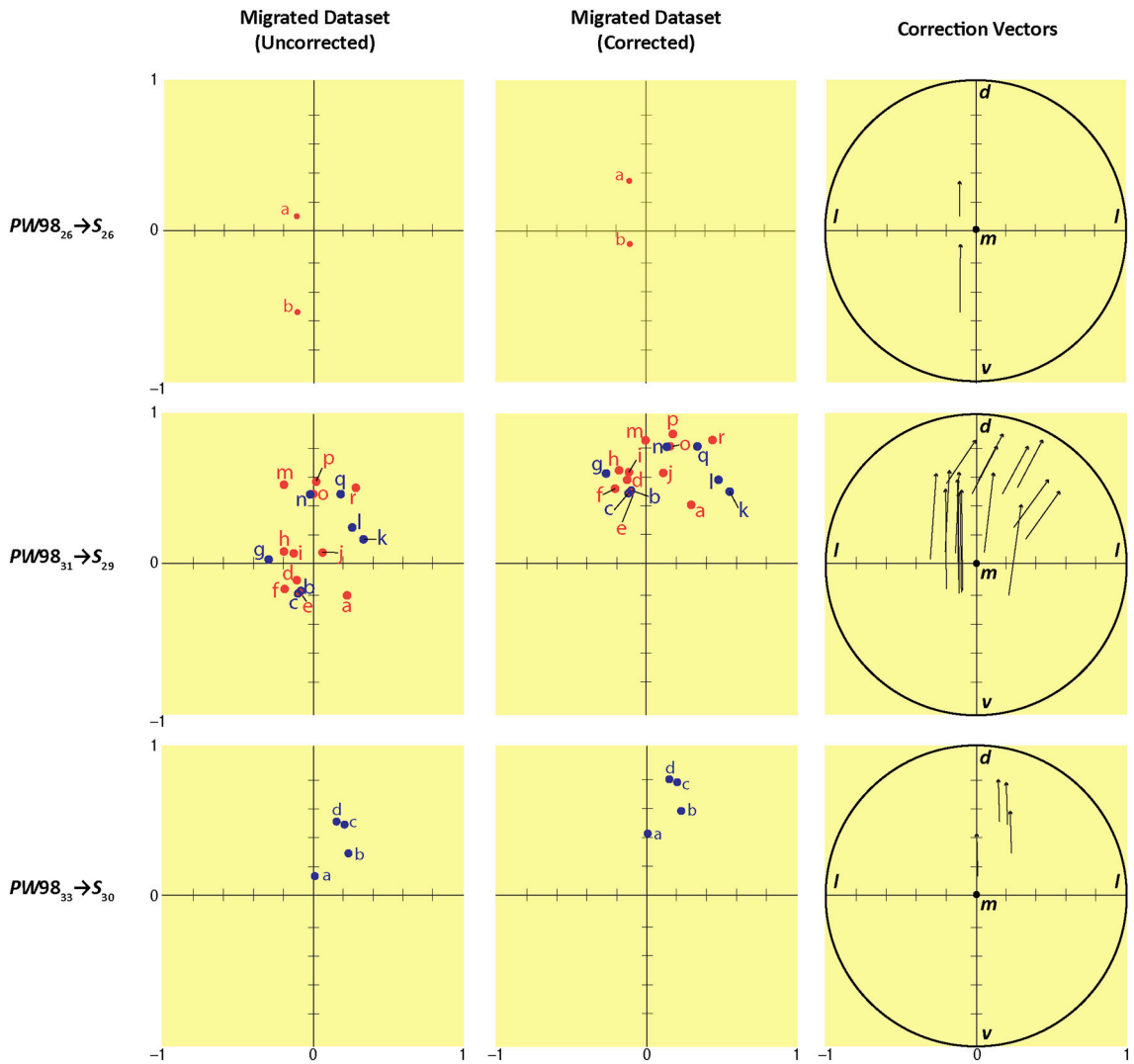

FIGURE 12 | Summary of migration analysis conducted by converting each *S* atlas reference quadrant into a Cartesian plane. Scales on the ordinate and abscissa are in millimeter units. Each row shows the migration from a different source *PW* level to its corresponding (most closely matching) level in *S* space: *PW*98$_{26}$→S_{26} (*top row*), *PW*98$_{31}$→S_{29} (*middle row*), and *PW*98$_{33}$→S_{30} (*bottom row*). The columns show the position of the migrated points in *S* space before (*left column*) and after (*middle column*) expert-guided mapping adjustments and corrections were made. *Right column*: the vectors for each pair of original and relocated points are shown within a unit circle with origin *m* (to denote the medial-most point), mediolateral *x*-axis (*l*, lateral), and dorsoventral *y*-axis (*d*, dorsal; *v*, ventral). Point-source data are shown by red dots, which have been shifted contralaterally; and blue dots, which have not been shifted.

some distortion. Second, within brain imaging and analysis, it has been recognized by the community that for many applications and tools developed to streamline registration of images, ground truth is either unavailable or difficult to come by (Prastawa et al., 2005; Bouix et al., 2007; Stevenson et al., 2014). This is due to a variety of reasons, including the difficulty in applying a consistent set of standard operating procedures across the highly variable parts of the brain to permit accurate inter-rater reliability scores that can serve as ground truth. We have experienced such challenges firsthand when attempting to create a basic standard for evaluating neuroanatomical datasets for animal taxa for which poor documentation currently exists (Hughes et al., 2016). A related issue is that the aforementioned 1.6 mm discrepancy that we observed between the craniometric and computer vision-derived values may not be a true "error," since this assumes that Bregma values serve as ground truth, an assumption that is not always valid but which depends on the types of comparisons being made (e.g., see Richard et al., 2014; Rangarajan et al., 2016). Third, despite the efficacy of our algorithm, there may be an upper limit to its ability to produce accurate matches that is governed by variability between the *PW* and *S* Nissl datasets themselves. As Simmons and Swanson (2009) describe in detail, the major sources of error that investigators encounter when comparing histological data between two brains include intrinsic variability, linear distortion, non-linear distortion, plane of section, and sampling error (see their **Figure 1**). The processes

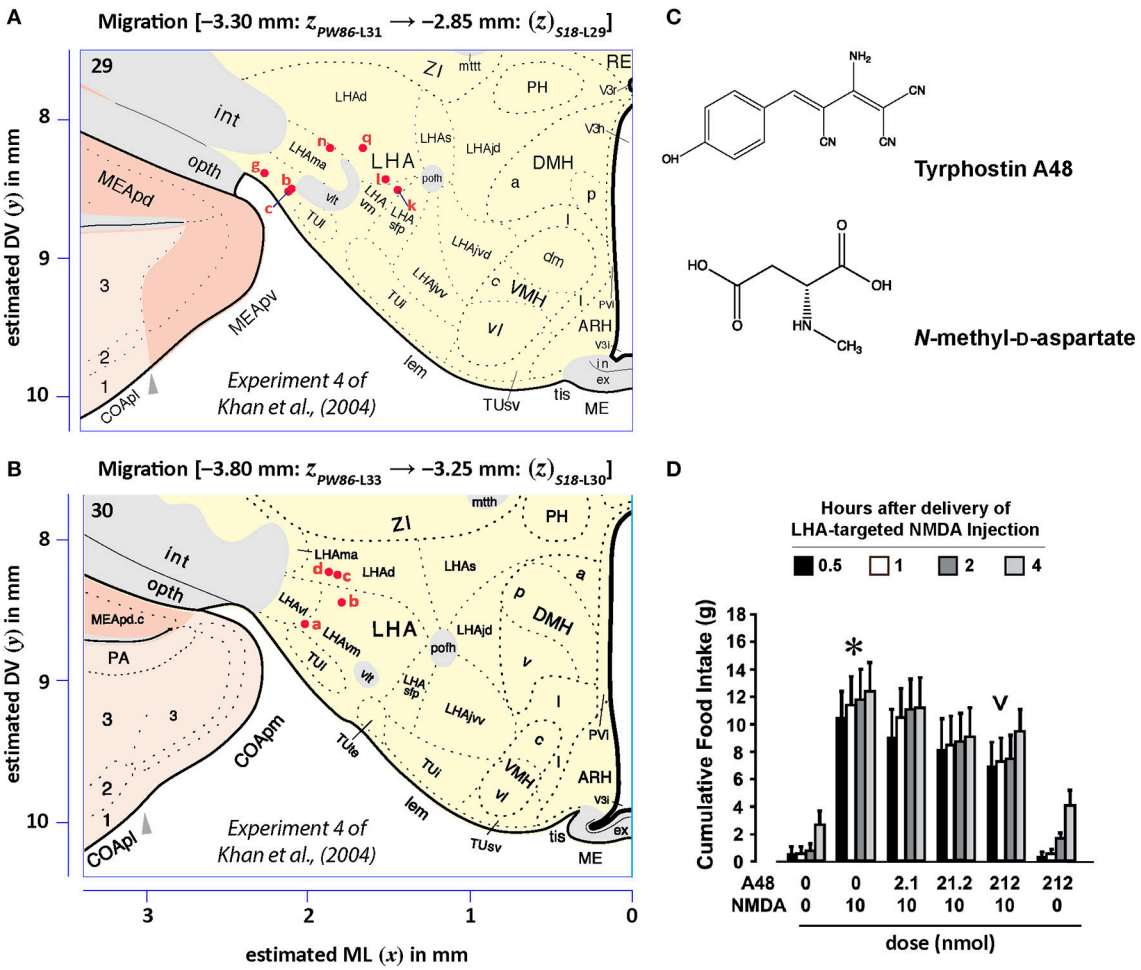

FIGURE 13 | Migrated data coded by behavioral experiment. The injection cases involving *Experiment 4* of Khan et al. (2004) are shown in their final migrated states. In **(A)**, the injection sites migrated from Paxinos and Watson (1986) (*PW86*), Level 31, are shown in their host reference space [(Swanson, 2018) (*S18*); Level 29]. In **(B)** the injection sites migrated from *PW86*, Level 33, are shown in *S18*, Level 30. The scales flanking these panels mark the estimated stereotaxic coordinates in the mediolateral (*x*) and dorsoventral (*y*) dimensions, derived from *PW86* and encoded within *S18*. The *x*-axis scale applies to both **(A,B)**. Note that expert-guided intervention was required to make adjustments of the injection sites to their final locations, and thus the plotted points should be considered as first-order approximations of the actual injection sites. The letters denoting each site refer to case numbers, which are found for the corresponding levels in **Table 7** for the "PW_{31}➔S_{29}" migration **(A)** and "PW_{33}➔S_{30}" **(B)**. **(C)** Structures of the reagents injected: the protein tyrosine kinase inhibitor, Tyrphostin A48 (A48), and the glutamate receptor agonist, *N*-methyl-D-aspartate (NMDA). **(D)** The behavioral results, adapted from Khan et al. (2004), associated with the injection sites mapped in A and B. Two injections were delivered to each site, 10 min apart, with A48 injected before NMDA. All injection volumes were 300 nl, containing the doses of the reagents as indicated. The asterisk marks significant overall cumulative food intake triggered by NMDA injection relative to vehicle injection, and the inverted carat denotes significant suppression of NMDA-elicited eating at the highest dose of A48 tested ($P < 0.5$). See Khan et al. (2004) for details. Permission to reproduce the data in **D** from Khan et al. (2004) has been provided under the permissions policy of *The Journal of Neuroscience*. Levels 29 and 30 from *S18* are reproduced from Swanson (2018) under the conditions of a Creative Commons BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/legalcode). For an explanation of the abbreviations on this figure, please see the List of Abbreviations, Swanson Nomenclature.

utilized to generate the Nissl-stained tissue sets for the *PW* and *S* reference spaces were not immune to these types of error, the extent of which will also vary between the two sets of tissue. Finally, the conclusions we draw about the reliability of craniometric alignments must necessarily be constrained by the limited number of atlas levels we evaluated in this study. A near-term goal for expanding this work would be to design and execute a large-scale test to draw statistically valid conclusions about the behavior of the algorithm, hopefully attaining a degree of agreement with an expert that is similar to the agreement between two different experts. Such an effort likely could also benefit from using deep learning approaches (Plis et al., 2014), including deep autoencoders and generative adversarial networks (Goodfellow et al., 2014), as have been applied to human brain MRI image data (e.g., Chen et al., 2016; Moeskops et al., 2017).

Data Migration

In addition to determining the reliability of a basic anteroposterior alignment of the reference spaces, we also

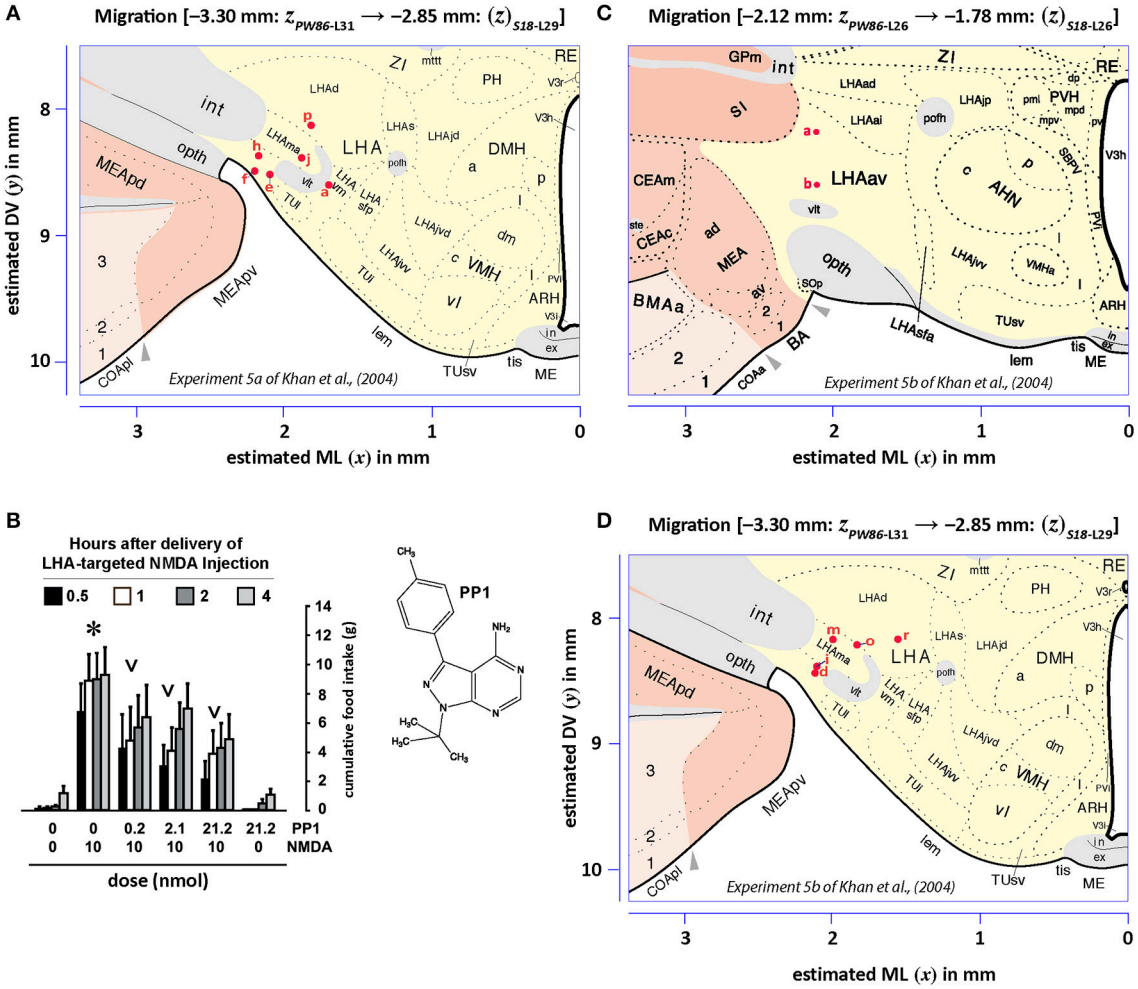

FIGURE 14 | Migrated data coded by behavioral experiment. The injection cases involving *Experiment 5a* and *Experiment 5b* of Khan et al. (2004) are shown in their final migrated states. In **(A)**, the injection sites for *Experiment 5a*, migrated from Paxinos and Watson (1986) (*PW86*), Level 31, are shown in their host reference space (Swanson, 2018 (*S18*); Level 29). In **(B)**, the behavioral data, adapted from Khan et al. (2004), are shown that are associated with the injection sites in **(A)**, along with the chemical structure of the protein tyrosine kinase inhibitor, PP1. Two injections were delivered to each site, 10 min apart, with PP1 injected before NMDA. All injection volumes were 300 nl, containing the doses of the reagents as indicated. The asterisk denotes significantly greater cumulative food intake overall relative to vehicle controls, and the inverted carats denote significant suppression of cumulative food intake relative to NMDA-elicited eating ($P < 0.5$). See Khan et al. (2004) for details. In **(C,D)**, the injection sites for *Experiment 5b*—and for an associated experiment not reported in Khan et al. (2004) (see cases marked with an asterisk in **Table 7**)—migrated from *PW86*, Level 26 and 31; are shown in *S18*, Level 26 and 29, respectively. Associated behavioral results for this experiment are not shown here, but can be found in the descriptive narrative of the Results section in Khan et al. (2004). The scales flanking these panels mark the estimated stereotaxic coordinates in the mediolateral (*x*) and dorsoventral (*y*) dimensions, derived from *PW86* and encoded within *S18*. The *x*-axis scale in **(A)** applies to both **(A,B)**. Note that expert-guided intervention was required to make adjustments of the injection sites to their final locations, and thus the plotted points should be considered as first-order approximations of the actual injection sites. The letters denoting each site refer to case numbers, which are found for the corresponding levels in **Table 7** for the "PW_{31}➔S_{29}" migration **(A)** and "PW_{26}➔S_{26}" and "PW_{31}➔S_{29}" migrations **(C,D)**. Permission to reproduce the data in **B** from Khan et al. (2004) has been provided under the permissions policy of *The Journal of Neuroscience*. Levels 26 and 29 from *S18* are reproduced from Swanson (2018) under the conditions of a Creative Commons BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/legalcode). For an explanation of the abbreviations on this figure, please see the List of Abbreviations, Swanson Nomenclature.

evaluated the feasibility of migrating the data representing central microinjection sites between the atlas spaces in the mediolateral and dorsoventral dimensions. Importantly, we sought to ascertain whether subject matter expertise was required for corrections to be made to the basic approach of anisotropically scaling the atlas levels and migrating the data based on stereotaxic coordinates alone. It is striking that all major deviations across the three pairs of *source level*: *destination level* migrations between the reference spaces were in the DV dimension. Although it remains unclear to what extent this trend generalizes for data migrated across all of the registerable levels for *PW* and *S* spaces, its recurrence in our data suggests that this deviation may be a systematic rather than random error when migrating point-source data between the spaces.

While further pairings and analyses are required to support this suggestion, a few possible reasons for this potentially consistent deviation are worth noting here. First, the histological to graphical transformation of the original injection sites, which required the use of a projection microscope, may have produced a distortion in the DV representation of the sites with respect to the size-matched figure of *PW*86 onto which they were projected. If this is the case, then the error may be peculiar to our dataset alone. Second, Swanson (1992) notes that there was a non-linear shrinkage of the tissue for the atlas brain used for *S* space in relation to that used for *PW* space; this shrinkage was greater in the DV dimension and may have been related to compression of the brain along the DV axis during sectioning. Moreover, *PW* space defines the DV zero point as a line tangent to the dorsal surface of the skull, a measure that was not obtained directly for *S* reference space. Some or all of these factors may have contributed to producing slight deviations from the grid of adapted *PW* coordinates designed to fit onto *S* atlas maps.

Significance of Data Migration to Behavioral Studies of the Hypothalamus

In the present study, we migrated unpublished central microinjection sites for a published behavioral study (Khan et al., 2004) from *PW* reference space to *S* reference space. For the benefit of the behavioral neuroscience domain experts, a few points about that study are noted here. Stanley et al. (1993a,b) reported that glutamate or its ionotropic receptor agonists, kainic acid, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), or *N*-methyl-D-aspartate (NMDA); could trigger eating when delivered by microinjection into the rat lateral hypothalamus. Moreover, glutamate receptor antagonists delivered into this region can suppress not only glutamate receptor agonist-elicited feeding, but also feeding triggered by an overnight fast or that triggered by the onset of the nocturnal cycle (Stanley et al., 1996). These results suggested that glutamate is a powerful endogenous controller of food intake. Prompted by reports that protein tyrosine kinases can regulate NMDA receptor function (Wang and Salter, 1994), one of us (AMK) began exploring the possibility that protein tyrosine kinase inhibitors (PTKIs) could alter the food intake triggered by NMDA receptor activation in the LHA. This was later demonstrated in Khan et al. (2004); also see Khan (2002). Specifically, the PTKIs, Tyrphostin A48 (a broad spectrum inhibitor; "*Experiment 4*" in Khan et al., 2004) and PP1 (an inhibitor of Src family tyrosine kinases; "*Experiment 5a*" and "*Experiment 5b*" in Khan et al., 2004), were able to suppress NMDA-elicited eating in a dose-dependent manner (the relevant data from these experiments are reproduced in **Figures 13D**, **14B**).

The data migration we performed in the present study revealed that sites where the PTKIs and NMDA were injected to influence food intake fell within the LHAav, LHAd, LHAma, LHAvm, and an area near the TUl. These regions appear to correspond to the general expanse of the LHA where Src family tyrosine kinases are reportedly expressed (Hirano et al., 1988; Ross et al., 1988; Walaas et al., 1988; Sugrue et al., 1990), and where NMDA receptor subunits are also expressed (Khan et al., 2000). A benefit of this migration is that these behavioral results can be contextualized with other datasets mapped in the same reference space. For example, it remains unclear which cell types within the LHA subregions just described are the substrates that respond to NMDA injections to help mediate the feeding response (or to PTKI inhibitors to suppress this response). Interestingly, several cell types have been mapped in *S* space within these same subregions, including neurons that express the neuropeptides hypocretin/orexin, melanin-concentrating hormone, and neurotensin (Swanson et al., 2005; Watts and Sanchez-Watts, 2007; Hahn, 2010). Thus, migrating the injection sites into a common space where other datasets are mapped allows us to develop new data-constrained hypotheses; in this case, about the possible cell types that might mediate the behavioral effects that we have reported previously. In contrast, many elegant studies that have reported the effects of glutamate microinjections into the LHA remain difficult to contextualize precisely with our current data in the same fashion, since they were not mapped to a reference atlas, yet contain very valuable spatial data that are effectively trapped within the study (e.g., Allen and Cechetto, 1993; Li et al., 2011). An important area for future expansion of brain atlas-based data migration efforts would be devising strategies to migrate and code trapped legacy datasets into extant reference spaces with graphical annotations of their relative positional uncertainty in relation to more precisely mapped datasets, assuming that such efforts are even feasible (also see section 4.6 in Khan, 2013).

Toward Formalizing Data Migration Between Stereotaxic Reference Atlas Spaces: Basic Steps for Neuroscientists

Data Migration as a Component of Formal Scientific Transcription

In this study, we demonstrate the stepwise transformation, from tissue section to atlas map, of unpublished hypothalamic injection sites to mapped data points across two distinct and widely used atlas reference spaces for the rat brain (*PW* and *S*). The metamorphosis of a 3-D material object (brain) into a 2-D diagram (map) exemplifies how scientists are frequently engaged in a form of literary "inscription" (which we extend here to also include "transcription"), an idea based on Derrida (1967) and first furnished by Latour and Woolgar (1986) after exploring how thyrotropin-releasing hormone was isolated and assayed from sheep hypothalamic extracts by Guillemin and colleagues (Guillemin and Lemke, 2013). Their sociological study marked the beginnings of treating data transformations in the neurosciences as a formal inscription process, and we emphasize this process here to underscore the importance of establishing a formal laboratory procedure for transforming and migrating spatial datasets between animal brain atlases, complementing efforts now underway, for example, to render human brain imaging datasets interoperable to permit large-scale neurogenomics studies (Medland et al., 2014). As Latour and Woolgar (1986) also argue, the process of inscription is essential in the construction of facts from initial conceptualizations.

Accordingly, in the interest of providing a basic operating procedure that can be deployed in most laboratories seeking to migrate various kinds of spatial datasets between *PW* and *S* or vice versa, we offer here a précis of steps generalized from the transformations described in the Methods. For this purpose, we assume that the data to be migrated already exist in published form in one of the two reference spaces. The steps are illustrated here for *PW* to *S* migration, but apply just as readily in the reverse direction. If behavioral neuroscientists keep these steps in mind in relation to their own spatial datasets, they can utilize them to create their own best practice in the lab, compare newer results with those found in previous studies, and/or interrelate data mapped in *PW* reference space with those mapped in *S* space or vice versa.

Basic Steps for Data Migration

Source:destination alignment

The enterprising behavioral neuroscientist should begin by consulting **Figure 2** to identify levels in *S* that correspond to the *PW* levels containing their mapped data. Either there will be *S* levels that match or are closely in register with the relevant *PW* levels by Bregma coordinate, or there will not be. If there are matching levels, the investigator should examine the *S* (destination) levels alongside the *PW* (source) levels, either using physical atlases or the atlas .ai files. The *S* and *PW* levels should appear closely similar based on the structures they contain—due to plane of section differences, etc., it is possible that only a portion of the levels will actually correspond by structure. To proceed with the migration it is important that the regions (for instance, the hypothalamus) of the *PW* levels wherein the mapped data reside should be structurally similar to the same regions in the *S* levels. When assessing structural correspondence, it is wise to give greater consideration to structures that are easily seen in a Nissl stain; whereas structures recognized only by *S* or only by *PW* should be given a lesser weight in the analysis. If the levels are similar in the regions containing data, the neuroscientist may proceed to the steps described below, in *Preparation of the AI Environment*.

If the levels are dissimilar by structure in the data-bearing regions, or if matching levels could not be identified in **Figure 2**, the neuroscientist is presented with a set of choices. The migration can be abandoned, dissimilar regions can be migrated across despite their differences, or the neuroscientist can explore adjacent *S* levels to identify candidates that do match *PW* by structure in the pertinent regions. For this latter goal, a preliminary analysis of the **Figure 2** dot plots, examining how *PW* and *S* levels may correspond based on structural features rather than Bregma coordinates, has been furnished by one of us (Wells, 2017) and may be of use. However, this fiducial analysis awaits validation and further refinement.

Preparation of the AI environment

Having identified the *S* levels that match with relevant *PW* levels, the neuroscientist will at this point require access to the .ai files of those levels. These files, at the time of this writing, are available in a few ways. First, *PW* files are downloadable from the publisher's website if the user has purchased a print edition of the atlas, and older CD-ROM-formatted files are available with older *PW* atlas editions. Second, *S* files are now available as open access files for the *S92*, *S98,* and *S04* editions from https://larrywswanson.com; *S18* files are available in Swanson (2018) as .pdf open access files which can be opened in Adobe Illustrator (AI) software (at the time of this writing, the latest version is AI Creative Cloud).

For any given set of matching levels the following file set-up operations should be performed. In the .ai file for the *PW* level, vector objects associated with the drawing of the *PW* level itself and with its coordinate grid should be grouped and copied into a new .ai file. Similarly, in the .ai file for the *S* level, vector-objects associated with the drawing of the *S* level itself and with its coordinate grid should be grouped and copied into a new layer within the new .ai file. This gives an .ai file containing both the *PW* (source) and *S* (destination) levels, neatly separated into different layers.

However, the levels will at this point be sized differentially. The simplest means of rectifying this is to scale the *S* level, by selecting it and dragging the selection edges, so that its coordinate grid aligns with the *PW* grid—the neuroscientist must take care that the *S* level vectors are grouped before attempting this scaling. The *Zoom* function may be used to increase the accuracy of scaling. It will be necessary to scale *S* independently in the *x*-axis and *y*-axis (i.e., anisotropically). Because *S* uses a derived stereotaxic coordinate system and because the atlas brain used as the basis for *S* was subject to more distorting manipulations (celloidin embedding) than the atlas brains used for *PW*, it is more reasonable to scale *S* to *PW* than vice versa—however, doing so would not be inherently incorrect. The *S* (destination) layer should now be hidden by clicking the *visibility ("eye") toggle* in the *Layers* window.

Now the mapped data must be imported into the .ai file, using the *Place* command. Assuming it is in a composite image format (i.e., both the mapped data and the underlying map or portion of the map are in the same image), it should be placed into a new layer underneath the *PW* layer, and then aligned and scaled to fit the *PW* level. If there are slight distortions in the image of the map, the image should be aligned so that it matches the *PW* level well in the region where the data occur. The data must then be rendered into vector format, again in a new layer. If the data are point-source, the *Circle Tool* may be used (while holding the SHIFT key) to create a circle that is then scaled as appropriate and copied repeatedly, and the copies superimposed over the data. If the data are two-dimensional, then either the neuroscientist can choose to represent them in point-source form, or the *Pencil Tool* may be used to trace them—vectors drawn using the *Pencil Tool* can be edited and readjusted until the fit is correct. While this migration method will be more successful using point-source data, 2-D data migration may be attempted as well with some reduction in accuracy. (Note that we have not yet validated the migration of 2-D data, but only point-source data at this time). The original map image should now be hidden by toggling off its layer's visibility.

Having followed these steps, the neuroscientist possesses a series of .ai files, one for each set of matching *PW* and *S* levels. Each file contains four layers. The *S* and *PW* levels are aligned by their stereotaxic grids, the original mapped data are aligned with

the *PW* level, and a vector representation of the data appears over the original. Migration can begin.

Migration

At this point, migrating the data based solely on stereotaxic coordinates is as simple as toggling off the visibility on the *PW* (source) level and the original map image, and toggling on the visibility on the *S* (destination) level, in each .ai file in the series. However, it will probably be necessary to take additional steps to account for finer differences in the exact disposition of structures between the *PW* and *S* levels.

Refinement

The neuroscientist should toggle on visibility to the *PW* level and study the relationship between the vector-formatted mapped data and nearby structures on *PW*, paying particular attention to any structures that are easily identified in Nissl-stained material, and paying less attention to any structures that are recognized only by *PW*. Then the visibility should be toggled off for *PW* and on for *S*, and the relationship between the data and nearby structures on *S* examined, noting any differences. This process should be repeated until the neuroscientist has a reasonable idea of the differences between the data's relationship to *PW* structures and their relationship to *S* structures. The original map image can be referenced for enhanced accuracy in this process.

The neuroscientist should then nudge the vector-formatted data until their positions vis-à-vis *S* structures matches their original positions vis-à-vis *PW* structures—the map image will be crucial here for assessing the original positions on *PW*. If the data are point-source, each vector circle should be nudged singly (unless some of the circles are very closely clustered; such clusters may be nudged as a group). **Figures 9–11** may be referenced for an example of this process, which will be highly individualized for each set of mapped data migrated. If the data are two-dimensional and not represented as point-source simplifications, it may well be necessary to scale them in addition to nudging. Here problems may arise: increasing the accuracy of the positioning of the migrated data may require significantly distorting their original shape, and increases in positional accuracy in one region of *S* may come only at the price of decreases in positional accuracy in another region. Resolving these difficulties would require the exercise of professional judgment regarding where accuracy may be sacrificed on a case-by-case basis. It is recommended that any and all transformations made to the data during migration be documented thoroughly.

FUTURE DIRECTIONS

Having described the basic steps of data migration, it is useful to consider the benefits of such a procedure in relation to the references spaces themselves. One benefit of registering reference spaces is that other brain atlases may already be registered to one or more of these spaces. For example, Leergaard et al. (2003) registered their manganese-enhanced MRI datasets with a digital 3-D reconstruction of Swanson reference space. A number of investigators have registered rat MRI or fMRI data with a Paxinos and Watson reference space (Schweinhardt et al., 2003; Schwarz et al., 2006; Lu et al., 2010; Johnson et al., 2012; Wisner et al., 2016). A new reference space for the mouse brain has been created that allows for interoperability among various online mouse brain resources within a common framework. Called *Waxholm Space* ("*WHS*"; Johnson et al., 2010; Bowden et al., 2011; Hawrylycz et al., 2011), *WHS* has also been created recently for the adult male Sprague-Dawley rat (Papp et al., 2014). The registration of multiple atlases with one another allows for greater interoperability between datasets (Toga and Thompson, 2001). This form of *model-to-model registration* (Zitová and Flusser, 2003) serves to ensure the lasting preservation and more widespread use of the hard-earned datasets produced from time- and labor-intensive experiments. Developing more robust pipelines to enable migration of mapped data across multiple reference systems will be a valuable means to further integrate the work of numerous investigators. One challenge that still remains is to enable data migration across reference spaces in a manner that takes into account differences in the boundary conditions of brain sub-regions in each reference space and across different scales (e.g., see Martone et al., 2008; Bohland et al., 2009).

A related challenge is the task of registering datasets in *PW* or *S* reference spaces, which are based on a "flat skull" orientation (i.e., no DV difference in the positions of a probe touching the Bregma or Lambda suture intersections), with older reference spaces such as the widely used de Groot rat atlases of the rat forebrain (de Groot, 1959a) and hypothalamus (de Groot, 1959b), and those of the rat brain by Pellegrino and colleagues (Pellegrino and Cushman, 1967; Pellegrino et al., 1979). These atlases utilized brains that were tilted in the stereotaxic frame to create a horizontal plane through the anterior and posterior commissures (+5.0 mm above the interaural line). The transverse plane atlas maps produced by this latter orientation differ markedly in the regional and sub-regional cytoarchitectonic boundaries they contain from those produced using a flat skull reference plane. A future expansion of the efforts set forth here could be to utilize computational methods to bring these atlases in register with *PW* and *S*, so data from valuable studies utilizing these reference spaces (e.g., see Chiappa et al., 1977) can be contextualized with *PW* and *S* datasets.

CONCLUDING REMARKS

In this study, we have demonstrated the first-order alignment and migration of point-source data consisting of central microinjection sites in the hypothalamus to the *S* atlas space from the *PW* space in three dimensions. It is anticipated that the approach for data migration outlined in this study will be useful to neuroscientists seeking to contextualize their datasets in these reference spaces with one another to generate new insights about structure-function relations in the brain. It should also prove useful as a starting point toward further work in atlas-based registration of experimental tissue.

AUTHOR CONTRIBUTIONS

AK and OF conceived of this project, with AK supervising its neuroanatomy component and OF supervising the computer

vision component. AK constructed the alignment tool and Cleveland plots, and performed the data migration analysis. JP developed the computer vision algorithm and performed the experiments to test its efficacy, with guidance from OF and AK. CW conducted the transformation and migration of the central microinjection data, with guidance from AK. OF wrote the pseudocode in **Figure 1**, with feedback from JP. AK wrote the manuscript, with contributions from all of the authors.

FUNDING

This project was supported by grants awarded to AK from the National Institutes of Health (DK08197 and GM109817) and from the UTEP Office of Research and Sponsored Projects (Grand Challenges Solicitation Award). Publication of this study has been supported by funds awarded to the Border Biomedical Research Center by the National Institute of Minority Health and Health Disparities (5G12MD007592). JP is supported by the BUILDing SCHOLARS program at UTEP (funded by the National Institute of General Medical Sciences of the National Institutes of Health under linked Award Numbers RL5GM118969, TL4GM118971, and UL1GM118970; PI: L. Echegoyen). CW has been supported by the UTEP SMARTS program funded by the National Science Foundation (DUE-1153832; PI: R. Aguilera, Co-PI: L. Echegoyen) and the UTEP PERSIST program funded by the Howard Hughes Medical Institute (PI: S. Aley; Co-PIs: L. Echegoyen, A. Khan, D. Villagrán, E. Greenbaum).

ACKNOWLEDGMENTS

We thank Dr. B. Glenn Stanley for providing access to original tissue slides. We are also grateful to Omid Hamzeinejad, Jennifer Ayonon, Jenel C. Lim, and Andrew Nguyen for assistance with tissue processing and injection site drawings at the University of California at Riverside. We thank Larry W. Swanson (University of Southern California, USC) for his feedback concerning our general data migration approach and for his helpful suggestions for framing our narrative. We also thank Dr. Sabiha Khan (UTEP) and Dr. Richard H. Thompson (USC) for stimulating discussions concerning this project and/or for also providing valuable feedback on an earlier draft of this manuscript. This interdisciplinary collaboration developed from a lecture delivered on 20 Mar 2015 by AK at the colloquium series of the UTEP Department of Computer Science: Deciphering the brain mapping enigma: Do we need a new Bletchley Park? The authors are grateful to Dr. Ann Q. Gates and Dr. Nigel G. Ward (both at UTEP) for arranging this lecture.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

LIST OF ABBREVIATIONS

Paxinos and Watson Nomenclature

3V, third ventricle; A11, dopamine cells; ACo, anterior cortical amygdaloid nucleus; AHA, anterior hypothalamic area, anterior part; Arc, arcuate hypothalamic nucleus; BAOT, bed nucleus of the accessory olfactory tract; DA, dorsal hypothalamic area; DM, dorsomedial hypothalamic nucleus; DMD, dorsomedial hypothalamic nucleus, dorsal part; F, nucleus of the fields of Forel; ic, internal capsule; LGP, lateral globus pallidus; LH, lateral hypothalamic area; MCLH, magnocellular nucleus of the lateral hypothalamus; MeAD, medial amygdaloid nucleus, anterior dorsal part; MEE, medial eminence, external layer; MEI, medial eminence, internal layer; MePD, medial amygdaloid nucleus, posterodorsal part; MePV, medial amygdaloid nucleus, posteroventral part; mfb, medial forebrain bundle; MGP, medial globus pallidus; mt, mammillothalamic tract; MTu, medial tuberal nucleus; opt, optic tract; Pa, paraventricular hypothalamic nucleus; Pe, periventricular hypothalamic nucleus; PeF, perifornical nucleus; PH, posterior hypothalamic nucleus; RCH, retrochiasmatic area; Re, reuniens thalamic nucleus; Rt, reticular thalamic nucleus; SI, substantia innominata; SO, supraoptic nucleus; SOR, supraoptic nucleus, retrochiasmatic part; sox, supraoptic decussation; SubI, subincertal nucleus; SubV, submedius thalamic nucleus, ventral part; TC, tuber cinereum area; Te, terete hypothalamic nucleus; VM, ventromedial thalamic nucleus; VMH, ventromedial hypothalamic nucleus; VRe, ventral reuniens thalamic nucleus; ZI, zona incerta; ZIV, zona incerta, ventral part.

Swanson Nomenclature

The nomenclature system here, based on Swanson (2015), consists of *standard term names*, followed by the last name of the author and the year in which the *standard term name* was first used *(author, date)*. Thus, a ***standard term*** is defined as *standard term name + (author, date)*. Terms named after 1840, but where the first use of the term has not been traced, are indicated as "*standard term name (>1840)*" to note this fact. A full listing of the sources of the *(author, date)* portions of each ***standard term*** can be found in the References, but note that these specific sources were not necessarily consulted directly by us, but are cited in Swanson (2015, 2018).

AHN, ***anterior hypothalamic nucleus (>1840)***; ARH, ***arcuate hypothalamic nucleus (>1840)***; BA, ***bed nucleus of accessory olfactory tract (Scalia and Winans, 1975)***; COAa, ***cortical amygdalar area anterior part (>1840)***; COApm, ***cortical amygdalar area posterior part medial zone (>1840)***; cpd, ***cerebral peduncle (Tarin, 1753); DMH, dorsomedial hypothalamic nucleus (>1840)***; em, ***external medullary lamina (>1840)***; I, ***internuclear hypothalamic area (Swanson, 2004)***; int, ***internal capsule (Burdach, 1822)***; LHA, ***lateral hypothalamic area (Nissl, 1913)***; LHAad, ***lateral hypothalamic area anterior group anterior region dorsal zone (Swanson, 2004)***; LHAav, ***lateral hypothalamic area anterior group anterior region ventral zone (Swanson, 2004)***; LHAjp, ***lateral hypothalamic area middle group medial tier juxtaparaventricular region (Swanson, 2004)***; LHAsfa, ***lateral hypothalamic area middle group perifornical tier subfornical region anterior zone (Swanson, 2004)***; ME, ***median eminence (Tilney, 1936); MEAad, medial amygdalar nucleus anterodorsal part (>1840)***; MEApd, ***medial amygdalar nucleus posterodorsal part (>1840)***; MEApv, ***medial amygdalar nucleus posteroventral part (>1840)***; mtt, ***mammillothalamic tract (Kölliker, 1896)***; opth, ***hypothalamic optic tract (Swanson, 2015)***; PH, ***posterior hypothalamic nucleus (>1840)***; pofh, ***hypothalamic postcommissural fornix (Swanson, 2015)***; PR, ***perireuniens nucleus (Brittain, 1988)***; PVH, ***paraventricular hypothalamic nucleus (>1840)***; PVi, ***periventricular hypothalamic nucleus anterior part intermediate zone (Swanson, 2018)***; RE, ***nucleus reuniens (Malone, 1910)***; SBPV, ***subparaventricular zone (Watts et al., 1987)***; SI, ***innominate substance (Schwalbe, 1881)***; SOp, ***supraoptic nucleus principal part (Swanson, 2018)***; ste, ***endbrain terminal stria (Swanson, 2015)***; suph, ***hypothalamic supraoptic decussations (Swanson, 2018)***; TUi, ***lateral hypothalamic area middle group lateral tier tuberal nucleus intermediate part (Swanson, 2004)***; TUsv, ***lateral hypothalamic area middle group lateral tier tuberal nucleus subventromedial part (Swanson, 2018)***; V3h, ***hypothalamic part of third ventricle principal part (Swanson, 2015)***; vlt, ***ventrolateral hypothalamic tract (Swanson, 2004)***; VM, ***ventral medial thalamic nucleus (>1840)***; VMH, ***ventromedial hypothalamic nucleus (>1840)***; ZI, ***zona incerta (>1840)***.

frontiers in Systems Neuroscience

MINI REVIEW
published: 16 April 2018
doi: 10.3389/fnsys.2018.00014

Lateral Hypothalamus as a Motivation-Cognition Interface in the Control of Feeding Behavior

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Converging evidence for an essential function of the lateral hypothalamus (LHA) in the control of feeding behavior has been accumulating since the classic work conducted almost 80 years ago. The LHA is also important in reward and reinforcement processes and behavioral state control. A unifying function for the LHA across these processes has not been fully established. Nonetheless, it is considered to integrate motivation with behavior. More recent work has demonstrated that the LHA is also required when cognitive processes, such as associative learning and memory control feeding behavior, suggesting it may serve as a motivation-cognition interface. Structurally, the LHA is well positioned within the cerebral hemisphere, with its extensive connectional network across the forebrain-brainstem axis, to link motivational and behavioral systems with cognitive processes. Studies that examined how learned cues control food seeking and consumption have implicated the LHA, but due to methodological limitations could not determine whether it underlies motivation, learning, or the integration of these processes. Furthermore, the identification of specific substrates has been limited by the LHA's extraordinary complexity and heterogeneity. Recent methodological advancements with chemo-and opto-genetic approaches have enabled unprecedented specificity in interrogations of distinct neurons and their pathways in behaving animals, including manipulations during temporally distinct events. These approaches have revealed novel insights about the LHA structure and function. Recent findings that the GABA LHA neurons control feeding and food-reward learning and memory will be reviewed together with past work within the context of the LHA function as an interface between cognition and motivation.

Keywords: lateral hypothalamus, learning, memory, motivation, cognition, feeding, circuitry

OPEN ACCESS

Edited by:
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Reviewed by:
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University of Franche-Comté, France
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Received: *25 January 2018*
Accepted: *28 March 2018*
Published: *16 April 2018*

Citation:
Petrovich GD (2018) Lateral Hypothalamus as a Motivation-Cognition Interface in the Control of Feeding Behavior. Front. Syst. Neurosci. 12:14. doi: 10.3389/fnsys.2018.00014

INTRODUCTION

Learning and motivation are both necessary for the survival of a mammalian organism and their neural substrates have been studied extensively. But these processes have been considered somewhat independently and how they are integrated at a neural circuitry level remains an important area of inquiry (Berridge and Robinson, 2003; Kelley et al., 2005). This mini-review article will outline the evidence that the lateral hypothalamus (lateral hypothalamic area, LHA; Swanson, 2004) functions as an interface between motivation and cognition in the control of feeding behavior. Recent insights gained with advanced methodological approaches will be examined within the context of classic perspectives on the LHA structure and function. The development of opto- and chemo-genetic methods has enabled unprecedented specificity in interrogations of distinct neurons and

their pathways. These methods have been used to manipulate the activity of selective, genetically defined neurons within functional circuits during temporally precise events in behaving animals (Fenno et al., 2011; Sternson and Roth, 2014). Novel insights gained with these approaches, particularly that the GABA LHA neurons control feeding and food-reward learning and memory, will be highlighted.

FROM A FEEDING CENTER TO AN INTEGRATIVE NODE WITHIN A DISTRIBUTED FEEDING NETWORK

A long history of structural and functional evidence supports a critical role of the LHA in the control of feeding behavior and in reward and reinforcement processes (Hoebel and Teitelbaum, 1962; for reviews see Elmquist et al., 1999; Stuber and Wise, 2016). Early work with electrolytic lesions and electrical stimulations identified the LHA as a "feeding center" in the brain (Hetherington and Ranson, 1940; Anand and Brobeck, 1951) and as one of the areas where reinforcing effects of electrical brain self-stimulations were the most potent (Olds, 1958; for a recent review see Stuber and Wise, 2016). Since then, as our understanding of the organization of the neural substrates underlying motivated behaviors has advanced, the concept of "centers" was replaced with neural networks. The LHA is now considered a core node within a distributed feeding network that integrates different feeding drives and motivates behavior accordingly (Stellar, 1954; Swanson, 2000; De Araujo et al., 2006; Berthoud, 2011; Clifton, 2017; Sternson and Eiselt, 2017).

The LHA is considered to mediate motivational processes underling feeding and other goal-directed behaviors necessary for survival, including translating motivation into action (Mogenson et al., 1980; Mahler et al., 2014; Stuber and Wise, 2016). In addition, the LHA may link motivation and cognition and facilitate their bidirectional influences, as a motivation-cognition interface. Early indication that the LHA can motivate learning comes from classic studies that demonstrated that electrical LHA stimulations enhanced responding for food and mastering a T-maze (Coons et al., 1965; Mendelson and Chorover, 1965). More recent work has shown that the LHA is also needed when cognitive cues drive food and drug reward seeking and consumption (Petrovich et al., 2002; Harris et al., 2005). The LHA is well positioned within the cerebral hemisphere to serve as a motivation-cognition interface. It has extensive connections with the brainstem and hypothalamic areas that process physiological and state signals from the body, as well as with the forebrain cognitive and hedonic systems and areas mediating stress and anxiety (recently reviewed in Reppucci and Petrovich, 2016). Through these connections, the LHA could inform cognitive processes based on organism's current physiological and behavioral state, and similarly could influence these states based on the outcome of cognitive processes (e.g., learning and memory). Within this framework, new evidence that the LHA is necessary in learning and memory is discussed next.

THE LHA IN REWARD LEARNING ACQUISITION, MEMORY RECALL AND BEHAVIORAL EXPRESSION

There is now strong support that in addition to the ability to influence learning, the LHA is important when learning and memory, in turn, influence the motivation to seek and consume food. In particular, two models of cognitive motivation to eat, cue-induced feeding and conditioned place preference preparations, have implicated the LHA (Petrovich et al., 2002; Harris et al., 2005). These preparations typically have two phases in order to test the influence of food-associated cues (discrete cues or contextual cues) on feeding behavior (food seeking or consumption). The first phase is learning, when cue-food associations are acquired and the second phase is behavioral expression, when the effects of these learned cues on feeding behavior are tested. The behavioral expression involves memory recall of the cue and subsequent induction of food motivation. At which stage during cognitive motivation acquisition and expression the LHA is necessary was not clear until recently. In a study that established the LHA necessity in cue-induced feeding, all manipulations occurred prior to any training and involved unilateral lesions of the LHA (Petrovich et al., 2002). Thus, that work could not determine whether the LHA is solely mediating the *expression* of cue-induced motivation to eat computed elsewhere within the network or whether it is also critical during the *acquisition* of cue-food associations and recall of that memory.

An indication that the LHA may be critically encoding information during the acquisition of reward learning came from a Fos induction imaging study that mapped forebrain recruitment across different stages of Pavlovian cue-food conditioning in rats (Cole et al., 2015a). The LHA was among a few forebrain areas selectively recruited in the learning group that received cue-food pairings compared to controls that received the cue (tone) and food presentations temporally and spatially separated. Thus, the LHA Fos induction patterns were predictive of its critical function during learning; however, until recently, methodological limitations precluded temporally selective manipulations that could establish causality. An exciting, recent study demonstrated that the LHA is necessary for the acquisition and memory storage of cue-food associations. Sharpe et al. (2017) used optogenetic methods in a novel GAD-Cre rat to selectively manipulate GABA neurons within the LHA during cue-food acquisition in a Pavlovian conditioning task. The optogenetic method allowed temporally selective manipulations during the cue (tone) presentations, while leaving food consumption undisturbed. Learning and memory was assessed by the cue's ability to drive food-seeking behavior (approach to the food receptacle). They showed that optogenetic inhibition of the LHA^{GABA} neurons selectively during cue presentations disrupted the *acquisition* and *memory* of cue-food association.

These findings are consistent with other recent evidence that the LHA^{GABA} neurons are critical in the control of feeding behavior (Jennings et al., 2013; Nieh et al., 2015;

O'Connor et al., 2015) and together suggest that a common substrate may mediate food learning and consumption. However, the LHA^{GABA} neurons are not homogenous and distinct subpopulations appear to mediate different aspects of food motivated behaviors (see "GABA Neurons" section). Additionally, a recent study found that some ORX neurons express GAD1 (Mickelsen et al., 2017), which is important for the interpretation of the above manipulations of GAD-neurons (Sharpe et al., 2017), given the ORX function in learning and motivation (see "ORX Neurons" section). Thus, identifying which subpopulation of the LHA neurons support learning and how they interact with other local neurons is imperative to our understanding of the LHA function. Equally important is identifying which specific inputs control the critical LHA neurons during reward learning and memory and which outputs mediate behavioral control.

THE LHA CONTAINS HETEROGENEOUS REGIONS AND NEURAL SUBSTRATES

The LHA is a large and complex structure and its different regions have distinct functions (Khan, 2013). There are also interspecies differences (e.g., Bittencourt et al., 1998; Chometton et al., 2014, 2016), and therefore the evidence from rodent studies discussed here should be interpreted with caution in regard to the LHA in primates and other species. Classic studies have shown that glutamate receptor agonists and neuropeptide Y (NPY) infusions into the perifornical area of the LHA stimulate feeding (Flood and Morley, 1991; Stanley et al., 1993, 2011), and at least the NPY effects are mediated via enhanced motivation to eat (Flood and Morley, 1991). That area contains neurons that express orexigenic neuropeptides, orexin/hypocretin (ORX) and melanin concentrating hormone (MCH), which when released centrally stimulate feeding behavior (Nahon et al., 1989; Qu et al., 1996; Broberger et al., 1998; Sakurai et al., 1998; de Lecea et al., 1998; Swanson et al., 2005). ORX is also critical for arousal and wakefulness, which is necessary for all goal-directed behaviors (Boutrel et al., 2010; Scammell et al., 2017). An overlapping area is also where reinforcing effects of electrical stimulations (brain stimulation reward) are sensitive to food deprivation and adipose-produced hormone leptin (Abrahamsen et al., 1984; Fulton et al., 2000). Thus, the effects of glutamate receptor agonists and NPY infusions likely mimic naturally occurring release when inputs relaying physiological or cognitive signals drive feeding behavior. Connectional data discussed next support this premise.

The perifornical region, including the suprafornical area for which detailed connections were recently established (Hahn and Swanson, 2010) could receive information regarding the energy balance and short-term, hunger and satiety signals, via inputs from the arcuate nucleus of the hypothalamus (ARH), including NPY neurons. It also receives inputs from other hypothalamic and brainstem areas, including behavioral state systems (e.g., dorsal raphe; Nectow et al., 2017), as well as inputs from the striatum (e.g., nucleus accumbens shell; O'Connor et al., 2015) and pallidum, particularly the bed nuclei of the stria terminalis (BST) and substania innominata (Dong and Swanson, 2004, 2006a,b; Hahn and Swanson, 2010). The ARH NPY neurons also express agouti-related peptide (AgRP) and GABA, which, interestingly, are differently controlling rapid (NPY and GABA) vs. prolonged (AgRP) feeding (Krashes et al., 2013). The perifornical region also receives inputs from cortical areas processing cognitive information, including the medial prefrontal cortex, the basolateral area of the amygdala (BLA) and the hippocampal formation (all presumed to be glutamatergic) and from the paraventricular thalamus (PVT), which is interconnected with these areas (Hahn and Swanson, 2010; Mena et al., 2013; Hsu et al., 2015; Kanoski and Grill, 2015; Sun et al., 2015; Reppucci and Petrovich, 2016). A third type of inputs to this area is from the regions well known for their role in stress and anxiety, the central nucleus of the amygdala (CEA) and BST, and their projecting neurons are GABAergic (Swanson and Petrovich, 1998; Dong et al., 2001; Hahn and Swanson, 2010; Kim et al., 2013; Reppucci and Petrovich, 2016). These parts of the CEA and BST, along with the LHA, are interconnected with the parabrachial nucleus (PB), including the areas that relay pain information (Bernard et al., 1993; Alden et al., 1994; Bester et al., 1997). This is notable, as the CEA and PB have been established in suppression of feeding (Petrovich et al., 2009; Carter et al., 2013; Cai et al., 2014).

How the LHA inputs are integrated and how local neurons are organized to compute specific outputs are fundamental questions that classic methods could not address, because heterogeneous LHA populations of neurons are intermingled. In addition to the ORX and MCH populations, the LHA contains GABAergic and glutamatergic neurons and distinct combinations of various receptors (e.g., leptin; Leinninger, 2011), peptides, and opioids (reviewed in Bonnavion et al., 2016). The burgeoning opto- and chemo-genetic methods, which enable cell-specific interrogations, are beginning to answer these questions, but they are also revealing additional complexity in the LHA organization.

GABA Neurons

Cell-specific manipulations have identified the LHA^{GABA} neurons as a critical substrate in the control of feeding behavior and reward associative learning and memory, as discussed above. Consistent with that notion, activation of the LHA^{GABA} neurons stimulated (Jennings et al., 2013), while their inhibition halted (O'Connor et al., 2015) feeding. However, relevant to the interpretation of these results regarding the LHA^{GABA} physiological function, chemogenetic manipulations found that they mediate non-specific consummatory behaviors directed at food and non-food items (Navarro et al., 2016). Furthermore, the LHA^{GABA} neurons are diverse and do not function in isolation (e.g., Jennings et al., 2015; Mickelsen et al., 2017; Qualls-Creekmore et al., 2017). Additionally, whether they are distributed or localized within a specific area (e.g., perifornical) is not addressed in these studies. Distinct subpopulations of LHA^{GABA} neurons (Vgat-expressing neurons that contain neither MCH or ORX) are believed to mediate food motivation (measured by approach behavior) vs. consumption, based on their distinct activation patterns (Jennings et al., 2015). Furthermore, selective manipulations of the Galanin-expressing

subpopulation ($GABA^{GAL}$) indicated that they mediate food seeking behavior (operant responding for sucrose reward) but not the chow consumption or compulsive locomotion that were observed after stimulation of total LHA^{GABA} neurons (Qualls-Creekmore et al., 2017).

There is also strong indication that different outputs from the LHA^{GABA} neurons mediate different aspects of feeding behavior. Activation of a subset of these neurons that project to the paraventricular nucleus of the hypothalamus stimulated feeding (Wu et al., 2015), while activation of the projections to the ventral tegmental area (VTA) produced more complex results (reviewed in this research topic (Tyree and de Lecea, 2017). Activation of the LHA^{GABA}–VTA neurons increased feeding duration and produced aberrant licking and gnawing behaviors (Nieh et al., 2015). Notably, the LHA $GABA^{GAL}$ neurons do not send direct projections to the VTA, the pathway hypothesized to mediate compulsive locomotion (Qualls-Creekmore et al., 2017). Instead, they innervate ORX neurons (Laque et al., 2015), which are important in behavioral state control and motivation (see "ORX Neurons" section). The results of manipulations of GAD-Cre rat LHA neurons projecting to VTA indicate that pathway conveys information about reward predictions for learning but not for behavioral control (Sharpe et al., 2017). Additional complexity was revealed recently, in that the LHA^{GABA}–VTA pathways can induce feeding or rewarding effects depending on the frequencies of their stimulation (Barbano et al., 2016). Interestingly, it was hypothesized that different behavioral outcomes were mediated by different neurotransmitters released with low and high stimulation frequencies from the same fibers (Barbano et al., 2016).

Glutamatergic Neurons

The glutamatergic (VGlut2-expressing) LHA neurons are critical in feeding (Stamatakis et al., 2016). The BST inhibitory inputs were demonstrated to innervate and suppress these neurons, which initiated consumption (Jennings et al., 2013). Similar to the LHA^{GABA}, glutamatergic LHA neurons are diverse. Notably, most ORX neurons are glutamatergic, while subpopulations of MCH neurons are glutamatergic or GABAergic (reviewed in Bonnavion et al., 2016). Thus, determining how different subpopulations of glutamatergic and GABA LHA neurons are integrated and which local and external inputs control them are important areas of future inquiries. In that regard, anatomical evidence suggests that in addition to the BST, converging inputs from the CEA could provide inhibition of the LHA glutamatergic neurons, including ORX neurons (Swanson and Petrovich, 1998; Reppucci and Petrovich, 2016; also the CEA-CRH neurons can activate ORX neurons; Winsky-Sommerer et al., 2004).

ORX Neurons

The ORX neurons are important for feeding as well as behavioral state control and motivation (de Lecea et al., 1998; Boutrel et al., 2010; Hurley and Johnson, 2014; Mahler et al., 2014; Sakurai, 2014), including arousal associated with changes in energy balance and feeding (Yamanaka et al., 2003; González et al., 2016). Relevant to the proposed LHA function as a motivation-cognition interface, ORX neurons are critical during reward associative learning as well as when learned food cues motivate feeding behavior. It is now well established that ORX neurons are activated in response to discrete and contextual food cues (Harris et al., 2005; Choi et al., 2010; Petrovich et al., 2012; Hassani et al., 2016) and their signaling via type 1 receptor (ORX-R1) mediates cue-induced feeding and operant responding for food (Nair et al., 2008; Borgland et al., 2009; Sharf et al., 2010; Cason and Aston-Jones, 2013; Cole et al., 2015b). They are also recruited during cue-food associative learning (Cole et al., 2015a) and ORX-R1 signaling modulates the acquisition and extinction of cue-food associations (Keefer et al., 2016). Thus, determining how external and local inputs control ORX neurons and how their outputs to different targets (Ho and Berridge, 2013), along with those from other LHA neurons, sum up to coordinate behavior is a pressing area of interest.

MCH Neurons

In addition to its essential function in the homeostatic regulation of food consumption and body weight the MCH is important in reward learning and memory (for reviews see Adamantidis and de Lecea, 2009 and in this topic Diniz and Bittencourt, 2017). The MCH regulates reward-seeking behavior and MCH-Receptor1 signaling is necessary for cue-induced feeding (Sherwood et al., 2015; Sita et al., 2016). Given the widespread distribution of its fibers and receptors, the MCH could affect multiple learning and memory systems (Diniz and Bittencourt, 2017). Notably, anti-MCH infusions in the hippocampal formation affected the latency to seek food in a working-memory spatial task, demonstrating a role in guiding learned behavioral responses (Sita et al., 2016).

INTEGRATIVE PROCESSING ACROSS LHA CIRCUITRY

The integration of food and energy sensory information may occur at multiple sites within the LHA circuitry. The ARH neurons, which are considered to relay energy-related sensory signals, appear to already integrate that information and respond in an anticipatory way (Chen et al., 2015) reviewed in Seeley and Berridge (2015). The activity of orexigenic, AgRP (NPY/GABA) neurons was high in fasted mice, as expected, but it decreased as soon as food was presented and eating began. The opposite was found for the anorexigenic, POMC/CART neurons. If food was removed during a meal, these patterns were reset and AgRP neurons increased activity, while the POMC/CART neurons decreased activity. These patterns suggest that the activity of the ARH neurons is regulated in anticipation of energy gain from a meal and that the incoming sensory information is continuously updated. The ARH and LHA bi-directionally communicate, and thus the LHA could update ARH neurons and guide their responding, in accordance with its proposed role in motivation-cognition integration.

Indeed, it is very likely that incoming sensory and processed cognitive information is continuously updated within the LHA circuitry. In that regard, the LHA may also contribute to the recently revealed circuitry underlying motivational state

(hunger/satiety) control over insular cortex (AI) processing during responding to food cues (Livneh et al., 2017). The LHA is well positioned to communicate across that system, as it is connected with each component of the AgRP-PVT-BLA-AI circuitry.

CONCLUDING REMARKS

Recent technologies, which enabled selective interrogations of specific neurons and their circuitries, have greatly advanced the field. These methods have revealed a novel LHA function in learning and memory and identified cell-specific substrates and their pathways in the control of feeding behavior. They are also revealing another dimension of LHA structural complexity. Future work will require thoughtful synergies across genetic, anatomical, and behavioral approaches to unearth the organization of the LHA structure and how it functions to control feeding and other motivated behaviors.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health, NIDDK (R01DK085721).

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Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Systems Neuroscience

REVIEW
published: 20 December 2017
doi: 10.3389/fnsys.2017.00094

Ventromedial Hypothalamus and the Generation of Aggression

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Aggression is a costly behavior, sometimes with severe consequences including death. Yet aggression is prevalent across animal species ranging from insects to humans, demonstrating its essential role in the survival of individuals and groups. The question of how the brain decides when to generate this costly behavior has intrigued neuroscientists for over a century and has led to the identification of relevant neural substrates. Various lesion and electric stimulation experiments have revealed that the hypothalamus, an ancient structure situated deep in the brain, is essential for expressing aggressive behaviors. More recently, studies using precise circuit manipulation tools have identified a small subnucleus in the medial hypothalamus, the ventrolateral part of the ventromedial hypothalamus (VMHvl), as a key structure for driving both aggression and aggression-seeking behaviors. Here, we provide an updated summary of the evidence that supports a role of the VMHvl in aggressive behaviors. We will consider our recent findings detailing the physiological response properties of populations of VMHvl cells during aggressive behaviors and provide new understanding regarding the role of the VMHvl embedded within the larger whole-brain circuit for social sensation and action.

Keywords: VMHvl, aggression, mouse, neural activity, neuromodulation

OPEN ACCESS

Edited by:
Menno R. Kruk,
Leiden University, Netherlands

Reviewed by:
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University of Groningen, Netherlands
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Received: *15 September 2017*
Accepted: *28 November 2017*
Published: *20 December 2017*

Citation:
Hashikawa Y, Hashikawa K, Falkner AL and Lin D (2017) Ventromedial Hypothalamus and the Generation of Aggression. Front. Syst. Neurosci. 11:94. doi: 10.3389/fnsys.2017.00094

AN ESSENTIAL ROLE FOR THE VMHVL IN AGGRESSIVE BEHAVIORS

Aggression is an innate social behavior essential for resource competition, settling disputes, defense, and protecting kin. It is a prevalent behavior across many species, including humans, and in a variety of species including cats, rats, chickens, and monkeys, electrical stimulation studies have demonstrated a causal role of the medial hypothalamus in expressing aggressive behaviors (Putkonen, 1966; Siegel and Skog, 1970; Lipp and Hunsperger, 1978; Kruk et al., 1979, 1983; Lammers et al., 1988; Siegel and Pott, 1988; Siegel et al., 1999; Halasz et al., 2002; Nelson and Trainor, 2007). Recent studies using more precise functional manipulation tools in mice have identified the ventrolateral part of the ventromedial hypothalamus (VMHvl), a small subnucleus in the medial hypothalamus, as a key region to drive inter-male aggression (**Table 1**). Silencing this area abolishes naturally occurring inter-male attack whereas optogenetic activation of the VMHvl but not its surrounding regions promotes the attack of suboptimal targets, including females and inanimate objects (Lin et al., 2011). The VMHvl is highly enriched in hormone receptors, including estrogen receptor alpha (Esr1) and progesterone receptor (PR) which co-express in nearly 100% of cells. Several converging studies have recently demonstrated that these hormone receptor-expressing cells appear to be key populations for mediating aggression in both males and females

(Yang et al., 2013, 2017; Lee et al., 2014; Hashikawa et al., 2017). Killing PR+ cells or inhibition of the Esr1+ cells suppressed naturally occurring aggression (Yang et al., 2013; Lee et al., 2014; Hashikawa et al., 2017). Conversely, optogenetic activation of the VMHvl Esr1+ cells elicited immediate attack and pharmacogenetic activation of the PR+ cells increases the frequency of attack (Lee et al., 2014; Hashikawa et al., 2017; Yang et al., 2017). Furthermore, the aggression-promoting effects of activating VMHvl PR+ cells were also observed in castrated males and also in males with defective olfactory inputs, suggesting that the VMHvl activation can "override" normal hormone and sensory requirements for aggression (Yang et al., 2017).

While these studies clearly implicated hormone-receptive populations in the generation of aggression, the role of the VMHvl in this behavior remained unclear. Is the VMHvl simply an "attack generator" or does it also promote flexible aggression seeking behaviors that lead to attack? As evidence for aggression-seeking behavior, it has been observed behaviorally from fish to primates that certain individuals will develop a strong preference for the context where they attacked a conspecific (Meisel and Joppa, 1994; Martinez et al., 1995; Golden et al., 2016) and will voluntarily seek the opportunity to attack a conspecific (Thompson, 1963; Cherek et al., 1973; Turnboug and Lloyd, 1973; Fish et al., 2002, 2005; May and Kennedy, 2009; Mitani et al., 2010; Golden et al., 2017). To test the role of the VMHvl in aggression seeking, we designed a self-initiated aggression-seeking (SIA) task during which the animals learn to voluntarily nose poke to gain access to a weaker male intruder (Falkner et al., 2016). Over weeks of training, the majority of trained males exhibited task-dependent learning. These animals demonstrated a clear preference for the nose port associated with the weak intruder, poked it repeatedly and attacked the intruder immediately after its introduction (**Figure 1**). We found that low-level optogenetic activation of the VMHvl cells reliably reduced the animals' latency to nose poke for an opportunity to attack. Conversely, inhibiting the VMHvl suppressed nose poking for the chance to attack but not for water reward, demonstrating a role for the VMHvl in aggression seeking in addition to attack (Falkner et al., 2016).

It is important to note that this area is not exclusively implicated in aggressive behaviors. In addition to aggression, the VMHvl is also well-known for its essential role in female sexual behaviors and to a lesser extent, in male sexual behaviors (Pfaff and Sakuma, 1979a,b; Yang et al., 2013; Lee et al., 2014; Hashikawa et al., 2017). Furthermore, the VMHvl may also mediate behaviors against an aggressor: immediate early gene mapping experiments have revealed strong activation of the area in subordinate animals after social defeat (Kollack-Walker et al., 1997; Motta et al., 2009; Pan et al., 2010; Silva et al., 2013) and re-activation of the defeat induced Fos population in the VMHvl elicits fearful responses (Sakurai et al., 2016). Thus, the VMHvl likely mediates multiple social behaviors and future studies will need to address how factors including social experience, hormonal state, and behavioral context influence which behaviors are generated.

THE ENCODING OF AGGRESSION-RELATED INFORMATION IN THE VMHVL

Among identified aggression-related circuits in the brain, the VMHvl is currently the best understood area, due in large part to our characterization of the response properties of neurons during social behaviors. We have performed extensive electrophysiological recording in the VMHvl in freely-moving, socially-interacting animals (Lin et al., 2011; Falkner et al., 2014, 2016; Wong et al., 2016). Based on these data, we propose that VMHvl cells encode at least three features of aggression-related information: (1) the overall aggressive state of the animal (*motivation*); (2) the detection of aggression-provoking sensory cues (*sensation*); and (3) the initiation and execution of attack and aggression-seeking behaviors (*action*) (**Figure 2**). We hypothesize that the aggressive state is encoded as the baseline spiking activity of the VMHvl cells whereas the sensory information and the motor actions are encoded by acute changes in VMHvl activity.

Aggressive Arousal State

Upon the intruder introduction, VMHvl activity quickly increases and is maintained at a high level. This elevation in spontaneous activity is not associated with any particular behavior and can account for ∼50% of the total VMHvl firing rate increase (Lin et al., 2011; Falkner et al., 2016; Hashikawa et al., 2017). In addition, VMHvl activity can remain at an elevated state for minutes after the intruder removal (Falkner et al., 2014). Increased VMHvl activity after intruder removal coincides with a heightened aggressive state. If a second intruder is presented shortly after removing the first intruder, both the latency to attack decreases and the probability of attack increases (Potegal et al., 1996). As further demonstration of this, in tests of the self-initiated aggression task, after the animal learned the task contingency, similar sustained increase in spiking activity was observed after the nose poking apparatus was introduced into the cage of the intruder but before any nose poking or attack (Unpublished data by Annegret Falkner). Consistent with a heightened aggressive state of the animal during the self-initiated aggression task, the resident male typically attacks the intruder immediately after the intruder becomes available. These electrophysiological and behavioral observations lead us to hypothesize that the spontaneous activity of the VMHvl signals the general aggressive state of the animal.

This property may be common to the hypothalamus and associated neural circuit. Other internal states such as hunger and thirst have also been shown to be encoded by the spontaneous activity of specific populations of neurons. For example, AgRP neurons in the arcuate nucleus promote feeding and food seeking when artificially activated and these neurons show sustained increase in neural activity in food deprived animals (Aponte et al., 2011; Krashes et al., 2011; Betley et al., 2015; Chen et al., 2015). Similarly, subfornical organ (SFO) neurons can promote drinking and seeking for water when artificially activated and show sustained increase in spontaneous activity in water deprived

TABLE 1 | VMHvl is essential for aggression.

	Manipulation	Sex	Population	Test	Behavior	References
Gain of function	ChR (20 ms, 20 Hz)	Male	VMHvl	R-I test	Attack	Lin et al., 2011; Falkner et al., 2016
	ChR2 (20 ms, 20 Hz), high intensity	Male	VMHvl Esr1+	R-I test	Attack	Lee et al., 2014
	ChR (20 ms, 20 Hz), low intensity	Male	VMHvl Esr1+	R-I test	Close investigation, mounting	Lee et al., 2014
	DREADDq (CNO, i.p. injection)	Male	VMHvl PR+	R-I test	Increase attack frequency	Yang et al., 2017
	ChR (20 ms, 20 Hz)	Female	VMHvl Esr1+	R-I test	Attack	Hashikawa et al., 2017
	ChR2 (20 ms, 5 Hz)	Male	VMHvl	SIA test	Shorten poke latency	Falkner et al., 2016
Loss of function	GluCL (IVM, i.p. injection)	Male	VMHvl	R-I test	Reduce attack	Lin et al., 2011
	DREADDi (CNO, i.p. injection)	Male	VMHvl	R-I test	Reduce attack	Falkner et al., 2016
	NpRH (continuous light)	Male	VMHvl Esr1+	R-I test	Block attack	Lee et al., 2014
	Caspase 3 (ablation)	Male	VMHvl PR+	R-I test	Reduce attack	Yang et al., 2013
	DREADDi (CNO, i.p. injection)	Female	VMHvl Esr1+	R-I test	Reduce attack	Hashikawa et al., 2017
	DREADDi (CNO, i.p. injection)	Male	VMHvl	SIA test	Reduce poke rate	Falkner et al., 2016

A summary of functional manipulation experiments that support an essential role of the VMHvl in conspecific aggression.

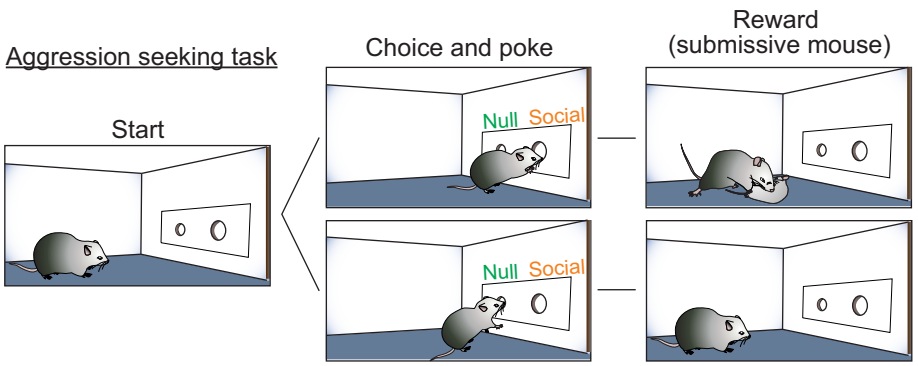

FIGURE 1 | Self-initiated aggression seeking task is utilized to study appetitive phase of aggression**.** Schematic illustration of the self-initiated aggression seeking task. By poking the social poke, subjects gain access to a submissive intruder and attack.

FIGURE 2 | The activity of VMHvl increases during social investigation, aggression seeking and attack, and when the animal is at a heightened aggressive state.

animals (Oka et al., 2015; Zimmerman et al., 2016). While in those cases, changes in spontaneous activity are caused by changes in interoceptive physiological signal, in our experimental condition, elevation in VMHvl spontaneous activity is triggered by external cues, such as conspecific intruders or aggression-associated object. However, an aggressive internal state can also be signaled internally through experience. For example, male mice that repeatedly encounter a male intruder at the same time of the day learn to anticipate the fighting as indicated by their increase in heart rate and body temperature prior to the scheduled fighting time (Tornatzky et al., 1998). Whether the internally generated aggressive state is also accompanied and/or caused by an increase in spontaneous VMHvl activity remains to be confirmed.

Aggression-Provoking Olfactory Cues

VMHvl cells also robustly respond to olfactory cues associated with aggression-provoking stimuli (Falkner et al., 2014). During

investigation of male intruders, male-responsive VMHvl cells acutely increase activity from an already elevated baseline. Moreover, when recorded males investigated urine from the intruder, VMHvl activity also increased in a subpopulation of cells (Lin et al., 2011; Falkner et al., 2014). Urine is a rich source of pheromones and carries important information regarding the sex, physiological, and social status of the animal (Dulac and Torello, 2003). Several volatiles and major urinary proteins have been identified in male mouse urine that can promote aggression when applied onto the intruder mouse (Novotny et al., 1985; Chamero et al., 2007). As a demonstration of this, castrated male intruders, whose urine contains fewer critical volatiles are less likely to be attacked by resident male mice (Mugford and Nowell, 1970) and urine from castrated male mice generate less activity from VMHvl cells in comparison to intact male mouse urine (Falkner et al., 2014). Male mice also rarely attack females, and female urine increases activity in only a small subpopulation of VMHvl neurons in aggressive males (Falkner et al., 2014).

Aggressive Actions

During free social interactions, the most prominent activity increase observed in VMHvl cells is from attack itself. Activity in responsive cells rises prior to attack (∼1 s), peaks at the onset of attack, sustains throughout the duration of attack, and quickly drops to the baseline level at the behavioral offset (Falkner et al., 2014). Importantly, movements unrelated to attacking do not correlate with or predict VMHvl activity (Falkner et al., 2014). In addition, several complimentary lines of evidence suggest that this increased activity during attack cannot be simply accounted by the increased activity from sensory cues. First, the response during attack is higher than the responses during investigation that precedes attack (Falkner et al., 2014; Hashikawa et al., 2017). Second, when we compare the isolated aggression trials (attack not preceded or followed by attack) and investigation trials (investigation not followed or preceded by attack), we find that attack responses are significantly higher during these isolated attack trials (Hashikawa et al., 2017). Third, in a linear regression model that explores the relationship between various behavioral parameters and VMHvl cell activity, we found that the inclusion of a "latency to attack" parameter can significantly improve model fit in a subpopulation of cells beyond what can be accounted for considering the distance between animals (an estimate of sensory input) and the movement velocity of the animal. This data quantitatively supports the hypothesis that the VMHvl activity carries information regarding the initiation of attack independently of these external cues (Falkner et al., 2014). Furthermore, using the self-initiated aggression seeking task as a way to separate actions associated with seeking and attack, we found that a subset of VMHvl cells increase spiking activity prior to and during nose poking once the animal learned the association between nose poking and future attack (Falkner et al., 2016). This shows that the VMHvl cells not only signal the initiation of physical attack but also flexible learned actions that lead to future attack.

Aggressive state, aggression-provoking sensory cues, and aggressive actions are encoded by highly overlapping populations of VMHvl cells. Approximately half of the VMHvl cells that respond during attack and investigation showed sustained increase in spontaneous activity in the presence of intruder (Lin et al., 2011). The cell responses during attack and social investigation are significantly correlated (Falkner et al., 2014). Approximately 80% of cells that increase activity during aggression seeking also increase activity during attack (Falkner et al., 2016). Thus, the activity of a single VMHvl cell at any given moment can encode multiple aspects of aggression-related information and these signals may be linearly summed to create the observed response profile.

Other hypothalamic subregions may encode similar multifaceted responses for other social behaviors. One well-studied example of this is the responses of cells in the medial preoptic area (MPOA) during sexual behaviors. *In vivo* extracellular recording from the MPOA in freely moving rats revealed that MPOA cells show sustained increase in activity after female introduction as well as acute increase in activity during female investigation and specific sexual actions, such as pursuing, mounting, and intromission (Oomura et al., 1983; Horio et al., 1986; Shimura et al., 1994). Additionally, an acute increase in the MPOA cell activity was observed every time when the male operated a lever to bring a female closer to him (Oomura et al., 1988). Thus, similar to VMHvl activity change during aggression, the MPOA cells signal the animal's sexual arousal, detection of sexual arousal cues, and specific preparatory and consummatory sexual actions. Taken together, we speculate that the same general coding principles are employed by hypothalamic networks to represent sensory, arousal (motivation) and action-related information essential for social behaviors.

Experience-Dependent Changes in VMHvl Cell Responses

Mounting evidence now suggests that the response properties of VMHvl neurons are not fixed as would be in a "hard-wired" innate circuit, but instead are constantly updated. Recently, Remedios et al. used microendoscopic calcium imaging to examine changes in VMHvl cell activity over days as the recorded males encountered male and female intruders and obtained social experience (Remedios et al., 2017). While the VMHvl responses during male and female investigation heavily overlap initially in naïve unexperienced males, brief sexual experience with females caused significant divergence of the VMHvl cell responses. Importantly, this divergence only occurred when animals started to show mounting and fighting, suggesting a potential causal link between the change in neural responses and the selection of appropriate social actions. Additionally, we also observed changes in VMHvl activity over the training of the self-initiated aggression seeking task (Falkner et al., 2016). In early training phase before the animals made the association between nose poking and the opportunity to attack, little response of VMHvl cells during poking was found. As the training went on and animals successfully learned the task contingency, VMHvl cells showed clear activity increase prior to, during and after the nose poking, supporting the capacity of VMHvl cells to change responses with experience.

Experience-dependent changes in VMHvl activity also appear to alter the efficacy of attack initiation. In early electric stimulation experiments in rats, it was found consistently that repeated attack induced by electric stimulation reduced the amount of current required to elicit attack in subsequent testing days (Kruk, 2014). This threshold-lowering effect requires the attack itself (i.e., it cannot be induced via stimulation in isolation) (Kruk et al., 1979; Kruk, 2014). More recently, Yang et al. examined the reliability of VMHvl activation-induced aggression in animals with different experience and under different testing environment (Yang et al., 2017). They expressed an engineered ligand (CNO) gated Gq coupled receptor, DREADDq, in the VMHvl and examined the CNO injection induced attack in animals that are single- vs. group-housed and in home territory vs. foreign territory. They found that while attack can be reliably chemogenetically induced in single-housed mice in both home and foreign territory, this same manipulation is only effective in inducing attack in group housed males when tested in home territory. The difference in attack induction efficacy between single-housed and group-housed animals is likely due to either to differences in the physiological properties of the VMHvl itself, or to changes in its inputs. In the VMHvl itself, intrinsic excitability may be higher in singly-housed animals than group-housed animals and VMHvl neurons in singly-housed animals are likely to fire more readily when activated using DREADDq. Alternatively, cues related to foreign territory may cause a stronger suppression of VMHvl cells in group-housed animals and prevent the cells from spiking in a foreign territory. Consistent with this second idea, a high efficacy of stimulation-evoked attack can be "revealed" in group-housed animals in a foreign environment when the olfactory inputs were blocked (Yang et al., 2017). Future experiments that examine the physiological and synaptic properties of VMHvl cells from animals with different experience and aggression level will help elucidate the dynamic range of VMHvl cell physiological properties and whether hyper-excitability of VMHvl cells could also be related to the exaggerated aggression observed under certain pathological conditions in human patients and animal models, such as autism, borderline personality disorder, and posttraumatic stress disorder (Kanne and Mazurek, 2011; Jiang-Xie et al., 2014; Wells et al., 2016).

MOLECULAR AND CIRCUIT MECHANISMS FOR DRIVING AGGRESSION-RELEVANT VMHVL ACTIVITY

Circuits for Olfactory Input

The VMHvl responses that signal aggressive state, sensory detection, and aggressive action may result from independent sources of activity which include both sensory inputs and changes in neuromodulatory tone. Among these potential molecular and synaptic drivers, the VMHvl responses to the olfactory cues are the best understood since pathways that convey olfactory information have been previously identified (**Figure 3A**). The VMHvl receives converging volatile and pheromone information from the medial amygdala (MEA) and bed nucleus of stria terminals (BNST) (Canteras et al., 1994). Volatile information passes through the main olfactory epithelium, main olfactory bulb, posterolateral cortical amygdala (plCOA) and arrives at the MEA and BNST whereas pheromone information is relayed through the vomeronasal organ (VNO), accessory olfactory bulb (AOB) and then also reaches the MEA and BNST (Hashikawa et al., 2016). Recent tracing studies also suggest that MOB mitral and tufted cells may directly project to the MEA (Pro-Sistiaga et al., 2007; Kang et al., 2009, 2011). Regardless, converged volatile and pheromone information at the MEA and BNST can then reach the VMHvl either directly or indirectly through the ventral part of the premammillary nucleus (PMv), a hypothalamic region that is situated posterior to the VMHvl (Canteras et al., 1992a, 1995; Pardo-Bellver et al., 2012). PMv inputs are likely to be critical for the responses of VMHvl cells to olfactory cues: immediate early gene mapping showed that the PMv is strongly activated by the odor cues from conspecifics (Donato et al., 2010; Soden et al., 2016) and when the PMv is inactivated, VMHvl response to an aggression-provoking conspecific is largely eliminated (Motta et al., 2013).

Neuromodulatory Inputs and Aggressive State

While the increase in the VMHvl spontaneous spiking during a heightened aggressive state may be partly due to the sensory inputs from the intruder, this is unlikely to account for all activity change, since these changes persist following the removal of aggressive stimuli (Lin et al., 2011; Falkner et al., 2014). We speculate that a relatively slow neuromodulatory or neuroendocrine mechanism may also contribute to the change in spontaneous activity (**Figure 3B**). Consistent with this hypothesis, *in vitro* extracellular slice recording demonstrated that VMHvl cells respond to a variety of bath-applied neuromodulators, including acetylcholine, norepinephrine, serotonin, and dopamine (Kow and Pfaff, 1985). Among them, serotonin and dopamine are mainly inhibitory possibly due to the strong expression of Gi-coupled serotonin receptor 1A (5HT1A) (Wright et al., 1995) and dopamine receptor D2 (D2R) in the area (Moss et al., 1985; Bouthenet et al., 1991; Weiner et al., 1991). In contrast, norepinephrine elicits both excitatory and inhibitory responses in the VMHvl cells, possibly due to the strong expression of Gq coupled α_{1a}–adrenergic receptor (Day et al., 1997) and to a lesser extent Gi coupled α_{2c}–adrenergic receptor (Wang et al., 1996). The effect of acetylcholine on the VMHvl cells is mainly excitatory, likely through both nicotinic and muscarinic acetylcholine receptors. More specifically, VMH cells express α7 nicotinic receptor (Clarke et al., 1985; Baddick and Marks, 2011) that has high Ca2+ permeability, and Gq coupled M1, M3, and M5 and to a less extent Gi coupled M2 and M4 (www.brain-map.org, experiment ID 73907497, 70560343, 79912556, 79591641, and 75826557) (Levey, 1993; Levey et al., 1994). The Muscarinic activation can generate plateau potential and persistent spiking activity in cortex and hippocampus (Egorov et al., 2002) and thus could be particularly relevant for generating the increased spontaneous activity in VMHvl cells. Consistent with a role of the acetylcholine in

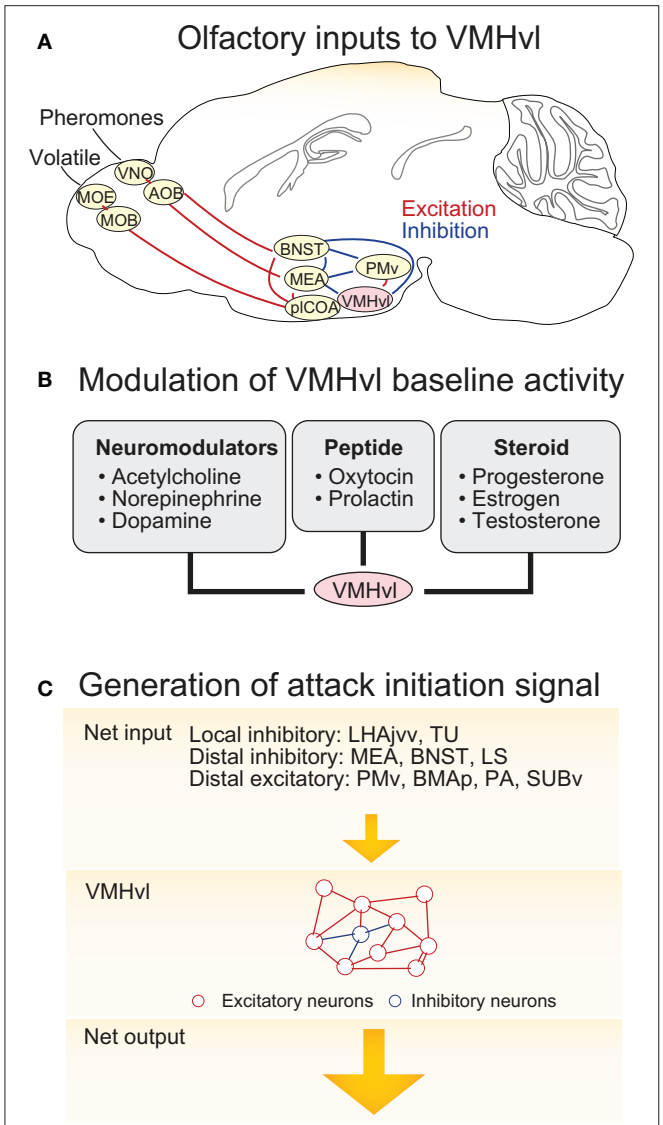

FIGURE 3 | Circuit mechanisms underlying VMHvl activity change during agonistic encounters. **(A)** The neural circuits upstream of the VMHvl that relay olfactory information. **(B)** Neuromodulators, neuropeptides, and neurosteroids that could potentially generate increased spontaneous activity in the VMHvl and cause sustained aggressive state. **(C)** Schematics illustrating a model responsible for activity increase at the VMHvl during attack initiation. In the model, inputs from the upstream regions bring the VMHvl activity to a threshold and then the recurrent excitatory network within the VMHvl quickly amplifies the signal to initiate attack and maintain it throughout the attack. OE, olfactory epithelium; VNO, vomeronasal organ; MOB, main olfactory bulb; AOB, accessory olfactory bulb; BNST, bed nucleus of stria terminalis; MEA, medial amygdala; pICOA, posterolateral cortical amygdala; PMv, ventral premammillary nucleus; VMHvl, ventromedial hypothalamus ventrolateral part. LHAjvv, lateral hypothalamic area, juxtaventromedial region, ventral zone; TU, tuberal nucleus; LS, lateral septum; BMAp, posterior basomedial nucleus; PA, posterior amygdala; SUBv, ventral subiculum.

enhancing VMH activity, microinjection of acetylcholine into the hypothalamus induces rage responses in cats, such as growling, hissing, and piloerection (Karmos-Varszegi and Karmos, 1977; Brudzynski, 1981; Siegel et al., 1999). Interestingly, the popular pharmacogenetics reagent, DREADD, is based upon muscarinic acetylcholine receptor, M3 (Dong et al., 2010). When DREADDq was virally expressed in the VMHvl PR+ cells, CNO (a selective ligand of DREADDs but see Gomez et al., 2017) mediated DREADDq activation significantly enhanced the probability and frequency of attacks in male mice, suggesting that the muscarinic activation in the VMHvl can potentially enhance aggressiveness of the animal.

Neuropeptides such as oxytocin, could also play a role in enhancing the spontaneous activity of the VMHvl. The oxytocin receptor is a Gq coupled receptor and expressed highly in the VMHvl (Young et al., 1997; Gould and Zingg, 2003) (www.brain-map.org, experimental ID: 75081001). *In vitro* slice recording showed that oxytocin can directly excite VMHvl cells when bath applied (Inenaga et al., 1991). The source of oxytocin to the VMHvl is not clear. Intriguingly, *in situ* hybridization revealed a cluster of OXT expressing cells lying along the ventral edge of the hypothalamus right against the VMHvl (www.brain-map.org, experiment ID: 112648396). Given that oxytocin can be released from not only axons but also dendrites (Ludwig, 1998), local oxytocin release from those ventrally situated oxytocinergic cells are likely to have strong influence on the adjacent cells in the VMHvl.

Estrogen is likely another potential contributor to alter spontaneous activity in VMHvl cells and it's actions can modulate activity on a variety of timescales. In the male brain, estrogen is believed to be synthesized by the action of the enzyme aromatase on testosterone (Ubuka and Tsutsui, 2014). Estrogen exerts its effect on cells through both slow genomic and fast membrane mechanisms. While the genomic actions of estrogen take hours for changes in protein expression to occur, non-genomic activation of estrogen occur within seconds to minutes through binding to the membrane estrogen receptor and kinase activation or calcium mobilization (Morley et al., 1992; Brubaker and Gay, 1999; Vasudevan and Pfaff, 2008). Given that the activity of aromatase can be regulated by phosphorylation within in seconds to minutes (Balthazart et al., 2001a,b), the local concentration of estrogen can rise rapidly and this can dynamically regulate the activity of cells that express membrane estrogen receptors on a similar timescale (Soma et al., 2004; Pradhan et al., 2010). The VMHvl is enriched with membrane estrogen receptor (Hazell et al., 2009) and patch clamp recording in slice preparation has shown that bath-applied estrogen can potentiate excitatory responses of VMHvl cells within 5 min (Kow et al., 2006). Behaviorally, estradiol supplements have also been shown to quickly enhance aggression. For example, orally administrated estradiol increases territorial aggression in male sparrow within 20 min (Heimovics et al., 2015), and subcutaneous administration of estradiol doubled male aggressive behavior within 15 min in two species of *Peromyscus* mice (Trainor et al., 2007, 2008).

While pharmacological studies, receptor expression patterns and *in vitro* recordings suggest that neuromodulatory mechanisms have the potential to change the VMHvl activity (**Table 2**), it remains unclear which mechanisms are utilized to facilitate VMHvl excitation under natural agonistic conditions. The development of genetically encoded protein sensors for

TABLE 2 | Neuromodulation at the VMHvl.

Region	Substrate	Subclass	Type	Neuralactivity	Reference
	Oxytocin	–	Gq	↑	Inenaga et al., 1991
	Prolactin	–	Type I cytokine receptor family	↑	Moss et al., 1985
	Acetylcholine	α-7 nicotinic	Nicotinic	↑	Baddick and Marks, 2011
	Acetylcholine	Ml,M3,M5	Gq	↑	Levey, 1993
VMHvl	Acetylcholine	M2,M4	Gi	↓	Levey, 1993
	Estrogen	Esr1	Membranelocalized	↑	Kow et al., 2006
	NE	α1a	Gq	↑	Day et al., 1997
	NE	α2c	Gi	↓	Wang et al., 1996
	Dopamine	D2	Gi	↓	Moss et al., 1985
	Serotonin	5HT1A	Gi	↓	Wright et al., 1995

A summary of receptors of neuromodulators, neurosteroids, and neuropeptides expressed in the VMHvl and their potential influences on the VMHvl activity.

biological compounds will be particularly useful in revealing the natural release of those compounds into the VMHvl (Kuner and Augustine, 2000; McLachlan et al., 2011; Marvin et al., 2013). Future studies that combine blocking or knocking down specific receptors and *in vivo* recording will be an effective way to reveal the contribution of a specific receptors to the increased activity of VMHvl cells during an aggressive state and to the behavior itself. The neuromodulators mentioned above are by no means complete, VMHvl expresses many other neuropeptide [cholecystokinin (Xu et al., 2012)] and hormone [e.g., progesterone (Furuta et al., 2010), androgen (Simerly et al., 1990; Mitra et al., 2003), prolactin (Chiu and Wise, 1994), glucocorticoid (Aronsson et al., 1988), and corticotropin-releasing hormone (Makino et al., 1998)] receptors and thus are likely under the influence of a cocktail of neurochemicals.

Circuit Mechanisms for Increasing VMHvl Activity to Initiate Attack

Optogenetic manipulations clearly demonstrate that increasing VMHvl activity can initiate attack (Lin et al., 2011; Lee et al., 2014). In addition, electrophysiological recording show that the VMHvl activity increases during attack and even starts to rise prior to attack onset (Falkner et al., 2014). What circuit mechanisms give rise to this excitatory response prior to and during attack under natural conditions? We speculate that it is the combined result of upstream inputs and local excitatory networks. The inputs to the VMHvl are diverse, coming from local cells in and surrounding the VMHvl, other regions of the hypothalamus, and beyond. To complicate matters, the VMHvl receives far more inhibitory inputs than excitatory inputs. The VMH itself is densely glutamatergic and the number of intermingled inhibitory neurons is extremely small. However, several regions surrounding the VMHvl, including the lateral hypothalamus, juxtaventromedial region, ventral zone (LHAjvv), and the tuberal nucleus (TU), are enriched of GABAergic cells and may provide direct inhibitory drive (Canteras et al., 1994) (For distribution of GABAergic cells: www.brain-map.org experiment 72081554; Glutamatergic cells: experiment 73818754). Tracing studies have revealed dense projections from the VMH surrounding regions to the VMH, suggesting a strong local control of the VMHvl activity by its surrounding zones (Canteras et al., 1994; Hahn and Swanson, 2015). Besides sources of local inhibition, antegrade tracing studies suggested that the VMHvl also receives strong inputs from the MEA (Canteras et al., 1995), BNST (Dong and Swanson, 2004), lateral septum (LS) (Risold and Swanson, 1997), and medial preoptic area (MPOA) (Simerly and Swanson, 1988), all of which contain mainly or nearly exclusively GABAergic cells. In support of a role for these inhibitory inputs in aggression, optogenetic activation of the MEA GABAergic cells elicits immediate attack, suggesting that the attack could be initiated upstream of the VMHvl although it is unclear whether the MEA activation induced attack is through its direct projection to the VMHvl or not (Hong et al., 2014; Padilla et al., 2016). In comparison to brain-wide sources of inhibitory inputs, excitatory inputs to the VMHvl are less studied. They include inputs from the ventral premammlinary nucleus (PMv) (Canteras et al., 1992a), the basomedial amygdala posterior part (BMAp) (Petrovich et al., 1996), posterior amygdala (PA) (Canteras et al., 1992b), and ventral subiculum (SUBv) (Canteras and Swanson, 1992; Tang et al., 2016). The roles of those glutamatergic regions in aggressive behaviors remain largely unclear.

Within the VMHvl, given that over 95% cells are glutamatergic (Choi et al., 2005) and these cells form numerous synaptic contacts with each other (Nishizuka and Pfaff, 1989), it contains the proper synaptic substrates to support recurrent synaptic excitation. Thus, we speculate that VMHvl cells may integrate inputs from the upstream regions until a "threshold" is reached and then the local recurrent VMHvl network may quickly amplify the activity to initiate attack and maintain this activity throughout the attack (Douglas et al., 1995; Schurger et al., 2012) (**Figure 3C**). Increases in the spontaneous activity in the VMHvl will help bring the activity closer to the "threshold" and thus increase the probability of the "threshold-crossing" event. In support of this hypothesis, pharmacogenetically increasing the spontaneous activity of the VMHvl cells significantly increases the frequency of attacks although it does not initiate attack immediately (Yang et al., 2017) while decreasing the spontaneous

activity of the VMHvl reduces the frequency of attack (Lin et al., 2011; Falkner et al., 2016).

THE INFLUENCE OF THE VMHVL ON CIRCUITS FOR SOCIAL PERCEPTION AND ACTION

Aggressive State and Perception of an Opponent

The motivational state of an animal influences the perception of relevant sensory stimuli. For example, the hunger state influences the attractiveness of food whereas the sexual arousal state influences the appeal of a potential mate. During subthreshold electric stimulation of hypothalamic attack area, it was noted that stimulation seems to" promote a shift from friendly social contact toward a more apprehensive and touchy social attitude" (Kruk, 2014). The neural mechanisms responsible for the perceptual change with the motivational state remain largely unknown. Recently, an elegant study by Livneh et al. showed that the responses of insular cortex to food reward are significantly modulated by the hunger state of the animal (Livneh et al., 2017) (**Figure 4**). While insular cortical neurons strongly responded to food in starved mice, the responses are virtually abolished under satiety. Importantly, activity of the AGRP neurons was found casually linked to the change in responses of insular cortical neurons: artificial activation of the AGRP neurons largely restored the responses of insular cortical neurons to food in satiated mice (Livneh et al., 2017). Detailed circuit mapping revealed that AGRP neurons project to the insular cortex through paraventricular thalamus (PVT) and basomedial amygdala (BMA). Taken together, this study revealed a potential circuit through which the motivational signal encoded in the subcortical region affects the perceptual responses in the cortex.

Intriguingly, PVT also receives strong inputs from the VMHvl. In fact, PVT is the only thalamic region to which the VMHvl projects (Canteras et al., 1994). In addition to the VMHvl and arcuate nucleus, PVT also receives inputs from a wide range of hypothalamic structures, including the dorsomedial, suprachiasmatic, paraventricular, suprachiasmatic nuclei as well as the preoptic, anterior, zona incerta, and lateral hypothalamic areas (Cornwall and Phillipson, 1988; Chen and Su, 1990; Kirouac, 2015). Thus, it is possible that PVT represents a common gateway through which the motivational and physiological signals encoded in the subcortical areas influence the perceptual processing in the cortex (**Figure 4**). During a heightened aggressive state, activity in VMHvl may excite the PVT neurons and influence the evaluation process of a potential opponent in the cortex. The influences of the PVT onto the cortex could be through the BMA as shown in Livneh et al. (2017) or via a direct projection to the prefrontal cortex (PFC) (Berendse and Groenewegen, 1991; Moga et al., 1995; Kirouac, 2015). Recent studies revealed that the neural activity in the PFC influences the dominance behaviors of an animal (Wang et al., 2011; Zhou et al., 2017). In a test that involves two male mice encountering each other in a narrow tube, the PFC activated animals are more likely to push the opponent while

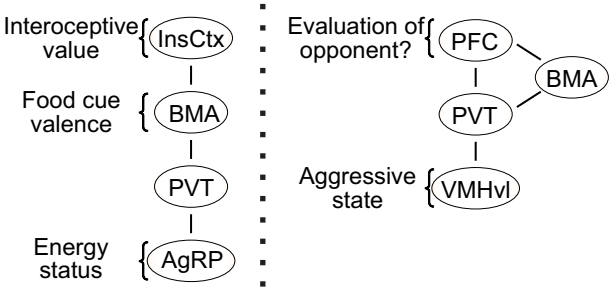

FIGURE 4 | The potential pathways for "bottom up" modulation. **(Left)** the key relays to transfer hunger state related information from AGRP neurons to the insular cortex; **(Right)** a putative pathway that transfers aggressive state related information from the VMHvl to the prefrontal cortex. InsCox, insular cortex; BMA, basomedial amygdala; PVT, paraventricular nucleus of thalamus; AgRP, agouti-related peptide in the arcuate nucleus; PFC, prefrontal cortex; VMHvl, ventromedial hypothalamus ventrolateral part.

the PFC inactivated animals are more likely to retreat (Zhou et al., 2017). In a warm-spot test, PFC activated animals occupied the warm spot in a cold arena for a significantly longer time in comparison to control animals (Zhou et al., 2017). Given that dominance behavior depends critically on evaluating self and opponent, changes in dominance behavior may reflect changes in perceived relationship of an opponent to oneself. Indeed, human fMRI studies revealed high activity in the medial PFC in a self-referencing task that requires the subject to compare oneself and others (Amodio and Frith, 2006; Mitchell et al., 2006). Taken together, we hypothesize that the PFC may represent a critical site for evaluation of an opponent in reference to self and a heightened aggressive state modulated from ongoing VMHvl activity may influence the activity of PFC through its projection to PVT and bias the evaluation process. Future studies that simultaneously manipulate the hypothalamic activity and monitor the cortical cell activity could be a general and fruitful strategy to understand the neural mechanisms underlying the motivational modulation of social perception.

Influences of the VMHvl on Midbrain Structures during Attack

The attack initiation signal in the VMHvl ultimately needs to activate premotor areas to drive the motor execution of attack. Among all the downstream regions of the VMHvl, the periaqueductal gray (PAG) represents the most likely relay between the VMHvl and the motor neurons in the spinal cord. PAG is a midbrain structure located around the cerebral aqueduct. PAG neurons project to the nucleus raphe magnus (NRM) and pallidus (NRP), the ventral part of the caudal pontine, the medullary reticular formation (Abols and Basbaum, 1981; Holstege, 1988; Mouton and Holstege, 1994; Cameron et al., 1995), which in turn project diffusely, but very strongly to all parts of the gray matter throughout the length of the spinal cord (Kuypers and Maisky, 1975; Tohyama et al., 1979; Holstege and Kuypers, 1982). The strength of this projection from the VMHvl to PAG has long been recognized (Beart et al., 1988;

Chung et al., 1990; Canteras et al., 1994). A survey of the Allan brain atlas (www.brain-map.org) illustrated the exceedingly high strength of this projection: in the list ranking the tracing results according to the largest terminal fields produced within the PAG, the VMH appeared in 6 of the 10 topmost positions. The 4 other top positions were occupied by areas adjacent to the VMH, such as the tubular- and the lateral hypothalamic area, which all receive strong inputs from the VMHvl. Thus, VMHvl projections to the PAG, either directly or indirectly, are likely to have strong impact on the PAG cell activity.

It is important to note that PAG is a massive and complex structure. In mice, it spans nearly 2.5 mm along the A-P axis (or ∼25% of the entire mouse brain) and is composed of multiple columns surrounding the midbrain aqueduct (dorsal, dorsolateral, lateral, ventrolateral, and ventral) (Bandler and Shipley, 1994; Bandler et al., 2000). Not surprisingly, PAG has been indicated in many social and non-social functions including defense, predation, lordosis, vocalization, nociception, analgesia, and cardiovascular control (Bandler and Shipley, 1994; Behbehani, 1995; Wang et al., 2015; Han et al., 2017; Motta et al., 2017). Due to the involvement of PAG in multiple behaviors, direct electric stimulation of the PAG has only been shown to induce attack with motor disturbance (Mos et al., 1982). More frequently, reports of direct PAG stimulation have elicited robust defense related motor patterns, including immobility, flight and escape jump (Bandler et al., 2000), though these studies do not rule out direct involvement of the PAG in attack initiation. Future studies that identify the molecular identities of the PAG cells that are relevant for aggression will be an essential step for understanding how VMHvl inputs influence PAG cells during aggression.

In addition to the PAG, there likely exist parallel pathways to generate attack, given that a large lesion that destroyed the entire PAG at one level only transiently impaired aggressive behaviors (Mos et al., 1983). The other midbrain region that has been recently implicated in aggression is the ventral tegmental area (VTA): optogenetic activation of the dopaminergic cells increased the time spent attacking male and female intruders (Yu et al., 2014). Microdialysis showed that the dopamine level in the nucleus accumbens (NA) increases when the animal anticipates attack and after attack although its release during the moment of attack remains unknown due to the low temporal sensitivity of microdialysis (Ferrari et al., 2003). D1 and D2 receptor antagonists effectively reduce attack and aggression seeking in mice (Nelson and Trainor, 2007; Couppis and Kennedy, 2008). In fact, D2 receptor antagonist risperidone is a commonly used drug to reduce aggressive behavior in patients with autism and schizophrenia (Soyka et al., 2007; Bronsard et al., 2010). Although the VMHvl and dopamine system are clearly both activated during aggression, they have been studied largely independently and the relationship between these two regions remain unclear. The VMHvl appears to project sparsely if at all to the VTA and *vice versa* (Canteras et al., 1994). However, the VMHvl does project densely to the MPOA which in turn projects to the VTA moderately (Simerly and Swanson, 1988; Canteras et al., 1994). The MPOA—VTA—NA pathway has been hypothesized as a key route for transferring the motivational signal in the hypothalamus to the striatal motor system to guide goal directed behaviors. Future circuit dissection studies will help elucidate the relevance of the VMHvl—MPOA—VTA—NA circuit in mediating aggressive behaviors.

CONCLUDING MARKS

After decades of relative quiescence, aggression research has regained its momentum. Recent studies using genetically precise, cell-type specific manipulation, tracing, and *in vivo* recording have quickly advanced our knowledge regarding the neural substrates relevant for aggression. Beyond the VMHvl and associated regions mentioned above, including the MEA (Hong et al., 2014), PMv (Motta et al., 2013), and VTA (Yu et al., 2014), aggression has also been shown to be modulated by GABAergic neurons in lateral habenula (Golden et al., 2016), serotonin cells in dorsal raphe (Niederkofler et al., 2016), GABAergic neurons in lateral septum (Wong et al., 2016), and pyramidal cells in prefrontal cortex (Takahashi et al., 2014). Although neural populations that can alter aggressive behaviors are being continuously discovered, efforts to understand the endogenous responses of the cells under natural behaviors remain limited. To date, the VMHvl remains the only region from which the electrophysiological responses during aggressive behaviors have been extensively studied. Such information is essential for interpreting the behavioral changes caused by the manipulation and understanding the role of these cells in the whole-brain aggression circuit. By combining physiology with connectivity, causality and correlation studies, we hope that a comprehensive and integrated aggression circuit will finally emerge.

AUTHOR CONTRIBUTIONS

DL wrote the manuscript. YH and KH made the figures. KH commented and AF edited the manuscript.

FUNDING

This research was supported 1R01MH101377 (NIMH) (DL), 1R21MH105774-01A1 (NIMH) (DL), Mathers foundation (DL), Irma T. Hirschl Career Scientist Award (DL), Uehara postdoctoral fellowship (KH), and K99MH109674 (AF).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Systems Neuroscience

REVIEW
published: 06 July 2017
doi: 10.3389/fnsys.2017.00050

Lateral Hypothalamic Control of the Ventral Tegmental Area: Reward Evaluation and the Driving of Motivated Behavior

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The lateral hypothalamus (LH) plays an important role in many motivated behaviors, sleep-wake states, food intake, drug-seeking, energy balance, etc. It is also home to a heterogeneous population of neurons that express and co-express multiple neuropeptides including hypocretin (Hcrt), melanin-concentrating hormone (MCH), cocaine- and amphetamine-regulated transcript (CART) and neurotensin (NT). These neurons project widely throughout the brain to areas such as the locus coeruleus, the bed nucleus of the stria terminalis, the amygdala and the ventral tegmental area (VTA). Lateral hypothalamic projections to the VTA are believed to be important for driving behavior due to the involvement of dopaminergic reward circuitry. The purpose of this article is to review current knowledge regarding the lateral hypothalamic connections to the VTA and the role they play in driving these behaviors.

Keywords: lateral hypothalamus, ventral tegmental area, motivated behavior, reward

OPEN ACCESS

Edited by:
Billy Glenn Stanley, University of California, Riverside, United States

Reviewed by:
Stephen Rayport, Columbia University, United States
Eugene A. Kiyatkin, National Institute on Drug Abuse, United States
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Received: *28 February 2017*
Accepted: *22 June 2017*
Published: *06 July 2017*

Citation:
Tyree SM and de Lecea L (2017) Lateral Hypothalamic Control of the Ventral Tegmental Area: Reward Evaluation and the Driving of Motivated Behavior. Front. Syst. Neurosci. 11:50. doi: 10.3389/fnsys.2017.00050

INTRODUCTION

The field of behavioral neuroscience has taken a keen interest in understanding the neural circuits driving motivated behaviors. Food- and drug-seeking behaviors have received significant attention due to a concentrated effort to develop new, more successful treatments for disorders such as drug-abuse and obesity. Motivation is considered to be the energizing and directing of an animal's behavior toward a specific reward or goal, giving the animal the energy or drive required to overcome the physical costs involved (i.e., climbing, fighting, hunting) as well as directing the animal's concentration to the relevant activity over other possible activities (for example, feeding, drinking, lever pressing). It is vital that researchers work to develop a greater understanding of motivated behaviors due to their importance for survival; when an animal's ability to successfully direct their energy toward important survival functions (such as eating, drinking and sleeping) is jeopardized, this can result in disordered states such as obesity/anorexia, drug addiction and sleep disruption.

A series of studies carried out by Anand and Brobeck (1951) brought attention to the lateral hypothalamus (LH) as a candidate neural structure involved in behavioral motivation. They showed that stimulating the lateral hypothalamic area resulted in increased food intake, and conversely, LH lesions caused aphagia and weight loss, which led the authors to label the LH a feeding center. Additionally, the LH projects densely to the ventral tegmental area (VTA), which is also known to play a role in not only food-reward, but reward in general and that these functions in the VTA rely on its population of dopaminergic neurons (Gallistel et al., 1985; Phillips et al., 2003; Grace et al., 2007) suggesting that these two structures are important for driving goal-oriented

behaviors. Rather than reviewing the many brain structures involved in the behavioral motivation circuit (for review see Bailey et al., 2016; James and Aston-Jones, 2016), this review article will focus on the role of the LH and LH projections to the VTA in driving motivated behaviors.

THE LATERAL HYPOTHALAMIC STRUCTURE AND FUNCTION

The LH has been implicated in numerous functions including sleep-wake transitions (Adamantidis et al., 2007, 2010; Carter et al., 2009), feeding (Anand and Brobeck, 1951), energy balance (Brobeck, 1946), stress (Bonnavion et al., 2015) and reward (Olds and Milner, 1954; Hoebel and Teitelbaum, 1962) and plays a critical role in maintaining physiological and behavioral homeostasis. As well as being dubbed a "feeding center" by Anand and Brobeck (1951), the LH has also been labeled as a "pleasure center" (Olds, 1970) after it was shown that electrode implantation into the medial forebrain bundle in the LH resulted in persistent intracranial self-stimulation (ICSS; Olds and Olds, 1965). It has been suggested that this behavior is a result of stimulation of the descending fibers in the medial forebrain bundle that feed into the VTA (Bielajew and Shizgal, 1986), likely triggering a reward response.

The LH is a part of the hypothalamus located in the midbrain, and is home to a heterogeneous population of neurons. These populations include both gamma-aminobutyric acid (GABA)-ergic and glutamatergic neurons as well as subpopulations of neurons expressing neuropeptides that have been linked to the modulation of motivated behaviors such as hypocretin (Hcrt, also known as orexin; de Lecea et al., 1998; Sakurai et al., 1998), melanin-concentrating hormone (MCH; Qu et al., 1996), cocaine- and amphetamine-regulated transcript (CART; Kristensen et al., 1998), neurotensin (NT; Luttinger et al., 1982), leptin receptor (LepRb; Leinninger et al., 2011) and galanin (Skofitsch et al., 1985; Melander et al., 1986). These neurons connect to other brain structures via efferent projections from the LH to multiple structures including the amygdala, hippocampal formation, thalamus, the pons, brainstem and spinal cord, as well as intra-structural projections within the LH to other hypothalamic subnuclei (Ricardo and Koh, 1978; Berk and Finkelstein, 1982; Ter Horst and Luiten, 1987; Ter Horst et al., 1989). The LH also projects densely onto the VTA (Phillipson, 1979; Watabe-Uchida et al., 2012).

The large amount of overlapping gene expression within the LH is matched by the complexity of LH inputs into its target structures as well as feedback signals from those structures. Indeed, Horvath et al. (1999) showed images of a single neuron in the arcuate nucleus expressing immunoreactivity for neuropeptide Y, Hcrt inputs (from the LH) and a receptor for satiety hormone leptin. This suggests that the LH targets are equally heterogeneous as the LH itself. The large number of inputs, outputs, neuron types and functions present within the LH suggest that this structure is very complex and hosts an extremely diverse population of neurons, with many neurons co-expressing multiple neuropeptides and projecting to numerous target neural structures (for a comprehensive review of these connections, see Bonnavion et al., 2016), moving forward it will be important to gain a more precise understanding of these neurons, projections and functions to better disentangle the many roles of the LH.

THE VENTRAL TEGMENTAL AREA STRUCTURE AND REWARD FUNCTION

The VTA is a semi-circular nucleus which lies along the midline in the midbrain, it is home to a heterogeneous population of neurons containing multiple neurotransmitters including NT (Kalivas and Miller, 1984), cholecystokinin (CCK; Studler et al., 1981) and dopamine (for a thorough neuroanatomical review, see Oades and Halliday, 1987). The VTA dopaminergic system in particular has been implicated in brain-stimulation reward and food reward, psychomotor stimulation, learning and memory formation (Yokel and Wise, 1975; De Wit and Wise, 1977; Berridge, 2007; Friedman et al., 2014; Popescu et al., 2016), and it has been shown that goal-directed behavior is promoted by dopamine release from VTA^{DA} neurons (Gallistel et al., 1985; Phillips et al., 2003; Grace et al., 2007). Studies have shown that both the synaptic connections and intrinsic excitability of DA neurons are highly plastic dependent on the experiences of the animals (Stuber et al., 2008; Mao et al., 2011; Collo et al., 2014; Friedman et al., 2014; Gore et al., 2014). This suggests the possibility for experience/outcome-based modulation of behavioral motivation to be mediated via VTA^{DA} neurons and a "directing" role for dopamine in goal-oriented behaviors.

VTA^{DA} neuron involvement in reward processing has been studied extensively in an attempt to understand how these neurons code for rewards and the mechanisms through which they are able to modulate animal behaviors. However the complexity of the VTA, as well as the LH inputs into the VTA, require equally complex methods to investigate specific neuron populations within such heterogeneous neuron populations. Notably Eshel et al. (2015) carried out a complex set of experiments using a multi-method approach combining computational modeling, extra-cellular recordings, optogenetics and viral injections to investigate the computational mechanisms by which VTA^{DA} neurons calculate reward prediction error. Performing extra-cellular recordings of DA neurons while delivering expected and unexpected rewards, and using subsequent optogenetic manipulations to investigate the importance of VTA^{GABA} neurons to normal VTA^{DA} function. They found that as the size of the reward the animal receives increases, so does the DA neuron response, which was consistent with previous results (Tobler et al., 2005; Cohen et al., 2012), they also found that expectation of a reward resulted in a suppression of the DA neuron response, and that this response fit to a subtractive computational model better than an alternative divisive model (Eshel et al., 2015). Then, Eshel et al. (2015) investigated the role of VTA^{GABA} neurons in their subtraction model of VTA^{DA} neuron suppression in expected rewards by

optogenetically mimicking normal VTA^{GABA} neuron firing patterns and observing VTA^{DA} activity. They found that VTA^{GABA} stimulation resulted in the suppression of DA responses to unexpected rewards in a similar pattern to that seen in animals receiving expected rewards. This VTA^{GABA}-induced suppression of DA responses also fit with a subtractive computational model. Additionally, they showed that inhibition of VTA^{GABA} neurons partially reversed the expectation-dependent suppression of VTA^{DA} reward responses. Taken together this suggests that VTA^{DA} neurons calculate reward-error using a subtractive model, and that VTA^{GABA} neurons play a role in the temporal expectation modulation of DA responses in a manner that is consistent with the ramping expectation function in some models of prediction error computational models (Hazy et al., 2010; Rivest et al., 2014). This modulation of reward response in the VTA may play an important role in directing motivated behaviors to rewards that are less predictable over rewards that are more regularly available. This also suggests a mechanism by which VTA^{DA} neurons can rationalize between multiple rewards within an environment by modulating the reward value of more reliable rewards to be less rewarding than unpredictable rewards to shift the animals drive to focus on less readily-available rewards. This series of experiments shows the multitude of benefits that can be gained by using a multi-method approach to investigate neural circuits, by using behavioral protocols, optogenetics, electrophysiology and computational modeling (as well as investigating the role of both DA and GABA activity for comparison) these researchers were able to gain a deeper understanding of VTA^{DA} activity by observing it from multiple angles.

THE LH → VTA CIRCUIT CONNECTIONS LINKED TO MOTIVATED BEHAVIORS

Connections between the LH and the VTA have been studied extensively regarding their role in motivation, particularly LH inputs into the VTA dopaminergic system. Both DA depletion and excitotoxic LH lesions have similar outcomes altering motivated behavior, including aphagia (Grossman et al., 1978; Stricker et al., 1978). Shizgal et al. (1980) showed that the majority of reward-relevant fibers in the LH (identified using a self-stimulation protocol) project toward the VTA showing a clear neurophysiological connection between these two structures relating to reward. Early electrophysiological studies in the LH showed that LH stimulation could trigger a variety of behaviors such as mating, feeding, drinking, nest-building and gnawing (Roberts and Carey, 1965; Caggiula and Hoebel, 1966; Mogenson and Stevenson, 1967). Interestingly, these different behaviors did not appear to correlate to topographically distinct stimulation regions within the LH (Wise, 1971) but instead to patterns that had developed over a number of trials according to what type of goal stimuli the animals were presented with Valenstein et al. (1968), Wise (1968). This suggests that perhaps this LH stimulation was initiating a more general drive response rather than initiating a specific goal-targeted behavior. Considering the role of VTA in calculating prediction errors and this could mean that the LH triggers the "drive" component of motivation and the VTA is playing the role of "directing" that motivation toward goals within the animal's environment, changing the focus of the motivation as the rewards within the environment change.

The development of new techniques and biomarkers has allowed a closer look at the roles of different populations of LH neurons in VTA function. For example, optogenetic stimulation of LH^{GABA} inputs to the VTA results in conditioned place preference (Barbano et al., 2016), reduces VTA^{GABA} activity, and drives nucleus accumbens dopamine release (Nieh et al., 2016). Nieh et al. (2016) additionally showed that optogenetic stimulation of glutamatergic LH inputs to the VTA results in conditioned place aversion. Interestingly, the behavioral response to optogenetic stimulation of VTA-innervating LH^{GABA} neurons differed depending on the stimulation frequency, with low frequency stimulation (5–10 Hz) resulting in increased feeding, and high frequency stimulation (40 Hz) appeared to trigger reward, resulting in a place preference (Barbano et al., 2016). It is possible that these two functions may be being mediated by two different neuropeptides co-expressed within LH^{GABA} neurons, or that this stimulation triggers a general "drive" and the stimulation frequencies result in the release of different neuropeptides in the VTA, resulting in a target for the "drive" response. This could suggest that the LH and the VTA are the "drive" and "focus" sources for motivated behaviors, respectively, with LH activation producing a general energizing of the animal to perform a behavior, and the VTA then directing that energy to a specific goal-oriented behavior—depending on which neurotransmitters are released, or the stimulation frequency, or some other determining factor. Additionally, the development of this gene-targeting methodology also opens up the possibility to investigate the multiple other neuron types in the LH that are known to project to the VTA to better understand how these neuron types differentiate between multiple input signals and determine which environmental goals to pursue.

A Role for Hypocretin in Motivation and Reward

Studies investigating connections between the LH and the VTA have also investigated whether there are specific populations of LH neurons underlying the connection with the VTA. One LH → VTA population of interest is Hcrt neurons, which are known to project densely to the VTA (Peyron et al., 1998; Fadel and Deutch, 2002) and are suggested to play an important role in the LH → VTA^{DA} reward circuit. Within the LH Hcrt-containing neurons (Hcrt-1 and Hcrt-2 also referred to as ORX-A and ORX-B, respectively), are concentrated particularly in the perifornical of the LH, and are known to project widely throughout the brain (de Lecea et al., 1998; Peyron et al., 1998; Sakurai et al., 1998, 2005; Marcus et al., 2001; Fadel and Deutch, 2002; Yoshida et al., 2006) and produce effects via actions at their receptors HcrtR1 and HcrtR2 (de Lecea et al., 1998; Peyron et al., 1998; Sakurai et al., 1998; Marcus et al., 2001; Fadel and Deutch, 2002).

Hcrt has been shown to play a prominent role in sleep-wake transitions (de Lecea et al., 1998; Hagan et al., 1999; Piper et al., 2000; España et al., 2001; Adamantidis et al., 2007, 2010; Carter et al., 2009) as well as reward/reinforcement (Boutrel et al., 2005; Harris et al., 2005; Smith et al., 2010) and previous studies have shown that $Hcrt^{LH}$ neurons have modulatory effects on VTA function (Harris et al., 2005; Borgland et al., 2006).

Pharmacological and genetic methods of inducing or inhibiting Hcrt activity have provided evidence that Hcrt is involved in DA reward processes particularly via connections with the VTA (España et al., 2010, 2011; Prince et al., 2014). There is also evidence that Hcrt directly affects dopamine activity and reward responses; it has been shown that Hcrt administration induces burst firing of DA neurons (Borgland et al., 2006) and results in increased cocaine self-administration (España et al., 2011). Chemical Hcrt activation and Hcrt infusions into the VTA reinstate previously extinguished drug- and food-seeking behaviors (Harris et al., 2005). Hcrt has also been shown to be necessary for normal behavioral and neural reward responses, Hcrt knock-out mice fail to develop a cocaine-conditioned place-preference, and showed diminished DA signaling following cocaine administration compared to wild-type controls (Shaw et al., 2016). Administration of Hcrt antagonist almorexant in the VTA attenuates ethanol self-administration (Srinivasan et al., 2012), and blocking Hcrtr1 using SB-334867 results in reduced excitation of DA neurons in the VTA (Moorman and Aston-Jones, 2010) and decreases motivation to obtain cocaine rewards (Borgland et al., 2006; España et al., 2010; Prince et al., 2014; Brodnik et al., 2015). Taken together these studies suggest that intact Hcrt function is necessary for both neural dopaminergic responses and behavioral responses to reward and reinforcement.

Considering the various roles for Hcrt in behavioral arousal, LH^{Hcrt} may initiate a general drive response, and the target behaviors of this increased drive may be determined in the targets of Hcrt neurons. Based on the evidence for Hcrt modulation of VTA^{DA} activity, it would appear that there is some link between LH^{HCRT} and VTA^{DA} neurons, however, studies investigating connections between these two neuron populations have discovered that this LH^{HCRT} modulation of VTA^{DA} neurons does not appear to be driven by a straightforward monosynaptic connection. It has been shown that few Hcrt axons in the VTA synapse directly onto VTA^{DA} or VTA^{GABA} neurons and the majority of Hcrt fibers appear to be passing fibers, possibly passing on to caudal brainstem structures (Balcita-Pedicino and Sesack, 2007). This suggests that these Hcrt → VTA inputs may be playing a modulatory role in the VTA via non-synaptic mechanisms or volume transmission, rather than direct monosynaptic inputs. Although the majority of reward-relevant fibers in the LH do project toward the VTA (Bielajew and Shizgal, 1986) and infusion of Hcrt-2 in VTA whole-cell patch-clamp recordings increases glutamatergic transmission to VTA neurons (Borgland et al., 2008), there have been findings which raise questions about the mechanisms of LH → VTA circuitry in mediating reward: paired-pulse studies of LH → VTA fibers have shown that both the refractory periods for fibers depolarized at the electrode tip and their conduction velocities are significantly faster than would be expected from the unmyelinated dopaminergic fibers connecting the VTA and LH (Yeomans, 1979; Shizgal et al., 1980; Gallistel et al., 1981). The finding that the timescales of these fibers do not line up suggest that the mechanism via which LH neurons mediate VTA^{DA} neurons may be more complex than previously considered and requires further investigation. These discrepancies can be further studied to develop models to determine possible mechanisms that would explain the divergent timescales. Tools such as optogenetics will be particularly useful for this purpose due to the rapid induction of neuronal activity and ability to manipulate neuronal activity with timed precision, this method has been used to investigate timescales of LH^{Hcrt} connections to the locus coeruleus and develop a network model accounting for variations in neuronal activity that have been observed in slice electrophysiology (Mosqueiro et al., 2014) this method could also be applied to the LH^{Hcrt} → VTA^{DA} circuit. It has previously been shown that Hcrt neurons take 30 s to peak in slice electrophysiology recordings (Ishibashi et al., 2015) understanding how this fits into a model of Hcrt interactions with the VTA will be important for determining mechanisms underlying this Hcrt modulation of DA signaling.

Other LH Neuropeptide Candidates Involved in Motivation and Reward

Timing discrepancies between Hcrt and DA signaling could also be due to the involvement of additional LH neurotransmitters that have been linked to driving motivated behaviors. A recent study highlighted CART as a candidate in the LH → VTA^{DA} circuit using ICSS with an electrode implanted in the LH. Somalwar et al. (2017) showed that ICSS resulted in increased activation of CART cells in the LH and that administration of CART into the posterior VTA enhanced the self-stimulation behavior. Additionally, they showed that animals avidly self-infused CART (55–102) into the posterior VTA via cannula, and that this behavior can be inhibited by administration of dopamine D1 receptor antagonist directly into the nucleus accumbens shell. Suggesting that CART may too play a role in VTA^{DA} reward processes. Another possible candidate is NT, which is known to project from the LH to the VTA (Leinninger et al., 2011) and has been shown to facilitate prolonged DA release (Patterson et al., 2015). It has also been shown that LH NT neurons synapse onto twice as many VTA^{DA} neurons as they do VTA^{GABA} neurons (Beier et al., 2015). The LH is home to many different neuropeptides and receptor types, and many LH neurons co-express multiple genes (for a comprehensive review see Bonnavion et al., 2016). For example, approximately 30% of LH NT neurons co-express the LepRb receptor for anorexigenic hormone leptin (Leinninger et al., 2011), and 95% of these neurons co-expressing NT and LepRb also co-localize with galanin (Laque et al., 2013).

FUTURE INVESTIGATIONS OF THE LH → VTA CIRCUIT: WHAT CAN WE LEARN?

While the broad connectivity between LH and VTA has been clearly elucidated, disentangling the precise roles of the relatively heterogeneous subpopulations of LH → VTA circuit neurons has made slow progress due to the topological and functional overlap of these LH neuronal populations, their various functions (illustrated in **Figure 1**), their diverse connections to the VTA, as well as feedback signals traveling from the VTA to the LH. These factors make investigating this circuit difficult, however the recent development of new technologies has provided researchers with more precise tools to investigate this circuit that should be taken advantage of.

Untangling the complexity of neurons co-expressing multiple genes can be investigated with gene-targeted methods such as optogenetics and fiber-photometry, methods which have been successfully used to investigate other LH circuits such as the LH-LC arousal circuit (Adamantidis et al., 2010). These methods could be used to investigate the role of each of these LH neuron populations in driving VTA^{DA} function, whether the subpopulations of NT or CCK neurons in the VTA are playing an important role in LH mediated VTA activity. Of particular interest will be the investigation of how LH^{Hcrt} neurons drive VTA^{DA} neurons, as it is apparent that there is some modulation in this direction, however the lack of direct $LH^{Hcrt} \rightarrow VTA^{DA}$ connections raises questions as to the mechanism behind this modulatory function. Additionally these methods could be used to study how LH inputs into the VTA and VTA inputs into the LH modulate this neural circuit. Researchers can also benefit from using multi-method approaches, such as those reported by Eshel et al. (2015) to develop a more nuanced understanding of the multi-faceted functions of the LH and VTA.

We have already seen how the transition from gross electrophysiological stimulation and recording to precise and neuron-type specific optogenetic stimulation and

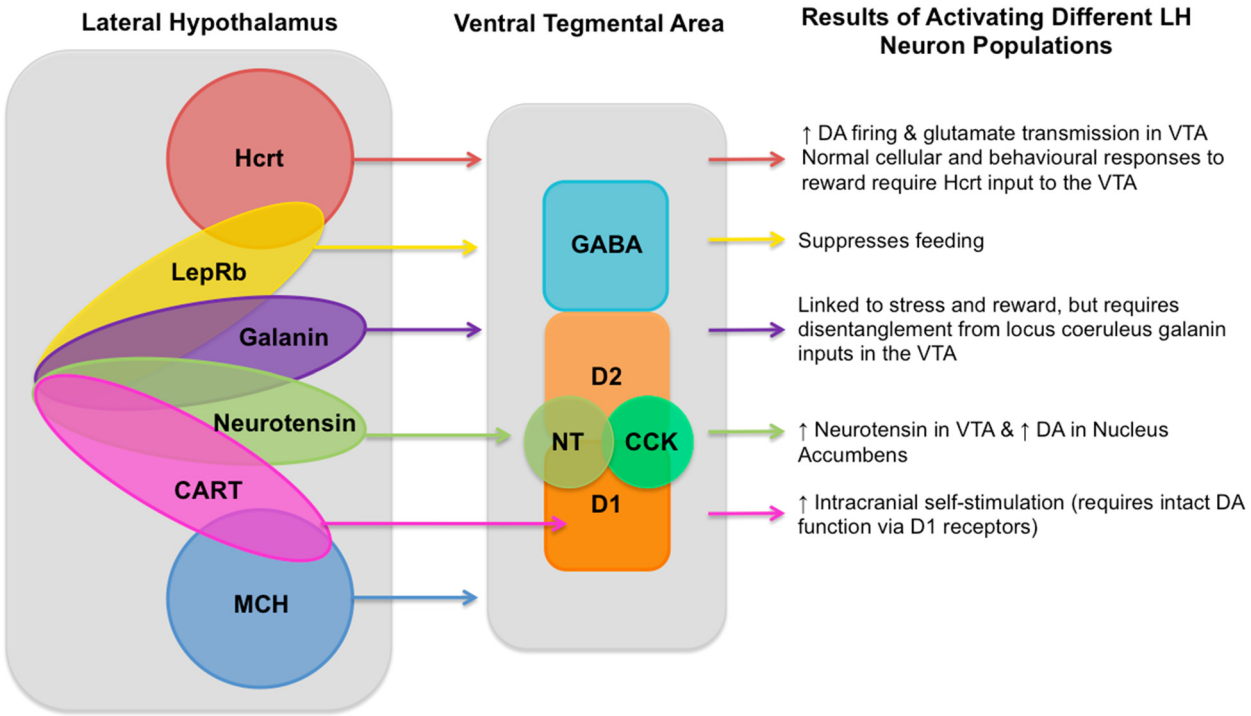

FIGURE 1 | Known lateral hypothalamus (LH) neuronal population inputs into the ventral tegmental area (VTA) and the functions linked to their activation. A diagram showing neuron populations in the LH, their currently known inputs into the VTA, and the behavioral and neural responses to their activation as described in the article text. LH cell types include: hypocretin (Hcrt; de Lecea et al., 1998; Sakurai et al., 1998) known to produce increased dopaminergic firing (Borgland et al., 2006) and glutamate transmission in the VTA (Borgland et al., 2008); leptin receptor (LepRb; Leinninger et al., 2011) which is involved in processing dietary fat intake and satiety (Leinninger and Myers, 2008; Vong et al., 2011); galanin (Skofitsch et al., 1985; Melander et al., 1986) which is linked to stress and reward (Picciotto et al., 2010), but requires disentangling from locus coeruleus galanin inputs to the into the VTA; neurotensin (NT; Luttinger et al., 1982) which produces increased NT in the VTA and subsequently increased dopamine in the Nucleus Accumbens (Patterson et al., 2015); cocaine- and amphetamine-regulated transcript (CART; Kristensen et al., 1998) which triggers increased intracranial self-stimulation (ICSS) which requires intact DA function via the D1 receptor (Somalwar et al., 2017); and melanin-concentrating hormone (MCH; Qu et al., 1996). As well as these LH neuron types there are also gamma-aminobutyric acid (GABA)-ergic and glutamatergic neurons, though the degree to which each of these different neuron types in the LH can be determined to be glutamatergic or GABAergic still requires further research and so is not represented in this diagram. VTA neuron types include GABAergic neurons, two types of dopaminergic neurons (D1 and D2), as well as NT (Kalivas and Miller, 1984) and cholecystokinin (CCK; Studler et al., 1981). Within the LH it is known that single neurons can co-express multiple different neurotransmitters, known overlapping populations are shown above, though the size of the overlapping circles is not intended to be representative of the size of the overlapping neuron populations and should be merely considered as illustrative.

fiber-photometry recordings has allowed researchers to genetically target (rather than topographically target) much more specific and well defined neuron populations than the prior large-scale neuron-type agnostic electrophysiological and pharmacological approaches. Additionally, the rapid development of next-generation sequencing approaches allows researchers to investigate heretofore unknown or indefinable single-cell gene-expression profiles (Macosko et al., 2015). These approaches are key in the identification of the different neuron types within a topologically heterogeneous and complex structure such as the LH, and in combination with optogenetics and fiber photometry will allow fine-grained dissection of the role of specific neuron populations in ways that were not previously possible. Indeed, a recently developed method for profiling cell-types by separating cells into Nano liter-sized droplets and sequencing each cells RNA (drop-seq) was recently used to identify 50 distinct cell types within the hypothalamic arcuate-median eminence complex (Campbell et al., 2017). A similar experiment in the LH will be crucial to give researchers the ability to cluster neurons into populations based on their gene expression. This could lead to a better categorization of different cell types within the diverse population of LH neurons and subsequent optogenetic investigations targeting these genetically distinct neuron populations could lead to a better understanding of the roles these neuron subpopulations play in functions such as motivating behavior.

CONCLUSION

The LH → VTA circuit clearly plays an important role in driving behavior. Initial studies showed the importance of the LH in motivating basic functions such as mating, feeding, drinking, nest-building and gnawing, and the evidence that lesioning the LH results in a loss of these behaviors such as dramatic weight-loss, has highlighted the importance of this circuit for survival. Understanding this circuit will be important for understanding how normal behavior is elicited, and what is going wrong when these behaviors become disordered in cases such as obesity, anorexia, drug-abuse, anhedonia, etc. Considering the two main facets of goal-oriented behavior are the energizing and directing of behavior and what is known of the LH and VTA, it would appear that the LH neurons may play more of a role in the "driving" motivated behavior whereas the VTA likely directs the behavior toward relevant goals/rewards via the dopaminergic system for example, by modulating the reward-value of different environmental rewards. Although there are still many outstanding questions regarding the LH → VTA circuit, the development of new research technologies are allowing researchers more promising opportunities to probe this circuit and gain a more specific understanding of the basis for the dysregulation of this circuit and the negative behavioral consequences associated with it, such as drug abuse and obesity.

AUTHOR CONTRIBUTIONS

This article was written by SMT with editorial help and guidance from LL.

FUNDING

SMT is currently funded by the Philip Wrightson Postdoctoral Fellowship, awarded by the New Zealand Neurological Foundation. LL is supported by grants from the National Institute of Mental Health (NIMH) (5R01MH087592-05; R01MH102638-01A1), the National Institutes of Health (NIH) (1R01AG047671-01), the US-Israel Binational Science Foundation (BSF #2011335), Merck and Johnson and Johnson.

ACKNOWLEDGMENTS

We thank Dr. Robert G.K. Munn for helpful comments on this manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Systems Neuroscience

REVIEW
published: 08 June 2017
doi: 10.3389/fnsys.2017.00042

Integrating Neural Circuits Controlling Female Sexual Behavior

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The hypothalamus is most often associated with innate behaviors such as is hunger, thirst and sex. While the expression of these behaviors important for survival of the individual or the species is nested within the hypothalamus, the desire (i.e., motivation) for them is centered within the mesolimbic reward circuitry. In this review, we will use female sexual behavior as a model to examine the interaction of these circuits. We will examine the evidence for a hypothalamic circuit that regulates consummatory aspects of reproductive behavior, i.e., lordosis behavior, a measure of sexual receptivity that involves estradiol membrane-initiated signaling in the arcuate nucleus (ARH), activating β-endorphin projections to the medial preoptic nucleus (MPN), which in turn modulate ventromedial hypothalamic nucleus (VMH) activity—the common output from the hypothalamus. Estradiol modulates not only a series of neuropeptides, transmitters and receptors but induces dendritic spines that are for estrogenic induction of lordosis behavior. Simultaneously, in the nucleus accumbens of the mesolimbic system, the mating experience produces long term changes in dopamine signaling and structure. Sexual experience sensitizes the response of nucleus accumbens neurons to dopamine signaling through the induction of a long lasting early immediate gene. While estrogen alone increases spines in the ARH, sexual experience increases dendritic spine density in the nucleus accumbens. These two circuits appear to converge onto the medial preoptic area where there is a reciprocal influence of motivational circuits on consummatory behavior and *vice versa*. While it has not been formally demonstrated in the human, such circuitry is generally highly conserved and thus, understanding the anatomy, neurochemistry and physiology can provide useful insight into the motivation for sexual behavior and other innate behaviors in humans.

Keywords: estrogen, progesterone, MOR, β-endorphin, dopamine, D1 receptors, dendritic spines, membrane estrogen receptor

OPEN ACCESS

Edited by:
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Received: *27 February 2017*
Accepted: *22 May 2017*
Published: *08 June 2017*

Citation:
Micevych PE and Meisel RL (2017) Integrating Neural Circuits Controlling Female Sexual Behavior. Front. Syst. Neurosci. 11:42. doi: 10.3389/fnsys.2017.00042

INTRODUCTION

Mating, a social behavior, is directly influenced by hormonal state, which transmits information about the internal state of the animal to steroid responsive circuits in the nucleus accumbens and hypothalamus. These circuits integrate the hormonal state of the animal with environmental/sensory cues to produce an appropriate response (Micevych and Ulibarri, 1992). Female sexual behavior is divided into three components: attractivity, proceptivity, and receptivity (Beach, 1976). The best studied of these are proceptivity and receptivity. The behavioral

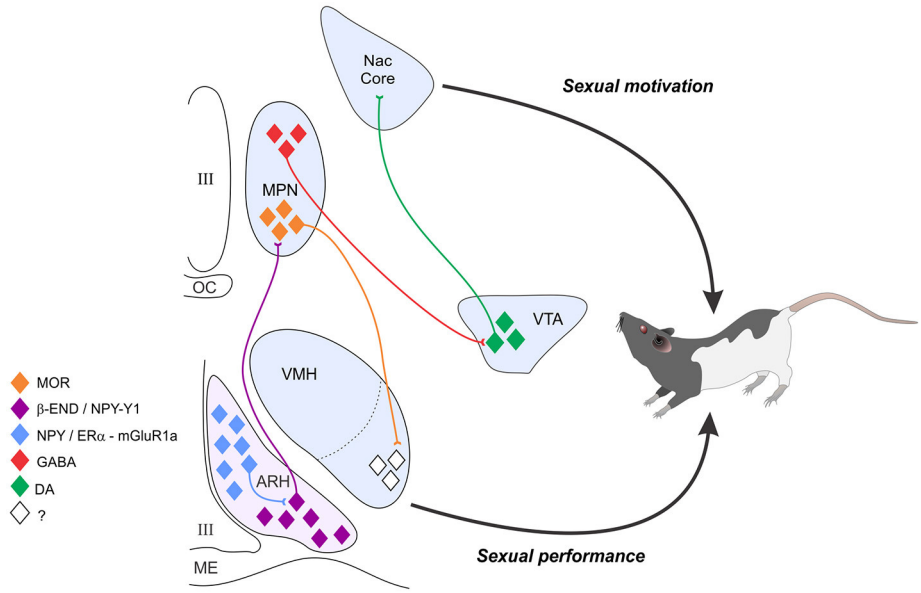

FIGURE 1 | The estradiol induction of sexual receptivity in the female rat is indicated by lordosis behavior. The CNS regulation of this global response to hormonal and sensory input is regulated by a diffuse circuit that extends from the limbic system to the spinal cord. Within this lordosis regulating circuit, estradiol acts rapidly through estradiol membrane signaling (EMS) to release neuropeptide Y (NPY) in the arcuate nucleus of the hypothalamus (ARH), which activates β-endorphin (β-END) projection neurons that extend to the medial preoptic nucleus (MPN). The MPN is an important integrative node receiving accessory olfactory and limbic input. β-END activates MOR, producing a transient inhibition of the MPN which is relieved by progesterone in the cycling female. The MPN MOR neurons in turn project to the ventromedial nucleus of the hypothalamus (VMH), the final common output of the hypothalamus. The EMS and resulting transient inhibition is necessary for the full expression of lordosis behavior in the rat. In addition to its VMH efferents, the MPN sends a GABAergic inhibitory projection to the VTA. Estrogen inhibition of the MPN contributes to dopaminergic activation of the nucleus accumbens, which both regulates sexual motivation and mediates the rewarding consequences of sexual behavior. Estradiol's actions on the combined circuits serve to initiate sexual motivation in the male's presence, modulate the expression of sexual behavior to tactile stimulation provided by the mounting male, and to feed forward, increasing the efficiency of future copulatory interactions in a way that presumably increases reproductive success.

manifestations of the motivation to copulate by a female are expressed as the female's willingness to accept the male's mount attempts and proceptive behaviors that include hopping, darting, and ear "wiggling" enticing the male to copulate. In the female rodent, developing ovarian follicles secrete estradiol into the peripheral circulation. This estradiol acts in mesolimbic circuits to increase motivation and in the hypothalamus to increase receptivity. Estradiol induces progesterone receptors (PR) within the hypothalamus (Parsons et al., 1979, 1980, 1981; McGinnis et al., 1981; Shughrue et al., 1997a; Alves et al., 2000) and stimulates synthesis of progesterone in astrocytes. This neuroprogesterone induces proceptive behaviors (Micevych and Sinchak, 2013), and the bolus of progesterone from the corpus luteum of the ovary acts on hypothalamic circuits to facilitate receptive behaviors since estradiol levels in the intact rat are insufficient to induce lordosis by themselves (Beach, 1948; Young, 1961; Powers, 1970; Sodersten and Eneroth, 1982). In addition to hypothalamic regions, motivation for mating behavior is heavily influenced by the release of dopamine (DA) in the nucleus accumbens. A persistent question in the field is the interaction of reward circuits and motoric circuits to elicit behavior, a model of which is summarized in **Figure 1**.

The mesolimbic motivational circuitry involves the classic reward circuit of projections from the ventral tegmental area (VTA) to the nucleus accumbens. Experiments in which the female rat paces the sexual encounter have demonstrated an increase in the release of dopamine in the reward pathway in anticipation of sexual contact (reviewed in Cummings and Becker, 2012) and female rats with basal forebrain damage including the nucleus accumbens resist the male's attempts to mount (e.g., Rivas and Mir, 1991).

The lordosis-regulating, receptive circuit involves limbic and hypothalamic nuclei including the posterodorsal medial amygdala (MeApd), the bed nucleus of the stria terminalis (BSt), medial preoptic nucleus (MPN), arcuate nucleus (ARH), and ventromedial nucleus (VMH) of the hypothalamus (Sinchak and Micevych, 2001; Sinchak et al., 2010; Polovin et al., 2012; reviewed in Micevych et al., 2015). It is in the ARH that estradiol has its initial behaviorally-relevant actions, which are mediated by membrane-initiated signaling. The VMH is the final common projection from the integrative hypothalamic and limbic circuits to the periaqueductal gray (PAG), reticular formation and vestibular nuclei, which in turn, send inputs to spinal motoneurons innervating trunk and neck musculature needed for the lordosis posture (reviewed in Pfaff et al., 1994)—a hallmark of sexual receptivity.

While, the common perception is that estradiol stimulates sexual receptivity, this is only the final result. The mechanisms in the interval between estradiol increasing in the blood and lordosis behavior are complicated and involve an inhibition in the hypothalamus but activation in the mesolimbic circuit. Ovariectomized (OVX) females are not sexually receptive with or without progesterone for approximately 24 h after estrogen (estradiol benzoate, EB) priming (Green et al., 1970; Quadagno et al., 1972; Sinchak and Micevych, 2001).

STEROID ACTIVATION OF SEXUAL RECEPTIVITY

The ability to copulate with a male regardless of the her motivational state was defined by Beach as sexual receptivity, physically manifested as the lordosis reflex (Beach, 1948, 1976 and reviewed in Micevych and Sinchak, 2007). This posture includes a stereotypic arching of the back, elevation of the hindquarters, dorsiflexion of the tail and extension of the neck. The lordosis quotient, a measure of sexual receptivity, is defined as the number of lordosis reflexes by the female in response to the number of mounts X 100. As Sternson indicates, lordosis is among the most dramatic behavioral responses in neuroscience (Sternson, 2013). What makes the lordosis reflex so valuable, however, is its reproducibility (i.e., the lordosis quotient is repeatedly achieved for a specific dose of estradiol or estradiol + progesterone).

In the intact cycling rat, the sequential release of estrogens and progesterone from the ovary tightly regulates sexual receptivity. Various doses and timing of treatments with estradiol as well as treatments with estrogens and progesterone have been used over the years to induce the lordosis reflex (described in Micevych and Sinchak, 2013). What all of these paradigms have in common is that they all require estrogen and that sexual receptivity lags behind the administration of estradiol. This refractory period lasts ∼20–24 h. Since the early 1980s, when it was demonstrated that gene transcription was needed for estrogenic induction of lordosis behavior, this lag-time was assumed to be due to the transcription of proteins necessary for lordosis behavior (Rainbow et al., 1980, 1982; Parsons et al., 1982). Among the first of these proteins to be discovered was PR (Parsons et al., 1979, 1980, 1981; McGinnis et al., 1981; Shughrue et al., 1997b; Alves et al., 2000). Indeed, progesterone was necessary to induce lordosis with low exogenous estradiol doses and at "early" (∼24 h post estradiol) time points (Parsons et al., 1980; Sinchak and Micevych, 2001). What this theory hypothesis does not explain is how estrogens alone induce lordosis, which required higher estrogen doses and a longer interval between estrogen treatment and the behavior (Pfaff, 1970 reviewed in Clemens and Weaver, 1985). What has emerged is the idea that different behavioral circuits are activated by estradiol only treatment compared with estrogen and progesterone (reviewed in Sinchak et al., 2015). In the ARH, estradiol-only facilitation of lordosis reflex requires the activation of the opioid receptor, ORL-1, but estradiol + progesterone does not (Borgquist et al., 2014). While the motivation of sexual behavior is driven by the pleasure derived from the copulatory act (e.g., Meisel and Mullins, 2006), reproduction requires the coordination of sexual receptivity with the production of a viable oocyte that can be fertilized. As mentioned above, this stimulus is estradiol which rises during the estrous cycle until it peaks on proestrus. Circulating levels of (ovarian) progesterone are elevated several hours after the female becomes sexually receptive (Moss, 1974; Sodersten and Eneroth, 1981). We tested the idea that in the intact rat both estradiol and progesterone were needed for sexual receptivity by treating OVX/ADX rats with 10 μg EB and then 48 h later with 17 β-estradiol. In this paradigm blockade of progesterone receptors or steroidogenesis did not attenuate sexual receptivity, but did block proceptive behaviors (Micevych et al., 2008; Micevych and Sinchak, 2011). These results support the idea that lordosis is sensitive to estradiol levels and progesterone is responsible for inducing proceptive behaviors (Tennent et al., 1980; Lumia et al., 1981) and demonstrate that neuroprogesterone mediates proceptive behaviors. Further, the study showed that neither progesterone nor progesterone receptors are needed for estradiol-only induced lordosis. Finally, progesterone terminates lordosis behavior. Thus, progesterone's action are bi-phasic, first it augments the estradiol induced lordosis behavior and then prevents it (Goy et al., 1966; Nadler, 1970; Sodersten and Eneroth, 1982; Meisel and Sterner, 1990).

ARH to MPN to VMH Circuit

The ARH to MPN neural subcircuit provides an excellent opportunity to examine steroid signaling that regulates sexual receptivity. Within the ARH, a population of β-endorphin (β-END) expressing neurons inhibit lordosis by acting on the μ-opioid receptor (MOR; Cheung and Hammer, 1995; Torii et al., 1999; Mills et al., 2004). β-END is one of several posttranslational proteins expressed in proopiomelanocortin (POMC) neurons. While much attention, in terms of metabolic control, has been lavished on the POMC neurons that project to the periventricular nucleus (Jacobowitz and O'Donohue, 1978; Bell et al., 2000; Melnick et al., 2007), another POMC population regulates sexual behavior through its projection to the MPN (Jacobowitz and O'Donohue, 1978; Cheung and Hammer, 1995; Bell et al., 2000; Ibrahim et al., 2003; Mills et al., 2004; Melnick et al., 2007). Reproductively important POMC neurons appear to have a distinct morphology, and sensitivity to MOR agonists and ATP-sensitive potassium (KATP) channel modulators (Jacobowitz and O'Donohue, 1978; Cheung and Hammer, 1995; Bell et al., 2000; Ibrahim et al., 2003; Mills et al., 2004; Melnick et al., 2007). An acute effect of estradiol treatment in OVX rats is the activation and internalization of MOR in the MPN, leading to inhibition of lordosis behavior (Sirinathsinghji et al., 1986; Pfaus and Pfaff, 1992; Sinchak and Micevych, 2001; Sanathara et al., 2011). Reversal of MOR activation produces a facilitation of sexual receptivity (Eckersell et al., 1998; Sinchak and Micevych, 2001; Micevych et al., 2003; Mills et al., 2004; Sinchak et al., 2005; Dewing et al., 2007).

Throughout the estrous cycle, the pattern of MPN MOR activation/internalization tracks the sexual receptivity of the female, that is, MOR are deactivated (internalized) on the evening of proestrus when the rat is sexually receptive and reactivated on

the morning of estrus when she is no longer receptive (Sinchak and Micevych, 2003; reviewed in Micevych and Sinchak, 2013; Sinchak et al., 2015).

A series of experiments sought to identify the ER mediating this transient inhibition. First, the MOR activation was not observed in ERαKO mice but was present–in ERβKO mice (Micevych and Sinchak, 2013). Second, in the ARH, a membrane impermeable estradiol-biotin conjugate induced MOR activation (Dewing et al., 2007) indicating a membrane ERα (mERα). Third, mERα forms a signaling complex with metabotropic glutamate receptor-1a (mGluR1a) (mERα-mGluR1a) that activates MOR (Dewing et al., 2007, 2008). Fourth, estradiol membrane-initiated signaling (EMS) through the mERα-mGluR1a complex activates PKCθ to induce internalization of MPN MOP and actively inhibit lordosis (Dewing et al., 2008). It is well established that estradiol activates the POMC from the ARH. Estradiol sensitive inputs appear to modulate POMC activity. In the rat, estradiol appears to act on NPY neurons, a subpopulation of which express ERα mRNA (Sar et al., 1990; Simonian et al., 1999). In N-38s immortalized hypothalamic neurons that express NPY, we have shown mERα, which mediates the estradiol activation of PKCθ, increases extracellular-signal regulated kinases 1/2 (ERK1/2) and intracellular free calcium (Micevych and Dominguez, 2009; Dominguez et al., 2013), results consistent with an estradiol-induced activation of NPY-Y1receptors on MPN-projecting POMC neurons, which inhibit lordosis behavior (Clark et al., 1985; Mills et al., 2004).

Estradiol controls the level of cell signaling in the ARH through modulation of levels of mERα. In primary cultured neurons, astrocytes, immortalized neurons and *in vivo*, estradiol treatment transiently increases trafficking of ER to the membrane (Bondar et al., 2009; Dominguez and Micevych, 2010). Plasma mER levels are determined by a balance of trafficking to the membrane, requiring ER palmitoylation and interaction with caveolin-1 (CAV1; Razandi et al., 2002; Meitzen et al., 2013), and internalization, requiring phosphorylation and β-arrestin-1 (Arrb1; Dominguez et al., 2009). Knockdown of CAV1 prevented the trafficking of ERα to the plasma membrane *in vivo*. Interestingly, ERαΔ4, a splice variant of ERα concentrated on the membrane in cultured cells from nervous tissue (Gorosito et al., 2008; Bondar et al., 2009; Dominguez and Micevych, 2010; Dominguez et al., 2013) was still trafficked to the plasma membrane (Christensen et al., 2011). To ascertain the role of β-arrestin-1 (Arrb1) in receptor dynamics, we studied ERα and ERαΔ in ARH tissue and N-38 neurons (Wong et al., 2015). As expected, Arrb1 was critical for ERα internalization following estradiol stimulation, but unexpectedly, trafficking of ERαΔ4 was also dependent on Arrb1. With siRNA, which reduced Arrb1 protein by 80%, membrane levels of ERαΔ4 were almost half of the control levels. Interestingly, previous studies have indicated that EMS requires ERα transactivation of mGluR1a, and that ERαΔ4 does not associate with mGluR1a (Bondar et al., 2009). The loss of ERK1/2 activation after Arrb1 siRNA indicates that Arrb1 helps organize the mER signaling machinery. Moreover, this loss of Arrb1-dependent signaling in the female ARH prevented EB induced lordosis behavior indicating that microcircuits in the ARH activated by estradiol need Arrb1 to function.

The microcircuits in the ARH mediating estradiol regulation of behavior are much more complex than indicated by the previous discussion. One indication of this is the role of GABA in this nucleus. $GABA_B$ receptors mediate both initial and sustained estradiol–induced activation of β-END release into the MPN (Sinchak et al., 2013). Inhibition of $GABA_B$ receptors in the ARH blocked estradiol-induced MPN MOR activation, which is needed for lordosis behavior, (Torii and Kubo, 1994; Torii et al., 1995, 1996, 1997, 1999). Knockdown of the GABA synthetic enzymes, GAD65 and GAD67, prevented facilitation of lordosis (McCarthy et al., 1994). Together these results indicate that estradiol-induced MOR activation is maintained at least in part by $GABA_B$ signaling. Antagonizing $GABA_B$ receptors 30 h after estradiol priming mimics the action of progesterone in this circuit (Sinchak and Wagner, 2012). The idea that progesterone acts through the silencing $GABA_B$ receptors is intriguing (Micevych and Sinchak, 2013). Nevertheless, these data indicate that the neurochemistry of sexual receptivity is a far from settled science and will require further research to unravel.

Mesolimbic Circuitry and Sexual Motivation

In contrast to the hypothalamic circuitry which regulates the expression of sexual receptivity—lordosis, the mesolimbic circuitry regulates behavioral processes key to the motivational control of the expression of female sexual behavior (Salamone et al., 2016). Bindra (1969) offered one of the early neurobiological conceptualizations of motivation in which an organism's internal physiological state interacts with environmental stimuli that have intrinsic value to create the "central motive state." This central motive state in turn induces postural adjustments (e.g., lordosis) and organized motor outputs (e.g., hopping and darting) that comprise the species typical actions related to each central motive state. Further, with experience motivated behaviors can be conditioned to increase the range of incentive stimuli and the animal's responses. Berridge (2007) refined this conceptualization of motivation to argue that mesolimbic dopamine primarily responds to incentive salience which increase the animal's "wanting" of rewarding stimuli. Though the literature on female sexual behavior is rather limited, it is notable that basal forebrain lesions of the mesolimbic pathway encompassing the nucleus accumbens increase the likelihood that a female rat will resist the male's mounting attempts or more rapidly escape the male (a decrease in "wanting" incentive stimuli), though if the male is able to forcibly apply tactile flank stimulation the lesioned female will exhibit the postural adjustments of the reflexive lordosis response (Dohanich and McEwen, 1986; Rivas and Mir, 1990, 1991; Guarraci et al., 2002). Such early studies formed the basis for conclusions that the mesolimbic system was associated with the incentive motivational properties of female sexual behavior. Our view of the female sexual behavior literature is that mesolimbic dopamine is involved in the rewarding consequences

of sex (or "liking") and incentive salience ("wanting"), both of which can be modified by the animal's experience.

The biological relevance of sexual motivation is seen in the modification of subtle sexual responses thought to impact reproductive success. Female rats display a series of approach and avoidance behaviors to males which regulate the temporal frequency of mounting attempts by the male (Bermant, 1961; Edmonds et al., 1972). Indeed, there is an optimal number of intromissions and periodicity of those intromissions needed to stimulate a set of neuroendocrine reflexes requisite for successful uterine implantation of embryos, though the specific sensory requirements differ among species. This pattern of vaginal stimulation was termed the "species vaginal code" by Diamond (1970) and typically is the optimal pattern of stimulation needed to produce a conditioned place preference (Paredes and Vazquez, 1999).

The highly active pattern of approach/avoidance regulates the receipt of intromission for female rats. Female hamsters have a very different sexual behavior pattern in that they are relatively immobile, maintaining the lordosis posture for up to 90% of the interaction with the male; seemingly the male determines the pacing of intromissions. However, the female hamster has subtle perineal movements that regulate the male's ability to achieve intromission (Noble, 1979, 1980). Anesthetizing the female hamster's perineum dramatically reduces intromissions, pointing to the female's control of the mating interaction (Noble, 1980).

Pacing of sexual interactions with the male is seen the first time female rats or hamsters are placed with a male. Still, sexual experience can modify these sexual interactions (Bradley et al., 2005; Meerts et al., 2014). In hamsters this change in copulatory efficiency was tested by giving females different levels of sexual experience and measuring the frequency of intromissions by the male. Giving female hamsters 6 weekly tests (but not 2 tests) for sexual behavior increased the percentage of the male's mounts that included intromission (termed "hit rate") on the following test (Bradley et al., 2005; Hedges et al., 2009). This was true whether that seventh test was conducted 1 or 6 weeks after the last sexual experience test (Bradley et al., 2005). Thus, sexual experience seems to increase the female hamster's ability to regulate intromission by the male (in Berridge's term, increases "wanting") and these effects of experience are persistent for at least several months without any further experience.

Direct tests of the rewarding (or "liking") consequences of female sexual behavior have most commonly used a conditioned place preference paradigm (Oldenburger et al., 1992; Meisel and Joppa, 1994; Paredes and Alonso, 1997). Here females are allowed to freely explore an apparatus containing two unfamiliar chambers, which determines the degree to which the female has an initial preference for either of the chambers. The female is then sequestered in one chamber during mating and placed alone in the other chamber over a series of conditioning trials. After the conditioning trials, the female is placed back in the apparatus alone and allowed to explore as in the preconditioning session. Importantly, control conditions in which females are successively placed alone (without the addition of mating or other stimuli) in both chambers are used to establish that simply repeated exposure to the chambers does not produce a change from the initial preference. A significant increase in the time spent in the chamber in which sexual behavior occurred in the postconditioning test compared with the preconditioning test is operationally defined as evidence for sexual reward.

Two other rather clever behavioral approaches have strengthened our understanding of the motivational control of female sexual behavior. One approach utilizes a bilevel chamber that capitalizes on the speed advantage of female rats to avoid and escape the male's approaches (Mendelson and Pfaus, 1989). The idea here is that the female can quickly change levels of the apparatus to pace her copulatory interactions with a male rat. Pfaus performed factor analyses on a number of female behaviors in this apparatus and was able to validate the distinction between appetitive/motivational responses and copulatory responses in female rats (Pfaus et al., 1990). Becker took a different approach (Cummings and Becker, 2012) by designing an operant chamber in which the male and female rats were separated by a sliding door. The female had the capability to make a nose poke response for the door to open to get access to the male. The male was tethered in a compartment on the other side of the door, which permitted him the freedom to mate with the female, but not to leave the compartment. Computer interfaced video tracking software recorded the location of the female in the apparatus, through which the computer closed the door when the female returned to her original compartment. In this way the female could control the pacing of access to the male.

For both rats and hamsters, pacing of the male's intromissions depends on estradiol and progesterone with the mesolimbic circuitry one target of these hormone effects.

Spinogenesis: A Common Feature of Hypothalamic and Mesolimbic Circuitry

Estradiol Induces Dendritic Spines in the ARH

At this point in time, it is well-established that estradiol regulates morphological plasticity in various parts of the brain (Matsumoto and Arai, 1979; Woolley and McEwen, 1993; Staffend et al., 2011; reviewed in Micevych and Christensen, 2012). In the VMH, the final common pathway out of the hypothalamus of information relevant to lordosis behavior, estradiol increased spine density and dendritic branching (Frankfurt et al., 1990; Meisel and Luttrell, 1990; Calizo and Flanagan-Cato, 2000, 2002; Madeira et al., 2001; Gonzalez-Burgos et al., 2015). Interestingly, estradiol also reduced the length of long primary dendrites that extend laterally out of the VMH the potential site of afferents from the MPN that are inhibited by β-END (Sinchak et al., 2010). These results suggest that as MOR inhibition wears off or is blocked with progesterone, excitatory afferents contact newly formed dendritic spines, activating VMH neurons.

In the ARH, estradiol-induced morphological plasticity was shown to be necessary for the induction of lordosis behavior (Christensen et al., 2011, 2012; Christensen and Micevych, 2012). Estradiol treatment increases dendritic spine density within 4 h, and it remains stable for 48 h. However, the composition of spines with different morphology changed. The early appearing spines were filopodial, morphology suggestive of immature, inactive

and unstable spines (Christensen et al., 2011). Such filopodial spines are highly labile, rapidly appearing and disappearing during intense neural activity until they are stabilized by contacting an appropriate presynaptic partner (Parnass et al., 2000; Grutzendler et al., 2002; Trachtenberg et al., 2002). At approximately 24 h, a time point at which lordosis can be elicited by prior progesterone treatment, the population has more mushroom-shaped spines. Mushroom-shaped spines appear to be stable and functional, with receptors and anchoring proteins that allow for synaptic transmission. Stabilization of spines requires mature postsynaptic spines with receptors anchored at the postsynaptic specialization by scaffold proteins (Srivastava et al., 2008; reviewed in Srivastava and Penzes, 2011; Micevych and Christensen, 2012), and a presynaptic element for synaptic communication.

An actin scaffold underlies dendritic spines. Indeed, spine formation requires rearrangement of the underlying actin cytoskeleton. In the ARH, an increase in β-actin immunoreactivity is correlated an increase in spines demonstrated with Golgi staining (Christensen et al., 2011). Estrogenic regulation of spinogenesis was shown to involve ERα-mGluR1a signaling leading to modulation of actin dynamics through phosphorylation of molecules important for spine formation including cofilin, an actin depolymerizing factor (for review see Sarmiere and Bamburg, 2004; Hotulainen and Hoogenraad, 2010; Sanchez et al., 2012). Cofilin must be deactivated (phosphorylated) to allow the formation of filamentous actin and new spines (Bamburg, 1999; Meng et al., 2002). Estradiol, within an hour, induces cofilin phosphorylation which can be inhibited by mGluR1a antagonism (Christensen et al., 2011). Cytochalasin D, which prevents β-actin polymerization, abrogated both estradiol-induced spine formation and lordosis behavior (Christensen et al., 2011). It has been proposed that estradiol rapidly induces labile spines but another stimulus is needed to stabilize them (Srivastava et al., 2008; reviewed in Srivastava and Penzes, 2011). On-going experiments point to membrane-initiated estradiol regulation of pre- and post-synaptic proteins, suggesting that, for stability, newly formed spines associate with a presynaptic element (Rudolph et al., 2016).

Sexual Experience Effects on Dendritic Spines in Nucleus Accumbens

Morphological changes in the mesolimbic system are dependent on both estradiol availability and on sexual experience. Estradiol treatment of either female rats or hamsters *decreases* spine density on medium spiny neurons in the core of the nucleus accumbens (Staffend et al., 2011; Peterson et al., 2015). The assumption is that estradiol exerts these effects on dendritic spine plasticity through membrane estrogen receptor interactions with metabotropic glutamate receptors (Micevych and Mermelstein, 2008). Consistent with this hypothesis are observations that pre-exposure to an mGluR5 antagonist blocks the estradiol effects on dendritic spines (Peterson et al., 2015).

Sexual experience *increases* dendritic spine density in medium spiny neurons of female hamsters, particularly in the core of the nucleus accumbens (Staffend et al., 2014). Dendritic spines receive excitatory, largely glutamatergic, inputs and have different morphologies which are thought to reflect biophysical properties impacting excitability of the neurons (Tonnesen and Nagerl, 2016). The increase in dendritic spines following sexual experience in hamsters was primarily associated with a change in filopodial spines, which have "silent synapses." Developmentally, silent synapses are enriched in glutamatergic NMDA receptors with an absence of AMPA receptors (Liao et al., 1999). In adulthood, silent synapses contain both NMDA and AMPA receptors, though with a preponderance of NMDA receptors (Huang et al., 2009). As a proxy for electrophysiological characterization of silent synapses, we measured AMPA and NMDA receptors in the nucleus accumbens of female hamsters following sexual experience. No changes in AMPA receptor gene expression or protein for either the GluA1 or GluA2 subunits were detected. Similarly, there were no changes in NMDA receptors measured by levels of the NR2B subunit, however, increased phosphorylation of tyr1472 of the NR2B subunit was observed. This specific phosphorylation site confers membrane stability to NR2B containing NMDA receptors (Chen and Roche, 2007), providing indirect evidence that female sexual experience increases NMDA-biased silent synapses in the nucleus accumbens. Clearly this idea needs to be confirmed electrophysiologically.

Dopaminergic projections from the ventral tegmentum synapse on nucleus accumbens medium spiny neurons that express either excitatory dopamine D1 receptors or inhibitory D2 receptors (Missale et al., 1998). In general, each of these neuronal phenotypes has a different pattern of efferent projections (Kupchik et al., 2015). Knowing the phenotype of medium spiny neurons affected by sexual experience can be informative for developing hypotheses about the functional consequences of these changes in dendritic spines. Not only were the effects of sexual experience restricted to the core of the nucleus accumbens, but changes in spines were localized to the D1 containing medium spiny neurons (Staffend et al., 2014). These anatomical observations link observations of plasticity in neural pathways associated with intrinsic fixed-action behavioral sequences (Kalueff et al., 2016) to the control of female sexual motivation. In this way dopamine neurotransmission may be the mediator of sexual motivation in females. As sexual experience produces changes in the motivational components of sexual behavior, these changes in behavior are paralleled by a corresponding change in neuronal plasticity.

Sexual Behavior Stimulates Mesolimbic Dopamine Release

Analysis of the mesolimbic dopamine system's role in sexual motivation began with microdialysis measurements of extracellular dopamine levels during sexual behavior in female rats and hamsters. Dopamine release in the nucleus accumbens of female rats during sexual encounters is associated with the female's ability to pace the mating interactions with the male (Mermelstein and Becker, 1995; Becker et al., 2001; Jenkins and Becker, 2001, 2003). Similarly, for female hamsters, dopamine is elevated in the nucleus accumbens during sexual interactions (Meisel et al., 1993) Dopamine release in the female hamster's

nucleus accumbens occurred during mating only if the male achieved intromission (Kohlert et al., 1997). To expand on these findings, we are now using fixed potential carbon fiber recording from the nucleus accumbens providing an ∼1 s temporal resolution of dopamine transients, which allows time locking the dopamine signal to specific components of the female's sexual interaction with the male. There is a strong concordance between the peak of the dopamine transients and the female's receipt of intromission by the mounting male. Collectively these results indicate that intromission is a salient signal for activation of the nucleus accumbens during sexual behavior in females and that this dopamine release does not depend on prior experience.

Further analyses of hamsters have tested the idea that sexual experience can potentiate the mesolimbic response to sexual stimuli in females. Our work indicated that with 6 (but not 3) prior sexual interactions there was an augmented release of dopamine relative to that seen in inexperienced female hamsters (Kohlert and Meisel, 1999), paralleling the change in hit rate noted previously. This "sensitized" dopamine response in hamsters was confirmed by c-Fos analysis in which mating increased the number of labeled neurons in the core of the nucleus accumbens, with an even greater elevation of labeled neurons in females with prior sexual experience (Bradley and Meisel, 2001).

Plasticity in Dopamine Signaling

Female sexual experience increases dopamine release in the nucleus accumbens during sex, and that increased dopamine release leads to changes in neuronal morphology. This raises the question of how changes in dopamine-mediated intracellular signaling underlie structural and behavioral plasticities? Female sexual experience does not affect the levels of either D1 or D2 receptors in the nucleus accumbens, nor does it impact D1 or D2 receptor binding (Staffend et al., 2014), yet there must be an enhancement of dopamine receptor signaling since c-Fos production is sensitized. Stimulating dopamine D1 receptors produces a greater cAMP response in homogenates from the nucleus accumbens of sexually-experienced vs. inexperienced female hamsters (Bradley et al., 2004). Though both Gpp(NH)p (a non-hydrolyzable GTP analog) and forskolin (a direct activator of adenylyl cyclase) increased cAMP accumulation in a concentration-dependent manner, the absence of any further augmentation by sexual experience on cAMP accumulation suggested that sexual experience either impacted the coupling of dopamine D1 receptors to G-proteins or modulated other G-protein regulators (e.g., RGS or AGS proteins). The observation that dopamine D1 signaling depends on interactions with caveolin-1 raises the possibility that sexual experience affects dopamine signaling by modulating caveolin-1 expression (Kong et al., 2007).

Several signaling events downstream from cAMP are impacted by sexual experience in female hamsters, particularly elements of MAP kinase signaling. MAP kinase signaling is relevant in this context since activity in this pathway is associated with neuronal plasticity (Sweatt, 2001). Sexual behavior testing does not impact MAP kinase signaling, as measured by levels of ERK 1/2 either in its phosphorylated state or as total protein. However, in sexually experienced females there is a dramatic increase in phosphorylated ERK1/2 soon after a subsequent test for sexual behavior (Meisel and Mullins, 2006). Thus ERK 1/2 phosphorylation is sensitized by sexual experience. This response of ERK 1/2 to sexual behavior may mediate the observed increases in c-Fos expression.

One important clue to potential molecular mediators of sexual experience on the nucleus accumbens came from behavioral results showing that the increase in copulatory efficiency in sexual interactions with male hamsters was maintained for over a month without further sexual experience. ΔFosB tuned out to be a good candidate as the molecular mediator of this long-term behavioral plasticity. This truncated variant of FosB confers a remarkable level of resistance to proteasome degradation (Ulery et al., 2006; Ulery and Nestler, 2007). No changes in pan-FosB immunocytochemical labeling were detected in the nucleus accumbens following an acute sex behavior test, but again in sexually experienced female hamsters there was an increase in FosB labeling (Meisel and Mullins, 2006). Further, overexpression of ΔFosB in the accumbens facilitated conditioned place preference in female hamsters given only two condition sessions (Hedges et al., 2009). Overexpression of ΔFosB in female hamsters also increased the male's ability to achieve intromission (i.e., increased hit rate) over control females given only two prior sex tests. Overexpressing ΔJunD, the dominant negative binding partner of ΔFosB (Winstanley et al., 2007), blocked the induction of a conditioned place preference after the requisite conditioning trials (Been et al., 2013). Collectively, these studies demonstrate that ΔFosB is a key molecular nexus for the effects of sexual experience on the long-lasting changes in sexual reward and the efficiency of copulatory interactions with a male.

INTEGRATING THE CIRCUITS

At the same time that the neural systems underlying the different components of reproduction in female rodents are separable, clearly these elements require integration for successful reproduction. One possibility is that the activation of ovulation, lordosis, and sexual motivation are simply temporally coincident. Alternatively, there are nodes through which the different circuits connect to execute this integration. The link between the hypothalamic and mesolimbic circuits historically has been rather mysterious, though recent work provides an intriguing (though currently untested) hypothesis that the MPOA could be a potential node for this integration (Coria-Avila et al., 2014). Dominguez (along with others) traced projections from the MPOA to the VTA (Tobiansky et al., 2013, 2016). They reported that the majority of these neurons were GABAergic, suggesting that the MPOA provided an inhibitory input to the VTA and in turn to the nucleus accumbens. This inhibitory control was revealed by the use of cocaine as a pharmacologic reinforcer, which increased the number of c-Fos stained neurons in the nucleus accumbens of MPOA lesioned animals and correspondingly produced a stronger conditioned place preference (Tobiansky et al., 2013).

An analysis of estrogen receptors provided a key extension of the research on the MPOA as an interface between the hypothalamus and mesolimbic dopamine system. Anatomically, the majority of the MPOA to VTA projecting neurons stained positively for either ERα (∼70%) or GPER (∼35%) (Tobiansky et al., 2016). The functional significance of these estrogen receptor containing neurons was demonstrated through intra-MPOA estradiol infusions which enhanced cocaine mediated dopamine release in the NAc (Tobiansky et al., 2016). These results support the idea that the MPOA is a source of inhibition to the mesolimbic dopamine system, which is released by estradiol acting on these GABAergic projection neurons.

Modulation of dopaminergic neurotransmission may be a mechanism through which estradiol modulates the inhibitory tone of the MPOA. Dopamine D1 receptors generally signal through excitatory G proteins, whereas D2 receptors are coupled to inhibitory signaling pathways (Nishi et al., 1989; Jaber et al., 1996). In this way regulating the balance of D1:D2 signaling can impact the level of excitation in MPOA neurons. The results of immunocytochemical staining, Western blot analyses and autoradiographic receptor binding converged on the conclusion that estradiol biased the ratio toward D2 signaling, presumably reducing the intrinsic excitability of MPOA neurons (Graham et al., 2015). The functional impact of this altered dopaminergic signaling balance was mirrored by pharmacological analysis. Amphetamine (which would stimulate both D1 and D2 dopamine receptors) infused into the MPOA increased the amount of time before the female rat returned to the male following mounts and ejaculation (Guarraci et al., 2008). Direct MPOA infusion of a dopamine D2 agonist increased while a dopamine D1 agonist reduced sexual motivation measured in bilevel chambers (Graham and Pfaus, 2010), indicating bidirectional effects of dopamine receptor subtypes on sexual motivation. Collectively these results identify dopaminergic efferents as a potential source of estradiol modulation of MPOA inputs to the VTA.

The desire to engage in sexual behavior and the performance of sexual behavior are both neurally and functionally separable (Georgiadis et al., 2012). The MPOA is both anatomically and functionally positioned to integrate the actions of estradiol on sexual motivation through the mesolimbic system, as well as on the overt expression of lordosis through hypothalamic circuitry. At the same time, continuing research on the hypothalamic and mesolimbic systems controlling female sexual behavior will undoubtedly develop a more detailed understanding of how these anatomical and functional circuits are integrated.

AUTHOR CONTRIBUTIONS

PM and RM each contributed background for this review.

ACKNOWLEDGMENTS

This work was supported by NIH Grants DA013185 & HD042635 to PM and NIH Grant DA013680 and NSF Grant IOS 1256799 to RM. We appreciate all the efforts of Drs. Melinda Mittelman-Smith and Lauren Rudolph.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Systems Neuroscience

REVIEW
published: 29 May 2017
doi: 10.3389/fnsys.2017.00032

The Melanin-Concentrating Hormone as an Integrative Peptide Driving Motivated Behaviors

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The melanin-concentrating hormone (MCH) is an important peptide implicated in the control of motivated behaviors. History, however, made this peptide first known for its participation in the control of skin pigmentation, from which its name derives. In addition to this peripheral role, MCH is strongly implicated in motivated behaviors, such as feeding, drinking, mating and, more recently, maternal behavior. It is suggested that MCH acts as an integrative peptide, converging sensory information and contributing to a general arousal of the organism. In this review, we will discuss the various aspects of energy homeostasis to which MCH has been associated to, focusing on the different inputs that feed the MCH peptidergic system with information regarding the homeostatic status of the organism and the exogenous sensory information that drives this system, as well as the outputs that allow MCH to act over a wide range of homeostatic and behavioral controls, highlighting the available morphological and hodological aspects that underlie these integrative actions. Besides the well-described role of MCH in feeding behavior, a prime example of hypothalamic-mediated integration, we will also examine those functions in which the participation of MCH has not yet been extensively characterized, including sexual, maternal, and defensive behaviors. We also evaluated the available data on the distribution of MCH and its function in the context of animals in their natural environment. Finally, we briefly comment on the evidence for MCH acting as a coordinator between different modalities of motivated behaviors, highlighting the most pressing open questions that are open for investigations and that could provide us with important insights about hypothalamic-dependent homeostatic integration.

Keywords: MCH, hypothalamus, energy homeostasis, feeding behavior, reward, foraging, metabolism

OPEN ACCESS

Edited by:
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Reviewed by:
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Received: *22 February 2017*
Accepted: *04 May 2017*
Published: *29 May 2017*

Citation:
Diniz GB and Bittencourt JC (2017) The Melanin-Concentrating Hormone as an Integrative Peptide Driving Motivated Behaviors. Front. Syst. Neurosci. 11:32. doi: 10.3389/fnsys.2017.00032

THE MELANIN-CONCENTRATING HORMONE[S]

The existence of the MCH was postulated in the 1930s as a factor that induces pallor in the skin of amphibians and participates in these animals' mechanism of background color adaptation (Hogben and Slome, 1935). It took almost 50 years, however, for MCH to be isolated for the first time from the chum salmon (*Oncorhynchus keta*) hypophysis, where it plays exactly its hypothesized role in skin pigmentation by triggering a concentration of melanin in melanophores that results in pallor (Kawauchi et al., 1983). Although the chronology of MCH discovery emphasized its role in skin pigmentation, it is now proposed that this function only emerged for the first time in the

last universal common ancestor of teleosts and holoceans, millions of years after the appearance of MCH synthesis in chordates (Kawauchi and Baker, 2004) .

It was later discovered that teleosts have two paralogs of MCH, termed MCH1 and MCH2 (Ono et al., 1988), that have different patterns of distribution in the CNS. MCH1 corresponds to the peptide isolated by Kawauchi et al. (1983) and is mostly associated with the control of skin pigmentation. Berman et al. (2009), working with the zebrafish (*Danio rerio*) hypothalamus, demonstrated that MCH1 immunoreactivity is largely impacted by background color, reflecting its role in skin pigmentation, while only MCH2 displays pronounced alterations in fasted animals. This correlation between hypothalamic MCH2 and the energy balance of the organism indicates that MCH may be linked to motivated behaviors and integrative functions at least since the divergence of tetrapods and ray-finned fishes at ∼440 million years ago (Amores et al., 2011).

Six years after the discovery of MCH1 in the salmon hypophysis, Nahon et al. (1989) and Vaughan et al. (1989) described for the first time the mammalian MCH coding gene and structure. Using rat samples, these researchers showed that there is a single mammalian MCH, with 19 amino acids and a cysteine bridge that confers it a cyclic structure, synthesized from a 165 amino acids precursor coded by the *Pmch* gene. This precursor also contains the sequence of two other peptides, NEI and NGE, and although some functions are now attributed to the former (for a review, see Bittencourt and Celis, 2008), the biological activity of the latter is largely unknown. Synteny, genomic structure, and sequence identity indicate that the mammalian MCH is an ortholog of teleost MCH2 (Berman et al., 2009) and shows remarkable conservation when compared to the single MCH of a cartilaginous fish, the scalloped hammerhead shark (*Sphyrna lewini*), with a single substitution at the C-Terminal (Mizusawa et al., 2012).

ANATOMICAL ASPECTS

The synthesis of MCH shows two remarkably conserved features: most, if not all, MCH-immunoreactive (MCH-ir) neurons are found in the hypothalamus, and those neurons have widespread projections throughout the CNS. The first anatomical characterization of MCH was made in the male and female rat by Bittencourt et al. (1992). In this species, most MCH-ir neurons are found in the LHA and, to a lesser extent, in the

Abbreviations: 3V, third ventricle; 5-HT, serotonin; 5-HT$_?$, unspecified serotonin receptor; 7N, nucleus of the cranial nerve VII; A10, ventral tegmental area dopaminergic group; A13, incerto-hypothalamic dopaminergic group; A8, retrorubral dopaminergic group; A9, *substantia nigra* dopaminergic group; ac, anterior commissure; Acb, *nucleus accumbens*; AcbC, *nucleus accumbens*, core subdivision; AcbSh, *nucleus accumbens,* shell subdivision; ACh, acetylcholine; ADX, adrenalectomized; AgRP, agouti-related protein; AH, adenohypophysis/anterior lobe of the pituitary; AHA, anterior hypothalamic area; AMPAR, AMPA ionotropic glutamate receptor; AMY, amylin; Amyg, amygdaloid nuclei; AON, anterior olfactory nucleus; AP, *area postrema*; Arc, arcuate nucleus; AV, anteroventral thalamic nucleus; AVP, vasopressin; AVPV, anteroventral periventricular nucleus; B7, dorsal raphe serotonergic group; B8, median raphe serotonergic group; BAT, brown adipose tissue; BNST, bed nucleus of the *stria terminalis*; CA, catecholamine; CA1, CA1 field of the hippocampus; CA2, CA2 field of the hippocampus; CA3, CA3 field of the hippocampus; CART, cocaine- and amphetamine-regulated transcript; CB_1, cannabinoid receptor type 1; cc, *corpus callosum*; CeA, central nucleus of the amygdala; CG, central gray of the spinal cord; CgCx, cingulate cortex; CORT, cortisol/corticosterone; cPPTg, caudal pedunculopontine tegmental nucleus; CPu, caudate putamen; CRF, corticotrophin-releasing factor; D_1, dopamine receptor subtype 1; D_2, dopamine receptor subtype 2; DA, dopamine; DG, dentate gyrus; DMH, dorsomedial hypothalamic nucleus; DR, dorsal raphe nucleus; DTM, dorsal tuberomammillary nucleus; Dyn, dynorphins; EB, estradiol benzoate; ERα, estrogen receptor type α; EW, Edinger–Westphal nucleus; EWcp, centrally projecting Edinger–Westphal nucleus; f, fornix; FOS, FOS proto-oncogene, AP-1 transcription factor subunit; $GABA_A$, GABA ionotropic receptor; GIRK, G protein-coupled inwardly rectifying K^+; GLUT3, glucose transporter 3; GnRH, gonadotropin-releasing hormone; GP, *globus pallidus*; Hab, habenular nuclei; *Hcrt*, Orexin/hypocretin gene; HF, hippocampal formation; HPA, hypothalamus-pituitary-adrenal; HPT, hypothalamus-pituitary-thyroid; Hyp, hypothalamic nuclei; IC, inferior colliculus; IHy, incerto-hypothalamic area; ImC, intermediolateral column of the spinal cord; Ins, insular cortex; IR, insulin receptor; K_{ATP}, ATP-sensitive potassium channel; LD, laterodorsal thalamic nucleus; LDTg, laterodorsal tegmental nucleus; *Lep*, leptin gene; LepR, leptin receptor; *LepR*, leptin receptor gene; LG, lateral geniculate nucleus; LH, luteinizing hormone; LHA, lateral hypothalamic area; LHAa, anterior part of the LHA; LHAp, posterior part of the LHA; LM, lateral mammillary nucleus; LPOA, lateral preoptic area; LS, lateral septal nucleus; mACh, metabotropic ACh receptor; MAS, masseter muscle; MCH, melanin-concentrating hormone; MCH1, teleost melanin-concentrating hormone subtype 1; MCH2, teleost melanin-concentrating hormone subtype 2; MCHR1, MCH receptor type 1; *Mchr1*, MCH receptor 1 gene; MCx, motor cortex; MD, mediodorsal thalamic nucleus; ME, median eminence; MEe, external layer of the median eminence; MEi, internal layer of the median eminence; mfb, medial forebrain bundle; MG, medial geniculate nucleus; mGlu, unspecified metabotropic GLU receptor; mGlu1, metabotropic GLU receptor subtype 1; mGlu5, metabotropic GLU receptor subtype 5; MidThal, midline thalamic nuclei; ml, medial *lemniscus*; MM, medial mammillary nucleus; MnR, median raphe nucleus; MoV, motor nucleus of the V nerve; MPOA, medial preoptic area; MRt, medullary reticular formation; MS, medial septal nucleus; MSN, medium spiny neuron; N/OFQ, nociceptin/orphanin FQ; NCX, Na^+/Ca^{2+} exchanger; NE, norepinephrine; NEI, neuropeptide E-I; NGE, neuropeptide G-E; NH, neurohypophysis/posterior lobe of the pituitary; NMDAR, NMDA ionotropic GLU receptor; NOP, nociceptin/orphanin Q receptor; NPY, neuropeptide Y; NREM, non-rapid eye movement sleep; NTS, nucleus of the solitary tract; Nts, neurotensin; OB, main olfactory bulb; opt, optic tract; ORX, orexins/hypocretins; ORX_A, orexin A/hypocretin-1; ORX_B, orexin B/hypocretin-2; ORXR, unspecified orexin receptor; $ORXR_2$, orexin receptor type 2; OT, olfactory tubercle; OXT, oxytocin; OXTR, oxytocin receptor; OVX, ovariectomized; P_4, progesterone; P2?, unspecified purinergic receptor; PAG, periaqueductal gray matter; PAGvl, ventrolateral periaqueductal gray matter; PAGdm, dorsomedial periaqueductal gray matter; PB, parabrachial nucleus; Pe, periventricular hypothalamic nucleus; PaF, parafascicular thalamic nucleus; PHA, posterior hypothalamic area; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; *Pmch*, Pro-melanin-concentrating hormone gene; PnRt, pontine reticular formation; Po, posterior thalamic complex; POMC, proopiomelanocortin; PPTg, pedunculopontine tegmental nucleus; Pr, prepositus nucleus; PreCBL, precerebellar nuclei; Pretect, pretectal nuclei; PV, paraventricular thalamic nucleus; PVH, paraventricular hypothalamic nucleus; PVHp, parvocellular subdivision of the paraventricular hypothalamic nucleus; PVHm, magnocellular subdivision of the paraventricular hypothalamic nucleus; Pir, piriform cortex; REM, rapid eye movement sleep; RIA, radioimmunoassay; rPPTg, rostral pedunculopontine tegmental nucleus; SAL, salivary glands; SuS, superior salivatory nucleus; SC, superior colliculus; scp, superior cerebellar peduncle; SHi, septohippocampal nucleus; SM, supramammillary nucleus; SN, *substantia nigra*; SpCd, spinal cord; SpV, spinal trigeminal nucleus; SSCx, somatosensory cortex; st, *stria terminalis*; SubThal, subthalamus; TRH, thyrotropin-releasing hormone; TRHR, unspecified thyrotropin-releasing hormone receptor; TRPC, transient receptor potential channel; TSH, thyroid-stimulating hormone; UCN1, urocortin 1; UCN2, urocortin 2; UCN3, urocortin 3; V1a, vasopressin receptor subtype 1a; VDCC, voltage-dependent calcium channel; Vest, vestibular nuclei; VL, ventrolateral thalamic nucleus; VMH, ventromedial hypothalamic nucleus; VTA, ventral tegmental area; YR, unspecified NPY receptor; ZI, *zona incerta*; α-MSH, α-melanocyte-stimulating hormone; αR, unspecified adrenergic receptor; $\alpha 2_A$, adrenergic receptor type 2A; κOR, opioid receptor type κ.

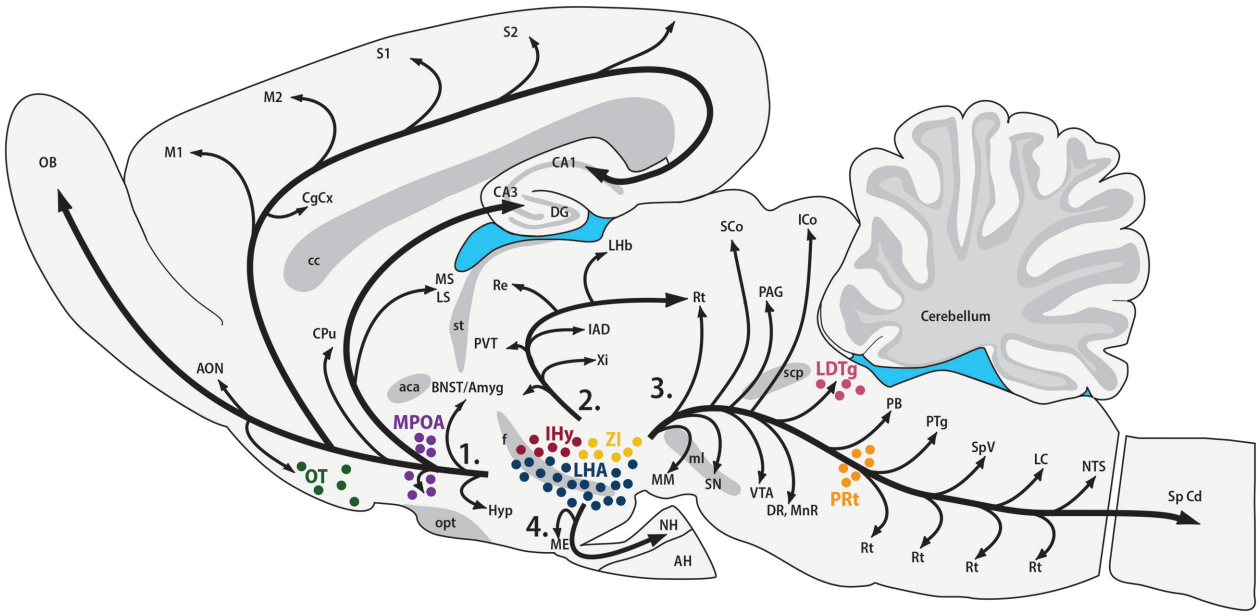

FIGURE 1 | MCH synthesis is found in discrete areas of the rat nervous system. Sagittal representation of the rat central nervous system illustrating the main areas where MCH-ir neurons are found. The hypothalamic areas of synthesis in all animals are: the LHA (blue) and the IHy (red). Extra-hypothalamic areas are: the ZI (yellow), the OT (green) and the paramedian PnRt (orange). Exclusively in female rats, one extra-hypothalamic site can be found, in the laterodorsal tegmental nucleus (pink). During the lactation period, MCH-immunoreactive neurons can be detected in the medial preoptic area (purple). The four main pathways of MCH-ir fibers are also represented: 1 – ascending pathway; 2 – periventricular pathway; 3 – descending pathway; and 4 – hypophysaire pathway.

AHA and PHA of the medial zone and in the dorsalmost area of the periventricular zone. A second group of rostral neurons is found in the IHy, previously known as rostromedial ZI (Bittencourt et al., 1992; Murray et al., 2000c), where MCH-ir neurons are intermingled with the DA neurons of the A13 group (Sita et al., 2003, 2007). Exclusively during the postpartum period, immunoreactivity to MCH can be detected in neurons of the MPOA and at the rostralmost aspect of the PVH, with an intensity that follows the progression of lactation and, with weaning, disappears (Knollema et al., 1992; Rondini et al., 2010). Outside the hypothalamus, MCH-ir neurons were described in the OT, ZI, and paramedian PnRt (Bittencourt et al., 1992) and in the LDTg of the female rat (Rondini et al., 2007). Little is known about the role played by MCH in these extra-hypothalamic areas. **Figure 1** summarizes the *loci* of MCH synthesis in the rat CNS and the main pathways formed by MCH-ir fibers.

Contrasting to the highly restricted pattern of MCH synthesis, MCH-ir fibers are found widespread throughout the whole CNS. Among the regions that contain fibers are olfactory areas, the septal nuclei, the basal nuclei, the HF, the neocortex, several diencephalic nuclei, the mesencephalic and PnRt, the PAG and all levels of the SpCd. The regions with the sparsest presence MCH-ir fibers are the cerebellum and some motor nuclei of the brainstem. In most of these areas, MCH-ir fibers are varicose and present many boutons *en passage* and boutons *terminaux*, suggesting an extensive synaptic activity (Bittencourt et al., 1992). **Figure 2A** is a visual representation of the MCH-ir fiber density in different areas of the rat CNS.

The distribution of MCH-ir cells has been studied in a wide range of animals, including agnathans (Mizuno, 1991; Bird et al., 2001), cartilaginous fish (*elasmobranchii*) (Vallarino et al., 1989; Mizusawa et al., 2012), teleosts (Naito et al., 1985; Batten and Baker, 1988; Powell and Baker, 1988; Bird and Baker, 1989; Minth et al., 1989; Gröneveld et al., 1995; Mancera and Fernandez-Llebrez, 1995; Amano et al., 2003; Huesa et al., 2005; Matsuda et al., 2009; Suzuki and Yamamoto, 2013), anurans (Andersen et al., 1986; Francis and Baker, 1995; Lázár et al., 2002), reptiles (Cardot et al., 1994), and birds (Cardot et al., 1998, 1999). In mammals, in addition to the rat (Bittencourt et al., 1992), MCH-ir cell bodies have been mapped in the mouse (Elias et al., 2001), Djungarian hamster (Khorooshi and Klingenspor, 2005), Syrian hamster (Vidal et al., 2005), tufted capuchin monkey (Bittencourt et al., 1998), humans (Elias et al., 2001), pigs (Chometton et al., 2014), sheep (Tillet et al., 1996), and cats (Torterolo et al., 2006). For a review of the anatomical aspects of MCH in mammals, see Bittencourt (2011).

Albeit there are some differences between these animals, the main *locus* of MCH synthesis in all of them is the hypothalamus or homologous structures, indicating that MCH-ir neurons reside this region at least since the emergence of the last vertebrate common ancestor. There is also a remarkable conservation in the widespread distribution of MCH-ir fibers. The hypothalamus, through its extensive projections, is a key structure for the execution of motivated behaviors, as understood as behavioral programs directed to exogenous factors that are executed by animals and result in a benefit to the survival of the organism or of its species (Swanson, 2005). This long phylogenetic history

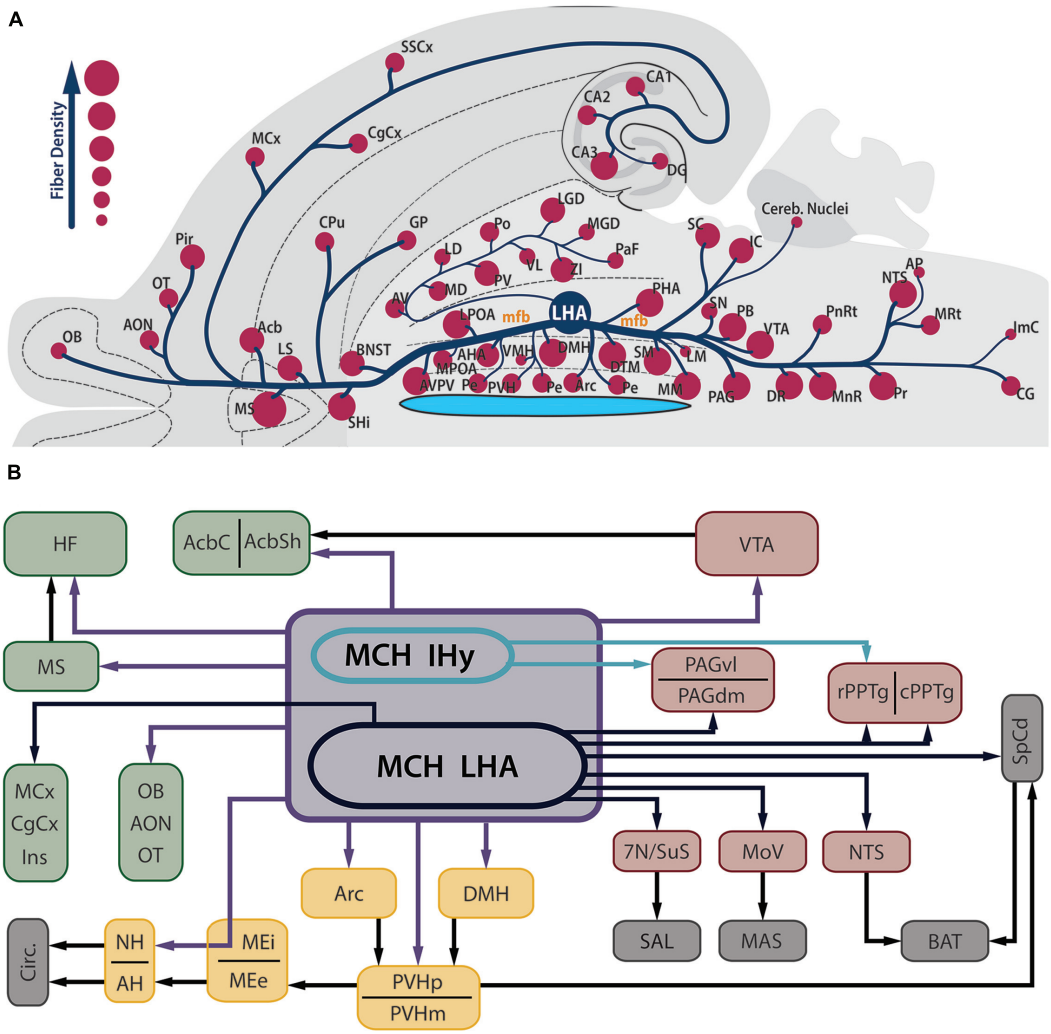

FIGURE 2 | MCH-immunoreactive fibers are profusely distributed in the neuroaxis and are found in several areas related to feeding behavior and energy homeostasis. (A) Flatmap representation of the rat central nervous system illustrating the main areas where MCH-ir fibers can be found. The relative density of MCH-ir fibers in each area is indicated by the size of the circles. Observe that septal, hypothalamic and brainstem areas are densely innervated by MCH neurons, while cortical and thalamic areas display a smaller number of fibers. Due to the position of the LHA, most of the MCH-ir fibers leave this region intermingled to the mfb, with the periventricular pathway as a notable exception to this rule. **(B)** Schematic representation of the main targets of MCH-ir fibers in the rat CNS. Dark blue arrows represent projections originated from MCH-ir neurons located in the LHA, while green arrows indicate projections preferentially originated from the IHy. Purple arrows indicate projections originated from both areas or a lack of hodological studies to pinpoint the origin of such projections. Dark arrows indicate indirect connections relevant to this circuit. The main targets of MCH innervation can be arranged in three groups: telencephalic projections, in green; hypothalamic-hypophysaire projections, in yellow; and brainstem projections, in red. Downstream targets of MCH action are represented in gray.

accounts, at least partially, for the numerous functions on which MCH has been suggested to play a role in, and may explain how MCH acquired an important integrative role in motivated behaviors centered on its hypothalamic localization supported by a widespread fiber distribution.

FEEDING BEHAVIOR

The most thoroughly described function of MCH is on the modulation of feeding behavior. The injection of MCH in the ventricle of rats (Qu et al., 1996; Rossi et al., 1997; Della-Zuana et al., 2002; Clegg et al., 2003; Duncan et al., 2005; Sakamaki et al., 2005) and mice (Gomori et al., 2003; Glick et al., 2009), as well as in the PVH (Rossi et al., 1997) or in the Acb of rats (Georgescu et al., 2005; Mul et al., 2011), provokes an increase in chow consumption followed by a gain of body weight and fat mass. Rossi et al. (1997), however, demonstrated that only acute injections of MCH can induce these effects, while chronic administration is unable to maintain it. Injections of MCH in the 3V also induce an increase in water and saccharin consumption in rats, independently of chow availability, indicating that MCH participates in hydric homeostasis processes (Clegg et al., 2003; Sakamaki et al., 2005; Furudono et al., 2006). This same

treatment, however, increases alcohol consumption, suggesting that MCH participates in hedonistic aspects of this behavior as well (Duncan et al., 2005). Mice deficient in leptin synthesis [$Lep^{ob/ob}$] with an obese phenotype display a higher expression of *Pmch* mRNA in the LHA when compared to WT mice, and this expression increases following fasting (Qu et al., 1996). MCH-overexpressing mice display higher chow consumption and increased body weight, as well as circulating levels of leptin and insulin resistance (Ludwig et al., 2001).

Data obtained from genetic models started broadening the roles associated to MCH. Shimada et al. (1998) report that $Pmch^{-/-}$ mice are leaner than $Pmch^{+}$ cohorts, but several evidence point to this leaner phenotype as the result of multiple physiological alterations. Not only $Pmch^{-/-}$ animals eat less, but they displayed increased oxygen consumption rates; their ability to overeat in response to starvation is not affected, although $Pmch^{-/-}$ animals have greater weight loss and higher mortality rate; and restricted food availability resulted in similar decreases in food intake between $Pmch^{-/-}$ and $Pmch^{+}$ mice, but deficient animals displayed increased weight loss. Another important evidence came with the work of Marsh et al. (2002), that reports that MCHR1-deficient mice have increased locomotor activity and energy expenditure that underlie an apparently contradictory hyperphagia observed in these animals. Finally, $Lep^{ob/ob}$ $Pmch^{-/-}$ mice are leaner than $Lep^{ob/ob}Pmch^{+}$, but their food consumption is not decreased (Segal-Lieberman et al., 2003). This leaner phenotype is attributed to factors linked to energy expenditure, with higher motor activity and basal temperature in response to cold. Taken together, these results indicate that MCH acts on energy homeostasis through multiple pathways, including energy expenditure, locomotor activity and reward, while increases in ingestive behavior promoted by MCH are secondary in nature. In this section, we will review the multiple aspects of MCH action over nutritional homeostasis.

Although variations in dietary parameters are capable of modulating MCH neurons, with fasting increasing *Pmch* expression, variations in this expression may also underlie the preference of some animals for a specific diet. For example, Morganstern et al. (2010b) identified a subset of Sprague-Dawley rats that are prone to overconsume a high-fat diet (HFD) when compared to animals that show a baseline consumption of HFD. These authors demonstrated that animals that ingest a higher amount of HFD during a 5-day period (as measured by caloric intake) have increased levels of *Pmch* expression in the LHA when compared to animals that were kept in the same diet but ingested a lower number of calories. This difference persists even after the animals have been maintained in regular chow for weeks, suggesting that this intrinsic variation in *Pmch* levels may generate the preference of these animals for the higher fat content or increased palatability of the diet. This is extremely relevant because a similar mechanism may underlie human pathologies related to the excessive consumption of fat-rich foods. To explore alterations in the MCH peptidergic system in other models of natural or induced preference may generate important knowledge on the human physiology of ingestive behaviors (Barson et al., 2012).

Inputs

Melanin-concentrating hormone neurons are influenced by many inputs, including other neurons in the LHA, adjoining hypothalamic nuclei, telencephalic and brainstem areas, in addition to circulating factors that act as metabolic cues. **Figure 3** summarizes the main homeostatic-relevant inputs to MCH neurons.

Lateral Hypothalamic Area

The LHA is classically characterized by two populations of neurons, one that is MCH-ir (Bittencourt et al., 1992) and one that is ORX-ir (Peyron et al., 1998), both intermingled with GABAergic and GLUergic interneurons (Gao and van den Pol, 2001) who provide extensive input to MCH neurons through ionic and metabotropic channels (Gao and van den Pol, 2001; Huang and van den Pol, 2007). These two populations coexist in the LHA but are almost completely segregated, with a minimal number of neurons that co-synthesize these two peptides (Broberger et al., 1998; Elias et al., 1998; Peyron et al., 1998). There are two orexin peptides, ORX_A and ORX_B, coded by the same gene (*Hcrt*) and involved in multiple functions related to homeostatic control and arousal (for recent reviews, see Mahler et al., 2014 and Sakurai, 2014). Both MCH and ORX neurons have profuse immunoreactive fibers in the LHA, contacting numerous neighboring cells (Bittencourt et al., 1992; Peyron et al., 1998). Although the high density of MCH-ir fibers close to MCH-ir cells could point to a recurrent effect of MCH on MCH cells, probably inhibitory due the nature of MCH neurons (Gao and van den Pol, 2001), there are no alterations in the electrophysiological properties of MCH neurons after MCH application in LHA slices (van den Pol et al., 2004).

MCH-ir neurons are densely contacted by ORX neurons, with some single MCH-ir cells receiving contacts by up to 30 individual ORX-ir boutons. Both ORX_A and ORX_B have a postsynaptic excitatory effect over MCH cells, depolarizing their membrane potential and increasing spike frequency (**Figure 4A**). ORX_B has also a presynaptic effect, increasing GLUergic input to MCH cells (van den Pol et al., 2004) (**Figure 5**). The similar response amplitude of MCH neurons to ORX_A and ORX_B suggests that this signaling occurs through $ORXR_2$, as $ORXR_1$ responds poorly to ORX_B (Sakurai et al., 1998). Apergis-Schoute et al. (2015), however, provided evidence that the relationship between ORX and MCH may be more complex, since optogenetic stimulation of ORX neurons in LHA slices promoted inhibition of some MCH neurons through a presynaptic mechanism, increasing GABAergic input on MCH cells, while other MCH neurons were excited by ORX stimulus. Regardless, all these results suggest that these two LHA populations are connected, with ORX influencing close MCH neurons in multiple ways.

While some MCH neurons co-synthesize the CART (Elias et al., 2001) and nesfatin (Brailoiu et al., 2007; Fort et al., 2008), almost all ORX neurons co-synthesize Dyn or N/OFQ, both endogenous opioids (Maolood and Meister, 2010). The application of Dyn or N/OFQ hyperpolarizes MCH cells, increasing their input resistance (Li and van den Pol, 2006; Parsons and Hirasawa, 2011), probably through κ-opioid (κOR) and NOP receptors present in these cells (Parks et al., 2014)

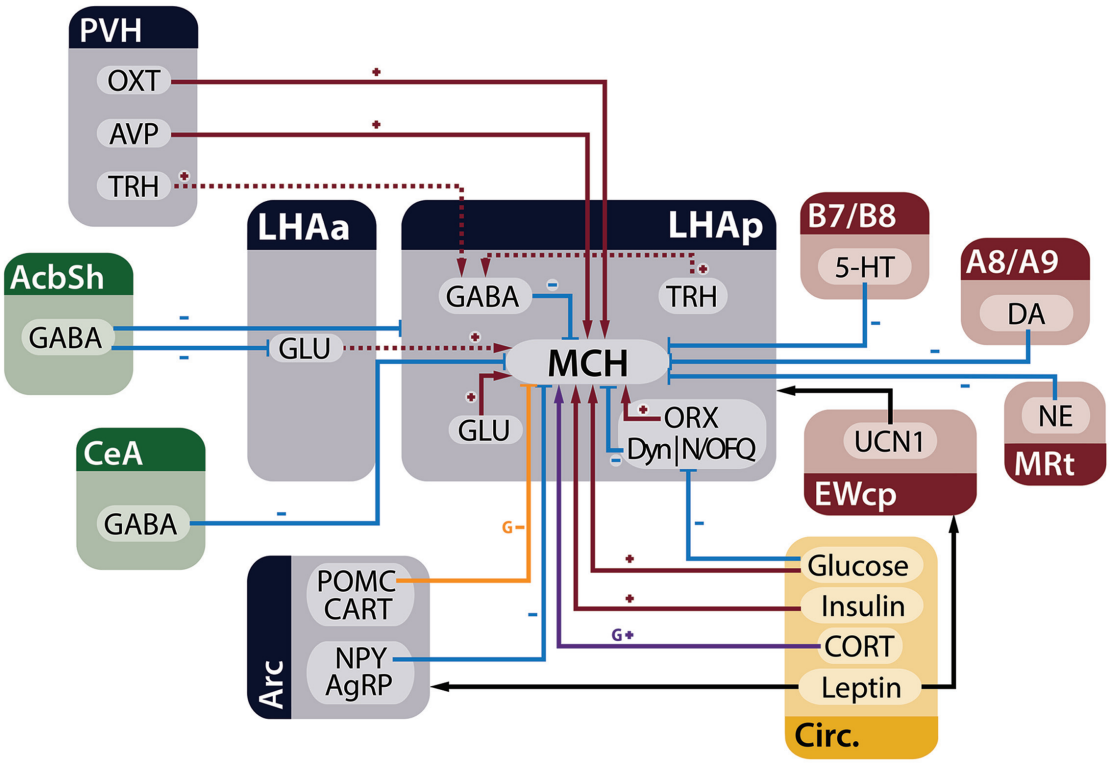

FIGURE 3 | MCH neurons receive several inputs relevant to the control of energy homeostasis. Neurons from the telencephalon (green), hypothalamus (blue), and brainstem (red), in addition to circulating factors (yellow), are capable of modulating electrophysiological and genetic expression properties of MCH neurons in the LHA. Red arrows with plus signs represent depolarizing inputs, blue arrows with minus signs represent hyperpolarizing inputs, black arrows indicate relevant indirect connections to the circuit, the purple arrow represents increased expression of the *Pmch* gene and the orange arrow an input that decreases gene expression. Dashed lines indicate connections that have ample physiological support but lack morphological demonstration or may be composed of multiple polysynaptic elements. Some presynaptic modulatory inputs to GABA- and GLUergic neurons were omitted for clarity.

(**Figure 4B**). These results suggest that MCH neurons could potentially be stimulated by ORX and inhibited by Dyn and N/OFQ originated from the same fibers. Li and van den Pol (2006) and Parsons and Hirasawa (2011) report that MCH cells quickly desensitize to Dyn and N/OFQ, while the response to ORX remains largely unaltered after sequential applications. Through this mechanism, Dyn and N/OFQ could damp the MCH response to brief spontaneous activation of ORX cells, since only constant ORX input would surpass the desensitized inhibitory effect of Dyn and N/OFQ and activate MCH cells. In other words, these endogenous opioids provide the ORX-MCH circuit with a mechanism for noise filtering.

In addition to the chiefly LHA-centered MCH and ORX populations, neurons with distinct chemical signatures and broader distributions are also found in the LHA. One example is the TRH-ir neuronal population located in the LHA, that does not co-localize with MCH or ORX (Horjales-Araujo et al., 2014) and can also be identified in the PVH, VMH, DMH, suprachiasmatic, Pe and Arc hypothalamic nuclei (Brownstein et al., 1974). Besides its role in thyroid function regulation through the AH, TRH is also associated with the control of energy homeostasis through central pathways: icv injections decrease feeding and increase thermogenesis and locomotor activity (for a review on TRH functions, see Lechan and Fekete, 2006). The central effects of TRH are all antagonistic to MCH actions, suggesting that TRH-mediated inhibition of MCH neurons could account, at least partially, for the observed results. Indeed, Zhang and van den Pol (2012) report that TRH inhibit MCH neurons by increasing presynaptic GABAergic input to MCH neurons (**Figure 5**). In conclusion, TRH may modulate feeding behavior and energy expenditure by inhibiting MCH neurons in the LHA, and the source of this TRH input may be from the LHA itself or from other hypothalamic nuclei linked to energy balance, as the PVH, VMH, DMH, and Arc.

Recently, another population of LHA neurons has emerged an important mediator of energy homeostasis: Nts neurons that also contain LepR, known as Nts-LepR. These neurons are found in the LHA (Leinninger et al., 2011) and make synaptic contacts with local neurons (Louis et al., 2010; Leinninger et al., 2011). Although Leinninger et al. (2011) have shown that Nts-LepR neurons make inhibitory synaptic contacts with ORX neurons, but not MCH neurons, it is possible that a polysynaptic circuit may link Nts-LepR neurons to MCH. The selective ablation of LHA Nts-LepR neurons leads to a phenotype of increased body weight, but these mice have only a slightly

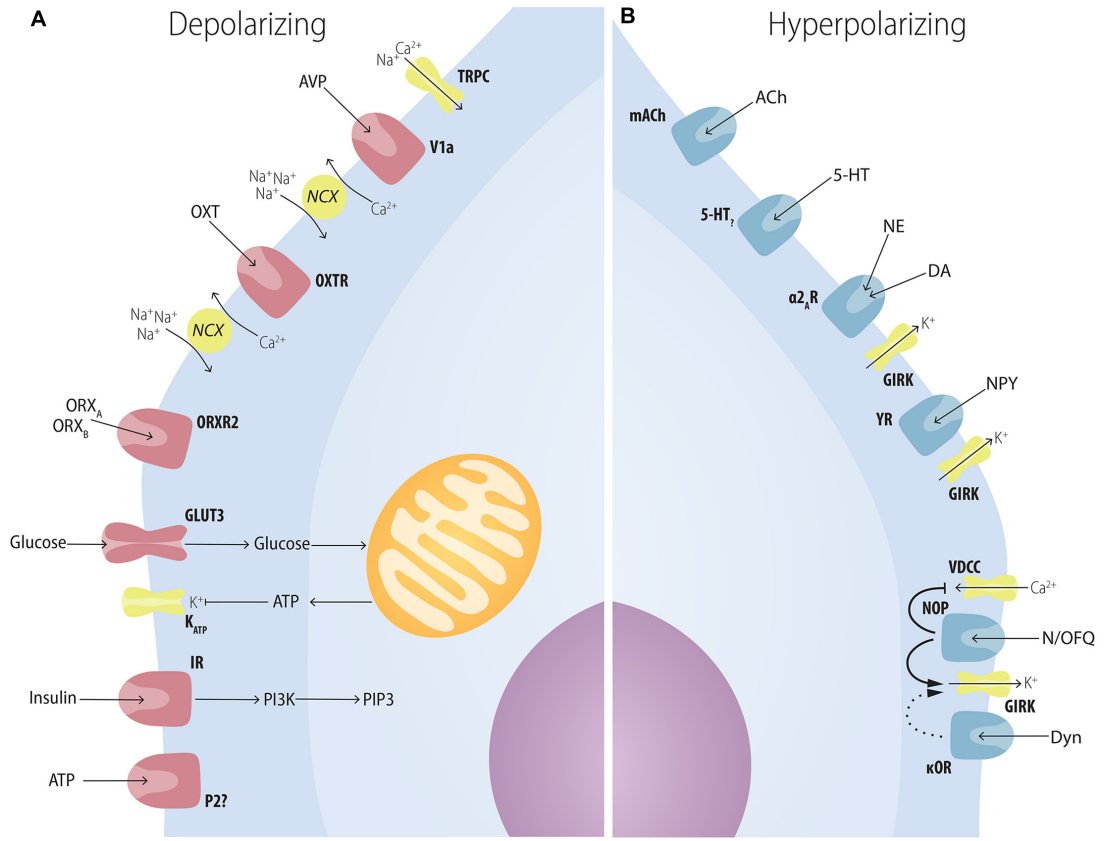

FIGURE 4 | Multiple factors have postsynaptic effects on MCH neurons. (A) Several neuropeptides increase the excitability of MCH neurons through postsynaptic mechanisms. AVP depolarizes MCH neurons through NCXs and TRPC; OXT increases Na^+ currents through NCXs; ATP in the extracellular medium increases MCH excitability through unknown mechanisms that may depend on P2?; orexins depolarize MCH neurons with special affinity for ORX_B, suggesting an $ORXR_2$-dependent pathway; glucose increases neuronal excitability in a mechanism similar to pancreatic β-cells: GLUT3 transporters increase the amount of glucose in the intracellular medium, boosting the amount of produced ATP, what blocks ATP-sensitive potassium channels (K_{ATP}); insulin excites MCH neurons and activates PIP3-dependent pathways. **(B)** Several neuropeptides decrease the excitability of MCH neurons through postsynaptic mechanisms. ACh and 5-HT, two important elements of the central arousal system, inhibit MCH neurons; both NE and DA are capable of decreasing MCH excitability through the activation of the adrenoceptor type 2A and resulting activation of K^+ currents through GIRK channels; NPY hyperpolarizes MCH neurons also through GIRK currents; opioids such as N/OFQ and Dyn hyperpolarize MCH neurons with convergent mechanisms through the blockage of VDCC and an increase in GIRK currents.

increased food consumption when young. On the other hand, these animals have increased adiposity and decreased VO_2 and ambulatory activity (Leinninger et al., 2011). This phenotype inversely mirrors that observed in *Pmch*$^{-/-}$ and *Mchr1*$^{-/-}$ mice, that show a reduction in chow consumption that is too small to explain their weight deficit, increased O_2 consumption and increased ambulatory activity (Shimada et al., 1998; Marsh et al., 2002). These energy expenditure alterations seen in Nts-LepR mice are more compatible with overactivity of MCH neurons than exclusively of ORX. As Nts-LepR neurons are predominantly GABAergic (Leinninger et al., 2009; Goforth et al., 2014), synaptic spilling or GABA-mediated decrease of GLUergic input to MCH neurons may explain how these neurons modulate MCH neurons. As leptin serves as a metabolic signal for high energy reserves, the inhibition of MCH neurons by Nts-LepR neurons may be a mechanism to decrease MCH-mediated energy conservation in states of positive energy balance.

Arcuate Nucleus

The Arc is a classical component of the feeding behavior circuitry, as lesions in this nucleus and the adjoining VMH by gold thioglucose promote hyperphagia and obesity in mice (Debons et al., 1962). This nucleus contains neuronal populations that produce anorectic peptides, such as the POMC-derived α-MSH (Jacobowitz and O'Donohue, 1978; Elias et al., 1998; Ludwig et al., 1998) and CART (Koylu et al., 1997, 1998; Kristensen et al., 1998), as well as orexigenic peptides as the NPY (Allen et al., 1983; Clark et al., 1984) and the AgRP (Ollmann et al., 1997; Shutter et al., 1997). This nucleus is also influenced by insulin (van Houten et al., 1980; Niswender et al., 2003), glucose (Leloup et al., 1994; Muroya et al., 1999; Parton et al., 2007), and leptin (Mercer et al., 1996; Schwartz et al., 1996; Cheung et al., 1997), all of them capable of conveying the short- and long-term energetic state of the organism from the CSF to the Arc through the ME (Woods et al., 1979; Banks et al., 1996), positioning the Arc as the ideal receptor for these circulating metabolic markers.

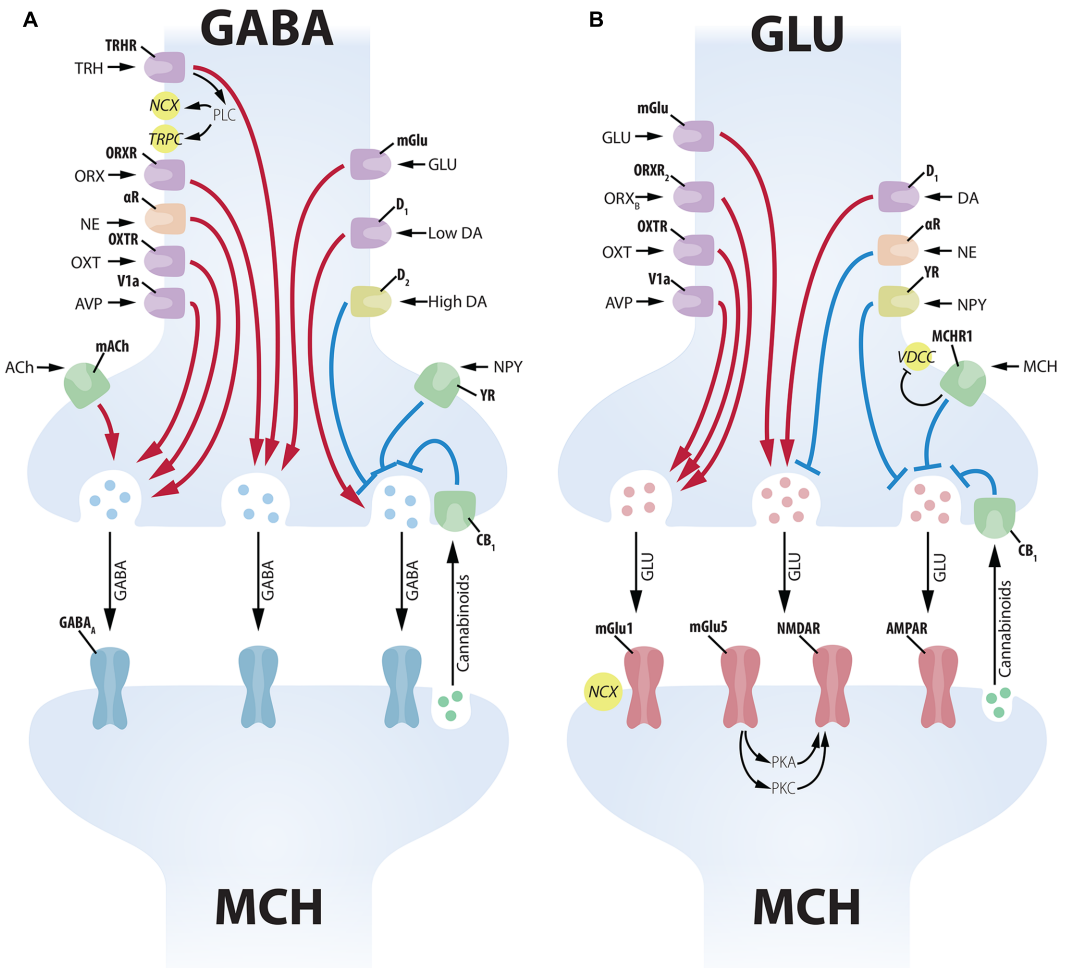

FIGURE 5 | Complex presynaptic modulation of MCH neurons. (A) Several neuropeptides increase GABAergic input to MCH neurons acting on the soma of GABA neurons, such as: TRH, ORX, NE, OXT, AVP, GLU and low levels of DA. ACh increases GABAergic input to MCH neurons acting on axon terminals. High levels of DA, on the other hand, promote an inhibition of presynaptic GABAergic input through D_2 receptors. NPY decreases inhibitory input acting on presynaptic terminals, while cannabinoids released by the MCH postsynaptic terminal decrease vesicle release (Huang et al., 2007). GABAergic input to MCH neurons is vehiculated mainly by $GABA_A$ receptors. **(B)** The GLUergic input to MCH neurons is similarly under the control of numerous inputs. GLU, ORX, OXT, AVP, and DA act on the soma of GLU cells to increase excitatory input to MCH neurons. NE and NPY have the opposite effect, decreasing GLU input to MCH neurons. MCH has an inhibitory presynaptic effect through an MCHR1-dependent decrease in Ca^{2+} currents. Cannabinoid activation of CB_1 has a similar inhibitory effect on vesicle release (Huang et al., 2007). GLU input to MCH neurons is conveyed by multiple receptors, including ionic (NMDAR and AMPAR) and mGlu1 and mGlu5. The activation of mGlu5 in MCH neurons leads to a potentiation of NMDAR responses through protein kinases A and C (PKA and PKC).

Elias et al. (1998) showed that both major populations of the Arc, NPY/AgRP and POMC/CART, project densely to the LHA, with immunoreactive fibers in close apposition to MCH neurons. van den Pol et al. (2004) report that NPY inhibits MCH neurons, decreasing spike frequency and hyperpolarizing membrane potential, both postsynaptically through GIRK currents and presynaptically inhibiting GLU and GABA release to MCH neurons (**Figures 4B**, **5**). To better understand these observations, it is important to consider that the LHA and the Arc present reciprocal connections. Direct injections of MCH in the Arc promote an increase in food consumption, placing this area as a site of MCH action (Abbott et al., 2003). Using hypothalamic cultures, these authors demonstrated that the addition of MCH to the culture induces a release of NPY, at the same time decreasing the amount of the anorectic α-MSH released by a hypothalamic explant. These data suggest that MCH projections from the LHA to the Arc may increase the release of the orexigenic peptide NPY and decrease the anorectic α-MSH, resulting in an overall increase in food consumption.

The results of Abbott et al. (2003) allow us to speculate that NPY inhibition of MCH neurons may be part of a negative feedback loop, with increased MCH activity on the Arc resulting in a depression of MCH neurons by NPY, avoiding excessive stimulation of this orexigenic circuit. Another possibility is that NPY–MCH interactions may be secondary to some other Arc-LHA circuits, as suggested by Segal-Lieberman et al. (2003) and van den Pol et al. (2004). Parallel to this negative feedback loop, a positive feedback loop may exist between MCH and

the anorectic peptides of the Arc. Although the addition of α-MSH agonists or antagonists has no major electrophysiological effect on MCH neurons (van den Pol et al., 2004), the selective deletion of the *Pomc* gene results in a marked increase in *Pmch* mRNA, suggesting that POMC-derived peptides are capable of inhibiting *Pmch* expression (Challis et al., 2004). In this case, inhibition of α-MSH neurons by MCH would result in decreased inhibition of *Pmch* expression, potentially increasing the stimulation of NPY by MCH and reinforcing the negative feedback loop. It is important to consider, however, that these circuits may be polysynaptic, since there is little evidence for NPY and melanocortin receptors in MCH neurons (Liu et al., 2003; Parks et al., 2014). Therefore, these circuits may employ interneurons or even ORX neurons, since these are susceptible to multiple regulatory mechanisms by NPY (Fu et al., 2004). Finally, another possibility is that Arc projections interact with MCH-ir fibers at the PVH, a target nucleus that display important effector actions regarding feeding behavior and energy homeostasis.

Paraventricular Hypothalamic Nucleus

The PVH is one of the better-described nuclei in the whole hypothalamus. It has eight major subdivisions, with three magnocellular subdivisions containing neurons that primarily synthesize OXT and AVP, and five parvocellular subdivisions containing neurons that synthesize CRF and TRH, although we now know that many other neurochemical populations are present in the PVH with distinct distributions (Swanson and Sawchenko, 1983; Simmons and Swanson, 2008, 2009). In many ways, the PVH can be regarded as the motor nucleus of the endocrine system, directly releasing OXT and AVP in the NH and CRF and TRH in the portal circulation to modulate AH activity and influence two of the major neuroendocrine axis in the brain, the HPT and the HPA axis. Projections from these neurons, however, are not restricted to the hypophysis, as they project densely to other areas of the brain and all of them show, to some extent, modulatory effects.

To investigate if OXT and AVP neurons could exert effects through MCH neurons, Yao et al. (2012) evaluated the electrophysiological response of MCH cells to these neuromodulators. AVP directly excites MCH neurons through the V1a receptor, depolarizing their membrane potential and increasing spike frequency through NCX and the opening of TRPC (**Figure 4A**). AVP also acts presynaptically increasing GLU- and GABAergic input to MCH neurons (**Figure 5**). OXT provides excitatory input to MCH neurons as well, through the OXTR, depolarizing their membrane potential and increasing spike frequency through NCX (Yao et al., 2012) (**Figure 4A**). In addition to OXT and AVP, neuropeptides from the PVHp may also contribute to MCH-dependent modulation of ingestive behavior. As discussed in the previous section, TRH modulates MCH function and it is not possible, for now, to specify the exact source of this input to presynaptic GABA neurons in the LHA. Moreover, little has been described about the effects of CRF-synthesizing neurons over MCH cells albeit that, if such mechanism exists, it must involve polysynaptic circuits, since Parks et al. (2014) report that CRF receptors are not significantly found in MCH neurons.

Limbic and Mesolimbic

The Acb is an important component of the ventral *striatum*, participating in several processes, including reward and aversion (for a review, see Carlezon and Thomas, 2009). The Acb can be divided into two subdivisions, a core (AcbC) and a shell (AcbSh), with distinct connectivity, cellular composition and function. AcbSh activity has been associated with feeding inhibition, as electrical and optogenetic stimulation of AcbSh MSNs are capable of quickly interrupting a feeding bout; a subset of AcbSh neurons decrease their activity immediately before the onset of a sucrose solution consumption; and photoinhibition of dopamine-sensitive (D_1) MSNs increases liquid fat intake (Krause et al., 2010; O'Connor et al., 2015).

AcbSh MSNs are GABA cells that project to the rostral parts of the LHA, suggesting the inhibition of orexinergic neurons of this area (MCH and ORX) as a putative mechanism for AcbSh-mediated inhibition of feeding. It is now understood, however, that AcbSh input to the LHA does not reach MCH or ORX neurons directly. Sano and Yokoi (2007) identified a group of GLU neurons in the anterior part of the LHA (LHAa) that receive dense projections from the AcbSh and project to the posterior part of the LHA (LHAp), suggesting that a GLU node exists between accumbal projections and neurons in the LHAp, where MCH neurons are located. O'Connor et al. (2015) identified a second pathway, reporting that D_1-MSNs from the AcbSh project to the LHA and inhibit GABA interneurons in this area to promote accumbal inhibition of feeding. As an AcbSh|GABA-LHA|GABA-LHA|MCH circuit would be ultimately excitatory for MCH neurons, it is likely that additional nodes are present in this pathway. Further experiments will be necessary to elucidate the intra-LHA circuit that conveys accumbal information to MCH and ORX neurons.

The inhibition of MCH neurons by accumbal projections agrees well with the theory proposed by Kelley et al. (2005) that the AcbSh functions as a "sensory sentinel," quickly inhibiting a feeding bout in response to an environment cue that may need the animal to respond to, such as a predator approaching, even if the animal's metabolic state would drive him to continue feeding. Since MCH neurons project to the MCx (Elias et al., 2008) and decrease locomotor activity, an inhibition of these neurons could, at the same time, decrease the drive to feed and disinhibit the MCx, prompting the animal to show a motor response to the environmental cue.

Amygdaloid input to MCH neurons may also pay a role in the inputs to this system. Nakamura et al. (2009) report that anterograde tracer injections centered in the CeA reveal numerous varicose fibers in the LHA, chiefly on its dorsolateral most area. These fibers often make contact to MCH-ir and ORX-ir neurons and these terminals are immunoreactive for a GABA marker, suggesting an inhibitory property for these CeA projections.

Midbrain and Hindbrain

The midbrain contains some of the most important nuclei containing DA-synthesizing neurons in the whole CNS, including the A8, SN – A9, and VTA – A10 groups (Dahlström and Fuxe, 1963). These groups project densely to the forebrain, including

the neocortex, the *striatum* and the diencephalon (Andén et al., 1966). Leibowitz and Brown (1980) showed that ventral A8 and A9 cells provide an important CA input to the perifornical area, and lesions of the A8 and A9 deplete the LHA of CA-ir, reduce the anorectic effect of the DA presynaptic drug amphetamine and potentiate the anorectic effect of DA itself. Alberto et al. (2011) and Conductier et al. (2011) report that DA produces hyperpolarization of MCH neurons through GIRK mediated outward currents, but DA receptors (D_1 and D_2) antagonists failed to induce similar currents, suggesting that the observed DA actions are not mediated by DA receptors (**Figure 4B**). Conductier et al. (2011) also found that DA can alter the presynaptic input to MCH neurons, with low concentrations of DA (1 μM) potentiating both GABA and GLU release through presynaptic D_1 receptors, while high concentrations of DA (100 μM) inhibits GABA release through D_2 receptors, with GABA effects more pronounced than GLU effects suggesting a predominantly inhibitory action for DA over MCH neurons (**Figure 5**). Both Alberto et al. (2011) and Conductier et al. (2011) found as well that the postsynaptic effect of DA depends on the adrenergic receptor α_{2A}, since specific antagonists blocks DA-induced currents and α_{2A} is present in MCH neurons (Modirrousta et al., 2005).

These results are particularly noteworthy because the α_{2A} is also the receptor through which NE inhibits MCH action. The LHA is moderately innervated by NE terminals originated from the brainstem (Palkovits et al., 1974), with an important contribution from the lateral tegmental NE system (Moore and Bloom, 1979). The work of van den Pol et al. (2004) has provided evidence that NE promotes a hyperpolarization of the membrane potential and a decrease in spike frequency of active MCH neurons by mobilizing GIRK currents, and this effect is blocked by a selective α_{2A} antagonist (**Figure 4B**). NE also acts on the synaptic input to MCH neurons, decreasing GLUergic and increasing GABAergic input, resulting in an overall inhibitory action (**Figure 5**). Taken together, the results of van den Pol et al. (2004), Alberto et al. (2011), and Conductier et al. (2011) point to a synergistic convergence of both DA and NE systems through the α_{2A}. This convergence may limit how much synergistic inhibition acts over MCH neurons, since DA inhibition would desensitize the receptors for NE inhibition and vice-versa. The DA and NE systems are, therefore, competitive elements instead of synergistic, even though both have inhibitory properties over MCH neurons.

Serotonin is a monoamine neurotransmitter synthesized in numerous groups of neurons of the brainstem and with widespread projections throughout the CNS (Dahlström and Fuxe, 1963; Steinbusch, 1981). Hypothalamic injections of 5-HT have been extensively correlated to a decrease in feeding (for a review, see Leibowitz and Alexander, 1998) through multiple receptors and different mechanisms (for a review, see De Vry and Schreiber, 2000). van den Pol et al. (2004) report that 5-HT hyperpolarizes MCH neurons and decreases spike frequency through postsynaptic mechanisms (**Figure 4B**). Taken into consideration, these data indicate that 5-HT may decrease feeding through the inhibition of MCH neurons in addition to 5-HT interactions with DA, although further experimental work is necessary to confirm this hypothesis and detect the specific 5-HT receptor subtypes responsible for this action.

The urocortins (UCN1, UCN2, and UCN3) are members of the mammalian CRF family, with a special affinity for the CRF receptor subtype 2 (Vaughan et al., 1995; Lewis et al., 2001; Reyes et al., 2001). UCN1 has been mapped in the albino rat by Kozicz et al. (1998) and Bittencourt et al. (1999), who have shown that the main sites of *Ucn1* mRNA expression and UCN1 synthesis are the EW and the superior olivary nucleus, both brainstem structures, with a broad distribution of UCN1-ir fibers throughout the CNS. Although the EW was initially recognized as a motor nucleus for vision related muscles, it is now recognized that the EW has two components: a preganglionic EW, responsible for this cholinergic motor control, and the centrally-projecting EW, involved in a series of functions such as feeding, stress and addiction (for a review in EW structure and function, we point the reader to Kozicz et al., 2011). Of special importance to us is the role of UCN1 in feeding behavior, as UCN1 acts as a powerful anorectic peptide in nanomolar concentrations (Spina et al., 1996) and diet/fasting can regulate *Ucn1* mRNA expression (Legendre et al., 2007; Xu et al., 2009). Júnior et al. (2015) have shown that UCN1-ir neurons or the EWcp send projections to the LHA and these fibers are in close proximity to MCH-ir neurons, so this UCN1|EWcp-MCH|LHA circuit could represent the pathway through which UCN1 influences feeding behavior. Although the low levels of CRF receptors in MCH could be taken as an evidence against this particular pathway, it is noteworthy that these receptors are found on the LHA (Chalmers et al., 1995), so the action of UCN1 could involve the modulation of GABAergic or GLUergic interneuronal input to MCH neurons. Further studies will be necessary to elucidate the physiological aspects of this circuit.

Circulating Factors

The MCH peptidergic system appears to be heavily influenced by leptin, a major circulating factor of satiety produced by adipocytes (Zhang et al., 1994; Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). Qu et al. (1996) were the first to identify a relationship between MCH and leptin, reporting that $Lep^{ob/ob}$ mice have twice the level of *Pmch* mRNA than WT animals, suggesting a leptin inhibition of *Pmch* expression. Agreeing to that, obese rats with a point mutation in the long form of the *LepR* gene (Zucker rats $LepR^{fa/fa}$) display higher levels of *Pmch* mRNA and decreased levels of *Mchr1* mRNA in comparison to lean rats, suggesting that deficient leptin signaling can upregulate the MCH system (Stricker-Krongrad et al., 2001). Likewise, Gavrila et al. (2005), studying humans, report that fasting (with a concomitant fall of circulating leptin levels) significantly increases serum levels of MCH, and supplementation with r-metHuLep prevents the raise of seric MCH. The mechanism through which MCH responds to leptin, however, must be polysynaptic, as studies using a reporter associated to the *Lepr* gene indicate that *Lepr*-expressing neurons are not MCH or ORX (Leinninger et al., 2009; Louis et al., 2010).

Other important factors for metabolism regulation are glucose and insulin. Although the periphery holds the machinery for glucose-mediated insulin production, and this machinery works

efficiently to keep the blood levels of glucose in a strict range, we now include the CNS as an important sensor for these metabolic cues and as an effector to keep their physiological levels (Myers and Olson, 2012). The IR is widely distributed in the CNS (Mayer et al., 1978), with deleterious effects on energy homeostasis occurring after its specific deletion (Brüning et al., 2000). Hausen et al. (2016) provide evidence that MCH neurons can respond to insulin, reporting that 200 nM of it is capable of activating PIP3-dependent pathways and invoking action potentials in 6 of 13 neurons tested, while 3 neurons displayed a decrease in firing and the remaining neurons showed no alteration, suggesting that subpopulations of MCH neurons are responsive to insulin with different dynamics (**Figure 4A**).

In addition to insulin, Burdakov et al. (2005) provided evidence that glucose is also able to modulate MCH neurons. Although not as high as plasma levels, the brain is subjected to variations in glucose concentration that mirror those of the plasma, with a physiological range that goes from ∼0.7–2.5 mM and may reach as low as 0.2 mM in conditions of hypoglycemia and 5 mM in hyperglycemia (Routh, 2002). A subset of approximately 70% of MCH-ir neurons is excited by high levels of glucose (5 mM) and dose-dependently hyperpolarizes as glucose levels reach 0.2 mM (Burdakov et al., 2005; Kong et al., 2010). Kong et al. (2010) demonstrated that glucose sensing by MCH neurons works in a similar fashion to pancreatic β-cells, in which an increase in intracellular glucose (driven by an increase in extracellular glucose carried by the GLUT3 transporter) results in increased ATP production which, in turn, induces the closure of K_{ATP} with consequent depolarization, and this effect is dependent of SUR1-containing K_{ATP}, establishing the SUR1 protein as a marker for glucose-sensitive MCH neurons (**Figure 4A**).

Although the activation of MCH in conditions of high glucose may seem counterintuitive, it is possible to fit it in a larger role for MCH in energy homeostasis. MCH is suggested to be involved in energy conservation, decreasing locomotor activity and metabolic parameters and increasing sleep in conditions of positive energy balance, thus its activation in response to high glucose (Burdakov et al., 2013). This led us to hypothesize that the orexigenic activity of MCH is not incompatible with that suggestion, when we consider the probable environment of an animal regarding food availability. It would be dangerous for animals to wait for their energy levels get low, as this could impair their capacity to obtain food, so it is beneficial for animals to eat periodically, even when their energy balance is positive. In most situations, animals have some awareness of their surroundings, and sources of food probably have been scouted, with animals preferentially occupying areas with abundant food availability. In that case, animals do not have to explore for food, so they may display decreased locomotor activity and longer periods of rest. Still, they must have some drive to feed periodically, and MCH could account for this effect, integrating not only glucose levels but also the other input discussed in this section. Food depletion of their environment or hazards (such as predators) may impair their ability to keep this constant state, in a situation that would lead to decreased levels of glucose. In this case, MCH would need to be shut off (low-glucose induced hyperpolarization) and a system that promotes food-seeking could be activated.

A possible component of this food circuit is ORX, as ORX-ir cells are excited by low levels of glucose (Burdakov et al., 2005) and their activation is also implicated in arousal (Alexandre et al., 2013), a necessary condition for active food-seeking. In this model, these two peptides could act in different aspects of feeding behavior: MCH is responsible for keeping a positive energy-balance state, while the ORX system "kicks in" when the animal reaches a negative energy balance and must return to a safer range. Since MCH is a "baseline stabilizer," avoiding big fluctuations in energy homeostasis, MCH inactivation would not promote big alterations in feeding, since other ingestive-controlling systems would act nevertheless when the animal entered deficient energy states, possibly consuming an amount of food similar to that of an MCH-preserved animal. This appears to be the case, since the selective ablation of MCH-neurons in adult mice by diphtheria toxin promotes leanness and hyperactivity, but the animals display only a non-significant tendency to eat less than control mice (Whiddon and Palmiter, 2013).

Another possible peripheral signal that may influence MCH neurons is CORT, a glucocorticoid produced by the adrenal gland that freely crosses the BBB to influence the regulation of energy balance, with central and systemic administration of glucocorticoids promoting an increase in food intake and body weight (Freedman et al., 1986; Green et al., 1992; Tataranni et al., 1996; Zakrzewska et al., 1999). Drazen et al. (2004) have shown that, in ADX animals, the icv injection of MCH has a less potent effect than in sham animals regarding both food and fluid intake, and this effect is reversed in ADX animals that receive CORT supplementation in their water. These authors also report that hypothalamic expression of *Pmch* mRNA is decreased in ADX animals. This is in good agreement with the results of Viale et al. (1997) that showed the partial sequence of a glucocorticoid response element in the 5′-flanking region of the *Pmch* gene, which could account for the mechanism through which the absence of CORT after ADX promotes a decrease in *Pmch* mRNA expression.

Amylin is a pancreatic peptide hormone, co-synthesized with insulin by β-cells in response to feeding (Leffert et al., 1989), although central AMY has been described as a lactation-related peptide synthesized in the MPOA (Dobolyi, 2009). AMY has an anorectic action, suppressing feeding in both rats and mice, suggesting a post-prandial satiety effect and long-term lipostatic signaling (Lutz et al., 2001; Rushing, 2003). The intraperitoneal injection in rats of salmon calcitonin, an AMY receptor agonist, decreases the levels of LHA *Pmch* mRNA (Barth et al., 2003). This effect is probably not direct, since there is little evidence for AMY binding in the LHA (Sexton et al., 1994), suggesting that AMY signaling reaches MCH neurons through circuits that may include the AP (Lutz et al., 2001), VTA (Mietlicki-Baase et al., 2013, 2015) and LDTg (Reiner et al., 2017).

Outputs

The regulation of feeding by MCH neurons is a complex mechanism that involves many aspects of this behavior, including the energy balance of the organism, metabolic rate, rewarding circuits, locomotor activity, arousal, spatial memory, olfactory cues and complex interactions with other peptidergic systems. In

this section, we will highlight the main actions of MCH and its underlying connectivity, illustrated in **Figure 2B**.

Locomotor Activity

MCH-ir neurons project diffusely to numerous areas of the isocortex, including the SSCx, MCx, infralimbic, and Ins, as shown by Saper et al. (1986) using an antibody for α-MSH that was, as we now know, cross-reacting to MCH. This pattern of MCH projections was confirmed by Cvetkovic et al. (2003) using an antibody for salmonid MCH and further explored by Elias et al. (2008), that showed extensive innervation of the MCx by MCH neurons of the LHA. These authors also reported that a subset of these MCH-ir MCx-innervating neurons also innervate the PPTg, what may also contribute to a motor regulation through the mesencephalic locomotor region of this area. This circuit supports an action of MCH decreasing locomotor activity as reported in *Pmch*$^{-/-}$ and *Mchr1*$^{-/-}$ mice (Segal-Lieberman et al., 2003; Willie et al., 2008), as well as progressive (Alon and Friedman, 2006) and sudden (Whiddon and Palmiter, 2013) adult ablation of MCH neurons. Through this pathway, MCH neurons decrease locomotor activity and energy expenditure to revert states of negative energy balance or sustain states of positive energy balance.

Energy Expenditure

Locomotor activity is not the only way the CNS can modulate energy expenditure, since thermogenesis also play an important role in this aspect of homeostatic balance. The first link between MCH and energy expenditure control was drawn by Shimada et al. (1998), that noticed a reduction in body weight that surpassed the reduction in food intake in *Pmch*$^{-/-}$ animals. Although these animals did not have a lower rectal temperature when compared to WT mice, their rate of O_2 consumption normalized for body weight was 20% higher. Mice lacking MCHR1 also display higher metabolic rates during the dark phase as measured by indirect calorimetry (Marsh et al., 2002).

Using a model of cold exposure, Pereira-da-Silva et al. (2003) showed that MCH is increased by almost 60% in cold-exposed animals. These animals display a series of physiological adaptations to the challenging temperature that include an increase in food consumption, fall of body weight and thermogenesis. These authors report that cold-exposed animals with impaired MCH synthesis have an increase in the molecular machinery necessary for BAT-dependent thermogenesis when compared to control animals, suggesting that MCH inhibits BAT thermogenic activity, potentially conserving energy for homeostatic purposes. To investigate the chemical signature of the hypothalamic circuits modulating BAT activity, Oldfield et al. (2002) injected a polysynaptic viral tracer in the BAT of adult rats combined to immunohistochemistry for different neuropeptides synthesized in the hypothalamus. Third-order MCH-ir neurons were found in the LHA, suggesting that MCH neurons provide input to the BAT through brainstem nuclei.

These results are reinforced by the works of Guesdon et al. (2009) and Chung et al. (2010). Guesdon et al. (2009) report that intraventricular injections of MCH decrease lipid oxidation and Chung et al. (2010) demonstrated that *Mchr1*$^{-/-}$ animals are resistant to weight gain when exposed to HFD due increased lipid metabolism. Furthermore, Verty et al. (2010), using temperature-sensitive telemetry probes, registered a significant increase in BAT temperature after intraventricular injection of an MCHR1 antagonist. All these data suggest that LHA MCH neurons polysynaptically decrease thermogenesis in BAT to reduce lipid metabolism and energy expenditure, an important mechanism to sustain a positive energy balance or revert a scenario of negative energy balance.

Autonomic Modulation

Saper et al. (1976) have shown that LHA neurons project both to autonomic centers of the brainstem, such as the dorsal motor nucleus of the vagus and the NTS, and directly to the SpCd, including the ImC in thoracic levels, suggesting a descending hypothalamo-autonomic pathway that access directly the preganglionic cells of the parasympathetic and sympathetic systems. Since MCH-ir fibers from the LHA also sparsely innervate the autonomic nuclei of the brainstem and all levels of the SpCd (Kohler et al., 1984; Bittencourt et al., 1992; Bittencourt and Elias, 1998), this represents a possible pathway through which MCH may integrate ingestive behavior to autonomic function, although further evidence is necessary to establish if there is a direct contact between ganglionic cells and MCH neurons, possibly through specific viral tracers. More recently, Pérez et al. (2011) injected a polysynaptic viral tracer in the SAL and MAS of mice and discovered that MCH-ir neurons of the LHA provide inputs to brainstem nuclei involved in the control of these structures (MoV – MAS innervation; 7N and SuS – SAL innervation). Through this innervation, LHA MCH-containing neurons can gate two important preparatory responses to feeding: salivation, an autonomic response necessary for bolus formation and swallowing, and mastication, a motor response which involves complex coordination between muscles of the jaw, face, and tongue. Another interesting aspect of this MCH input is the fact that both salivation and mastication are under the control of higher-order cortical centers (Thexton, 1992; Hubschle et al., 1998), and MCH could potentially integrate this higher-center input and provide the output to lower brainstem nuclei.

An indirect mechanism for this feeding-autonomic coupling is through the PVH. An important number of labeled cells are found in the PVH after retrograde tracer injection in the SpCd, suggesting that this nucleus also acts over autonomic systems (Hancock, 1976; Saper et al., 1976; Ono et al., 1978; Blessing and Chalmers, 1979; Hosoya and Matsushita, 1979). The PVH neurons that project to the SpCd and to the dorsal vagal complex are concentrated in the dorsal, lateral, and medial parts of the PVHp (Swanson and Kuypers, 1980), regions that receive more MCH-ir innervation than the PVHm (Bittencourt et al., 1992). Since injections of MCH restricted to the PVH without spilling to the ventricular system promote an increase in feeding behavior (Abbott et al., 2003), this could represent another node through which MCH integrates feeding behavior and autonomic function,

triggering the necessary autonomic preparatory processes to feeding.

Thyroid Modulation

Kennedy et al. (2001) suggest that MCH could be part of the peptidergic modulation of the HPT axis. After icv injections of MCH, there is an *in vivo* decrease in the plasmatic levels of TSH (10 and 60 min after injection). *In vitro*, the addition of MCH and NEI in hypothalamic cultures promotes a decrease in the levels of TRH, and MCH co-applied with TRH decreases the secretion of TSH in cultures of hypophysaire cells when compared to TRH alone. MCH may act both through a direct and an indirect pathway to control the HPT axis activity. Directly, MCH-ir fibers from the LHA could innervate TRH-ir neurons in the PVH, decreasing their secretory activity. In the indirect pathway, MCH-ir projections to the Arc could potentially act over the α-MSH/CART and NPY/AgRP systems to promote downstream alterations in TRH synthesis. There is anatomical support for both pathways. Both MCH-ir fibers and MCHR1 immunoreactivity are found in the PVH of rats (Bittencourt et al., 1992; Hervieu et al., 2000; Saito et al., 2001) and a reporter to associated to *Mchr1* can be detected in TRH-ir cells of the PVH (Chee et al., 2013). An Arc mediated pathway is supported by the observation that the neonatal ablation of the Arc abolishes the decrease in PVH| TRH resulting from fasting (Legradi et al., 1998). NPY-ir and AgRP-ir nerve terminals contact TRH-ir neurons in the PVH, and the main source for this innervation is the Arc (Legradi and Lechan, 1998, 1999). A similar innervation of pro-TRH mRNA expressing-neurons is also observed for α-MSH and CART fibers (Fekete et al., 2000). Regardless of the pathway, considering the energy conservation effects of MCH, it is likely that this peptide acts on the HPT axis to decrease energy expenditure.

Circulation

There are few studies that examined the levels of MCH in the bloodstream. Evaluating the possibility that MCH was produced and released by adipocytes, Bradley et al. (2000) did not detect *Pmch* mRNA in these cells, but they detected MCH in the plasma through a RIA. Stricker-Krongrad et al. (2001) evaluated the amount of MCH in the serum of Zucker rats and found that MCH levels were increased in the obese animals compared to the lean ones. On the other hand, in Wistar rats with obesity resulting from electrolytic VMH or PVH lesions, there was a decrease in the intensity of MCH staining in the LHA (9.3% for VMH lesions and 21.9% for PVH lesions), but the decrease in serum MCH levels did not reach statistical significance (Sun et al., 2004). It is important to consider, however, that Sun et al. (2004) used densitometry in LHA slices after immunohistochemistry to quantify the alterations in MCH immunofluorescence, a method that allows the detection of general differences but renders impossible to define if subsets of neurons had alterations. Therefore, it is still possible that some neurons displayed a decrease in MCH synthesis in response to the lesions, but the neurosecretory neurons kept stable levels of synthesis.

Gavrila et al. (2005) examined serum levels of MCH in humans, evaluating correlation factors for diverse parameters such body fat, leptin, estrogen and testosterone, dietary patterns and fasting. These authors found a direct correlation between serum MCH and fat mass and fat percentile, but no correlation could be drawn between MCH levels and estrogen, testosterone or dietary parameters. Working also with humans, Carnier et al. (2010) evaluated obese adolescents with eating disorders after 6 months and 1 year of interdisciplinary therapy, and observed a short-term increase of MCH in the blood of these patients followed by a decrease after 1 year of therapy. These authors suggest that the short-term increase in energy demand upregulate the release of MCH and the long-term correction of dietary habits promote a lower MCH baseline. During the preparation of this review, a new study about circulating MCH in humans was published by Naufahu et al. (2017). In this study, a reliable RIA was developed and tested in over 200 humans of both sexes and different body fat profiles, revealing a plasma MCH baseline of 19.5–55.4 pg/ml and some differential regulation of MCH that depends on both gender and adiposity, although further studies are necessary to fully elucidate how that regulation occurs.

The data regarding circulating MCH must be examined with caution. One major caveat is that Waters and Krause (2005), in a letter to the editor addressing the work of Gavrila et al. (2005), report that the commercial RIA used in most of these studies for MCH has non-specific reactivity with serum elements, since samples from $Pmch^{-/-}$ mice resulted in a positive signal. However, it is now hard to dispute that MCH is found in physiological levels in the bloodstream after the experiments ran by Naufahu et al. (2017). Another important aspect about circulating MCH is that its source is still unknown. Several peripheral tissues synthesize MCH (Hervieu and Nahon, 1995), and the role of this synthesis is, in many cases, still not associated with specific functions. Viale et al. (1997) showed that peripheral MCH has a different structure when compared to central MCH, as it is processed differently and its sequence combines central MCH and NEI. Since many peripheral tissues display MCHR1 mRNA (Saito et al., 1999; Hill et al., 2001), it is highly likely that MCH released in the bloodstream from one or more sources can reach those tissues and play modulatory roles. The study of MCH as a secreted factor in the bloodstream represents, therefore, a prolific field of MCH study.

Reward

In an oversimplification, feeding behavior is stimulated by two main drives: a metabolic drive, guided by homeostatic cues aiming to keep the energy balance of the animal, and a reward drive that involves complex emotional aspects and is directly linked to the subjective experience of pleasure. The reward aspect of feeding is a major component of that behavior, playing a preeminent role in our modern society. Rats are willing to expose themselves to aversive stimuli, such as foot-shock, painful heat and intense cold to obtain palatable food, such as candies and soda (Cabanac and Johnson, 1983; Foo and Mason, 2005; Oswald et al., 2011). As the complex relationship between metabolic and reward aspects has been thoroughly examined by others (Saper et al., 2002; Berthoud, 2011; Ferrario et al., 2016), in this review, we will focus on the role that MCH may play coordinating these different drivers.

The motivation and reward system involves a complex circuit with many relevant nodes, including the prefrontal cortex, *striatum*/Acb and the VTA. One of the regions with highest *Mchr1* mRNA expression is the Acb (Hervieu et al., 2000; Saito et al., 2001) and MCH fibers are found in this area (Bittencourt et al., 1992), especially numerous in the septal pole of the AcbSh (corresponding to the dorsocaudomedial AcbSh) (Haemmerle et al., 2015). Haemmerle et al. (2015) also report that accumbal MCH-innervation originates from neurons in all major hypothalamic areas containing MCH-ir neurons, with the highest rate of neurons simultaneously labeled for MCH and retrograde tracer found in the IHy. These results are in good agreement to those of Kampe et al. (2009) that report AcbSh-projecting MCH neurons in the lateral hypothalamus that also project to the CgCx/Ins.

MCH influences feeding behavior through the AcbSh, as MCH injected in this area promotes an increase in chow consumption, while an MCHR1 antagonist injected in this area decreases feeding (Georgescu et al., 2005). These authors also report that MCHR1 co-localizes to enkephalin and Dyn in the AcbSh, suggesting that MCH may influence the opioid system through these cells, a possible crossroad between MCH and the reward system (Holtzman, 1974; Frenk and Rogers, 1979). These results are supported by the work of Mul et al. (2011) that, using the only published model of $Pmch^{-/-}$ rat, found that the MCH signal on the AcbSh is important to keep the motivational aspects of the feeding behavior. Therefore, this hodological aspect between the LHA, the AcbSh and limbic areas is fundamental for the characteristic control of energy balance and to increase motivational or incentive-related aspects of food consumption.

A new facet of MCH actions on reward was provided by Karlsson et al. (2012). Using an MCHR1 antagonist combined with self-administration of sucrose (caloric and sweet) or saccharin (just sweet), these authors found that antagonist administration reduces sucrose self-administration, but not saccharin-reinforced lever-pressing, indicating that MCH conveys rewarding signals from the caloric content of consumed food. The antagonist administration also decreased cue-induced reinstatement of sucrose seeking, confirming that MCH participates in the rewarding properties of sucrose.

Domingos et al. (2013) extended these result employing optogenetic manipulation of MCH neurons in mice. Photostimulation of MCH neurons is able to revert the innate preference of mice for sucrose over saccharin and stimulate DA release in the *striatum*. A similar effect is observed in sweet-blind mice, suggesting that taste is not necessary for MCH-dependent preference for caloric ingestion. Contributing to these effects may be an activation of DA neurons in the VTA, as MCH ablation reduced FOS synthesis in this area. The VTA is densely innervated by MCH (Bittencourt et al., 1992) and MCHR1 is found in low (Saito et al., 2001; Pissios et al., 2008) to moderate (Hervieu et al., 2000) numbers in this area. CART/MCH terminals are found in the VTA, and CART-ir fibers contact DA neurons, suggesting that MCH and CART may work in tandem to modulate DA release by VTA neurons (Dallvechia-Adams et al., 2002), although electrophysiological data suggests otherwise (Korotkova et al., 2003). One possible explanation is that CART and MCH may not act directly on DA neurons, but modulate their auto inhibition. CART impairs DA-D_2 binding when co-applied with stimulatory substances (Jaworski et al., 2003; Kim et al., 2003; Moffett et al., 2011), and DA neurons are auto inhibited through D_2 (Johnson and North, 1992; Momiyama et al., 1993), suggesting that CART may decrease DA-dependent inhibition of DA neurons, increasing their activity. Since MCH can depress the presynaptic machinery of release (Gao and van den Pol, 2001), MCH released with CART in the synapse could depress further CART release, preventing too much disinhibition of DA neurons. A similar mechanism to this has an experimental basis in the work of Yang and Shieh (2005), on which MCH impaired CART stimulation of DA activity in the Acb. Further experimental evidence, however, is necessary to test if this is the mechanism of action for MCH, CART, and DA in the VTA.

The interactions between MCH and DA are not limited to the VTA, however, as these two may also interact directly on the Acb. When Chung et al. (2009) applied MCH or D_1 and D_2 agonists separately in the AcbSh, there was no change in animal behavior, but when the three were co-applied there was a potentiation of the cocaine rewarding response. Furthermore, $Mchr1^{-/-}$ animals displayed resistance to the rewarding effects of cocaine, in a similar way to the pharmacological blockade of this receptor. More work will be necessary to fully elucidate the interaction between MCH and DA, however, as Smith et al. (2005) observed upregulation of D_1 and D_2 in the AcbSh of $Mchr1^{-/-}$ mice and hyper sensibility to amphetamine, while methamphetamine responses were attenuated after injections in the AcbSh of MCH and amplified after MCHR1 agonists injections (Sun et al., 2013). Albeit Chung et al. (2009) suggest that these apparent contradictions can be explained by the different mechanisms of cocaine and amphetamine action, more evidence is necessary to confirm this and to allow us to understand the role that MCH may play in reward and addiction.

As a final consideration about MCH and reward, although alcohol consumption is not the theme of this review, both drug and food consumption have a common ground that is the reward motivation. Alcohol is a particularly interesting model, as it is both a stimulatory factor of the reward system and a source of caloric nutrient. Duncan et al. (2005) found an increase in 10% alcohol and isocaloric sucrose solutions intake after 3V MCH injections, suggesting that MCH may drive the pursuit of these substances for their rewarding or caloric value. Morganstern et al. (2010a) developed on these results, demonstrating that injections of MCH restricted to the PVH and Acb were able to increase alcohol consumption, suggesting those nuclei as downstream targets of MCH action on alcohol consumption. The exact mechanism by which this MCH modulation happens was, however, contentious, as some reports suggested that MCHR1 was not involved in the alcohol consumption-promoting effect of MCH (Duncan et al., 2006, 2007), while others found a potent decrease in alcohol consumption and reinstatement after an MCHR1 antagonist treatment (Cippitelli et al., 2010). Recently, Karlsson et al. (2016) reported that MCH and MCHR1 have a dual role in the regulation of alcohol intake through mechanisms related both to caloric intake and reward

motivation. Further experiments on MCH alterations linked to alcohol consumption, however, are still necessary to provide an overarching explanation for all results reported in the literature. Understanding the mechanisms through which MCH modulates alcohol consumption may provide insightful views on addictive and excessive behaviors.

Foraging/Predation

The participation of MCH in the pre-ingestive steps of feeding is perhaps its most elusive aspect so far. Albeit the *ad libitum* diet used in most experimental designs is convenient to standardize parameters, it also creates a highly artificial setup for the animal. In its natural environment, the animal must constantly make decisions regarding food acquisition while factoring elements such as its current energetic availability compared to other needs, foreseen energetic necessity, proximity to food sources and the risk of being eaten by a predator or injured by another environmental hazard. Some animals must also decide when to hunt for prey, since predation may reward it with metabolic needs, if successful, but may also incur in wasted energy if a failure.

Among the many complex elements of foraging and predation, there are three aspects to which MCH may be associated to: sensory integration, decision-making, and memory. The first role proposed for MCH in the mammalian brain was over the modulation of auditory stimuli, by Miller et al. (1993). Using an auditory gating paradigm, these authors have shown that the suppression of a redundant auditory signal is abolished after MCH addition to the CSF, suggesting that MCH can induce a state of higher vigilance at the cost of decreased focus on a single signal. Besides auditory stimuli, Adams et al. (2011) provided evidence that MCH participates in the integration of olfactory stimuli through numerous MCH-ir fibers that are found in olfactory-linked structures such as the Pir and the OB. Using *Pmch*$^{-/-}$ mice, these researchers observed a decreased capacity for them to find food using olfactory cues, suggesting that MCH may display an important role in the search for food. MCH is, therefore, an important actor in the gating of sensorial information, altering perceptual properties of the animal (such as vigilance and focus) depending on the available energy stores.

Regarding decision-making, there is a paucity of functional information relating it to MCH. Projections arising from MCH neurons in the IHy and tuberal LHA densely and diffusely reach the PAG (Elias and Bittencourt, 1997). Comoli et al. (2003) have shown that the lateral aspects of the PAG are involved in predatory behavior, as FOS synthesis in this area is increased in rats after cockroach predation compared to control animals. It is possible, therefore, that MCH-ir projections to the PAG may influence predatory behavior, but experimental evidence is necessary to establish the PAG-mediated actions of MCH.

The third important aspect of foraging is memory, as animals that can adequately remember the location of food sources will have a survival advantage. The HF has been thoroughly implicated in memory (Scoville and Milner, 1957; Zola-Morgan et al., 1986; Squire, 1992) and is one of the major targets of MCH-ir fibers and MCHR1 synthesis in the rat brain (Bittencourt et al., 1992; Hervieu et al., 2000; Saito et al., 2001). Lima et al. (2013) provided a more detailed description of the MCH input to the HF, describing a higher density of MCH-ir fibers in the dorsal HF, especially in the CA3 field, and the presence of MCH-ir fibers plexuses around GABAergic basket cells in CA1 and CA3, in addition to apparent contacts between MCH-ir fibers and HF-projecting cholinergic cells of the MS.

Several authors have provided evidence that MCH plays a role in HF modulation. After CA1 MCH injections, rats show: improved acquisition and consolidation in an inhibitory avoidance test (Monzon et al., 1999); a decrease in the levels of nitric oxide and its second messenger cGMP (Varas et al., 2002a); and a facilitation in long-term potentiation through alterations in the NMDA receptor-gated channel (Varas et al., 2002b, 2003). Adamantidis et al. (2005) provided further evidence for these memory-facilitation actions of MCH by reporting that *Mchr1*$^{-/-}$ mice have worse memory retention of aversive stimuli and decreased response of pyramidal cells to NMDA in the CA1. Pachoud et al. (2010) expanded the roles of MCH in HF plasticity, reporting that *Mchr1*$^{-/-}$ mice have decreased output from excitatory GLUergic Schaffer Collaterals from the CA3 to the CA1 through both AMPA- and NMDA-mediated responses. In summary, these results point to MCH influencing GLUergic transmission in the CA1 field through both presynaptic mechanisms and the increase of NMDAR and AMPAR.

While most studies focused on the effects of MCH in the CA1 field, Sita et al. (2016) investigated the effects of MCH immunoneutralization centered on the CA3. Animals with impaired MCH signaling in the CA3 were more effective recalling the place where food was buried during the training phase, while control animals took longer and dug in other sites before attempting the right quadrant. The most reasonable explanation for the observed results is that animals could perceive olfactory cues indicating that there was no food in the test trial, so control animals searched for food in the arena while MCH-impaired animals were unable to incorporate these cues to the previously stored information.

What circuit could underlie the observations of Sita et al. (2016)? According to Easton et al. (2012), there is a dichotomy between the encoding of new afferent information into preexisting memories, a phenomenon dependent of the entorhinal cortex and the CA1 field, and the retrieval of memories, based on CA3–CA1 interactions. Therefore, the inhibition of MCH in the CA3 may have favored this latter circuit, allowing the animal to better recruit the location of the buried empty petri dish, but impairing its ability to perceive the absence of food, indicating that normal MCH activity in the CA3 is necessary for regular integration of sensory information to acquired memories, in a similar fashion to cholinergic input (Easton et al., 2012).

OTHER BEHAVIORS

The actions of MCH on behaviors not related to energy homeostasis are less understood, although recent developments

began to shine some light in these other actions (**Figure 6**). There is ample morphological support for MCH actions over sexual behavior, as MCH-ir fibers are found in multiple relevant areas such as the AVPV, the MPOA, the AHA, and the ME (Bittencourt et al., 1992), where they may contact GnRH-ir neurons (Williamson-Hughes et al., 2005; Ward et al., 2009; Wu et al., 2009; Skrapits et al., 2015) and fibers (Williamson-Hughes et al., 2005; Ward et al., 2009). This MCH–GnRH interaction may underlie the modulation of MCH over LH release, although the exact mechanism through which this modulation occurs is still contentious (Gonzalez et al., 1997; Murray et al., 2000a, 2006; Tsukamura et al., 2000; Wu et al., 2009). In this regard, the MPOA and the IHy seem to play an important role (Murray et al., 2000b,c, 2006).

Not only MCH appears to impact the sexual physiology, but steroidal hormones seem to influence the MCH peptidergic system as well. In OVX rats supplemented with EB, the orexigenic effects of MCH are impaired in comparison to male and OVX-only rats, an observation that can be extended to physiological conditions (Messina et al., 2006; Santollo and Eckel, 2008, 2013). These effects of E2, however, appear to be dependent on polysynaptic mechanisms, since MCH neurons are close to ERα-containing neurons, but they do not co-localize (Muschamp and Hull, 2007; Santollo and Eckel, 2013). Steroidal cues also play a role in MCH fiber density, as the number of MCH-ir fibers in the external layer of the ME (MEe) is increased in the diestrus and proestrus, in sharp contrast to what is observed during estrus or in males (Chiocchio et al., 2001; Gallardo et al., 2004).

In conclusion, although there is an important amount of information regarding the relationship between MCH and sexual behavior, especially through the modulation of gonadotropin release, further experiments will be necessary to understand the exact mechanism of MCH action in animals in their natural environment, in light of the complex influence that steroidal hormones and sites of action appear to exert. It is noteworthy, as others have pointed (Naufahu et al., 2013), that MCH actions on sexual behavior probably incur in important survival gains by better coordinating the time for animals to engage in reproductive behaviors, since doing so in states of negative energy balance could incur in danger to the animal's life and their potential offspring.

There is also evidence for MCH actions on maternal behavior and the lactation period. The very first functional clue was presented by Parkes and Vale (1993), that applied MCH and NEI in a NH cell culture and found that the amount of OXT secreted increases around 188 and 245%, respectively. Adams et al. (2011) described that female *Pmch*$^{-/-}$ mice show a higher litter postpartum mortality (31.1 ± 13.6% *Pmch*$^{-/-}$ vs. 7.3 ± 4.2% WT), an observation that they attribute to the reduced capability of the female to integrate olfactory stimuli. Alvisi et al. (2016) report that pup-suckling stimulus does not elicit MCH neurons activation or increase the number of MCH-ir neurons in the MPOA, suggesting that hormonal and other sensorial cues have prevalent interactions modulating the MCH system during this period. Recently, Benedetto et al. (2014) and Alachkar et al. (2016) demonstrated that MCH has an important role in the expression of maternal behavior through the MPOA, acting as a promoter of maternal behavior in the early postpartum period and a selective inhibitor of appetitive components of this behavior at late stages. It is noteworthy that Alachkar et al. (2016) describe impaired maternal aggression in *Mchr1*$^{-/-}$, while Adams et al. (2011) report that *Pmch*$^{-/-}$ animals have increased levels of aggression when on a group and faster initial aggressive response. Besides those two works, very little is known about a possible role for MCH in aggression and defensive behavior, so further experimental investigation is necessary to better elucidate this aspect of the MCH peptidergic system.

Although not motivated behaviors *per se*, sleep and arousal are essential processes with an important interplay with motivated behaviors, especially through hypothalamic circuits (Saper et al., 2001, 2005). Intraventricular injections of MCH increase the amount of time spent by rats in REM and slow-wave sleep (Verret et al., 2003). Likewise, the subcutaneous treatment with an MCHR1 antagonist decreases the amount of REM sleep (Ahnaou et al., 2008). Recently, Vetrivelan et al. (2016) demonstrated that MCH neuronal activity increases REM sleep, but MCH neurons are necessary for the normal wake-REM sleep rhythm. MCH actions on sleep depend on projections to the DR (Lagos et al., 2009, 2011), oral pontine reticular nucleus (Torterolo et al., 2009), horizontal limb of the diagonal band of Broca (Lagos et al., 2012), ventrolateral preoptic area (Benedetto et al., 2013) and to the *locus coeruleus* (Monti et al., 2015). Sleep-promoting actions of MCH may be important to reduce activity and conserve energy in states of negative energy balance, when the animal has a propensity to increase arousal to maximize foraging. The results of Willie et al. (2008) suggest that this is the case, as fasted *Pmch*$^{-/-}$ animals have increased activity and exaggerated REM sleep time reduction.

CONCLUDING REMARKS

In this review, we highlighted the circuitry that underlies the integrative functions of MCH. The MCH circuitry is an extremely relevant tool because it allows us to dissect some of the multiple inputs that reach the hypothalamus and the many targets that can be affected by this system. However, this exactly plethora of connections makes this peptidergic system overwhelmingly hard to study and to comprehend, as each experimental manipulation affects multiple physiological variables. It is not uncommon, therefore, to observe slightly distinct experimental approaches resulting in widely different results, sometimes to the point of an apparent contradiction between obtained data. Although diverse reasons can be pointed to explain this, it is remarkable to us that studying the MCH neuron as a single entity may be hampering the field. Studies like those of Burdakov et al. (2005) and Hausen et al. (2016) tell us that subsets of MCH neurons differ in respect to their electrophysiological responses to varied stimuli, while studies like those of Elias et al. (2008) and Kampe et al. (2009) provide evidence for differential projection fields among MCH neurons. This may be in the heart of our inability to turn MCH into a valid pharmacological target to treat obesity or psychiatric disorders (Méndez-Andino and Wos, 2007; Högberg et al., 2012), as the desirable effects of MCHR1

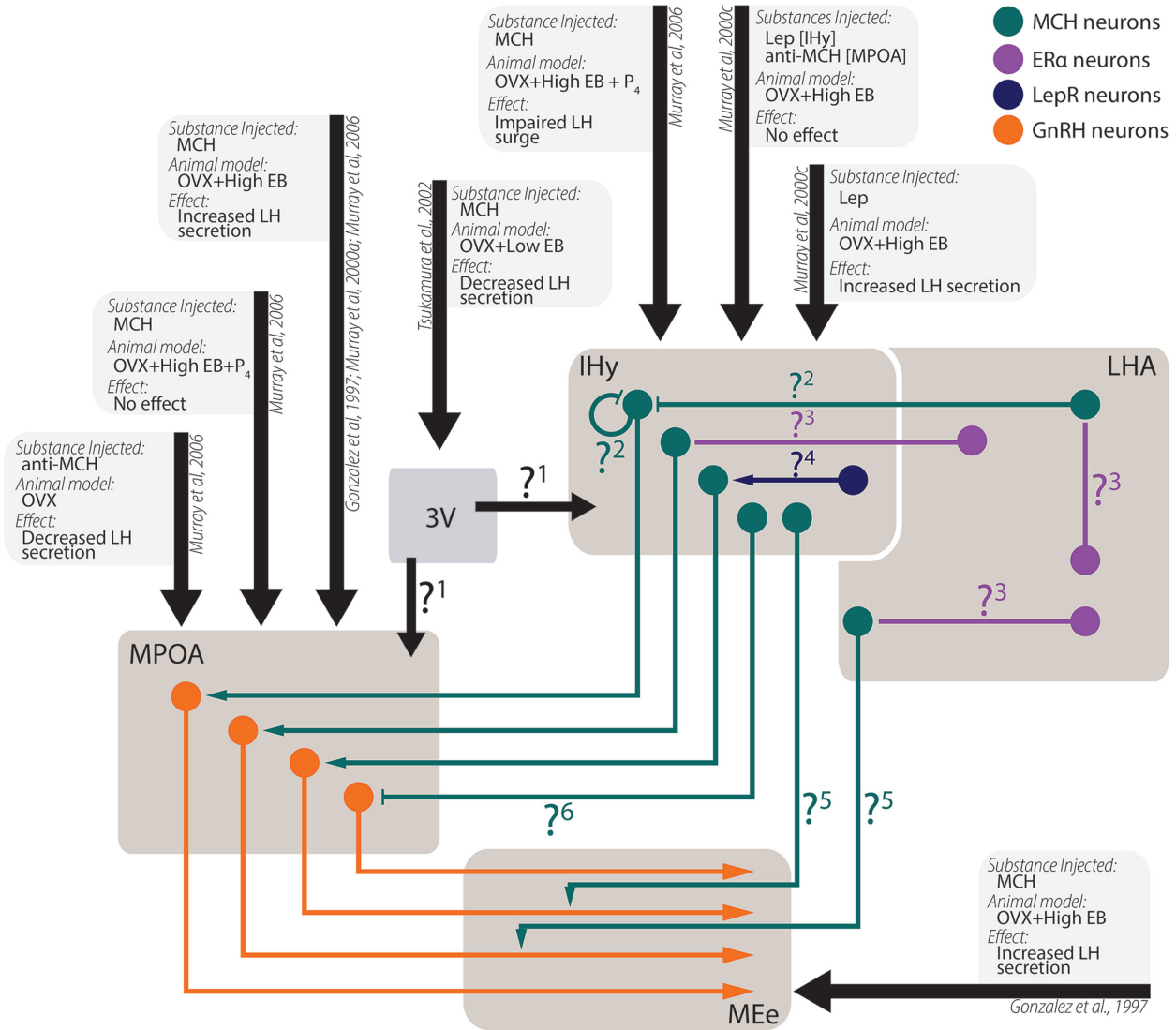

FIGURE 6 | Experimental data from *in vivo* experiments regarding MCH actions on sexual physiology. Simplified schematic representation of the main findings regarding MCH actions after intracerebroventricular injections in areas related to sexual physiology and the circuit underlying these observations. Several questions remain open regarding the pathways necessary for the observed results: **1**. After intraventricular injections, a decrease in LH secretion was observed, although several other authors report increases in LH secretion after intranuclear injections. It is unknown, at this time, if this contradictory observation stems from MCH action on areas that may negatively modulate LH secretion or if the low EB milieu employed by the authors may have contributed to the observed differences; **2**. MCH injections centered on the IHy impair the LH surge expected after OVX+EB+P_4 treatment, suggesting that auto-receptors or extra-IHy MCH inputs may negatively modulate LH release; **3**. Although physiological E_2 levels are necessary for MCH-dependent modulation of LH secretion, MCH neurons do not co-localize with ERα, suggesting that E_2 action over MCH neurons depend on polysynaptic circuits. These circuits are yet to be demonstrated; **4**. Leptin injected in the IHy increases MCH dependent-LH secretion, but MCH neurons do not co-localize to LepR, suggesting that polysynaptic circuits also convey leptinergic information to MCH neurons; **5**. MCH-ir axons are found near GnRH fibers, and the density of MCH fibers in the MEe vary according to the estrous cycle of female, but the source of these MCH-ir fibers is still unknown; **6**. Although several groups reported that MCH increases LH secretion by GnRH cells, electrophysiological data indicated that MCH hyperpolarizes GnRH neurons through postsynaptic mechanisms, therefore the exact mechanism for MCH-dependent LH secretion is uncertain.

antagonism are overshadowed by unwanted side effects. Not everything is grim in the future of MCH study, however, as works that better characterize the morphology, the hodology and the chemical signatures of the LHA, such as those of Swanson et al. (2005), Hahn (2010), and Hahn and Swanson (2015), are important steps in the better characterization of the intrinsic properties of subsets of MCH neurons. Once we understand their variability, we may be able to design drugs to act only on the desired aspects of the MCH peptidergic system and preserve those that must not be manipulated. Accurate morphological data, nevertheless, continues to be an essential tool for the better understanding of peptidergic systems and of the integrative properties of the hypothalamus as a whole.

AUTHOR CONTRIBUTIONS

GD and JB contributed with the writing of this article and approved it for publication.

FUNDING

This article was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (São Paulo Research Foundation – FAPESP) grants n° 2016/02748-0 (GD) and n° 2010/52068-0 (JB). We would also like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Agency for the Advancement of Higher Education – CAPES, Grant #848/15). JB is an Investigator with the Conselho Nacional de Desenvolvimento Científico e Tecnológico (National Council for Scientific and Technological Development – CNPq).

ACKNOWLEDGMENT

We would like to thank Dr. Luciane Sita for her contributions revising some sections of this article, especially those regarding reproductive behavior.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer SEK and handling Editor declared their shared affiliation, and the handling Editor states that the process nevertheless met the standards of a fair and objective review.

frontiers in Systems Neuroscience

ORIGINAL RESEARCH
published: 14 November 2016
doi: 10.3389/fnsys.2016.00092

Evidence of a Role for the Lateral Hypothalamic Area Juxtadorsomedial Region (LHAjd) in Defensive Behaviors Associated with Social Defeat

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Our understanding of the extrinsic connections of the lateral hypothalamic area (LHA) has deepened in recent years. In particular, a series of studies using neural pathway-tracing methods to investigate the macroconnections of histologically differentiated LHA regions, have revealed that the neural connections of these regions are substantially distinct, and have robust connections with neural circuits controlling survival behaviors. To begin testing functional associations suggested by the distinct LHA region neural connections, the present study has investigated the role of the LHA juxtadorsomedial region (LHAjd) in the control of social defeat (a socially-relevant defensive behavior). Male rats received bilateral cytotoxic lesions targeted to the LHAjd. A resident-intruder paradigm was then employed to investigate the effect of these lesions on defensive behavioral responses. Behavioral data were collected during three phases of testing: (1) pre-encounter habituation to testing context; (2) encounter with a dominant conspecific in the testing context; and (3) post-encounter context. Statistical analysis of behavioral measures revealed a significant decrease in risk assessment behaviors during post-encounter context testing in lesioned intruders compared to sham-lesioned and intact rats. However, changes in defensive behavioral measures during the habituation, or during resident-intruder encounters, did not reach significance. We discuss these data in relation to LHAjd (and neighboring LHA region) neural connections, and in relation to current advances in understanding of the neural control of defensive behaviors. A refined model for the neural circuits that are central to the control of socially-relevant defensive behaviors is outlined. We also consider possible broader implications of these data for disorders of behavioral control.

Keywords: LHAjd, social defeat, hypothalamus, lateral hypothalamic area, behavior, animal

OPEN ACCESS

Edited by:
Agnes Gruart,
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Reviewed by:
Alexander C. Jackson,
University of Connecticut, USA
Cristina Marquez,
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Received: *05 August 2016*
Accepted: *31 October 2016*
Published: *14 November 2016*

Citation:
Rangel MJ Jr., Baldo MVC, Canteras NS and Hahn JD (2016) Evidence of a Role for the Lateral Hypothalamic Area Juxtadorsomedial Region (LHAjd) in Defensive Behaviors Associated with Social Defeat. Front. Syst. Neurosci. 10:92. doi: 10.3389/fnsys.2016.00092

INTRODUCTION

In rats, as in other animals, social defeat can occur as a consequence of social conflict with a dominant aggressor (Blanchard et al., 1977). In this scenario, a sequence of stereotyped behavioral interactions occurs, leading ultimately to behavioral expressions of social defeat in the subordinate animal (Blanchard et al., 1977). In addition to the expression of innate defensive

behavioral responses occurring at the time of a stressful social conflict and defeat, socially defeated animals also typically express post-conflict defensive behaviors in the absence of the original stressor that are elicited by learned conflict-associated contextual cues (Motta et al., 2009; Faturi et al., 2014).

The hypothalamus plays a critical role in the control of survival behaviors (for reviews see Risold et al., 1997; Swanson, 2000), and our understanding of the organization and relations of hypothalamic neural circuits underlying behavioral responses to stressful stimuli has increased markedly in recent decades (Simerly and Swanson, 1988; Canteras and Swanson, 1992a,b; Canteras et al., 1992a,b, 1994, 1995, 1997; Risold et al., 1994; Chiavegatto et al., 1998; Canteras and Goto, 1999). Over the past decade, attention has refocused on the spatially-extensive and poorly understood lateral hypothalamic area (LHA), leading to a series of high spatial resolution neural pathway-tracing studies that have determined the extrinsic macroconnections of the LHA medial- and perifornical tiers (Goto et al., 2001, 2005; Goto and Swanson, 2004; Hahn and Swanson, 2010, 2012, 2015). Among the numerous connections identified, it emerged that two neighboring LHA medial tier regions (the LHA juxtaparaventricular—LHAjp, and especially the LHA juxtadorsomedial—LHAjd) are connected to a previously identified social threat responsive hypothalamic circuit involving the medial preoptic area (MPO), medial preoptic nucleus (MPN—embedded within the MPO), ventromedial hypothalamic nucleus (VMHvl; ventrolateral part), tuberal nucleus (TU) and the ventral premammillary nucleus (PMv; Motta et al., 2009; Hahn and Swanson, 2012; Faturi et al., 2014). The LHAjd and LHAjp also have robust connections with the dorsal premammillary nucleus (PMd; in particular, they provide an input to a dorsomedial subregion of the PMd; Hahn and Swanson, 2010, 2012) that is indicated to play a critical role in innate defensive responses (Canteras et al., 1997, 2008; Markham et al., 2004; Blanchard et al., 2005; Cezario et al., 2008; Motta et al., 2009).

These correlated data coalesced in two recent articles: the first of these reported that socially-defeated rats had a robust increase in the expression of the immediate early gene product cFos in the LHAjd after exposure to a social defeat-associated context (Faturi et al., 2014); more recently, a significant increase in LHAjd and LHAjp cFos was reported to occur in response to the social defeat itself, and also to the stress of entrapped immobilization (Motta and Canteras, 2015). Taken together, these data suggest a role for the LHAjd (and LHAjp) in both learned and innate defensive behavioral responses to more than one type of threat. Given the accumulating neuroanatomical and neuroactivational evidence implicating the LHAjd in the control of innate and learned defensive behavioral responses, notably in relation to socially-relevant fear-associated stimuli, in the present study we have investigated this possibility further with a series of experiments employing a territorial resident-intruder paradigm and cytotoxic lesions targeted to the LHAjd.

MATERIALS AND METHODS

Animals

Animals were maintained in accordance with the guidelines of the Brazilian Association for Laboratory Animal Science (Sociedade Brasileira de Ciência em Animais de Laboratório; COBEA), and the Guide for the Care and Use of Laboratory Animals (National Research Council, USA, 2011). In addition, all experimental procedures involving animals were approved by the Committee on Care and Use of Laboratory Animals of the Institute of Biomedical Sciences, University of São Paulo, Brazil (Instituto de Ciências Biomédicas, Universidade de São Paulo; Protocol number 130/2005). For resident-intruder behavioral experiments, male Wistar rats (n = 32, 3–4 months old, approximately 300 g) were used as intruders; Long Evans males (n = 4, 9–12 months old, approximately 600 g) were used as residents (the latter were housed with Long Evans female rats, n = 4, 3–5 months old, approximately 300 g). Long Evans rats are commonly used as residents in a resident-intruder paradigm because they display high levels of aggression toward young male conspecifics (Thor and Flannelly, 1976). All animals were obtained from local breeding facilities, and were housed in dedicated animal housing facilities under controlled temperature (23°C) and illumination (12/12-h light/dark cycle), and with unrestricted access to food (standard laboratory diet) and water.

LHAjd NMDA and Sham Lesions

Male Wistar rats (n = 20) were deeply anesthetized with sodium pentobarbital (40 mg/kg, IP; Cristália: Itapira, SP, Brazil) and received bilateral *N*-Methyl-D-aspartate (NMDA; 0.15 M) injections targeted stereotaxically to the LHAjd (typical coordinates: 2.2 mm caudal to bregma, 0.8 mm lateral to the middle of the superior sagittal sinus, 7.8 mm ventral to surface of cerebral cortex). The NMDA was injected iontophoretically from glass micropipettes (approximate tip diameter 20 μm) using a constant current source (Model CS3, Midgard Electronics) with the following parameters: 10 μA (negative polarity), 7 s current on/off, 10 min/side. Additional rats (control groups, n = 12) either received saline injections (sham lesion, n = 5), or were not injected (intact; n = 7). Rats were allowed a 2-week post-surgery recovery period before they were used in resident-intruder behavioral testing.

Resident-Intruder Behavioral Experiments

Methods for the resident-intruder behavioral experiments followed those described previously (Ribeiro-Barbosa et al., 2005; Faturi et al., 2014). Wistar rats were housed individually; male and female Long Evans rats were housed together in pairs for 3 weeks prior to use of the Long Evans males as residents in behavioral testing. Two weeks prior to pairing, the Long Evans females were sterilized by severing their uterine horns (partial hysterectomy), to prevent pregnancy while retaining ovarian function and sexual behavior—this surgical procedure was performed under deep anesthesia (mixture of ketamine and xylazine; 1:2 v/v; 1 ml/kg body weight). Animals were housed in

transparent acrylic (Plexiglas) home cages (25 cm cube with a 12.5 cm width vertically sliding access panel positioned centrally on one side). All behavioral experiments were video recorded for subsequent analysis.

Habituation to Context

For 10 days, each Wistar rat (NMDA lesion, sham lesion, or intact) was isolated in its home cage. At the beginning of the light phase the rat was transferred in its home cage from a housing room to an adjacent procedure room. The home cage access panel was then raised for 10 min, allowing egress and free exploration of an enclosed Plexiglas corridor (100 cm length × 30 cm height × 12.5 cm width) and (at the other end of the corridor) a second cage of identical construction to the home cage, into which were placed food pellets the rat could obtain. A small amount of fresh bedding was placed in the testing apparatus (corridor and second cage) prior to habituation. After the 10-min habituation period the rat was returned in its home cage to the housing room. The corridor and second cage of the apparatus were cleaned between each habituation session.

Resident-Intruder Encounter

After 10 days of habituation to context, on the next day, the second cage (food compartment) was replaced with the Long Evans pair resident home cage (with the female removed for the duration of the encounter). The Wistar male intruder was allowed access to the resident home cage following the habituation protocol of the prior 10 days, and once inside the resident's cage, the access panel was lowered to prevent egress. Only experienced resident males were used for resident-intruder encounters. If a clear attack (bite) occurred within the first 10 min of an encounter, the resident and intruder were allowed to remain together for a further 10 min after the first attack; if an attack did not occur in the first 10 min, the pair were separated (and these intruders were excluded from subsequent testing and analysis, $n = 1$ from the experimental group, none from control groups).

Post-Encounter Context

On the day after an encounter and social defeat, socially-defeated intruders were allowed to explore the testing context for 5 min. That is, a shortened version of the habituation protocol was followed, with the resident's home cage placed at the other end of the connecting corridor, and with the resident removed from its home cage for the duration of the experiment.

Histology

Ninety (90) minutes after the start of the post-encounter context testing, rats were deeply anesthetized (sodium pentobarbital 40 mg/kg, IP), and then perfused transcardially with ice-cooled 0.9% saline, followed by ice-cooled 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4. The perfusion-fixed brains were removed and placed overnight in a solution of 20% sucrose in 0.1M phosphate buffer pH 7.4 at 4°C. They were then frozen on dry-ice and sectioned on a sliding microtome in the transverse (coronal) plane into four stepwise collated series (40 μM thickness). One of the series was processed for detection of Nissl substance (thionine stain) to confirm cannulae placement and cytotoxic lesion extent. For additional analysis of the lesions a second series of sections was processed for immunohistochemical (IHC) detection of NeuN (Anti-NeuN, MAB377, clone A60, Millipore, USA); the remaining series of sections were transferred to an anti-freeze solution and stored at −20°C for future use. For NeuN IHC, (in brief) the sections were incubated overnight in primary antibody (1:1000 dilution), then for 90 min at room temperature in a solution of biotinylated goat anti-mouse IgG (Vector Laboratories, Burlingame, CA, USA; 1:200 dilution). The sections were then exposed to an avidin–biotin horseradish peroxidase (HRP) reagent (ABC Elite Kit; Vector Laboratories) for 90 min. To visualize the location of the bound NeuN antibodies, the sections were exposed for 10-min to a solution containing 0.02% of a chromogen (3,3-diaminobenzidine tetrahydrochloride—DAB; Sigma, St Louis, MO, USA) and 0.3% nickel–ammonium sulfate in 0.05 M Tris–buffer (pH 7.6), followed by the addition of hydrogen peroxide (1:3000 dilution) and a further 10 min incubation, resulting in a dark blue-black product. The reaction was stopped by extensive washing in potassium phosphate-buffered saline pH 7.4 (KPBS). Sections were mounted on gelatin-coated slides, air-dried, dehydrated through an ascending series of alcohols, cleared with xylene, and coverslipped with DePeX (Sigma). During antibody incubation steps sections were refrigerated; antibodies were diluted in KPBS, that was also used for multiple washes between the incubation steps.

Data Quantification

Data were quantified cumulatively for the intruder for a period sufficient for quantification for the three phases of the experiment (habituation, encounter and context re-exposure). Measurements for the first 5 min of habituation to context, and 5-min post-encounter context testing, included: (1) spatiotemporal measurements: time spent in home cage, corridor and second cage; and (2) duration of the following behaviors: risk assessment, exploration, rearing and grooming. Measurements for 10-min of resident-intruder agonistic encounters included the duration of following behaviors: passive defense, active defense, locomotion, grooming and social investigation. Data quantification (behavioral scoring) from video recordings was done by a trained observer using dedicated analysis software (The Observer, version XT; Noldus, Netherlands). Only intruders that had suffered a clear social defeat were used in the present analysis. The criteria used for scoring encoded behavioral measures are as follows:

- Risk assessment: (1) crouch-sniff (animal immobile with its back arched but actively sniffing and scanning the environment); and (2) stretch postures (animal's body stretched forward, either motionless or moving slowly toward the second/resident cage).
- Exploration: (1) fearless locomotion (locomotion with arched back); and (2) upright position (animal actively exploring the

environment, standing over its rear paws and leaning on the wall with the fore paws).
- Rearing: animal standing over its rear paws without wall contact.
- Grooming: self-cleaning behavior.
- Social investigation: intruder animal sniffing and making light exploratory paw contact with the body of the resident animal.
- Locomotion: forward movement.
- Passive defense: animal motionless and supine (on-the-back submissive posture).
- Active defense: including (1) intruder animal pushing away the resident animal; (2) assuming an upright position with sparse boxing; and (3) attempts to flee from the resident.

Statistical Analysis

After testing for homogeneity of variance (Levene's test), the behavioral data were analyzed using a parametric a univariate analysis of variance (ANOVA) for each dependent variable followed by a *post hoc* analysis using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$) to isolate respective effects. Due to the number of dependent variables, we applied the Bonferroni's correction to the significance level in the ANOVA ($\alpha = 0.007$ for the behavioral measurements during the last day of habituation and during exposure to the context associated with social defeat; $\alpha = 0.01$ for behavioral measurements associated with social defeat during encounter with aggressive conspecific).

RESULTS

For the rats that received NMDA lesions targeted to the LHAjd, only those with bilateral lesions substantially restricted to the LHAjd were included in the present analysis ($n = 5$; **Figure 1**). Spatiotemporal and behavioral measurements were taken on the last day of habituation to the testing environment, on the next day during encounter with the conspecific aggressor, and again on the day following the encounter and social defeat. Over the first few days of the habituation, risk assessment behaviors were observed frequently; however, by day 3 (typically) these behaviors diminished, and by the end of the habituation phase (day 10) were not observed. These observations concur with the results of a recent study using the same experimental paradigm (Faturi et al., 2014). As shown in **Table 1**, during exposure to the testing environment on the last day of habituation, the ANOVA revealed no significant differences among the groups for either the spatiotemporal ($F_{(2,14)} < 2.82$, $p > 0.093$) or behavioral ($F_{(2,14)} < 2.83$, $p > 0.092$) measurements.

Behavioral interactions during the resident-intruder encounter were comparable to those described previously: a typically short (<30 s) latency to first attack by the dominant aggressor (resident), and predominantly passive subordinate (intruder) defensive responses (remaining mainly motionless; Faturi et al., 2014). The ANOVA revealed no significant differences among the groups for the behavioral measurements ($F_{(2,14)} < 3.31$, $p = 0.066$; **Table 2**). However, during exposure to the social defeat-associated context, the ANOVA revealed a significant main effect for risk assessment measurements ($F_{(2,14)} = 10.16$, $p = 0.0018$). Moreover, in animals that received bilateral LHAjd lesions, there appeared to be an increase in the time of fearless exploratory locomotion ($F_{(2,14)} = 3.92$, $p = 0.044$; **Table 3**), but after applying Bonferroni's correction ($\alpha = 0.007$) this behavioral measurement did not differ significantly among the groups. For the other spatiotemporal and behavioral measurements, the ANOVA revealed no differences among the groups during exposure to the social defeat-associated context (**Table 3**).

Post hoc pairwise comparison revealed for the animals exposed to the social defeat-associated context that bilateral LHAjd lesions significantly decreased risk assessment responses compared to the other experimental groups ($p < 0.007$, Tukey's HSD test; see also **Table 3**; **Figure 2**). Overall, the present results suggest that bilateral LHAjd lesions do not have a significant impact on innate social defensive behavioral responses, but do significantly impact contextual responses, in particular risk assessment behavior in the environmental context associated previously with a stressful social defeat event.

DISCUSSION

The experimental paradigm used in the present study was established in a recent study that showed rats exposed to a single social defeat event, and then re-exposed to the defeat-associated context, displayed robust and reproducible defensive behavioral responses (Faturi et al., 2014). In the previous study it was also reported that the socially-defeated animals had a marked increase in their levels of LHAjd cFos expression compared to controls, and further that muscimol blockade of $GABA_A$ receptors in either the PMd or the dorsal division of the periaqueductal gray (PAGd) immediately prior to re-exposing them to the defeat-associated context, resulted in a significant attenuation of risk assessment behavior (Faturi et al., 2014). Here we have reported a comparable significant attenuation of risk assessment behavior resulting from cytotoxic (NMDA) lesion of LHAjd neurons that provide a moderately robust input to the PAGd, and a very robust input to the PMd (Hahn and Swanson, 2012).

The PMd and PAGd are both extensively characterized key nodes for the control of defensive behavioral responses to different types of threat stimuli (Cezario et al., 2008; Motta et al., 2009; Sukikara et al., 2010; Motta and Canteras, 2015). Thus, like the LHAjd, the PMd and PAGd both show increased expression of cFos in rats re-exposed to the context of a social defeat (Faturi et al., 2014). Moreover, increased PMd and PAGd cFos expression is also seen directly after social defeat, and also after exposure to the threats posed by a predator (Motta et al., 2009), and entrapped immobilization (Motta and Canteras, 2015). Conversely, passive defensive behavioral responses (typified by "freezing", and supine posture) associated with exposure to a predator are blocked by cytotoxic lesion of the PMd (Cezario et al., 2008) and PAGd (Sukikara et al., 2010); PMd lesions also abolish passive defensive responses associated with context re-exposure (Cezario et al., 2008). The present data therefore add to, and are consistent with related earlier pathway-tracing, neuroactivational and behavioral data that collectively

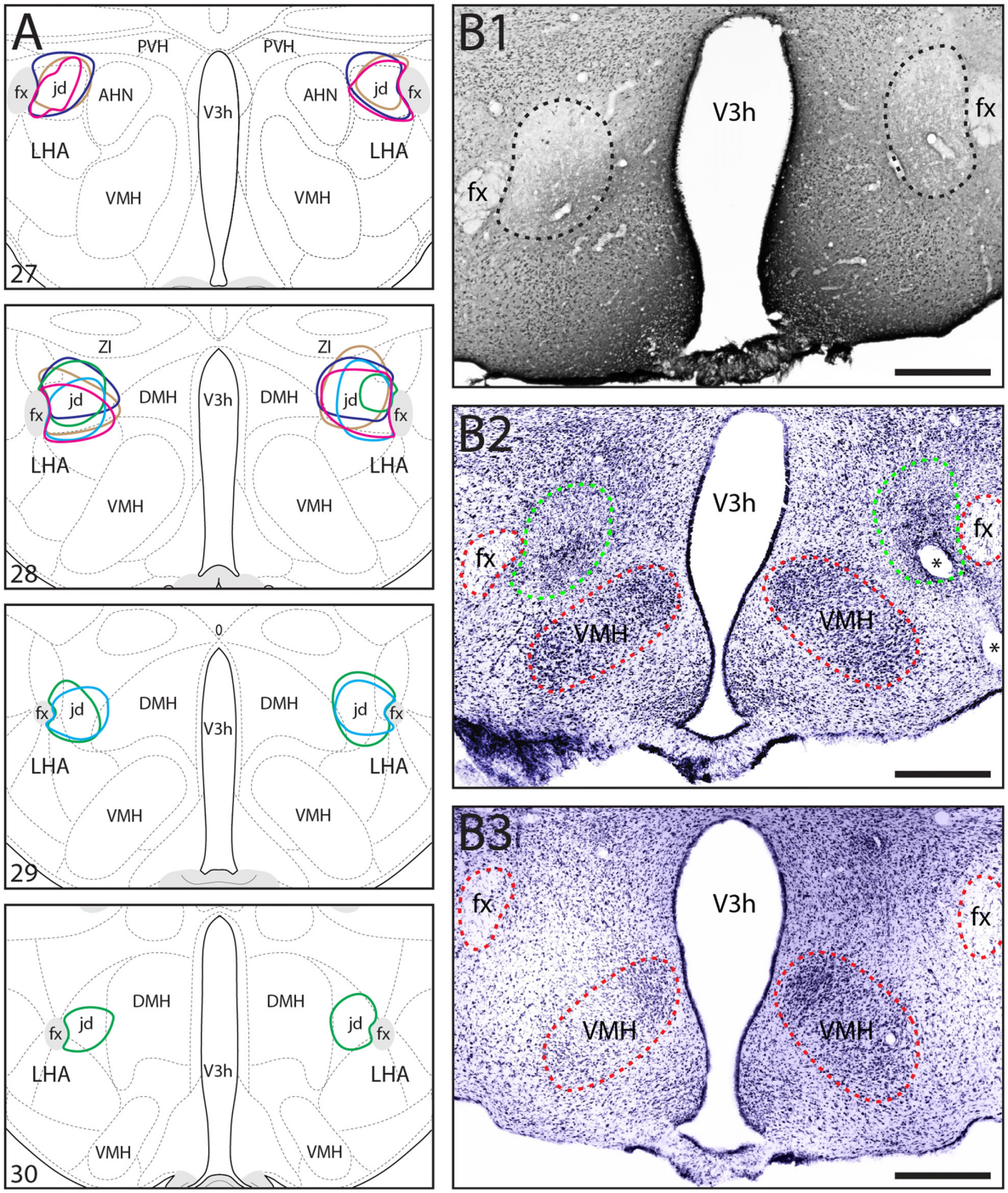

FIGURE 1 | (A) Location and extent of bilateral *N*-Methyl-D-aspartate (NMDA) lesions including the lateral hypothalamic area (LHA) LHAjd in five socially-defeated rats that were used for behavioral analysis. The approximate location and extent of each lesion was determined by analysis of Nissl-stained cytoarchitecture (each is indicated by a different color). For comparison, the data are plotted on a reference rat brain atlas (numbered atlas levels are indicated; Swanson, 2004). **(B1,B2)** Representative digital photomicrographs of NeuN-labeled **(B1)**, and Nissl-stained **(B2)** cytoarchitecture indicating the general region of a bilateral NMDA lesion including the LHAjd (corresponds to region indicated by light blue polygon in **A**); **(B3)** shows the Nissl-stain for a sham-lesioned (vehicle injected) control at a similar rostro-caudal level. The approximate boundary of the lesion in **(B1,B2)** is indicated by a dashed line (black in **B1** green in **B2**); red dashed lines indicate additional fiducial markers (asterisks in **B2** indicate the location of a blood vessel). Images adjusted for brightness/contrast, Nissl images pseudocolored from grayscale. Abbreviations: AHN, anterior hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus; fx, fornix; LHAjd, lateral hypothalamic area, juxtadorsomedial region; PVH, paraventricular nucleus of the hypothalamus; V3h, third ventricle, hypothalamic part; VMH, ventromedial hypothalamic nucleus; ZI, zona incerta. Scale bars = 200 μm.

TABLE 1 | Spatiotemporal and behavioral measurements during habituation to context (10th day).

	Experimental groups			
	Intact (*n* = 7)	**LHAjd lesion (*n* = 5)**	**Sham lesion (*n* = 5)**	**Statistics ($F_{(2,14)}$, *p*)**
Spatiotemporal measurements				
Home cage	61.4 ± 13.7	23.4 ± 10.6	55, 4 ± 10.5	2.82, 0.093
Corridor	86.1 ± 9.5	107 ± 12.5	112.8 ± 14.7	1.39, 0.280
Resident cage	152.7 ± 12.6	169.8 ± 20	132 ± 7.7	1.5, 0.255
Behavioral items measured				
Risk assessment	3.3 ± 1.6	12.4 ± 6.5	11.2 ± 5	2.83, 0.092
Exploration	274.2 ± 9.9	271.8 ± 5.4	281.2 ± 6.3	0.3, 0.743
Rearing	3 ± 1.1	3.4 ± 1.4	3 ± 3	0.34, 0.712
Grooming	20 ± 10.4	12.4 ± 5.4	4.4 ± 3.4	0.92, 0.422

Values are mean ± SEM of the time in seconds during a 5-min observation period.

TABLE 2 | Behavioral measurements during encounter (11th day).

	Experimental groups			
	Intact (*n* = 7)	**LHAjd lesion (*n* = 5)**	**Sham lesion (*n* = 5)**	**Statistics ($F_{(2,14)}$, *p*)**
Behavioral items				
Passive defense	525.5 ± 20.9	360.6 ± 82.4	438.6 ± 53.4	2.73, 0.099
Active defense	58.5 ± 19.5	182.0 ± 50.8	120.8 ± 39.5	3.31, 0.066
Locomotion	11.9 ± 4.37	17.8 ± 13.8	21.1 ± 11.1	0.23, 0.791
Grooming	0.2 ± 0.2	4.0 ± 2.8	8.7 ± 4.2	1.7, 0.217
Social investigation	3.8 ± 3.8	35.4 ± 23.8	10.4 ± 4.8	1.41, 0.275

Values are mean ± SEM of the time in seconds during a 10-min observation period.

TABLE 3 | Behavioral measurements during context re-exposure after social defeat (12th day).

	Experimental groups			
	Intact (*n* = 7)	**LHAjd lesion (*n* = 5)**	**Sham lesion (*n* = 5)**	**Statistics ($F_{(2,14)}$, *p*)**
Spatiotemporal measurements				
Home cage	98.1 ± 29.7	101.0 ± 19.9	114.8 ± 47.8	0.06, 0.93
Corridor	154.9 ± 29.1	94.4 ± 15.6	107.8 ± 32.8	1.08, 0.364
Resident cage	47.0 ± 25.7	104.8 ± 29.9	77.6 ± 20.7	1.6, 0.234
Behavioral items				
Risk assessment	180.9 ± 7.7	81.0 ± 8.9*	182.0 ± 38.3	10.16, 0.0018
Exploration	107.3 ± 10.4	207.4 ± 7.0	111.2 ± 36.2	3.915, 0.044
Rearing	0.6 ± 0.3	1.8 ± 0.8	1.2 ± 0.4	1.02, 0.382
Grooming	9.9 ± 4.4	9.6 ± 5.1	4.6 ± 2.0	0.40, 0.677

*Behavioral measurements during encounter (11th day). Values are mean ± SEM of the time in seconds during a 5-min observation period. *Differs significantly from sham-lesioned and intact control groups ($p < 0.01$, Tukey HSD test).*

support a role for LHAjd in the control of defensive behavioral responses.

In addition to the LHAjd, PMd and PAGd, several other interconnected gray matter regions (within and outside of hypothalamus) show increased cFos expression in response to social defeat, or in response to re-exposure to the context in which a social defeat occurred (Motta et al., 2009; Motta and Canteras, 2015; those with direct connections to the LHAjd are shown in **Figure 3**). Five of these regions—the MPO, MPN (embedded within the MPO), VMHvl, TU and PMv—are notable because the available data indicates their increase in cFos expression is striking and significant in response to perceived social threats (Motta et al., 2009; Motta and Canteras, 2015), but not in response to the potentially existential threats of entrapped immobilization (Motta and Canteras, 2015), or predator exposure (Canteras et al., 1997).

Furthermore, the MPO, MPN, VMHvl, TU and PMv, in addition to being implicated by cFos analysis in socially-relevant defensive responses (Motta et al., 2009; Motta and Canteras, 2015), have longer been implicated in other socially-relevant behaviors (for review, see Canteras, 2012). These include aggressive (Kollack-Walker and Newman, 1995; Veening et al., 2005), sexual (Kollack-Walker and Newman, 1995; Veening et al., 2005), and reproductive (Beltramino and Taleisnik, 1985; Risold et al., 1997) behaviors. The MPN and MPO are additionally implicated in maternal (Stack and Numan, 2000), and also paternal (Dulac et al., 2014) behaviors. This plurality of indicated social behavioral roles for the same hypothalamic

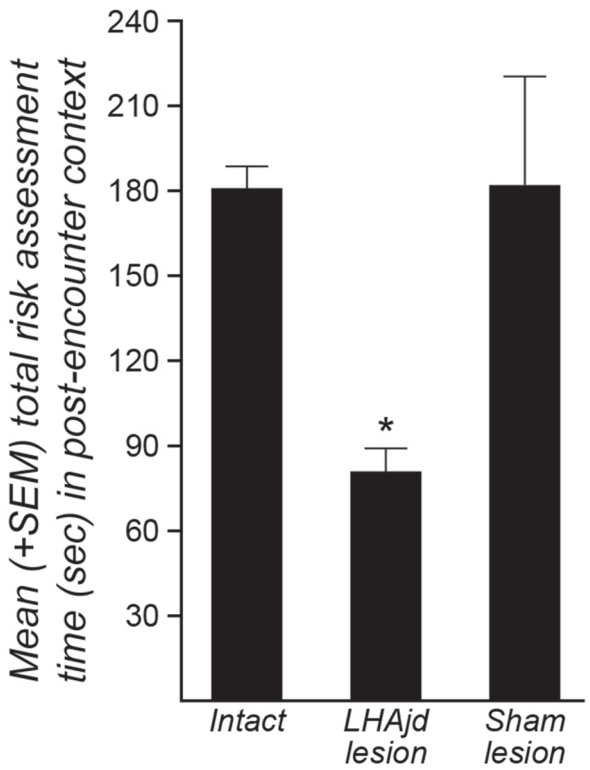

FIGURE 2 | Mean (+SEM) risk assessment behavior duration for three groups of rats (intact, *n* = 7; bilateral NMDA LHAjd lesion *n* = 5; bilateral [vehicle injected] sham lesion *n* = 5) quantified for a 5-min period during exposure of socially-defeated intruders to the environmental context in which they experienced a social defeat on the previous day. (*p = 0.0018).

regions that are themselves highly interconnected lends support to their current inclusion in a conceptual "conspecific/social-responsive hypothalamic circuit" (Motta et al., 2009; Motta and Canteras, 2015; **Figure 3**). It is noteworthy that a strong association with reproductive behavior and function for this circuit led to its earlier conception as a "medial hypothalamic reproductive system" (Canteras, 2002).

Taking the present results together with the indicated similarities in responses to social defeat, in terms of neuroactivational responses of the LHAjd and the conspecific/social-responsive hypothalamic circuit (Motta et al., 2009; Motta and Canteras, 2015; **Figure 3**), it suggests that the LHAjd might also participate in other socially-relevant behavioral functions of this circuit. Consistent with this hypothesis, it is noted that the LHAjd has robust connections with the MPO, and receives a moderate input from the VMHvl (Hahn and Swanson, 2012; **Figure 3**). However, unlike the conspecific/social-responsive hypothalamic circuit, the LHAjd shows a significant increase in cFos expression to entrapped immobilization (Motta and Canteras, 2015; **Figure 3**), suggesting a wider more general defensive behavior related role.

In support of a possible general role for the LHAjd in defensive behavioral control, in addition to direct connections with the conspecific/social-responsive hypothalamic circuit, the LHAjd also connects directly with a conceptual predator-responsive hypothalamic circuit involving the anterior hypothalamic nucleus (AHN) and the VMH dorsomedial part (VMHdm; see Figure 11 in Motta et al., 2009; Hahn and Swanson, 2012). Furthermore, the LHAjd also connects with regions that appear to be part of an earlier alluded to common pathway for the expression of defensive behaviors in general. Foremost among these regions is the PMd (Blanchard et al., 2003, 2005; Canteras et al., 2008; Cezario et al., 2008), with which the LHAjd has strong bilateral and bidirectional connections (Hahn and Swanson, 2012). The organization of connections between the LHAjd and PMd is noteworthy because while the LHAjd primarily targets a dorsal/dorsomedial PMd subregion, it receives input primarily from a ventral/ventrolateral subregion. This topographic connectional difference has relevance in relation the pattern of PMd cFos occurring in rats in response to social defeat (dorsal/dorsomedial PMd) or predator exposure (ventral/ventrolateral PMd). Thus the LHAjd appears well placed to integrate information relevant to defensive responses to different types of threat.

In the present study an additional and intriguing finding was an increase in active- and decrease in passive defensive responses in LHAjd lesioned intruders during resident-intruder encounters. Although these behavioral changes did not quite reach significance (**Table 2**), they nevertheless echo a similar yet significant result in the same paradigm after bilateral cytotoxic PMd lesions (Motta et al., 2009). Together these findings not only reinforce a view of the PMd as part of a common pathway for the expression of defensive behavioral responses, but also suggest the LHAjd may be an important upstream component of that pathway. The latter suggestion is also supported by a significant increase in LHAjd cFos occurring in response to entrapped immobilization (Motta and Canteras, 2015). As to why the effects of PMd lesions produce a more pronounced effect than LHAjd lesions, this might be explained by considering a possible contribution of the LHAjp, which is rostrally adjacent to the LHAjd, and has a similar pattern of connections (both input and output), including a dense innervation of the dorsal/dorsomedial PMd (Hahn and Swanson, 2010). Furthermore, the LHAjp also shows a significant increase in cFos expression following social defeat and entrapped immobilization (Motta and Canteras, 2015; **Figure 3**).

Continuing a consideration of a potentially broad role for the LHAjd in control of defensive behavioral responses, in addition to LHAjd connections with the PMd, and to the previously mentioned PAGd, the LHAjd also has robust downstream connections with several other PAG divisions, notably including the PAG lateral (PAGl) and precommissural (PRC) divisions (Hahn and Swanson, 2012). As noted at the start of this discussion, muscimol blockade of $GABA_A$ receptors in the PAGd (and also PMd) in socially defeated rats immediately prior to re-exposing them to the defeat-associated context, results in a significant attenuation of risk assessment behavior (Faturi et al., 2014). Additionally, a robust increase in PAGd cFos expression

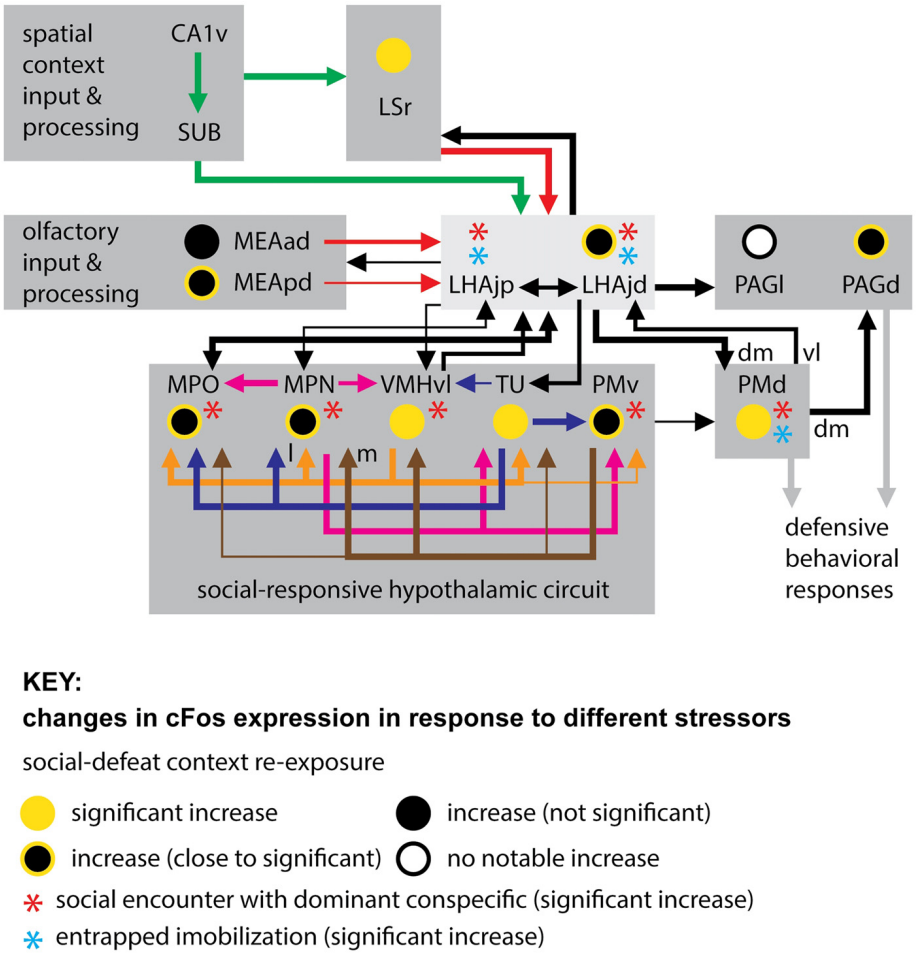

FIGURE 3 | Connections of the LHAjd and LHAjp with regions indicated to play a key role in the expression of defensive behavioral responses, in particular to socially-relevant threats (stressors). Also indicated are comparative changes in the levels of cFos expression associated with three different stressors: entrapped immobilization (restraint; Motta and Canteras, 2015), encounter with a dominant conspecific (Motta et al., 2009; Motta and Canteras, 2015), and re-exposure to social defeat-associated context (Faturi et al., 2014; see figure Key for explanation of symbols). The cFos data is shown only for the LHAjd and LHAjp and their connected regions that were included in previous analysis. The connections shown are based on data obtained from previous pathway tracing studies (not all connections are shown): (Simerly and Swanson, 1988; Canteras and Swanson, 1992a; Canteras et al., 1992a, 1994; Risold and Swanson, 1997b; Comoli et al., 2000; Goto et al., 2005; Cenquizca and Swanson, 2007; Motta et al., 2009; Hahn and Swanson, 2010, 2012). Abbreviations: CA1v, Hippocampal region, Field CA1, ventral part; LHAjp, Lateral hypothalamic area, Juxtaparaventricular region; LHAjd, Lateral hypothalamic area, Juxtadorsomedial region; MEAad, Medial amygdalar nucleus, anterodorsal part; MEApd, Medial amygdalar nucleus, posterodorsal part; MPN, Medial preoptic nucleus; MPO, Medial preoptic area; PAGd, Periaqueductal gray, Dorsal division; PAGl, Periaqueductal gray, Lateral division; PMd, Dorsal premammillary nucleus; PMv, Ventral premammillary nucleus; SUB, Subiculum; TU, Tuberal nucleus; VMHvl, Ventromedial hypothalamic nucleus, Ventrolateral part; dm, dorsomedial; l, lateral; m, medial; vl, ventrolateral.

is seen in these animals after context re-exposure compared to non-defeated controls (Faturi et al., 2014); a similarly robust cFos increase is seen in the PAGl immediately after encounter with a dominant conspecific (Motta et al., 2009). Furthermore, a significant increase in PRC cFos is reported following predator exposure (Canteras and Goto, 1999). However, a specific role for the LHAjd in relation to predator threat remains to be investigated.

Turning from LHAjd downstream connections to those upstream, three sites are prominent: within the striatum the rostral part of the lateral septal nucleus (LSr), and medial nucleus of the amygdala (MEA), and within the cerebral cortex the subiculum (SUB; dorsal and ventral, but mostly its intermediate part—SUBi; see Figure 11 in Hahn and Swanson, 2010, 2012). The MEA is a major recipient of olfactory sensory information, especially behavior-relevant pheromone signals (Swanson and Petrovich, 1998), and in addition to having bidirectional connections with the LHAjd (Hahn and Swanson, 2012), it also provides substantial input to both the conspecific/social-responsive and predator-responsive hypothalamic circuits (Canteras et al., 1995). The major MEA connection with the LHAjd is with the MEA anterodorsal

part (MEAad), with a lesser MEA posterodorsal part (MEApd) connection, and weak to very weak connections with the other parts of the MEA (Hahn and Swanson, 2012). All parts of the MEA are interconnected (Canteras et al., 1995), and all show increased cFos expression in socially-defeated rats re-exposed to the defeat context (Faturi et al., 2014); however, in socially-defeated rats the most notable MEA cFos increase is reported to occur in the MEApd and MEAad, in response to the social defeat (Motta et al., 2009), and also in response to re-exposure to the defeat context (Faturi et al., 2014). Furthermore, socially-defeated animals exposed to a similar spatial context but with fresh bedding (i.e., lacking the olfactory cues associated with the aggressor) do not show conditioned defensive responses (Faturi et al., 2014). The present results are consistent with these findings, and with a role for MEA-LHAjd connections in the processing of behaviorally relevant olfactory information.

More broadly, it is worth noting that significantly increased MEAad and MEApd cFos associated with agonistic encounters was previously reported in Syrian hamsters in both dominant and subordinate males, and also following copulation (Kollack-Walker and Newman, 1995). However, while increases of MEA cFos did not differ between dominant and subordinates, a significant difference was reported between the copulatory group (less cFos) and the agonistic encounter group (more cFos), specifically in the caudal MEApd (Kollack-Walker and Newman, 1995). With respect to this difference in neural activation of the MEApd, it is noteworthy that the moderate MEApd connection with the LHAjd and LHAjp is mostly with its rostral half (with little to no connection to the most caudal level of the MEApd; Hahn and Swanson, 2010, 2012). In fact, this correlation is consistent with a model in which differences in the connections of the hypothalamus with the parts of the amygdala that receive input from the accessory olfactory bulb (relaying pheromone signals from the vomeronasal organ) are thought to reflect differences in the genetically-encoded mechanisms that enable different behavioral responses (such as reproductive or defensive) to different olfactory cues (Choi et al., 2005).

In addition to the MEA, the other part of striatum with robust LHAjd connections is the LSr. The LSr in turn receives a major input from the Ammon's horn and SUB (Risold and Swanson, 1997b), and the latter, in particular the SUBi, provides a major input to the LHAjd (Hahn and Swanson, 2012). The LHAjd also receives an input from the ventral part of CA1; however, this is relatively weak compared to a more substantial ventral CA1 (and ventral SUB) input to the LHAjp (Kishi et al., 2000; Hahn and Swanson, 2010, 2012). The organizational pattern of these connections extends to a general topographic organization between Ammon's horn and the subiculum, the lateral septal nucleus (LS), and the hypothalamus (Risold and Swanson, 1996, 1997b; Cenquizca and Swanson, 2006, 2007).

Given the close connectional associations between the LSr and LHAjd, and the results of the present study, it is salient to note that socially-defeated rats re-exposed to the defeat context show a significant increase in LSr cFos expression (Faturi et al., 2014). This aligns with earlier data indicating that electrolytic LS lesion reduces behavioral measures of anxiety (Menard and Treit, 1996). Along similar lines, cytotoxic (ibotenic) lesions of the HPF (including ventral CA1 and SUBi) reportedly reduce unconditioned risk assessment behaviors to predator odor cues (an effect not seen with visual cues, or with lesions restricted to the dorsal part of Ammon's horn and the subiculum; Pentkowski et al., 2006). A consideration of the underlying circuit properties speaks to these effects: the predominant output of the LSr, like other parts of the LS complex (and the striatum in general), is inhibitory (GABAergic; Risold and Swanson, 1997a); whereas hippocampal outputs (like other outputs of the cerebral cortex) are excitatory (glutamatergic; Swanson, 2000). With respect to behavioral responses relevant to defensive behaviors, the connectional relations and differing neurochemistry of the HPF and LS is highlighted by experiments reporting that activation of $GABA_A$ receptors in the HPF produces an anxiolytic effect that is blocked by glutamate activation of the LS (Menard and Treit, 2001). However, inclusion of the LHAjd in a model of these behaviorally-relevant circuits should take account not only of the HPF input to the LHAjd, but also of the highly bi-directional (and indicated reciprocal) connections between the LS and LHAjd (Hahn and Swanson, 2012).

A consideration of hippocampal neural connections in defensive behavioral responses was revisited in a recent article that compared and contrasted the pattern of cFos expression in male rats associated with the stressful threats posed by either entrapped immobilization, or an encounter with an aggressive conspecific (Motta and Canteras, 2015). A novel possibility was raised that one role of the subiculum (and LS) to LHAjd (and LHAjp) pathway may be to transmit behaviorally relevant threat-associated spatial boundary information, following recent work further characterizing the role of subicular neurons that respond to environmental boundaries (Stewart et al., 2014). The potential importance of a direct hippocampal to LHAjd (and LHAjp) connection that could integrate spatial information with specific sensory cues to modulate behavioral control was recognized in the first pathway tracing study of the LHAjp: "...one could predict that lesion of the LHAjp may have an effect on spatial (contextual) learning and navigation, and especially in relation to defensive behavior that has an olfactory component." A similar prediction was subsequently made for the LHAjd: "locational information relayed by hippocampal neurons to the LHAjd could have obvious relevance if the LHAjd is an integral part of a system for the control of defensive behavior." The present results are consistent with the earlier predictions. More generally, these perspectives inform a growing understanding of regional and topographic hippocampal division of labor with respect to processing and integration of different types of sensory information and the major mnemonic and spatial computation roles of the HPF (Dong et al., 2009; Strange et al., 2014).

As a final point of discussion, and by way of considering possible broader implications of the present results, one might ask how convergent hippocampal-septal and amygdala input to the LHAjd may contribute to the control of defensive behavioral responses? As noted previously, in socially-defeated rats re-exposed to the defeat context, visuospatial context cues alone

do not appear sufficient to elicit conditioned defensive responses, which also require the presence of (at least) olfactory cues previously associated with the dominant aggressor (Faturi et al., 2014). Therefore, the LHAjd is in a position to integrate spatially-relevant information (presumably relayed by the subiculum), as well as olfactory-relevant information (presumably relayed by the MEA). In a general sense, the integration of dual sensory input streams conveying threat-relevant information by a hypothalamic region (LHAjd) implicated in behavioral control, may have relevance to (for example) neuropsychiatric diseases in which there is a dysfunction of context-appropriate behavioral responses.

One possible example is post-traumatic stress disorder (PTSD) that is typified by context and cue disassociated ("inappropriate") defensive (or aggressive) behavior following a traumatic (highly stressful and threatening) event or episode (American Psychiatric Association, 2013). The hippocampus and amygdala are both implicated in PTSD (Shiromani et al., 2009), and an underlying thread common to both appears to be their (indirect) role in, and (direct) responsiveness to, neuroendocrine signaling associated with the stress response—in particular, indirect stress-associated modulation of the hypothalamic-pituitary-adrenal (HPA) axis (Herman et al., 2003), and direct responsiveness to glucocorticoid hormones (Sapolsky et al., 1984; Reul and de Kloet, 1985; Han et al., 2014). It is noteworthy that CA1 and SUB, which provide the single most abundant source of input to the LHAjd (and LHAjp; Hahn and Swanson, 2010, 2012), are the same hippocampal regions that have the highest expression of corticosterone receptors (also highly expressed in the LS nucleus; Reul and de Kloet, 1985); moreover, the expression levels of glucocorticoid receptors in ventral CA1 and SUB is more than double that in the dorsal parts of these regions (Reul and de Kloet, 1985). With regard to the amygdala in relation to PTSD, much attention has been paid to the role of the lateral amygdala in models of fear-conditioning (Debiec and LeDoux, 2009), while the medial amygdala has received less attention. Nevertheless, repeated restraint stress in mice appears to cause a reduction in dendritic spines on MEA neurons (Bennur et al., 2007). More generally, restraint stress and social-defeat in rats both generate a robust increase in cFos expression in the medial parvicellular part of the hypothalamic paraventricular nucleus (indicative of HPA axis activation; Faturi et al., 2014; Motta and Canteras, 2015). Also of broader relevance to the organization of the underlying neural circuits, a substantial input to the amygdala (including a light to moderate input to the MEAad) from the ventral subiculum is noted (Canteras and Swanson, 1992b).

CONCLUSION

The present results provide the first direct evidence of a functional role for the LHAjd in the control of socially-relevant defensive behavioral responses, and conditioned context-dependent responses in particular. Additionally, the results are consistent with a previous report indicating increased activation of the LHAjd in the same behavioral model (Faturi et al., 2014). The results are also supported by a more recent study that further suggests the LHAjd and LHAjp may play a broader role in control of behavioral responses to different types of threat (Motta and Canteras, 2015). A key neural circuit node in these behavioral responses is suggested to be the subiculum, which provides a major input to the LHAjd. Future studies will be necessary to determine the role of the LHAjd and subiculum in relation to the existing model, and also in relation to models employing different threat stimuli (stressors). More broadly the results suggest that additional investigations into the role of the LHAjd, and other LHA regions whose connections have been described in recent years (Goto et al., 2005; Hahn and Swanson, 2010, 2012, 2015), may have relevance to a wide range of neuropsychiatric diseases that involve disordered behavioral control.

AUTHOR CONTRIBUTIONS

JDH and NSC designed the experiments. JDH and MJR carried out the experiments. MJR did most of the analysis (with additional input from NSC and JDH). MVCB did the statistical analysis. JDH wrote the article (with editorial input from NSC and MJR).

ACKNOWLEDGMENTS

This work was supported by a grant to JDH from the University of Southern California (USC) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Br), and by a grant to NSC from FAPESP (2014/05432-9). Additional support for NSC, and MVCB was provided by the National Council for Scientific and Technological Development (Brazil). MJR was supported by a FAPESP fellowship (2012/13804-8).

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