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*Nat Neurosci.* 2016 February ; 19(2): 198–205. doi:10.1038/nn.4220.**Lateral Hypothalamic Circuits for Feeding and Reward****Garret D. Stuber<sup>1,2,3,\*</sup> and Roy A. Wise<sup>4</sup>**<sup>1</sup>Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA<sup>2</sup>Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA<sup>3</sup>Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA<sup>4</sup>Intramural Research Program National Institute on Drug Abuse, NIH/DHHS, Baltimore, MD 21224, USA**Abstract**

In experiments conducted over 60 years ago, the lateral hypothalamic area (LHA) was identified as a critical neuroanatomical substrate for motivated behavior. Electrical stimulation of the LHA induces voracious feeding even in non-restricted animals. In the absence of food, animals will work tirelessly, often lever-pressing 1000's of times per hour, for electrical stimulation at the same site that provokes feeding, drinking, and other species-typical motivated behaviors. Here we review the classic findings from electrical stimulation studies and integrate them with more recent work that has utilized contemporary circuit-based approaches to study the LHA. We identify specific anatomically and molecularly defined LHA elements that integrate diverse information arising from cortical, extended amygdala, and basal forebrain networks to ultimately generate a highly specified and invigorated behavioral state conveyed via LHA projections to downstream reward and feeding specific circuits.

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The hypothalamus, while accounting for only ~3% of brain tissue, has direct control over essential homeostatic functions and primitive behavioral states. The hypothalamus can readily be divided based on gene expression<sup>-</sup>, function, or classical anatomical boundaries<sup>-</sup>, but a large portion of the hypothalamus consists of an extended field of neurons and fibers with substantially less anatomical definition<sup>-</sup> referred to as the lateral hypothalamic area (LHA). As studies continue to uncover the precise circuitry and cellular phenotypes within the LHA that encode and orchestrate behavior, it is important to revisit many of the well-described findings using classical anatomical and electrical stimulation methods previously used to elucidate LHA function. In this article, we review some of these seminal findings from the 1950's–80's, and integrate them with more recent discoveries utilizing optogenetic neurocircuit approaches. A holistic synthesis of these findings paints an emerging picture of multiple, well-defined neurocircuit elements, embedded within the LHA, that interface with downstream systems to ultimately generate specific motivational and actionable states.

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## Classic experiments on LHA function

The LHA is a richly heterogeneous structure residing posterior to the preoptic area and anterior to the ventral tegmental area. The LHA contains a number of genetically distinct cell populations (for review see) and forms a bed nucleus through which the fibers of the medial forebrain bundle pass. Lesion studies conducted in the 1940's – 1980's described the effects of electrolytic or chemical ablation of the LHA and subsequent effects on feeding and drinking. This work collectively demonstrated the importance of the LHA for homeostatic physiology and behavior. Electrolytic lesions of the LHA suppresses feeding, and drinking while lesioning of the nearby VMH promotes feeding and body weight gain. Later studies that utilized chemical lesions to destroy catecholaminergic fibers containing either norepinephrine or dopamine demonstrated that these fibers of passage contained within the median forebrain bundle are important components controlling feeding and drinking. Anand and Brobeck suggested that fibers of passage, but also fibers of origin from somata distributed throughout the LHA are also important for controlling feeding. Chemical lesions that ablate LHA somata but spare passing fibers also suppressed feeding and drinking. The pioneering early studies that utilized electrical stimulation of the LHA in rodents showed that gross electrical activation of this region produces voracious feeding behavior, as well as reinforced lever-pressing behavior to gain additional stimulation, (Fig. 1). This suggests that the LHA and associated brain regions are not only critical for feeding and other drive-like effects, but also reinforcement processes. Intra-LHA injection of neurotransmitter agonists or antagonists further demonstrated that glutamate receptor activation can also induce feeding, while GABA agonist can suppress it. These studies show that modulation of neurotransmission within the LHA can generate feeding responses similar to those observed following electrical stimulation or lesions. However, it is worth noting that electrical stimulation of the LHA can become aversive if its intensity is too strong or its duration too long. Additionally, electrical stimulation at sites in this and adjacent levels of the medial forebrain bundle motivate a variety of species-typical behaviors—e.g., feeding, drinking, copulation, gnawing and nest building, in addition to reinforcement.

## Lateral hypothalamic electrical stimulation

Electrical stimulation of the LHA and other portions of the medial forebrain bundle can motivate a variety of species-typical, biologically primitive behavior patterns including eating, drinking, and gnawing in sated animals. With respect to feeding and copulation, there seems to be clear anatomical separation between systems. For feeding, gnawing, and predatory attack, the systems appear to overlap. Because the LHA had been implicated in feeding and drinking by lesion studies as described above, a good deal of attention was paid to the motivational effects of lateral hypothalamic stimulation. Such stimulation does not elicit specific motor responses, but rather establishes a state of heightened responsiveness to a variety of environmental stimuli. Stimulation in this region might produce different reactions in animals such as feeding in one animal, drinking in another, gnawing of wood in another, or predatory attack in yet another. These differences are not due to differences in stimulation region within the LHA, but rather are the result of response patterns that develop during the stimulation trials. That is, responsiveness to a given goal object increases with

repeated stimulation trials and the dominant response of a given animal can change as a function of what goal objects are offered. In the case of feeding and drinking, the animal behaves in much the same way it would under food and water deprivation. First, electrical LHA stimulation motivates the learning of food-reinforced instrumental responses, as well as the performance of such responses as were previously learned under conditions of deprivation. The LHA evoked feeding is also influenced by unconditioned and conditioned taste aversions. In cats, LHA stimulation appears to motivate goal-directed behavior involving response sequences such as, in the case of predatory attack, visual stalking, approach, pouncing and bringing the prey to the mouth, mouth opening, and, finally, snapping shut of the mouth. Each act in the sequence has its own environmental triggering stimulus, and the effect of stimulation is to make the animal more responsive to the triggering stimulus. The behavior observed in a given experiment depends to a great extent on what stimuli are available for interaction in the testing chamber. This suggests that the activated substrate is more a general arousal system than a set of specific motivational pathways. Against this view are the findings that stimulation at different points along the medial forebrain bundle are differentially sensitive to modulation by food restriction and leptin (LHA at the A-P level of the ventromedial nucleus) on the one hand and testosterone (posterior hypothalamic MFB) on the other, that stimulation-induced eating and drinking are preferentially responsive to low and high (respectively) stimulation frequencies. Morgane suggested that even the feeding response results from activation of two LHA “hunger-motivational” subsystems, one slightly lateral to the other. Because electrical stimulation activates neurons near the electrode tip rather indiscriminately, and because 50 or more fiber systems share this region, the question of one or multiple systems has not been resolved by electrical stimulations studies.

Whereas the effects of lesions and stimulation led to the labeling of the LHA as a “hunger system” a “feeding center” and a “drinking center”, the discovery that stimulation of this region was rewarding led also to the label of a “pleasure center”. The fact that rats would work for stimulation of a brain region where stimulation appeared to make them hungry, was termed the “drive-reward” paradox and raised the issue of whether a single arousal system or two independent systems mediated the drive-like effects and the rewarding effects of the stimulation.

Pharmacological studies of stimulation-induced feeding and LHA brain stimulation reward suggested an important role for the forebrain-projecting midbrain dopamine systems. However, parametric studies of brain stimulation reward soon falsified the hypothesis that the rewarding effects of stimulation were primarily due to the depolarization, at the electrode tip, of dopaminergic fibers of passage. First, the dopamine system was insensitive to changes of stimulation frequency over the range that altered the rewarding impact of stimulation. Paired pulse studies showed that the refractory periods for the directly stimulated “first stage” fibers (the fibers depolarized at the electrode tip) were too fast to reflect direct activation of the ascending dopamine fiber system. At least two sub-populations were implicated, one of which was undefined and one was an ultra-fast sub-population that was sensitive to cholinergic receptor blockade; each, however, appeared to contribute to both the feeding effect and the rewarding effect of LHA electrical stimulation.

Dual electrode paired-pulse studies followed and showed that despite the fact that the LHA and VTA were connected by reward-relevant fibers, their conduction velocities were, like the refractory periods, too fast to reflect a significant contribution of the small unmyelinated dopaminergic fiber system. Finally, by challenging the effects of cathodal stimulation at one level with anodal stimulation at another, Bielajew and Shizgal showed that the bulk of the reward-relevant fibers of the LHA project caudally, toward, not away from, the ventral tegmental area (VTA). Subsequent studies showed axonal connectivity between the lateral preoptic area and the VTA, suggesting that the reinforcing effects of lateral hypothalamic stimulation were likely due to activation of descending fibers of passage originating in or rostral to the anterior hypothalamus. Taken together, these studies suggested that brain stimulation reward resulted from activation of descending medial forebrain fibers of passage that activated, directly or indirectly, the VTA dopaminergic system that had been implicated not only in brain stimulation reward but also in the rewarding effects of food and psychomotor stimulants.

The paired-pulse parametric techniques that were developed to characterize the substrate of brain stimulation reward were also used in studies to characterize the substrate of stimulation-induced feeding and to explore whether a common substrate might mediate stimulation-induced feeding and reward. As in the case of LHA brain stimulation reward, in the case of stimulation-induced feeding there again appeared to be two non-overlapping sub-populations of contributing first-stage fibers: an ultrafast subpopulation with refractory periods between 0.4 and 0.6 msec, and a non-overlapping slower subpopulation with refractory periods between 0.7 and approximately 2.0 msec. Stimulation-induced feeding was induced by stimulation not only of the LHA but also by stimulation of the VTA and intervening levels of the medial forebrain bundle; and evidence for the same non-overlapping sub-populations of first-stage fibers was seen at each of these levels. Single electrode refractory period findings suggested that both effects were mediated by activation of two sub-populations of ultrafast and fast fibers of the medial forebrain bundle that extended at least from the lateral hypothalamus to the region of origin of the mesolimbic dopamine system.

Dual electrode studies suggested further evidence for a common substrate or substrates. Here, one electrode was aimed at the LHA and another was aimed more posterior at the VTA. The findings concluded that stimulation induced feeding and reward were each found with both placements. The two electrodes were thus inferred to be aligned along the path of the same axons whenever the effects of stimulation at one electrode cancelled the effects of stimulation of the other at short inter-pulse intervals. In those cases where alignment was found for stimulation-induced feeding, it was also found for reward. As with the refractory periods for the fibers mediating the two behaviors, the conduction velocities were very similar. These findings do not rule out the possibility that different subsets of fibers are involved in the two responses to stimulation, but the common sites, trajectories, refractory period distributions and conduction velocities continue to point to the possibility of a common neural substrate.

## Molecular phenotypes and functions of LHA neurons

The LHA encompasses a plethora of genetically and functionally distinct cell types that utilize various signaling modalities, including various neurotransmitters and neuropeptides<sup>−</sup>. Vesicular glutamate transporter type 2 (*Vglut2*; a marker for glutamate neurons) mRNA expression is abundant in the LHA<sup>+</sup> (Fig. 2), suggesting that numerous LHA neuronal subpopulations synthesize the excitatory neurotransmitter, glutamate. In addition to glutamate, the LHA is enriched with GABAergic neuronal markers<sup>+</sup> (Fig. 2), which are largely segregated from *Vglut2*-expressing LHA cells. Some LHA neurons also produce several important neuropeptides, including orexin/hypocretin (Orx), melanin-concentrating hormone (MCH), neurotensin (Nts), and galanin (GAL). While these neuropeptide expressing cell populations likely play an important role in regulating feeding and reward (see below), it is worth noting that some of these cells groups not only regulate feeding, but also metabolism, likely through different circuits.

Neurons that synthesize and release the neuropeptide orexin/hypocretin (~3,500 – 5,000 total in rodents) are restricted to the LHA and also have been reported to express *Vglut2*. Orx neurons are thought to primarily regulate arousal, but also feeding and reward-related behaviors. Consistent with this, injections of the peptide into the lateral ventricle increases food intake, while Orx receptor antagonists and genetic removal of Orx decrease consumption. Furthermore, chemical activation of Orx cells as well as infusions of the peptide into the VTA, an anatomical target of LHA Orx neurons, reinstates drug- and food-seeking behaviors. However, these neurons are also heavily involved with arousal, as optogenetic stimulations of Orx neuron increases wakefulness, while genetic ablation of the cells causes narcolepsy. Therefore, they are likely a contributor, but not primary determinant of motivated behavioral output mediated by the LHA. For further review of Orx neuronal function see.

Melanin-concentrating hormone (MCH) producing neurons are also predominantly found in the LHA, project widely throughout the brain, and are distinct from Orx neurons<sup>−</sup>. Some MCH neurons express markers for GABA (glutamic acid decarboxylase; GAD67) while others express markers for glutamate (*Vglut2*)<sup>−</sup>, suggesting that MCH neurons are composed of subsets of inhibitory and excitatory cells. MCH neurons have also been implicated in the regulation of feeding and sleep-wakefulness balance. Intracerebroventricular injections of the peptide increases feeding and body weight in rodents. Further, recent genetic studies revealed that overexpression of MCH results in hyperphagia and obesity, while mice lacking MCH neurons or MCH are hypophagic and lean<sup>−</sup>. In contrast to Orx neurons, activation of MCH neurons promotes REM sleep, consistent with an opposing role of these cells to Orx neurons in controlling arousal states. For additional reviews of the neurocircuitry of Orx and MCH LHA neurons see<sup>−</sup>. Collectively, these studies demonstrate a role for LHA ORX and MCH neurons in regulating arousal and sleep in addition to feeding and body weight. Thus, and interesting possibility is that the feeding phenotypes associated with these cell types are more related to an animals natural behavioral patterns that would normally occur in particular states of arousal.

A separate neuropeptide-containing cell population concentrated in the preoptic and anterior hypothalamic region but overlapping with the LHA, Neurotensin (Nts) producing neurons, have been hypothesized to be involved with negative energy balance. Peripheral and central administration of Nts suppresses feeding, and both the genetic ablation of a subset of Nts neurons, as well as the removal of the Nts receptor (NTR1), result in hyperphagia and obesity. Nts neurons highly co-localize with galanin expressing neurons (~95% overlap), but not with MCH and Orx cells. Interestingly, LHA neurons that express vesicular GABA transporters (Vgat-ires-Cre) show little to no co-localization with neurons that are immunopositive for either MCH or Orx (Fig. 3). This suggests at least some of the LHA neurons that have been previously targeted for manipulation in the Vgat-ires-Cre line may also be Nts expressing neurons, although this will need to be fully investigated in future studies.

### Optogenetic studies to delineate LHA function

The introduction of optogenetic stimulation methods has provided a powerful new tool for identifying the substrates of motivation and reward. Electrical stimulation preferentially activates fibers of passage and does not differentiate between fibers from arising at the stimulation site and fibers of passage with distal origins. Electrical stimulation allows only crude differentiation of different fiber types and provides little information as to the type of fibers activated. Optogenetic techniques make it possible to activate only fibers of origin from a confirmed cell group of interest and to trace and selectively activate only the fibers that arise from that cell group and project to or through a given target area. Whereas electrical stimulation activates a set of fibers by causing the opening of cation channels that are voltage-sensitive and expressed in the membranes of all neuronal elements, optogenetic stimulation activates fibers by causing the opening of cation channels that are light sensitive and that are expressed only by neurons originating in a particular brain regions and expressing particular gene used to at least partially delineate a cellular phenotype. Thus, optogenetic studies have already begun to unravel which of the numerous molecularly defined neuronal fibers that originate in or pass through the LHA contribute to motivational and reward function. Cell-type specific optogenetic targeting approaches have begun to ascribe functional roles for distinct LHA populations for orchestrating feeding and reward.

Direct optogenetic activation of VGat expressing LHA neurons produces voracious feeding and optical self-stimulation behavior, a phenotype that is strikingly reminiscent of that seen with electrical stimulation of the LHA. Interestingly, optogenetic stimulation of VGlut2 expressing LHA neurons has the opposite effect; it reduces feeding in hungry mice, as well as producing an aversion to locations where stimulation of these cells occurs. Consistent with the idea that VGat and VGlut2 expressing LHA neurons exert opposing behavioral effects, selective genetic ablation of VGat expressing LHA neurons reduces feeding, body weight gain, and motivation to obtain palatable caloric rewards, while ablation of VGlut2 expressing LHA neurons enhances both feeding and body weight gain. Thus, perhaps VGat and Vglut2 expressing LHA neurons produce a bidirectional output signal, which is then directly and indirectly conveyed to VTA dopamine neurons to homeostatically invigorate behavioral output (Fig. 4). Second, complex, but reoccurring environmental representations are likely encoded in upstream cellular networks in the cortex and hippocampus, which in

turn convey representational information to LHA neuronal circuits. However, in order to mechanistically understand how LHA signals are processed; first consider the neural circuit input architecture.

## Input circuitry to the LHA

The LHA receives multiple excitatory and inhibitory inputs from both cortical and subcortical structures. Direct, electrical stimulation of the medial prefrontal cortex produces many distinct mono and polysynaptic activity patterns in LHA neurons. Monosynaptic excitatory fibers arriving via the fornix, also likely provide important hippocampal information related to the ongoing processing of space and context. Inhibitory GABAergic subcortical fibers innervate the LHA from the lateral septum and much of the basal forebrain and extended amygdala including the nucleus accumbens shell, the BNST/preoptic area, the ventral pallidum and nucleus basalis/substantia innominata. Midbrain and brainstem inputs to the LHA are more sparse but arise from classical processing centers of autonomic function including the parabrachial nucleus and periaqueductal grey. Neuromodulators including dopamine, norepinephrine, and serotonin, are also released within the LHA where they can act to further sculpt circuit dynamics. Furthermore, intra-hypothalamic connectivity providing input to the LHA from regions such as the arcuate nucleus, periventricular hypothalamus, and ventral medial hypothalamus have also been described. Importantly, optogenetic stimulation of Arcuate<sup>AGRP</sup>-LHA or PVH<sup>GABA</sup>-LHA pathways are capable of evoking feeding behavior. Collectively, these findings suggest that arcuate nucleus circuitry directly controls homeostatic feeding in response to energetic demands while LHA circuits drive compulsive and/or hedonic feeding, due to the tight linkage to the VTA reward circuitry.

The functional input architecture from regions of the extended amygdala that interface with definable LHA neurons is beginning to emerge. GABAergic neurons from the ventral BNST and related structures send monosynaptic inputs that preferentially inhibit postsynaptic LHA glutamate neurons (Fig. 4). Direct optogenetic stimulation of the vBNST<sup>GABA</sup>-LHA<sup>Glutamate</sup> circuit produces robust feeding behavior that is initiated rapidly, correlated with stimulation frequency, and directed towards the most palatable, calorically dense foods available. Furthermore, mice readily engage in optical self-stimulation of this circuit, and self-stimulation output is strongly modulated by food deprivation or satiety states, consistent with a dual role of the LHA to orchestrate both motivation and feeding behaviors. Inhibitory input to the LHA from the nucleus accumbens shell arises from both D1 and D2 expressing medium spiny projection neurons (O'Connor et al., in press) (Fig. 4). Functionally, the importance of this pathway was first described by Ann Kelley and colleagues who reported that AMPA receptor antagonism or GABA-mediated inhibition in the NAc shell elicits feeding that is dependent on the LHA<sup>-</sup>. Circuit input from the NAc shell to the LHA was recently investigated in more detail (O'Connor et al., in press). The majority of NAc shell MSNs that project to the LHA are D1 expressing MSNs, with only a minority arising from the D2R expressing MSN population. Fibers from D1R expressing MSNs innervate the more ventral lateral aspects of the LHA where they functionally target LHA GABAergic neurons, and not Orexin or MCH producing neurons. Optogenetic stimulation of the NAcshell<sup>D1R</sup>-LHA<sup>GABA</sup> pathway suppresses licking for a palatable reward, while optogenetic inhibition

of postsynaptic LHA<sup>GABA</sup> neurons suppresses consumption of food. Collectively, it appears likely that distinct subcircuits arising from various extended amygdala and neighboring structures provide inhibitory input that preferentially targets molecularly distinct LHA postsynaptic neurons to regulate feeding and reward (Fig. 4).

## Output circuitry of the LHA

Given that multiple populations of functionally and genetically classifiable neurons exist in the LHA, it is also of importance to consider the projection target structures of these cells. Some of the most well described outputs from classical anatomy studies demonstrated the existence of multiple projection specific outputs to brain regions such as the VTA, periventricular thalamus, lateral habenula, and many others. A recent study by Nieh et al., demonstrated that both glutamatergic and GABAergic LHA fibers functionally innervate both VTA GABA neurons and VTA dopamine neurons. It seems likely that these inhibitory fibers may preferentially innervate VTA GABA neurons, as does the pathway from the BNST to the VTA, as optogenetic stimulation of LH<sup>GABA</sup>-VTA pathway also produces feeding behavior, and as mice will readily engage in optical self-stimulation of this pathway. This could occur by transiently increasing VTA dopaminergic neuronal activity via a disinhibitory mechanism to thus control motivation (Fig. 4). Consistent with this, brief optogenetic stimulation of VTA GABAergic neurons suppresses cue-evoked licking for a caloric reward and is aversive.

In addition to the glutamatergic projection to the VTA, Vglut2-expressing neurons in the LHA also project to the lateral habenula to excite LHb neurons that likely project to VTA/RMTg GABAergic neurons, which in turn can inhibit VTA dopamine neurons (Fig. 4). Consistent with this, optogenetic inhibition of the LHA<sup>Vglut2</sup>-LHb pathway enhances licking for a caloric reward and produces aversion when optogenetically activated. Intriguingly, optogenetic stimulation of glutamatergic projections from the neighboring endopeduncular nucleus (EP) is also aversive, suggesting that glutamatergic neuronal populations in the LHA, the zona inserta, and EP may share a common behavioral/circuit function. In our studies, we have also observed substantially weaker LHb innervation from LHA GABAergic neurons. However, LHA<sup>VGat</sup> neurons appear to innervate midline thalamic structures just ventral to the LHb such as the periventricular thalamus (PVT), a brain region shown to produce GABA-mediated feeding. Both LHA GABAergic and glutamatergic neurons project heavily to the parabrachial nucleus (PBN). While it is still functionally untested, it seems feasible that LHA excitatory and inhibitory signals can also tune PBN circuits shown to play an important role in regulating feeding and taste aversion. In addition, LHA projections to the arcuate nucleus have also been documented, which could likely play an important role in generating feeding. Clearly, additional studies are required to more fully elucidate the functional wiring output of the LHA (Fig. 4). It is also worth noting that a dearth of Cre-driver mouse lines exist to selectively parcel out LHA cellular function. Furthermore, as this circuit architecture is deduced, we can begin to consider the dynamic nature of these systems during ongoing innate and learned behavior.



## Neuronal encoding dynamics of LHA neurons

Much of the previously described work has focused on contemporary circuit mapping and optogenetic approaches to identify the functions of LHA components. However, it is important to note that approaches that have recently revolutionized systems neuroscience cannot yet detail circuit dynamics, and thus are largely a way to imply circuit function based on imprecise and artificial activity patterns. For example, while LHA neurons are capable of firing up to or even beyond 20 Hz, the collection of LHA cellular subtypes likely do not fire in highly synchronous patterns generated by bulk optogenetic methods. Even with inhibitory optogenetic and chemogenetic approaches, neuronal activity is suppressed over extended time epochs, which is also likely inconsistent with neurotypical signaling dynamics, and may also increase activity if sufficient light is delivered to heat the tissue. Thus, an ongoing area of intense research is to examine the endogenous neural encoding properties of distinct LHA neuronal networks and populations. These rich datasets, collected from 100s of LHA neurons, can in turn be used to accurately test which aspects of LHA spatiotemporal signaling are critical for motivated behavioral states.

Early *in vivo* electrophysiological studies in rodents, rabbits, and primates demonstrated that individual LHA neurons are responsive to rewarding, aversive, and associated conditioned stimuli<sup>7</sup>. Ono et al identified populations of LHA neurons that were responsive to rewarding or aversive stimuli presentation. LHA neurons that are responsive to primary rewards, tended to not respond to aversive stimuli, but if they did, they tended to respond in the opposite direction (i.e. excited by rewarding, inhibited by aversive stimuli). Additionally, LHA neurons that displayed activity changes in response to a caloric reward displayed similar response patterns to electrical stimulation that could evoked ICSS, suggesting that distinct types of rewards can engage the same LHA neurons. Moreover, a subset of LHA neurons also respond to conditioned stimuli presentation, but these cells are largely distinct from the LHA neurons that responded to primary rewards<sup>8</sup>. While these early studies demonstrated the existence of distinct LHA neuronal populations that respond to aspects of feeding and reward, it is difficult to designate the functional properties of these neuronal subtypes due to the inability at the time to record from identified LHA neurons.

Two recent studies have begun to further unravel the signaling properties of defined subsets of reward-relevant LHA neurons based on their projection targets or genetic specificity<sup>9</sup>. Nieh, Mathews, et al. studied LHA neurons based on their connectivity with the VTA. Specially, they introduced channelrhodopsin-2 into LHA neurons that projected to the VTA using retrograde Cre-encoding virus strategy (this strategy did not differentiate whether these targeted cells were glutamatergic and GABAergic). Neurons could then be identified as directly projecting or as polysynaptically connected with the VTA based on the electrophysiological response to intra-LHA blue light pulses. Some LHA neurons that were classified as directly-projecting to the VTA tended to respond with excitations while others responded with inhibitions when mice entered a reward retrieval port. In contrast, LHA neurons that were classified as polysynaptically connected to the VTA responded to nose pokes and cues that predicted rewards as well as to reward port entry. Using microendoscopic calcium imaging Jennings, Ung et al. selectively imaged neuronal activity from 100's of LHA Vgat-expressing (putative GABAergic) neurons in behaving mice.

Individual LHA GABAergic neurons displayed changes in their activity time-locked to either nose-pokes required to produce the delivery of a caloric reward or to the first lick following reward delivery, but very few LHA GABAergic neurons responded to both nose-pokes and lick events. These data suggest that LHA neurons that respond to primary rewards, aversive stimuli, or conditioned stimuli may be dissociable based on their circuit connectivity or molecular phenotype.

## Future outlook

While a detailed description of LHA circuitry and cellular encoding properties is far from complete, emerging evidence generated with contemporary techniques coupled with historic findings are beginning to reveal reoccurring themes and principles of these circuits. For example, both classical electrical stimulation and recordings studies as well as cell type specific optogenetic studies have suggested that discrete LHA circuits not only play an important role in reward and feeding, but can also produce aversive states<sup>17</sup>. While there is limited (but some) information detailing how a few defined LHA neural populations encode rewards and predictive cues, there is currently no published data on whether subsets (or all) of these same cell types also respond to aversive stimuli, or whether these are encoded by different cell populations. Recent studies have begun to define LHA neuronal subtypes based on the small molecular neurotransmitters they are capable of utilizing or by the production of a few select neuropeptides<sup>18</sup>, that are involved in motivation and reward. While this is an ideal first pass approach to study LHA circuits, a good deal more must be done to accurately define circuit architecture and function. An even more fundamental problem is that it remains to be determined precisely how many neuronal phenotypes are present within the LHA. High throughput single-cell transcriptional profiling strategies appear to be rapidly emerging, and have already begun to elucidate the number of cell subclasses in other parts of the central nervous system. If these methodologies are applied to 100,000 or more LHA neurons, it may be possible to accurately identify the number of neuronal phenotypes based on quantitative genetic data. Thus, coupled with state-of-the-art circuit targeting strategies, delineation of LHA circuits will continue to evolve.

While a clearer picture of LHA neuronal classes and connectivity will continue to emerge, it is not certain whether ‘rewarding’ and ‘feeding’ phenotypes that are readily engaged by neuromodulation of the LHA are actually dissociable from each other, and thus drive the reward paradox, with respect to the LHA, still remains unresolved. Current evidence suggests that bulk optogenetic modulation can activate or inhibit up to 1 mm of brain tissue, or up to 10,000 LHA neurons and their associated circuit components, while the spatiotemporal activity dynamics of LHA neurons are highly complex even at the cellular level. Thus, accurately recording and modulating LHA network dynamics to living neurocircuits will require single-cell optogenetic modulation capabilities. Another perhaps more immediately testable scenario is that LHA circuit dynamics are largely based upon their input and output circuitry. Stimulus evoked activity patterns can be conveyed from one individual cortical neuron to another, and thus aggregate afferent input from the extended amygdala, basal forebrain, cortex, and other hypothalamic nuclei may ultimately provide more specified ensemble information to LHA circuits which in turn generates appropriate behavioral drive.

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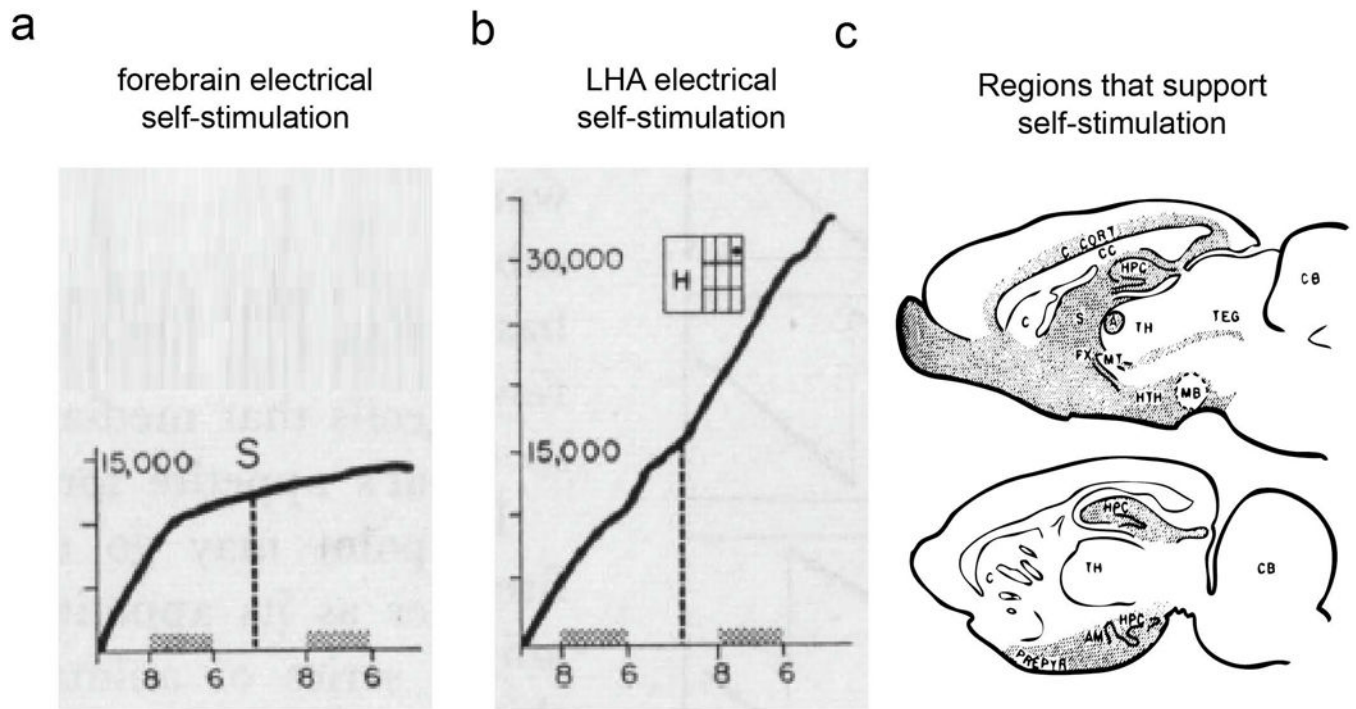
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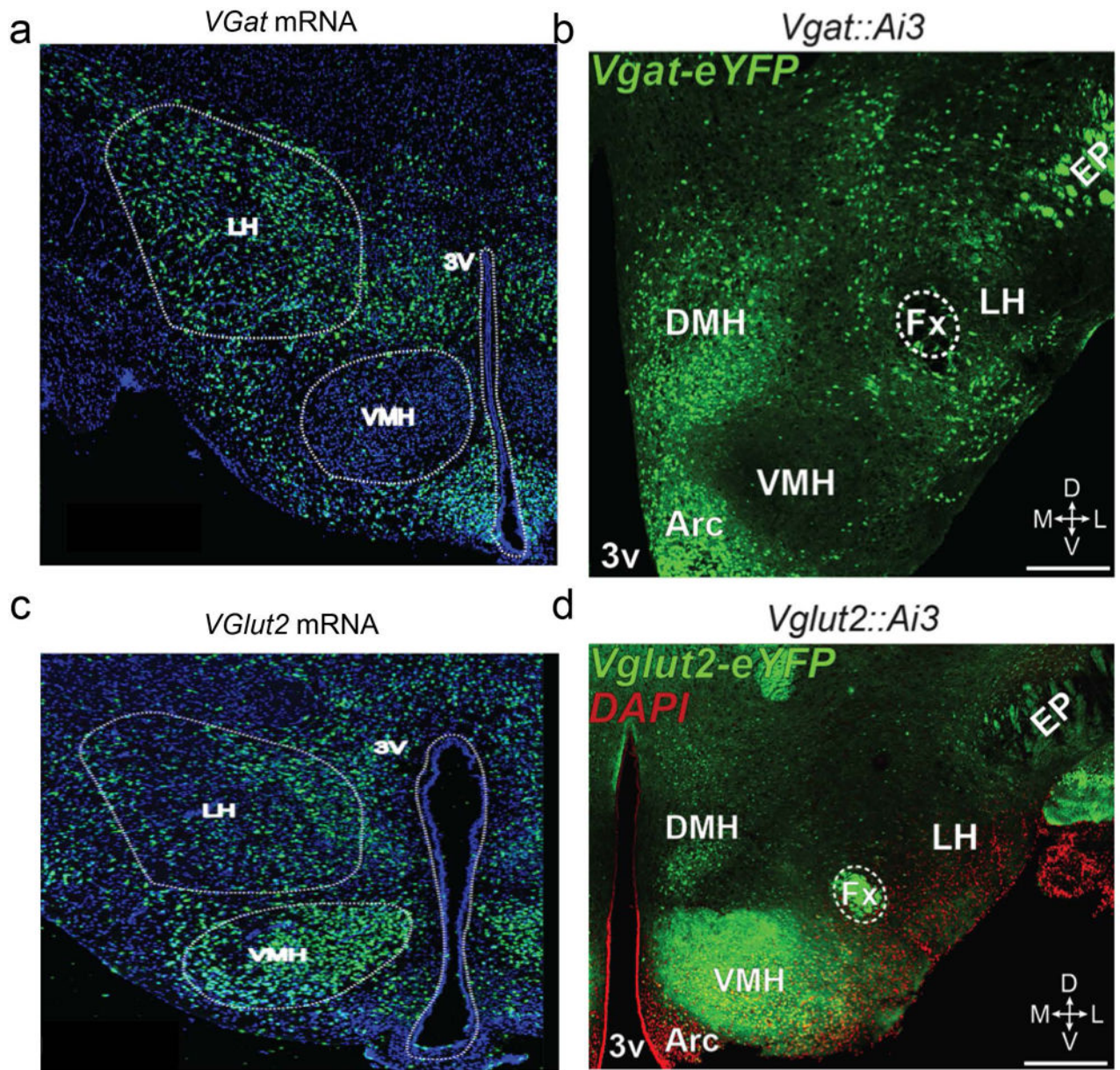


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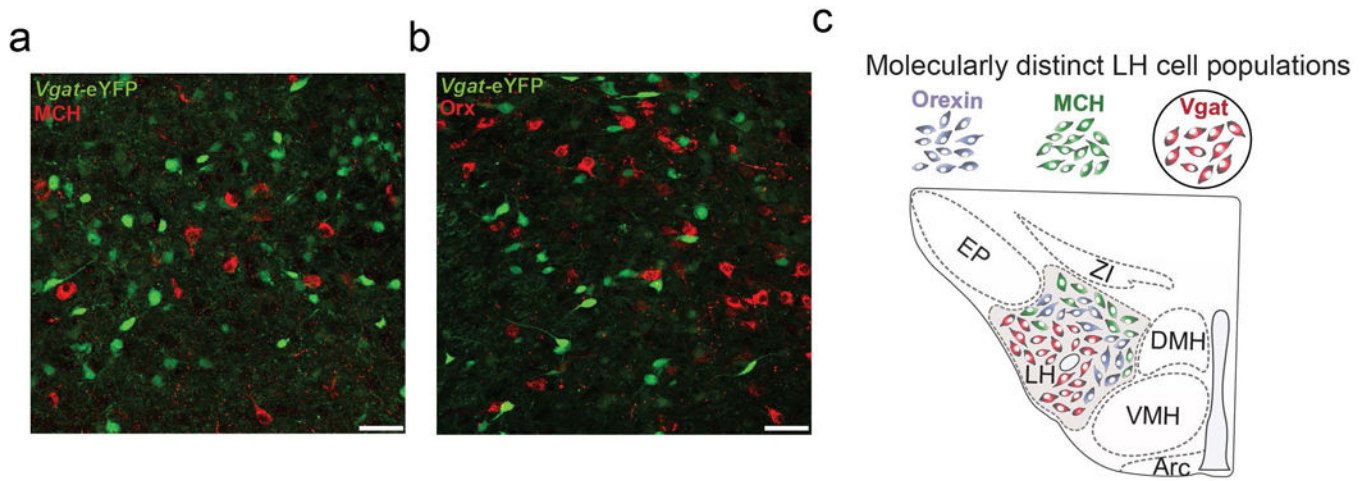


**Figure 1. Electrical stimulation of the LHA produces reinforcement**

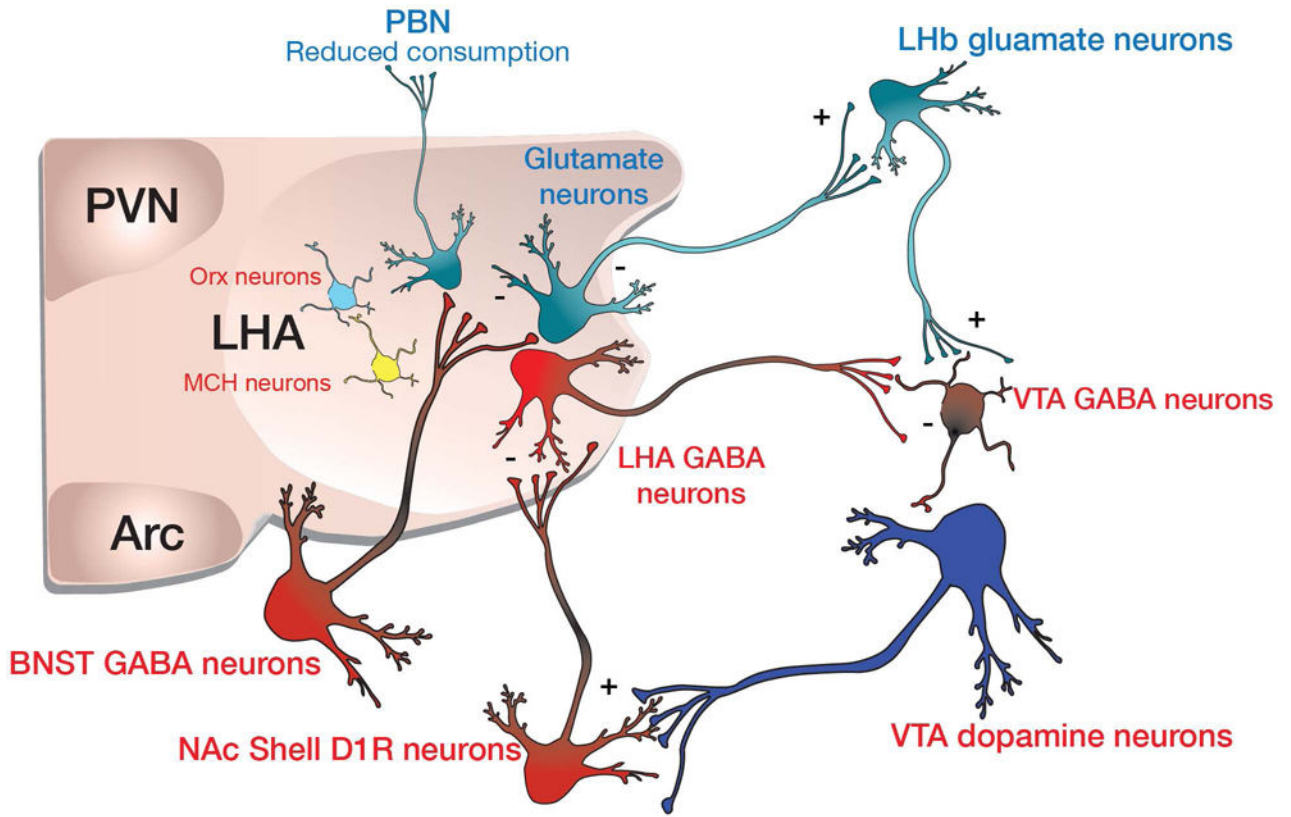
**a.** Animals will self-stimulate in many regions of the ventral forebrain, but only the LHA electrical self-stimulation is largely insatiable (**b**). **c.** Illustration showing that the forebrain and hypothalamus sites (shaded) that supports electrical self-stimulation. Adapted from.



**Figure 2. The LHA contains a mixture of inhibitory and excitatory neurons**  
**a.** In situ hybridization image of LHA *Vgat* expression. **b.** *Vgat* targeted neurons in the *Vgat-ires-Cre* mouse line. **c.** In situ hybridization image of LHA *Vglut2* expression. **d.** *Vglut2*-targeted neurons in the *Vglut2-ires-Cre* mouse line.



**Figure 3. Vgat-targeted neurons are distinct from MCH and Orexin producing LHA neurons**  
**a.** YFP expressing Vgat neurons (green) and MCH immunopositive neurons (red) in the LHA. **b.** YFP expressing Vgat neurons (green) and Orexin immunopositive neurons (red) in the LHA. **c.** Vgat target LHA neurons thus represent a distinct population of LHA cells that mediate feeding. Data adapted from.



**Figure 4. Proposed neurocircuit-wiring diagram based on optogenetic studies**

LHA GABAergic neurons inhibit VTA GABAergic neurons to disinhibit VTA dopamine neurons. Dopamine is released within the NAc where it excites D1R expressing MSNs and induces plasticity. These inhibitory signals then feedback to inhibit LHA GABAergic neurons to terminate feeding bouts. BNST GABAergic neurons preferentially inhibit LHA Glutamate neurons, some of which may project to the lateral habenula.