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Critical Review

Neural Integration of Reward, Arousal, and Feeding: Recruitment of VTA, Lateral Hypothalamus, and Ventral Striatal Neurons

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Summary

The ability to control neuronal activity using light pulses and optogenetic tools has revealed new properties of neural circuits and established causal relationships between activation of a single genetically defined population of neurons and complex behaviors. Here, we briefly review the causal effect of activity of six genetically defined neural circuits on behavior, including the dopaminergic neurons DA in the ventral tegmental area (VTA); the two main populations of medium-sized spiny neurons (D1- and D2-positive) in the striatum; the giant Cholinergic interneurons in the ventral striatum; and the hypocretin- and MCH- expressing neurons in the lateral hypothalamus. We argue that selective spatiotemporal recruitment and coordinated spiking activity among these cell type-specific neural circuits may underlie the neural integration of reward, learning, arousal and feeding.

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Abbreviations VTA, ventral tegmental area; ARC, arcuate nucleus; LH, lateral hypothalamus; NAc, nucleus accumbens; MSN, medium spiny neurons; MCH, melaning concentrating hormone; Hctr+, hypocretin neurons; DA, dopamine; ChAT, choline acetyl transferase; ChR2, channel rhodopsin 2; AGRP, Agouti-related peptide; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; CPP, conditioned place preference

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OPTOGENETICS

The recent development of optogenetic probes allows millisecond light-control of neural circuits and has resulted in impressive advances in understanding brain function in awake, behaving animals. Using genetic targeting strategies, microbial opsin genes can be introduced into the brain and selectively expressed in a subset of genetically defined neurons. These microbial opsins are single-component light-sensitive ion channels and pumps with millisecond-timescale activation and inactivation kinetics. When expressed on neuronal membrane, microbial opsins can directly convert flashes of light illumination into changes in the neuronal membrane potential.

Optogenetic probes are broadly classified in two main categories: a set of light-activated channels that induce membrane depolarization, and a second class that hyperpolarizes the cell. The light-gated ion channel, Channelrhodopsin-2 (ChR2), comes from the algae *Chlamydomonas reinhardtii*, and is a cation channel [permeable to $H^+ > Na^+ > K^+ > Ca^+$ (*1*)] that opens when activated by blue light (~470 nm). ChR2 allows the passage of cations across the membrane in both directions but always along the electrochemical gradient of the transported ions. Thus, expressing ChR2 in neurons allows light to generate large photocurrents sufficient to trigger individual action potentials with millisecond precision (*2, 3*). Moreover, ChR2 can be chronically expressed in transgenic mice at high levels without adversely affecting fertility and causing no significant changes in neuronal morphology or intrinsic electrical properties (*4*). Several gain-of-function mutants of ChR2s have been developed with improved temporal precision (*e.g.*, CHEF, CHIEF, CHETA) (*5, 6*) or long-lasting membrane potential modulation (SFOs) (*7, 8*). Additionally, the channelrhodopsin-1 from *Volvox carteri* exhibits red-shifted activation spectra (*9*).

In contrast, inhibition of neuronal activity using light can be achieved by expressing the chloride-pumping *Natronomonas pharaonis* halorhodopsin (NpHR) (*10*). NpHR inhibits electrical activity by pumping chloride ions into neurons after yellow light stimulation. Thus far, three generations of NpHR have been developed. The first-generation NpHR can inhibit neuronal firing (*10*), however, this NpHR is retained in the endoplasmic reticulum when expressed at high levels in mammalian neurons. Fusing an endoplasmic reticulum export motif onto the C-terminus of NpHR, created an "enhanced" NpHR (eNpHR2.0) that improves membrane targeting of NpHR by reducing endoplasmic reticulum retention. Furthermore, a third-generation NpHR (eNpHR3.0) offers increased potency of optical inhibition without requiring increased light power (*11*). Importantly, eNpHR3.0 enables optogenetic control with red/far-red light (680 nm), which allows deeper penetration of light and therefore achieves inhibition of a larger volume of brain tissue. In summary, channelrhodopsins and halorhodopsins enables rapid and bidirectional control of neuronal activity. Thus far, optogenetic tools have been used to study a number of genetically-identified cell types (*12–16*), including the dopaminergic (DA) neurons in the ventral tegmental area (Fig. 1).

THE VENTRAL TEGMENTAL AREA (VTA) DOPAMINE NEURONS

The VTA consists of a heterogeneous group of cells lying together close to the midline on the mesencephalon. The group A10 of cells produces dopamine and it represents 27–29,000 DA neurons in the rat (*17*). VTA DA neurons normally fire in a low tonic firing rate mode. However, DA neurons respond to reward stimuli in phasic burst of action potentials. DA neurons phasic activity has been found to encode reward prediction errors. E.g., extracellular recordings in non-human primates have found that DA neurons initially (before learning) fire in burst to unexpected rewards, when errors were frequent and rewards unpredictable. However, the burst spiking progressively reduced, as a function of learning, when rewards became more predictable. Moreover, DA neurons depressed its firing rate when rewards were omitted at the predicted times, but whether the reward is presented at unexpected times then DA neurons fire in burst again (to the unpredicted earlier reward). Thus, DA neurons encode a teaching signal in the form of phasic spiking each time that a prediction about an impending reward is violated. In sum, phasic firing of DA neurons, but not tonic mode, correlates with the prediction error signal used in reinforcement learning theory (*18*). Recently, the use of optogenetics enable scientists with the tools to determine the causal impact that phasic DA spiking have on behavior. To control VTA DA neurons Tsai et, al, 2009 used optogenetic tools, a Cre-inducible adeno-associated virus (AAV) vector carrying gene encoding ChR2 and a tyrosine hydroxylase (TH)-Cre transgenic mouse (*15*). After delivering the vector in VTA region, blue light pulses reliably evoked action potentials in DA neurons. In fact, by varying the frequency of blue light pulses it was possible to mimic tonic or phasic firing of DA neurons. For example, 1–5 Hz light pulses drove a tonic single spike reliably, whereas burst spiking was achieved after 20–50 Hz blue light pulses stimulation. Finally, they used a conditioned place preference (CPP) behavioral paradigm, in which animals learned to associate an environment (e.g., left compartment of a chamber) with tonic (1 Hz light pulses) or in the right chamber with phasic blue light flashes (50 Hz). Surprisingly, mice developed a clear preference to the side paired with burst firing of DA neurons, but not to the side paired with tonic firing, demonstrating for the first time that phasic firing of DA neurons by *itself* was sufficient to induce place preference. As, the VTA DA neurons are the origin of the mesolimbic dopaminergic pathway that connects the limbic system via Nucleus Accumbens (NAc, see below), they rationalized that phasic firing of DA neurons may induce place preference as a result of a stronger dopamine transient release in the NAc. As expected, they found that 50-Hz light pulses evoked a stronger dopamine transient peak in NAc than 1-Hz blue light stimulation, and thus they elegantly correlated NAc dopamine release with development of conditioned place preference.

MEDIUM SPINY NEURONS IN THE VENTRAL STRIATUM

The ventral striatum comprises the NAc and the olfactory tubercle, the latter is located just ventral to NAc (*19, 20*). Since the NAc-olfactory tubercle complex is the main target of VTA DA neurons. We note that the rewarding effects induced by the optogenetic stimulation of VTA DA neurons could also be mediated by increasing the dopaminergic tone in the olfactory tubercle, or other downstream outputs, but this hypothesis remains to be experimentally tested. In particular, the NAc is subdivided in two subregions, the core and the shell. The GABAergic medium-sized spiny neurons (MSNs, 10–11 μm round soma; Fig. 1) constitute the major cell type, comprising about 90–97% of striatal neurons in rodents (*21*). The remaining 3–10% of neurons are composed of aspiny interneurons, which have been classified as giant cholinergic interneurons (12–50 μm soma; Fig. 1) and three other types of GABAergic interneurons that expressed somatostatin (8–12 μm, elliptic soma), parvalbumin (Fast-Spiking, 9–11 μm slightly elongated soma; Fig. 1) and calretinin (6–10 μm), respectively (*22*). The MSNs located in NAc core are similar to those found in the dorsal striatum and comprise two spatially intermingled populations distinguished by their downstream projection outputs. Those expressing dopamine D1+ receptors (D1+ dominant) project preferentially to the substantia nigra pars reticulata (SNr; direct-pathway), whereas those expressing dopamine D2+ receptors (D2+ dominant) project preferentially to the dorsolateral part of the ventral pallidum (VP; indirect-pathway). A subpopulation of NAc core, MSN D1+ dominant neurons located in the patch region selectively project to VTA DA

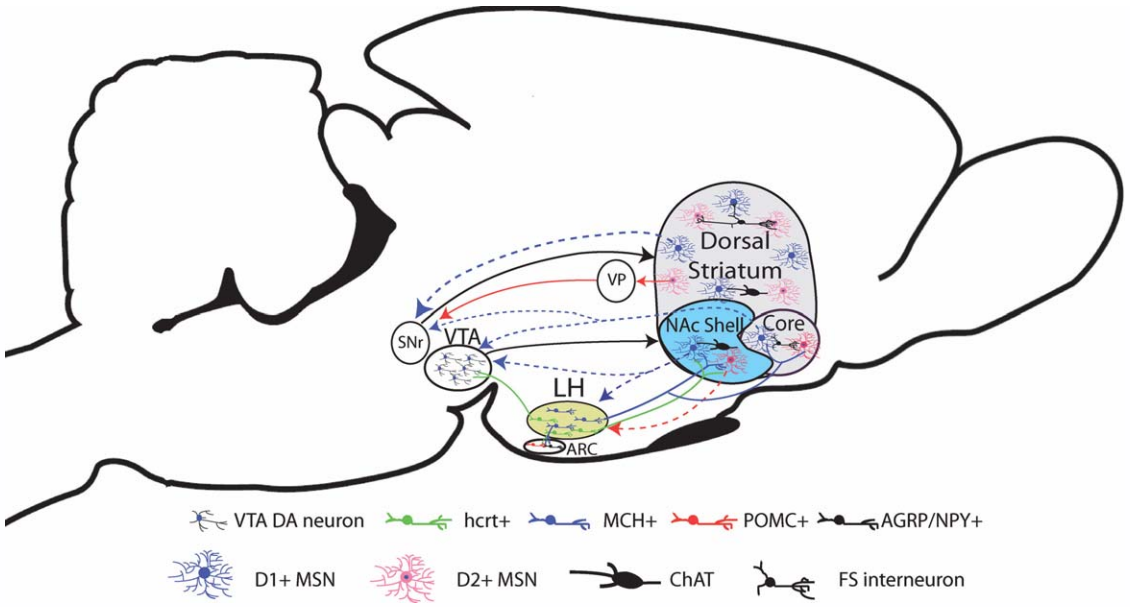

Figure 1. Schematic representation of genetically defined neuronal circuits in VTA, ARC, LH, and Striatum. See main text for details.

neurons. In contrast, the NAc shell forms a circuit unique within the striatum. Compared to the rest of striatum, its MSNs are smaller, have a lower spine density, and have more dopaminergic terminals on their dendritic shafts (*21*). However, like the core, there is a distinction in projection targets of the D1+ and D2+ dominant MSN populations, although this distinction is not that clear since the shell contains 17% of MSNs co-expressing detectable levels of both D1 and D2 receptors much higher than in the dorsal striatum (5%) and in NAc core (6%) (*22*). Nevertheless, the NAc shell D1+ dominant MSNs send presumably inhibitory projections to VTA DA neurons. However, both the D1+ and/or D2+ dominant populations project to ventral pallidum and to the lateral hypothalamus (Fig. 1) (*21*).

The NAc plays a crucial role in mediating the rewarding effects of drugs of abuse, however, it was unknown the specific role that the two major type of MSNs, the D1+ and D2+ expressing cells, play in reward behaviors. Recently, Lobo, et. al. 2010 used optogenetic tools and transgenic mice (D1-Cre and D2-Cre mice) to selectively control the firing rate of D1+ or D2+ NAc expressing neurons. Unlike VTA DA neurons, the selective activation of D1+ or D2+ MSNs in the NAc was not sufficient by *itself* to induce conditioned place preference in the absence of cocaine. However, they demonstrated that activation of D2+ expressing neurons suppressed place preference triggered by cocaine reward, whereas activation of D1+ expressing cells enhances the rewarding properties of cocaine as reflected by a stronger cocaine place preference. Thus, at the striatal and downstream outputs these two cell types exert an opposite and antagonist role in reward behaviors modulated by drugs of abuse (*12*).

At the intracellular signaling level, Lobo et. al. 2010 also found that loss of TrkB (the receptor for Brain Derived Neurotrophic Factor- BDNF) in each type of MSN mimics the optogenetic control over place preference induced by cocaine reward. Specifically, they show that in D2+ MSNs this is likely regulated by higher firing rates of D2+ MSNs after loss of TrkB and enhanced c-Fos expression (a maker for neuronal activity) induction in D2+ MSNs after cocaine exposure. Finally, they found a potent decrease in the activated form of extracellular signal related kinase (ERK), a downstream BDNF signaling molecule, in the NAc shell after D1+ MSN activation suggesting a link between BDNF signaling and neuronal activation in this cell type. These results provide insight into the molecular control of D1+ and D2+ neuronal activity as well as the circuit-level contribution of these cell types to cocaine reward.

INTERNEURONS IN THE VENTRAL STRIATUM

As noted, the giant cholinergic interneurons, owing to their large soma size (12–50 μm) comprise less than 1–2% of the total neuronal population in striatum and they are the source of ACh for the entire neostriatum (*23*). We note that ~80% of cholinergic interneurons besides expressing ChAT they also co-express D2 receptors (*23*). These neurons fire in a tonic irregular spike pattern with an average rate of 2–10 Hz, they are also known as Tonically Active Neurons (TANs). TANs respond in a biphasic form first with an inhibition followed by brief excitatory response to sensory stimuli associated with rewards and with aversive stimuli (*24*). They send local projections to Fast-Spiking GABAergic interneurons, but mainly project to striatal MSNs. TANs, although small in number, may play an essential role in modifying the activity of surrounding MSN projecting neurons (*25*). However, the causal effect of the

tonic spiking of ChAT interneurons was just recently uncovered. To control the spiking activity of cholinergic interneurons Witten et. al. 2010, used a transgenic mouse line expressing Cre-recombinase under the Choline Acetyl Transferase (ChAT) promoter and a Cre-adeno-associated virus (AAV) vector carrying the blue-light gated cation channel ChR2 (to excite) or to inhibit they expressed eNpHR3.0 (*26*). Despite that cholinergic interneurons represent less than 1–2% of the entire neuronal population on the striatum their activity seems to have a profound impact in the local microcircuit activity of majority of MSNs. In fact, optogenetic stimulation (with ChR2) of ChAT interneurons enhanced inhibitory post-synaptic currents (IPSCs) of surrounding MSNs projecting neurons. Likewise, activation of ChAT interneurons *in vivo* evoked a strong inhibition of MSNs spiking activity. In contrast, selective inhibition of ChAT interneurons produces a robust activation of MSNs, thus ChAT interneurons have a bidirectional modulatory role for the striatal microcircuits.

At the behavioral level, selective driving of ChAT spiking with blue light pulses was not sufficient to induce conditioned place preference by *itself* or to enhance cocaine place preference. In contrast, they found that optogenetic silencing of the Cholinergic interneurons in the NAc (that in turn disinhibited most MSNs and probably others MSN's downstream outputs) blocked cocaine conditioned place preference, in which animals learned to associate an environment with cocaine administration (*26*). It is possible that attenuation of cocaine place preference after silencing Cholinergic interneurons may be due, at least in part, to an indirect and stronger activation of D2+ MSNs neurons than D1+ MSNs type.

Electrophysiological studies showed that fast-spiking (FS) GABAergic interneurons in ventral striatum can also exert a powerful somatic inhibition even a single spike in one interneuron can delay or inhibit spiking in populations of MSNs (Fig. 1). In fact, a single FS interneuron extensively synapse (at the soma) ~126–568 MSNs (*27*). Moreover, FS neurons can achieve firing rates over 200 Hz allowing temporal summation of synaptic inhibition. Optogenetic tools may now be used to uncover the behavioral impact of the spiking activity of these FS interneurons (*13*).

NEURONS IN THE LATERAL HYPOTHALAMUS (Hcrts+ AND MCH+)

As noted both types of MSNs in the NAc shell are the only cell-types in the entire striatum that send projections to the Lateral Hypothalamus (LH) (*21*). The LH plays an important role for the integration of feeding behavior with arousal and reward and these functions are mediated via the activity of, at least, two complementary neuronal populations, one expressing hypocretins (hcrt+) and the other expressing Melanin Concentrating Hormone (MCH+). The activity of these two spatially overlapping neuron types has been shown to participate in sleep regulation, energy homeostasis, drug craving, hyperarousal, and feeding.

The hypocretins (hcrt), also known as orexins (*28, 29*), are two neuropeptides derived from a same preprohypocretin precursor, produced in a few thousand cells located at the lateral and perifornical hypothalamus (comprising ~5% of all neurons in LH). Hcrt+ neurons widely project to several parts of the brain including midbrain structures, and wake promoting brain regions such as dorsal raphe and locus ceruleus and to a lesser extent to reward-associated brain regions such as VTA DA neurons and NAc Shell (Fig. 1). Since hcrt+ neurons are absent in narcoleptic patients, and the hallmark symptom of narcolepsy is the intrusion of sleep episodes into wake states (*30*), the Hcrt system has been associated to the maintenance and stability of wakefulness. Hcrt+ neurons are silent during sleep, but maximally discharged during active wake states or during the transitions between sleep and full awakening (*31*), suggesting that they can also initiate wakefulness. Recently, the group of de Lecea et. al. has clearly and convincingly demonstrated that selective activation, with optogenetics, of hcrt+ neurons increased the probability of transition to wakefulness from either slow wave sleep or rapid eye movement sleep (*16*). Thus, the spiking activity of a small group of hcrt+ neurons was sufficient to drive awakening from sleep states (*16*). Interestingly the effectiveness to trigger awakening was attenuated as a function of sleep pressure, e.g. after 2 or 4 h sleep deprivation, indicating that sleep is regulated by a distributed neural network that can override hcrt waking-up signal under higher sleep pressure (*32, 33*). Besides their role of hcrt+ neurons in stabilizing awakening and promoting arousal, the hyprocretinegic system has been related to reward and addiction. For example, despite that narcoleptic patients are normally treated with amphetamine-like compounds it is rare that they develop drug dependency. Moreover, i.c.v. infusions of hcrt-1 peptide elevate intracranial electrical self-stimulation threshold, indicating that hcrt+ neurons can blunt the sensitivity of the reward system (*34*). In addition, overactivation of hcrt+ neurons can lead to a hyperarousal state propitious to induce drug (food) craving (*35*). Boutrel et. al. 2005 has shown that this hyperarousal state is mediated by the interaction between stress-brain pathways, because infusions of the antagonist of the corticotropin-releasing factor severely attenuated hcrt-1 reinstatement of extinguished cocaine seeking behaviors (*34*). Additional observations have noted that the number of hcrt+ neurons activated in LH during a conditioned place preference positively correlates with the amount of place preference evoked by cocaine, amphetamine and food rewards (*36*). Furthermore, the direct activation of hcrt+ neurons reinstates an extinguish drug seeking behavior (*36*). Hence, in addition to the hcrt role in stress and drug relapse, the interaction between hcrt+ neurons and VTA DA neurons seems to be also relevant to mediate the hcrt+ participation in reward processing because, direct administration of hypocretin-1 peptide into VTA also reinstate amphetamine drug-seeking behavior (*36*).

MCH+ expressing neurons are selectively located in LH and zona incerta and send extensive projections to the cortex, the hippocampus, basolateral amygdala and NAc Core and Shell

(Fig. 1). They also project to neurons in arcuate nucleus and to hcrt+ neurons in LH. Juxtacellular recordings of MCH+ neurons across the sleep-wake cycle have shown that MCH+ expressing neurons discharge in a reciprocal profile than hcrts+ neurons. Specifically, MCH+ neurons are silent during wake and sparsely fire during slow-wave sleep, whereas they discharged maximally during paradoxical sleep. In contrast, hcrts+ neurons fired maximally during active awake periods and few seconds before the transitions between sleep to awake. Thus activation of MCH+ neurons promotes sleep whereas hcrts+ evokes arousal propitious to enhance alert and attentional states (*31*). The MCH system defined as the MCH peptide and its receptors (there are 2 receptors MCHR1 and MCHR2, but the latter is not functional in rats) has been link with enhancing cognitive functions, most likely during a sleep memory consolidation mechanism (although this remains to be tested) (*37*). In addition to learning, MCH+ and hcrts+ neurons can also serve as metabolic sensors and thus they may be relevant to coordinate arousal as a function of metabolic needs (*38*). For example, hyperarousal state is normally triggered by a reduced food availability period (*38*). The particular relevance of the metabolic and arousal interaction is the fact that hcrt+ neurons are sensitive to leptin and other food related hormones. Leptin is an adipose releasing-hormone involved in satiety and energy expenditure that mainly act throughout POMC (anorexigenic) and AGRP/NPY (orexigenic)-expressing cells in the arcuate nucleus of the hypothalamus (ARC, Fig. 1) (*39*). Moreover, hcrt+ neurons in LH are inhibited by leptin and glucose [they are glucose-inhibited neurons (*40*)], but excited by ghrelin (a food stimulating hormone) (*38*). Thus, it is expected that hcrt+ and/or MCH+ neurons can provide a crucial link between energy balance, arousal and sleep, explaining at least in part why sleep and metabolism are intrinsically related, *i.e.* a lack of sleep alters metabolism, down regulates leptin, and up regulates the appetite-stimulating hormone, ghrelin, and thus sleep-loss increased hunger and suppresses satiety signals (*41*).

NEURAL INTEGRATION OF FEEDING

At its simplest description the neural control of feeding can be explained as the reciprocal inhibition and opposing effects on appetite of two genetically defined groups of neurons located in the hypothalamic arcuate nucleus. The first group of neurons expresses POMC and its activation suppress appetite, whereas the other cell-type expresses both AGRP/NPY and its activation promote feeding (*39*). Recently Aponte et. al. 2011 used optogenetic tools and transgenic Cre-mice to definitely confirm that the selective activation of AGRP/NPY neurons and its downstream outputs are sufficient to evoke voracious feeding and without previous training (*42*). They found that food intake increased as a linear function of the number of AGRP neurons activated by blue light, and the activation of at least 300 AGRP neurons were necessary to increase food intake. Interestingly, continuous photostimulation was required to maintain food intake, since feeding rapidly stopped after photostimulation offset, which indicates that AGRP neurons were not only a trigger of feeding, but their continuous activity was required to sustain consumption. Selective stimulation of POMC neurons gave the opposite behavioral outcome. Photostimulation of POMC neurons inhibited food intake and induced body weight-loss after constant 24 h stimulation. This impressive results convincingly demonstrate that a complex behavior such as feeding can be evoked (or inhibited) by the selective activity of a genetically defined group of neurons and its downstream outputs. Moreover, they also pave the way to potential new therapeutic strategies for obesity (*43*).

Beyond the ARC feeding network composed by AGRP+ and POMC+ neurons, we note that several, if not all, of the genetically defined neuronal populations described in this manuscript are also recruited during feeding, however, so far no optogenetic studies had directly evaluated their role in feeding behavior. Nevertheless, these neuronal circuits, at least in part, may modulate some attributes of feeding behavior (*44*). For example, direct infusions of leptin in VTA inhibits the firing rate of DA neurons and decreases food intake, suggesting that VTA DA neurons integrate signals from both metabolic hormones and the reward system (*45*). Despite that pharmacological manipulations of the dopaminergic activity in the NAc does not alter feeding, lesions of VTA DA neurons dramatically impaired free-feeding (*46*). In addition, both D1+/D2+ MSNs in NAc shells contain a high concentration of MCHR1 receptor and infusion of MCH promotes feeding. Moreover, an MCH antagonist inhibits feeding (*47*). More importantly direct infusion of MCH into the NAc Shell increases feeding by globally decreasing the firing rate of both types of MSNs (*47*). Previous evidences have also found that global inactivation of NAc shell promotes feeding. E.g., recordings in freely moving rats have found that a subpopulation of putative MSNs are inhibited during ingestive behavior (probably D2+MSNs) (*48, 49*), although we have observed that a similar proportion of putative MSNs are selectively excited during free-feeding (probably D1+MSNs) (*50*), suggesting that during physiological conditions, feeding behavior induces a balance of excitation and inhibition of putative MSNs. Nevertheless, temporary global inactivation of NAc Shell (e.g., by infusing Muscimol) promotes abnormal over-feeding in sated rats, similar to that induced by exogenous MCH infusions (*51*). The opposite effect was evoked after electrical activation of NAc shell since it refrained feeding behavior (*52*). All these evidence have supported the "inhibitory gating hypothesis" that propose that a general pause in the NAc activity is necessary to initiate and maintain feeding behavior (*52*). We suggest that pharmacological inactivation of the NAc shell produces a non-physiological brain state that promotes over-feeding in sated rats. This mechanism might be relevant to understand the neurobiological basis of obesity, since obesity may induce a hypoactive state or blunted response on the striatum (*53*). One current theory dictates that obesity arises from alteration of the activity of reward brain areas by down-regulating expression of D2

receptors in striatum (*54*), which in turn can trigger compulsive eating behavior (*55*). However, it is currently unknown whether the spiking activity of D1+ or D2+ expressing MSNs in NAc plays a causal or correlative role on feeding behavior (*49*). We can only speculate that under a diet-induced obesity context, selective silencing of D2+ MSNs by optogenetic tools will result in compulsive overeating and, in the long term, body weight gain, mimicking the behavioural effects induced by downregulating the expression of D2 receptors in striatum (*55*). Finally, it has been shown that NAc shell can control food intake by direct coordination with LH neurons (*51*). Both hcrt+ and MCH neurons project to NAc and the interaction between the MSNs and either hcrt+ or MCH+ neurons may be relevant to feeding behavior or to process cues-associated with food intake. For example, muscimol inactivation of NAc shell induces abnormal over-feeding, but also activates hcrt+ neurons in LH (*51*), however optogenetic activation of hcrt+ neurons by *itself* was not sufficient to modulate feeding behavior (*16*). Though, it remains possible that hcrt+ neurons can still be important to induce a stress-like food craving state relevant to compulsive eating.

CONCLUSION AND PERSPECTIVES

The constant refinement of optogenetic tools will no doubt increase our power to control simultaneously the spatiotemporal activity patterns of several genetically-defined neuronal circuits that are necessary to encode higher brain functions (*56*). The combined use of optogenetics and electrophysiological recording technics such as high-density multielectrode arrays (*57, 58*) or calcium imaging (*59, 60*) will allow us for the first time not only to identify the neuronal correlates of behavior, but also to determine its sufficiency and causality. The current impressive advances, foretells a better understanding of the neural integration and coordination of several brain functions such as reward, arousal and feeding. The challenge now it is to identify the neural codes hidden on the coordinated spiking activity in these and other neural circuits that plays a causal role in behavior.

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