Review

Optogenetic dissection of neural circuits underlying emotional valence and motivated behaviors

Edward H. Nieh\textsuperscript{a}, Sung-Yon Kim\textsuperscript{b}, Praneeth Namburi\textsuperscript{a}, Kay M. Tye\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a}Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA
\textsuperscript{b}Department of Bioengineering, Neurosciences Program, Stanford University, Stanford, CA, USA

\textbf{Article info}

Article history:
Accepted 2 November 2012
Available online 8 November 2012

Keywords:
Optogenetics
Systems
Circuit dissection
Behavior
Emotion
Motivation
Brain
Projections
ChR2
NpHR
Opsin
Valence

\textbf{Abstract}

The neural circuits underlying emotional valence and motivated behaviors are several synapses away from both defined sensory inputs and quantifiable motor outputs. Electrophysiology has provided us with a suitable means for observing neural activity during behavior, but methods for controlling activity for the purpose of studying motivated behaviors have been inadequate: electrical stimulation lacks cellular specificity and pharmacological manipulation lacks temporal resolution. The recent emergence of optogenetic tools provides a new means for establishing causal relationships between neural activity and behavior. Optogenetics, the use of genetically-encodable light-activated proteins, permits the modulation of specific neural circuit elements with millisecond precision. The ability to control individual cell types, and even projections between distal regions, allows us to investigate functional connectivity in a causal manner. The greatest consequence of controlling neural activity with finer precision has been the characterization of individual neural circuits within anatomical brain regions as defined functional units. Within the mesolimbic dopamine system, optogenetics has helped separate subsets of dopamine neurons with distinct functions for reward, aversion and salience processing, elucidated GABA neuronal effects on behavior, and characterized connectivity with forebrain and cortical structures. Within the striatum, optogenetics has confirmed the opposing relationship between direct and indirect pathway medium spiny neurons (MSNs), in addition to characterizing the inhibition of MSNs by cholinergic interneurons. Within the hypothalamus, optogenetics has helped overcome the heterogeneity in neuronal cell-type and revealed distinct circuits mediating aggression and feeding. Within the amygdala, optogenetics has allowed the study of intra-amygdala microcircuitry as well as interconnections with distal regions involved in fear and anxiety. In this review, we will present the body of optogenetic studies that has significantly enhanced our understanding of emotional valence and motivated behaviors.

This article is part of a Special Issue entitled Optogenetics (7th BRES)

\textsuperscript{*}Corresponding author.
E-mail address: kaytye@mit.edu (K.M. Tye).

0006-8993/$ - see front matter \textcopyright 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2012.11.001
1. Introduction

If evolution were a game, the objective would be to spread your genes to as many surviving offspring as possible (Darwin, 1909). The strategy would be straightforward: take actions that will support, and avoid actions that will threaten, the survival of you or your descendants. The challenges are (1) fierce competition and (2) an ever-changing environment overflowing with both familiar and novel sensory stimuli. To be competitive, you must filter out unimportant information, identifying whether certain cues are likely to have a positive or negative impact on your objective and subsequently select an appropriate behavioral response. To win, you must do this quickly and accurately.

Evolution has produced many winning designs, but all of these designs share the critical ability to differentiate between good and bad stimuli (Tooby and Cosmides, 1990). Emotions have been hypothesized to be a biological strategy for rapidly integrating previously recorded data (weighted for significance), assigning a motivational value to the stimulus, and orchestrating an appropriate behavioral response (Cosmides and Tooby, 1987; Barkow et al., 1995). In vertebrates, particularly mammals, the neural circuits thought to be important for this ability are remarkably well-conserved (MacLean, 1990). However, the precise neural mechanisms underlying the differentiation between positive and negative emotional valence are still poorly understood.

Despite the importance of understanding valence processing, technical, experimental, and practical obstacles have impeded progress in this field. First, for the field of behavioral neuroscience, studying sensory and motor systems (more amenable to discrete inputs and outputs) may have been a logical prerequisite. Second, regions involved in emotional valence processing are comprised of many different cell-types with heterogeneous functional roles that are not spatially segregated from each other within these “primitive” subcortical structures. Indeed, many regions such as the amygdala (LeDoux, 2000; Paton et al., 2006; Tye et al., 2008; Shabel and Janak, 2009; Pape and Pare, 2010), ventral tegmental area (Wise and Rompre, 1989; Schultz, 1998; Tan et al., 2012; van Zessen et al., 2012), and hypothalamus (Atasoy et al., 2012; Harris and Aston-Jones, 2006; Aponte et al., 2011; Lin et al., 2011) have been implicated in both positive and negative valence processing. This organization makes classical techniques that involve spatially-defined manipulations alone difficult to interpret on a mechanistic level. Third, valence processing involves a spatially distributed network and a multitude of parallel circuits, which demands projection-specific circuit-level control for functional dissection (Fig. 1). The indispensable nature of these functions may have led to the diffuse spatial distribution and system redundancy, which provide an evolutionary advantage in the face of accidents or injuries.

Optogenetic tools that allow for cell-type (Boyden et al., 2005; Zhang et al., 2007; Atasoy et al., 2008) and even projection-specific (Tye et al., 2011; Stuber et al., 2011) manipulation of neural activity with precise temporal control have given us the ability to overcome many of these obstacles. Although emotions are difficult to quantify, motivated behaviors provide a measurable output that summarizes many factors, including emotional state. Optogenetic tools have accelerated our understanding of a vast array of neural phenomena (Tye and Deisseroth, 2012). Here, we focus on the burst of recent insights towards understanding the neural circuits encoding emotional valence and motivated behaviors.
2. Breaking down reward: Classical views of ventral tegmental area function and new insights into the mesolimbic dopamine system

The mesolimbic dopamine system consists of the ventral tegmental area (VTA) and the regions it provides dopaminergic innervation to, including the nucleus accumbens (NAc), amygdala, hippocampus (HPC), and prefrontal cortex (PFC) (Fields et al., 2007). Dopamine neurons in the midbrain have long been thought to serve a central role in reward prediction (Romero and Schultz, 1990; Mirenowicz and Schultz, 1994). In a seminal study, Schultz et al. (1997); Schultz (1998) showed that putative dopamine neurons of the VTA are activated by presentations of unexpected rewards and inhibited by omissions of expected rewards, evidence that neuronal activity reflects a model of learning based on divergences from expectations (Rescorla and Wagner, 1972). Sugrue et al., (2005) proposed an actor-critic conceptual model for the decision-making process, in which the VTA plays a role as the critic, alongside the ventral striatum, judgingvaluations of higher order processes by taking in predictions about the value of a stimulus and outputting error signals comparing these predictions with the actual reward obtained.

The significant contribution of the VTA to processing motivation and hedonia makes it a region likely to be perturbed in pathological states, such as addiction and other neuropsychiatric disorders (Wise, 2002, 2005; Everitt and Robbins, 2005; Kalivas, 2005; Hyman et al., 2006; Nestler and Carlezon Jr, 2006; Fields et al., 2007; Grace et al., 2007; Schultz, 2007; Lüscher and Malenka, 2011). Although the VTA has long been implicated in addiction, reward processing, and learning (Koob, 1992; Bonci and Malenka, 1999; Di Chiara, 1999; Nestler, 2001; Piccolotto and Corrigall, 2002; Wise, 2004), the advent of optogenetics has clarified the synaptic, cellular, and circuit mechanisms underlying these processes as discussed below.

2.1. Optogenetic tools aid in the identification of dopamine and GABA neurons and their functions in the ventral tegmental area

The VTA is a heterogeneous structure containing dopamine neurons (~65%), GABA neurons (~30%), and glutamate neurons (~5%) (Margolis et al., 2006; Yamaguchi et al., 2007; Nair-Roberts et al., 2008; Dobi et al., 2010). Historically, researchers have used electrophysiological criteria based on waveform characteristics and firing rates to identify dopamine neurons (Grace and Bunney, 1980,1983; Wang, 1981; Grace and Onn, 1989), but recent studies have shown that there may be non-dopaminergic neurons in the VTA that may also fit into these criteria (see Ungless and Grace, 2012 for an in-depth review). Ungless and Grace conclude in their review that using extracellular criteria is sufficient to identify dopamine neurons, but acknowledge that there is still a possibility of misidentification. However, Margolis et al. (2006) offer an opposing viewpoint, concluding that dopamine neurons cannot be distinguished from other VTA neurons by size, shape, or various electrophysiological criteria.
The recent advent of optogenetic tools has provided a new method for characterizing neurons by cell-type, essentially eliminating the need to classify dopamine neurons by electrophysiological markers (Cohen et al., 2012). Several studies have combined the use of transgenic animals expressing Cre-recombinase with Cre-inducible viral vectors to selectively target dopamine neurons with optical stimulation (Tsai et al., 2009; Witten et al., 2011; Cohen et al., 2012). Given that dopamine neurons express tyrosine hydroxylase (TH), one strategy first employed by Tsai et al. (2009) to selectively target dopamine neurons was to deliver a Cre-inducible adeno-associated viral (AAV) vector carrying the light-activated cation channel channelrhodopsin-2 (ChR2) into the VTA of TH::Cre transgenic mice to limit expression of ChR2 to TH-positive neurons.

Using a similar cell-type specific targeting technique with dopamine transporter (DAT)::Cre mice and GABA transporter (VGAT)::Cre mice, Cohen et al. (2012) were able to selectively activate dopamine and GABA neurons in vivo. Following electrophysiological recordings during a Pavlovian conditioning task, the authors measured each neuron’s response to photostimulation. By examining which units responded in either the DAT::Cre or VGAT::Cre mice, they were able to identify recorded units as either dopaminergic or GABAergic, an approach commonly referred to as “phototagging” (Lima et al., 2009). The authors confirmed that dopamine neurons are involved in reward-prediction error (Schultz et al., 1997), increasing their firing rates during reward-predicting cues and decreasing their firing rates when expected rewards are omitted. These results fit well into the traditional views of the VTA as a critic, with dopamine neuronal activity reflecting reward prediction error.

With respect to GABA neuronal function, Cohen et al. (2012) found that GABA neurons showed persistent activity during the delay between a reward-predictive cue and the reward that reflected the value of the upcoming reward (big, small, or none). Combined with the lack of modulation by the delivery or omission of the reward, these data suggest that GABA neurons encode reward expectation, and thus are not affected by the reward itself. In addition, GABA neurons were also excited by aversive stimuli, potentially suppressing dopaminergic activity in response to aversive events. In fact, many addictive drugs are known to inhibit GABA neurons (Hyman et al., 2006; Tan et al., 2010; Lüscher and Malenka, 2011), likely leading to the sustained reinforcement of addiction (Redish, 2004; Cohen et al., 2012).

Two other studies also aimed to elucidate the function of GABA neurons in the VTA (Tan et al., 2012; van Zessen et al., 2012). Tan and colleagues selectively expressed ChR2 in GABA neurons using glutamate decarboxylase (GAD)::Cre...
transgenic mice. From recordings of neuronal activity in the VTA, the authors found that not only are dopamine neurons inhibited by GABA neurons, activation of GABA neurons expressing Chr2 or direct inhibition of dopamine neurons by halorhodopsin (NpHR), a light-activated chloride pump, elicited conditioned place aversion (CPA) (Tan et al., 2012) (Fig. 2). While some studies use the acronym eNpHR3.0 to refer specifically to the third variant of NpHR (Gradinaru et al., 2010), many studies use the acronym NpHR interchangeably. Taking a similar approach, van Zessen and colleagues selectively expressed Chr2 in GABA neurons of VGAT::Cre transgenic mice. Activation of VTA GABA neurons was shown to disrupt the reward consummatory behavior by inducing early termination of licking for sucrose during a cue-evoked reward-seeking task (van Zessen et al., 2012). In addition, GABA neuronal activation was shown to directly suppress the activity and excitability of dopamine neurons in the VTA and the release of dopamine in the NAc (Fig. 1A and Fig. 2). The authors also showed that while optogenetic stimulation of GABA neurons in the VTA could directly affect reward consummatory behavior, stimulating their inputs into the NAc could not (van Zessen et al., 2012), indicating that intra-VTA GABA inhibition of dopamine neurons likely mediates the interruption of reward consummatory behavior. One important caveat to note is that when illuminating axon terminals, which are more spatially dispersed than cell bodies, it can be difficult to evoke behavior due the smaller volume of illumination. This may be another possible explanation for why GABA cell-body stimulation in the VTA was able to evoke a behavioral change, while GABA terminal stimulation in the NAc was not.

2.2. Functional heterogeneity of dopamine neurons—reward, aversion, and salience

If dopamine neuron activity truly mirrored reward prediction error, we would expect aversive stimuli to inhibit dopaminergic activity. While some studies show data that dopamine neurons in the midbrain (Kaufling et al., 2009). Jhou et al. (2009) showed robust excitatory input from the Lh to the rMTg and inhibitory input from the rMTg to the VTA (Jhou et al., 2009; Matsui and Williams, 2011), suggesting a LHb-RMTg-VTA pathway that mediates responses to aversive stimuli. Stamatakis and Stuber (2012) expressed Chr2 in LHb neurons of mice and implanted optical fibers above LHb terminals in the posterior VTA and the rMTg. Behavioral experiments showed that optical stimulation of LHb terminals caused CPA, was capable of providing negative reinforcement, and also disrupted positive reinforcement (Fig. 1B and C, and Fig. 2). Shabel et al. (2012) examined projections from the entopeduncular nucleus (EP) to the LHb by expressing Chr2 in the EP and optically stimulating terminals in the LHb (Fig. 1D). The authors found that animals developed a clear aversion to optical stimulation of this pathway. In addition, serotonin, a neuromodulator that plays a major role in depression, was shown to suppress activity in this pathway, providing an insight into how serotonin may help to modulate reward processing (Shabel et al., 2012).

The laterodorsal tegmentum (LDT) is another brain region that has been shown to innervate the VTA (Cornwall et al., 1990). In addition, electrical stimulation was shown to elicit dopamine release in the NAc, mediated by glutamatergic and cholinergic receptors in the VTA (Forster et al., 2002), suggesting a LDT-VTA-NAc pathway involved in processing reward. In a recent study, Lammel et al., 2012 used a combination of tracing techniques and retrograde/anterograde delivery of ChR2 to study both the projection targets of VTA dopamine neurons and afferent projections to the VTA. By infusing a retrograde rabies virus (RV) containing Chr2 into the VTA and differentially targeting two groups of animals with optical fibers in LDT or LHb, the authors induced conditioned place preference (CPP) and conditioned place aversion (CPA) by optical stimulation of these two pathways, respectively. In addition, by combining retrobead injection in the mPFC or NAc with anterograde delivery of Chr2 into the LDT or LHb, the authors could perform whole-cell recordings of retrogradely-labeled VTA neurons that could be optically stimulated ex vivo. Using this technique, LDT neurons were found to preferentially synapse onto dopamine neurons in lateral VTA projecting to the NAc lateral shell, while LHb neurons were found to preferentially synapse onto both dopamine neurons in medial VTA projecting to the mPFC and rostromedial tegmental nucleus (rMTg) GABA neurons. Moreover, optical stimulation of the LHb induced inhibitory postsynaptic currents (IPSCs) in dopamine neurons projecting to NAc lateral shell. These data indicate a distinct separation of two circuits in the VTA—while the LDT-Lateral VTA-NAc lateral shell pathway encodes reward (Fig. 1A and E, and Fig. 2), the LHb-medial VTA-mPFC pathway and disynaptic inhibition of dopamine neurons projecting to NAc lateral shell (likely via RMTg neurons) encode aversion (Lammel et al., 2012) (Fig. 1B and F, and Fig. 2).

In fact, evidence of opposing functions of dopamine neurons in the VTA may also explain how addictive drugs
differentially hijack neural circuits. Koo et al. (2012) found that although brain-derived neurotrophic factor (BDNF) is a positive regulator of neural plasticity in dopamine neurons to promote the actions of cocaine and other stimulants (Horger et al., 1999; Hall et al., 2003; Graham et al., 2007; Lobo et al., 2010), it plays an opposite role in the effects of morphine. BDNF knockdown in the VTA enhanced the ability of morphine to increase dopamine neuron excitability and promote reward, and optogenetic stimulation of ChR2-expressing VTA dopamine neurons in NAc reversed the suppressive effect of BDNF on morphine. These data imply that drugs of abuse may act in different ways on the VTA or recruit different subsets of VTA neurons.

Together, these studies suggest that classical approaches of organizing brain function by neurotransmitter or brain region are not sufficient. While it appears that some dopamine neurons respond as predicted by traditional models of reward processing, newer data has found parallel components involved in aversion and salience processing. Revisiting the critic role of the VTA in an actor-critic model, it is possible that the salience circuit is used to “weigh” the valuation of a stimulus rather than to simply increase or decrease it. For example, a rewarding and salient stimulus would be assigned a high value, but a rewarding and non-salient stimulus would only be assigned a moderate value. In addition, two separate streams for reward and aversion processing allows for the separation of the omission of a rewarding stimulus from the presence of an aversive stimulus. A system with these extra parallel processors would be more effective and efficient in evaluating stimuli than a system where the only processor is for reward prediction error (Fig. 3).

3. From motor to motivation: Distinct circuits within the ventral and dorsal striatum

The striatum is a large subcortical area in the forebrain that is associated with a broad set of behavioral processes ranging from goal-directed learning to basic motor functions (Mogenson et al., 1980). In many ways, the striatum is the brain’s central meeting point as information from the cortex, thalamus, and dopaminergic innervation from the midbrain all converge (Smith et al., 1994; Kincaid et al., 1998; Bolam et al., 2000). The striatum has been conceptualized as the final site of information integration before the processing of motor output. The cognitive, motor, and limbic systems give rise to functionally distinct areas of the striatum (Berendse et al., 1992; Voorn et al., 2004; Schilman et al., 2008), though identifying homogenous regions has proven to be difficult. Behaviorally, differences in striatal subregions do not obey clear demarcations and instead follow various gradients (Voorn et al., 2004). However at the highest level, the striatum is commonly segregated into two halves because of their functional contributions to behavior (Voorn et al., 2004). Unlike the ventral striatum, which is anatomically connected to limbic structures and involved in reward-related learning,
(Kelley and Domesick, 1982; Kelley et al., 1982; McGeorge and Faull, 1989; Cardinal et al., 2002), the dorsal striatum receives inputs from the substantia nigra and cortex and is primarily involved in action selection and movement (Albin et al., 1989; Graybiel et al., 1994; Chang et al., 2002; Lauwereyns et al., 2002; Haber, 2003; Balleine et al., 2007). Here, we discuss how these striatal subregions contribute to valence processing and how optogenetics has helped advance our understanding of striatal mechanisms and circuitry.

### 3.1. Reward processing in the nucleus accumbens by medium spiny neurons and cholinergic interneurons

Approximately 95% of the striatum is made up of GABAergic medium spiny projection neurons (MSNs), in addition to cholinergic interneurons (~1%) (Kemp and Powell, 1971; Phelps et al., 1985; Rymar et al., 2004; Kreitzer, 2009). MSNs in the NAc are segregated into two subclasses—MSNs expressing D1 receptors that are part of the “direct” pathway (dMSN) and those expressing D2 receptors that make up the “indirect” pathway (iMSN) (Gerfen et al., 1990; Gerfen, 1992; Kawaguchi, 1997; Gerfen and Surmeier, 2011). As a result of their differential expression of dopamine receptors and projection targets, dopamine is thought to have opposing effects on MSNs, exciting dMSNs and inhibiting iMSNs (Albin et al., 1989). The existence of these two separate pathways acting in functional opposition to shape behavioral output (Alexander et al., 1986; Graybiel, 2000) has long been an accepted model, but establishing a causal relationship between activation of each subtype and its effect on behavior has proved to be challenging.

Only recently with optogenetics and Bacterial Artificial Chromosome (BAC)-transgenic mice has it been possible to isolate and selectively activate dMSNs or iMSNs (Gong et al., 2003, 2007; Shuen et al., 2008). While the NAc makes up only a portion of what is considered to be the ventral striatum, we focus solely on the NAc for the purposes of this review. Lobo et al. (2010) combined optogenetic strategies with cocaine treatment to show that activating dMSNs in the NAc enhances, whereas activating iMSNs in the NAc suppresses, the effects of cocaine reward (Fig. 1G and Fig. 2), validating the idea that the two subtypes have opposing influences on reward processing. In addition to a differentiation of cell-type, the source of excitatory input into NAc also appears to affect cocaine reward. In a study investigating various excitatory afferents to the NAc, Britt et al. (in press) found that ventral hippocampus (vHPC) input to the NAc was predominantly and selectively potentiated from cocaine exposure compared with projections from the basolateral amygdala (BLA) and the PFC. However, activation of each of these discrete pathways was able to invoke CPP and optical intracranial self-stimulation (ICSS) (Fig. 1H–J and Fig. 2). These findings suggest that cocaine may selectively activate only one of several excitatory inputs into the NAc, and future experiments should aim to determine when each of these distinct pathways are active and how they might individually

![Image](image-url)
shape behavior. In an earlier study, Stuber et al. (2011) also showed that stimulating BLA terminals in the NAc could evoke ICSS (Fig. 1) and Fig. 2), but that stimulating mPFC terminals could not. This contrast with data obtained from Brit and colleagues may be explained by the coordinates of the viral injection site, optical fiber placement, or illumination parameters. In addition, the authors showed that ICSS required D1, but not D2, signaling in the NAc and that inhibition of NpHR-expressing BLA projections to the NAc reduced cue-evoked intake of sucrose (Stuber et al., 2011). Striatal MSNs also express muscarinic acetylcholine (ACh) receptors and are modulated by ACh from local cholinergic interneurons (Bolam et al., 1984; Weiner et al., 1990). Pin-pointing the functional role of cholinergic interneurons has been a challenge, as they only make up ~1% of the neuronal population (Kemp and Powell, 1971; Phelps et al., 1985; Rymar et al., 2004; Kreitzer, 2009). To resolve this debate, Witten et al. (2010) found that optically inhibiting cholinergic neurons by expressing NpHR in choline acetyltransferase (ChAT)::Cre mice blocked cocaine conditioning (Fig. 2). More recently, two separate studies have also shown that cholinergic activation by optogenetic stimulation elicits dopamine release (Cachope et al., 2012; Threlfell et al., 2012), suggesting that ACh modulates MSN activity by regulating dopamine content.

3.2. Cocaine-induced synaptic plasticity in the nucleus accumbens

In addition to using optogenetic tools to study circuit activity, optogenetics can also be used to investigate the mechanisms of drug-induced synaptic plasticity. Pascoli and colleagues were able to examine the effects of cocaine at the synaptic level by expressing ChR2 in infralimbic cortical cells and optically stimulating terminals in the NAc (Fig. 1H and Fig. 2). The authors optogenetically depotentiated cortical inputs to the NAc to abolish cocaine-induced locomotor sensitization, demonstrating a causal link between cocaine-evoked plasticity and behavioral adaptation (Pascoli et al., 2012). Their findings also demonstrated that cocaine-evoked potentiation relies on extracellular signal-regulated kinase (ERK)-dependent long-term potentiation (LTP) in dMSNs, but not iMSNs (Pascoli et al., 2012).

Optogenetic tools provide new techniques for “remote control” of neural activity, without rupturing the cell membrane, thus allowing a more physiologically relevant LTP induction protocol (Zhang et al., 2008). Extracellular control of neural activity offers the added advantage of manipulating a cell for longer time periods compared to whole-cell patch-clamp, making it possible to study homeostatic plasticity mechanisms that operate over longer time scales (Goold and Nicoll, 2010). One potential technique for studying spike timing dependant plasticity (STDP), is to use ChR2, in combination with the red-shifted ChR1/VCr1 chimaera opsin variant (C1V1) (Yizhar et al., 2011) to allow remote induction of STDP by millisecond-scale manipulation of activity of both the presynaptic and postsynaptic neurons. This can be achieved by selectively stimulating axon terminals of the presynaptic neuron expressing ChR2 with blue light and stimulating the postsynaptic neuron expressing C1V1 with yellow light (Fig. 4).

3.3. Integrating the actor-critic model with the ventral striatum

Looking back at the actor-critic model of decision-making (Sugrue et al., 2005), it is important to note that the ventral striatum may also play a significant role as a critic (Montague et al., 2004; O’Doherty et al., 2004; van der Meer and Redish, 2009), as human functional magnetic resonance imaging (fMRI) data have shown that ventral striatum activity correlates with prediction error during both Pavlovian and instrumental conditioning (O’Doherty et al., 2004). In an extension of the model, Atallah et al. (2007) suggest that the ventral striatum may actually serve as a “director” in an actor-director–critic model of decision-making (Fig. 3). While VTA dopaminergic inputs to the NAc serve as input from the critic, the ventral striatum learns the relative task demands and directs the dorsal striatum to perform an action. Thus, the role of ventral striatum may actually be to facilitate the effect of the critic on the actor, possibly through modulating activity in the orbitofrontal cortex, which provides top-down control of the dorsal striatum (Joel and Weiner, 1994; Frank and Claus, 2006), or through projections to the substantia nigra, which sends dopaminergic projections back to the dorsal striatum (Haber et al., 2000; Joel and Weiner, 2000).

3.4. Diverse functions in the dorsal striatum

Since the dorsal striatum receives its dopamine inputs from the substantia nigra pars compacta (SNc) (Heimer and Wilson, 1975; Grace and Bunney, 1983; Graybiel et al., 1994; Hikosaka et al., 2000), evidence that degeneration of SNc dopamine neurons causes motor deficits in both Parkinson’s disease (PD) and Huntington’s disease (Graybiel, 2000; DeLong, 2007) supports the motor-centric role of dorsal striatum. By expressing ChR2 separately in dMSNs and iMSNs in the dorsal striatum, Kravitz et al. (2010) showed that activation of dMSNs reduced freezing and increased locomotion, while activation of iMSNs induced a Parkinsonian state with increased freezing, bradykinesia, and decreased locomotor initiations (Fig. 1K). These data suggest that degeneration of SNc dopamine neurons likely acts by creating an imbalance in dMSN and iMSN activity. However, it is important to note that PD patients not only suffer from severe motor impairments, but also display a learning bias, showing enhanced learning from negative feedback and impaired learning from positive feedback. Following dopamine medication (L-Dopa, D2 receptor agonists, and/or monoamine activity enhancers), this disparity was reversed and PD patients became more responsive to positive outcomes than negative ones (Frank et al., 2004). These data would suggest that motor and reinforcement may not be so easily separable. In a more recent study, Kravitz et al. (2012) again expressed ChR2 in dMSNs and iMSNs and revealed that dorsal striatal MSNs also show similar opposing influences on reinforcement that were shown in the NAc (Lobo et al., 2010). The authors showed that illuminating ChR2-expressing dMSNs in the dorsomedial striatum was sufficient to induce ICSS, whereas activating iMSNs punished the same behavior (Kravitz et al., 2012) (Fig. 1K). This discovery that neurons in dorsal striatum may have similar function to those in ventral striatum shows that our model of striatal function and the
dorsal/ventral separation may need to be revisited. In another surprising study, Tritsch et al. (2012) found that dopamine neurons projecting from SNc to dorsal striatum co-release GABA, providing an inhibitory influence on striatal activity. In combination with studies showing glutamate co-release from dopamine neurons projecting to the NAc (Stuber et al., 2010; Tecuapetla et al., 2010), these findings show an interesting dichotomy between dopamine neurons that innervate dorsal versus ventral striatum.

4. Fighting and feeding: Investigating behavioral circuits in the hypothalamus

The hypothalamus is a prime example of functional, structural, and cellular heterogeneity, making it an ideal target for optogenetic approaches to gather new insights linking cell- or projection-specific circuits with behavior. Indeed, some of the seminal in vivo optogenetic studies have been performed in the hypothalamus, providing novel insights into the remarkably diverse set of behaviors central to survival, including aggression (Lin et al., 2011), feeding (Aponte et al., 2011; Domingos et al., 2011; Atasoy et al., 2012), and sleep (Adamantidis et al., 2007, 2010; Carter et al., 2009). Keeping with the theme of emotional valence and motivated behaviors in this review, we will discuss the body of literature encompassing aggression and feeding, and respectfully note that there are significant optogenetic studies on sleep and arousal that we are unable to review here.

4.1. The role of the ventromedial hypothalamus in aggression

More than four decades ago, electrical stimulation studies in cats provided evidence that there may be a specific hypothalamic subregion that mediates aggression (Siegel and Skog, 1970; Chi and Flynn, 1971). This work, replicated in rats, also supported the existence of a hypothalamic attack area (HAA) (Kruk et al., 1983; Lammers et al., 1988; Kruk, 1991; Siegel et al., 1999; Hrabovszky et al., 2005) that serves as the aggression locus in the rodent brain. However, Lin et al. (2011) reported that electrical stimulation in this same region failed to elicit attack behaviors in mice. This circuit is further complicated by evidence that aggression and mating may recruit neurons from the same neural structures, or neurons in close spatial proximity (Kollack-Walker and Newman, 1995; Veening et al., 2005).

By delivering ChR2 into the ventrolateral subdivision of the ventromedial hypothalamus (VMHvl) using an AAV that infects neurons preferentially without retrogradely infecting cells from uptake at axons terminals, Lin et al. (2011) could selectively activate cell bodies at the injection site, while sparing axons of passage. The authors found that optical stimulation of the VMHvl was able to acutely evoke aggressive behavior in mice (Lin et al., 2011) (Fig. 1L and Fig. 5). In addition, pharmacological silencing of the VMHvl was able to inhibit aggression. Lin and colleagues explain that failure to evoke the same behavior with electrical stimulation is likely due to overlapping populations of neurons controlling aggression and mating. Distinct neurons, such as those showing increased firing rates during aggression, were inhibited during mating, suggesting opposing inhibition. Electrical stimulation could have stimulated both aggression and mating neurons simultaneously, leading to a zero-sum effect on behavior.

4.2. AGRP, POMC, and leptin control of feeding behaviors

Agouti-related protein (AGRP) neurons and pro-opiomelanocortin (POMC) neurons are two cell populations with competing functions found in the arcuate nucleus (ARC) of the mediobasal hypothalamus. While AGRP neurons promote feeding behaviors (Ollmann et al., 1997), POMC neurons suppress
them (Yaswen et al., 1999). Evidence has also been shown that AGRP neurons inhibit POMC neurons (Cowley et al., 2001; Roseberry et al., 2004) and that AGRP directly blocks melanocortin receptors (Ollmann et al., 1997). This has led to a proposal that AGRP neurons might serve as a modulatory signal, blocking the melanocortin pathway to promote feeding (Cowley et al., 2003). However, evidence also exists showing that AGRP’s effect on feeding may be independent of the melanocortin signaling pathway (Wu et al., 2008).

Aponte et al. (2011) were able to express ChR2 into AGRP and POMC neurons separately in AGRP::Cre and POMC::Cre transgenic mice. AGRP stimulation alone was able to induce feeding within minutes, and as expected, POMC stimulation reduced feeding and led to weight loss (Fig. 1M and Fig. 5). In mice expressing an agouti protein that blocks melanocortin receptors, stimulation of POMC neurons was unable to reduce feeding, indicating that POMC-mediated hypophagia required melanocortin signaling. In contrast, AGRP stimulation in these mice evoked similar feeding behaviors as in the AGRP-ChR2 mice, suggesting that melanocortin receptors are not necessary for AGRP function, evidence that AGRP neurons have direct access to the feeding pathway, in addition to modulating feeding through inhibition of POMC neural activity. In a more recent study, Atasoy et al. (2012) found evidence that AGRP suppression of POMC activity instead modulates food intake on the order of hours, rather than acute feeding. In addition, by expressing ChR2 in AGRP neurons and targeting an optic fiber over axons in paraventricular hypothalamus (PVH), the authors also showed that feeding could be evoked by AGRP terminal stimulation in the PVH through the suppression of PVH oxytocin neurons (Fig. 1N and Fig. 5).

Leptin is a peripheral hormone secreted by adipocytes heavily connected in the feeding circuit with POMC and AGRP neurons that signals the status of energy reserves to the brain, in order to modulate appetite and feeding behavior (Elia et al., 1999; Meister, 2000; Leinninger et al., 2009). Evidence suggests that leptin may modulate food intake by suppressing the reward value of food (Fulton et al., 2000; Figlewicz et al., 2007). In humans, fMRI studies have shown that patients with congenital leptin deficiency give higher ratings to food images and that ratings could be lowered with leptin treatments (Fulton et al., 2000; Figlewicz et al., 2007). Domingos et al. (2011) developed an assay where animals were given choices between a combination of sweeteners (sucrose, sucralose, and water) and optical stimulation of ChR2-expressing VTA dopamine neurons. Mice were found to prefer optogenetic stimulation to sucralose, but not sucrose, and sucralose with stimulation over sucrose. From here, the authors showed that when the animals were food restricted, they increased their preference for sucrose over stimulation with sucralose. However, when treated with leptin, animals experienced a decrease in preference for sucrose, showing that leptin and food restriction elicit opposite effects on the value of sucrose (Domingos et al., 2011).

5. The many facets of fear: Dissection and control of fear and anxiety

The circuits mediating fear and anxiety are remarkably well-conserved between humans and rodents (Adolphs et al., 1995; Morris et al., 1996; LaBar et al., 1998; De Bellis et al., 2000; Thomas et al., 2001; Chen et al., 2006; Kienast et al., 2008; Etkin et al., 2009). Anxiety disorders, which comprise the most common class of psychiatric disorders, with a lifetime prevalence of ~28%, include more specific disease states such as generalized anxiety disorder, social anxiety, panic disorder, post-traumatic stress disorder (PTSD), and specific phobias (Kessler et al., 2005). Although related, fear and anxiety are distinctly different—fear is an emotional reaction triggered by an immediate threat, while anxiety is a state of heightened apprehension in the absence of an immediate threat (Davis et al., 2010).

Decades of studies have focused on the amygdala and established its central role in the processing of fear and anxiety (LeDoux, 2000; Maren and Quirk, 2004; Pape and Pare, 2010). Among the many subnuclei of the amygdala, most researchers have focused on the lateral nucleus of the amygdala (LA), which is an early site of convergence for sensory information about both conditioned and unconditioned stimuli (LeDoux et al., 1990). The LA is thought to send projections to the central amygdala (CeA), which in turn activates output neurons to trigger the expression of fear or anxiety (LeDoux, 2000; Maren and Quirk, 2004; Pari et al., 2004). Studies employing focal lesions, pharmacological manipulations, and electrophysiological recordings, have established a fundamental model of amygdala microcircuitry (LeDoux, 2000; Maren and Quirk, 2004; Ehrlich et al., 2009; Pape and Pare, 2010; Johansen et al., 2011). However, the causal role of specific circuit elements has, due to technical limitations, largely remained elusive. With the advent of optogenetics, it has become possible to manipulate specific elements of fear or anxiety circuitry and probe the neural mechanisms underlying these behaviors.

5.1. Plastic changes in the lateral amygdala mediate fear conditioning

Pavlovian fear conditioning is the most widely used behavioral paradigm to study fear in rodents. In this paradigm, an initially neutral cue, such as a tone or a context (conditioned stimulus; CS), and an aversive stimulus, such as a footshock (unconditioned stimulus; US), are presented to the subject, usually paired with a brief US co-terminating with a long CS (Maren, 2001). After pairing the CS with the US, the CS acquires the aversive property of the US and can alone evoke fear responses, such as freezing. The association of CS and US signals are thought to recruit Hebbian plasticity in LA neurons, where the synaptic inputs that convey weak sensory information about both conditioned and unconditioned stimuli (LeDoux et al., 1990). Although related, fear and anxiety are distinctly different—fear is an emotional reaction triggered by an immediate threat, while anxiety is a state of heightened apprehension in the absence of an immediate threat (Davis et al., 2010).

However, the causal role of this postsynaptic depolarization had never been directly tested until Johansen et al. (2010) substituted the shock-evoked US with direct optogenetic depolarization of LA neurons. The authors expressed ChR2 in the pyramidal neurons of the LA and found that optical stimulation alone was sufficient to evoke a small amount of unconditioned freezing (~10% increase from the baseline) (Fig. 10 and Fig. 6). After sixteen paired stimulations with a CS, the CS alone could evoke the conditioned freezing
response, which indicates that optogenetic depolarization of LA neurons could replace the shock and therefore serve as a "teaching signal" for the CS-US association. However, the authors also noted that LA stimulation was only able to elicit very low levels of freezing behavior, suggesting that nonspecific depolarization of LA neurons is not the only mechanism by which fear conditioning occurs.

CS information can reach the LA via many routes, but in the case of auditory fear conditioning, tone information is conveyed to the LA from both the thalamus and the cortex, including the posterior intralaminar nucleus (PIN), the medial sector of the medial geniculate nucleus (MGm), and the temporal association cortex (TeA) (LeDoux et al., 1984; Romanski and LeDoux, 1992; Campeau and Davis, 1995; Antunes and Moita, 2010). Morozov et al. (2011) directly examined the inhibitory gating in the cortico-amygdala pathway using optogenetics (Fig. 6). The authors expressed ChR2 in the TeA and recorded from GABAergic neurons in the external capsule (EC), where excitatory responses were elicited by laser pulses. Then they recorded from LA neurons, where cutting off the EC or applying picrotoxin (a GABA_A receptor antagonist) enabled the induction of LTP by a STDP protocol (Fig. 6). Therefore, to induce LTP in the TeA-LA pathway, feed-forward inhibition from the EC needs to be blocked, which suggests that the TeA-LA CS input pathway is under the control of this inhibition from the EC. In contrast, when the authors expressed ChR2 in the anterior cingulate cortex (ACC), LTP in LA neurons did not require inhibition of GABA_A receptor-mediated transmission (Morozov et al., 2011).

5.2. Disinhibitory microcircuits in the central amygdala regulate the expression of conditioned fear

LA neurons project to the central amygdala (CeA), either directly or indirectly via the basal nucleus (BA) or the intercalated cells of the amygdala (ITC) (Paré et al., 2004; Pape and Pare, 2010). The CeA is mostly composed of GABAergic medium spiny neurons and is further subdivided into the lateral division (CeL) and the medial division (CeM) (McDonald, 1982; Ciocchi et al., 2010). Emerging evidence suggests that the CeL is critical for the acquisition of conditioned fear, whereas the CeM is the main output structure of the amygdala (Wilensky et al., 2006; Ciocchi et al., 2010). Ciocchi and colleagues expressed ChR2 in the CeM and found that optical stimulation evoked unconditioned freezing (Fig. 1P and Fig. 6). Notably, a larger freezing response was evoked (40–60% increase from the baseline) than the freezing response obtained from stimulation of the LA, consistent with the idea that the CeM is the main output of the amygdala. Given the GABAergic content of the CeM (McDonald, 1982) however, it remained unclear how GABAergic output from the CeA affects downstream structures to trigger conditioned motor and autonomic responses.
Using in vivo single unit recording in awake, behaving mice, Ciocchi et al. (2010) also discovered two functionally distinct types of units in the CeL that acquired opposite responses to the CS, namely CeL\textsubscript{on} and CeL\textsubscript{off} units. In the companion paper, Haubensak et al. (2010) found that a subpopulation of GABAergic neurons within the CeL, marked by the expression of protein kinase C-delta (PKC-\(\delta\)) corresponded to CeL\textsubscript{off} units. Optogenetic stimulation of PKC-\(\delta\) CeL neurons directly inhibited CeM neurons projecting to the periaqueductal gray (PAG)–a region implicated in freezing behavior (LeDoux et al., 1988; Kim et al., 1993; De Oca et al., 1998) and also inhibited PKC-\(\delta\) CeL neurons (Fig. 6). Haubensak and colleagues further provided anatomical evidence that PKC-\(\delta\) GABAergic neurons also project to PKC-\(\delta\) neurons. Based on these results, the authors proposed a model where a CS presentation activates PKC-\(\delta\) CeL neurons, which in turn inhibits PKC-\(\delta\) CeL\textsubscript{off} neurons, thereby disinhibiting CeM output neurons that trigger conditioned fear responses.

The amygdala is heavily influenced by neuropeptide inputs (Veinante and Freund-Mercier, 1997; Asan, 1998; Cassell et al., 1999; Grace and Rosenkranz, 2002; Fuxe et al., 2003; Muller et al., 2007; Jungling et al., 2008; Pinard et al., 2008; Ehrlich et al., 2009), and one such neuropeptide is oxytocin (OT), which has been implicated in social behaviors, attachment, fear, and anxiety (Lee et al., 2009). Huber et al. (2005) observed that OT activates a subpopulation of CeL neurons expressing OT receptors (OTRs) and inhibits CeM neurons. More recently, Knobloch et al. (2012) expressed ChR2 in OT-labeled regions of the hypothalamus and found that stimulating OT terminals in CeL decreased contextual freezing after fear conditioning (Fig. 6). Stimulation was also shown to increase action potential frequencies in CeL neurons and IPSC frequencies in CeM neurons that were abolished by OTR antagonists. These data demonstrate that OTR-expressing CeL neurons inhibit CeM neurons when activated by OT released from hypothalamic terminals.

5.3. **Amygdala circuits for anxiety and their outputs to the bed nucleus of the stria terminalis**

The functional processing of anxiety was also recently discovered in amygdala circuitry (Tye et al., 2011). Based on prior lesion and pharmacology studies, it was proposed that the expression of acute cued fear and sustained anxiety-like responses are mediated by the CeA and the bed nucleus of the stria terminalis (BNST), respectively (Davis et al., 2010). The most common and well-established assays for anxiety include the elevated plus maze test and the open field test (Carola et al., 2002), which were used by Tye and colleagues. The authors expressed ChR2 in the pyramidal neurons of the BLA, which collectively refers to the LA and the BA, and implanted an optical fiber over the CeL. Photostimulation of BLA terminals in the CeL demonstrated an acute and reversible decrease in anxiety-like behavior, whereas the opposite behavioral change was induced by inhibiting the BLA-CeL projection using NpHR (Tye et al., 2011) (Fig. 1Q and Fig. 6). Byrestricting light illumination to the CeL, the authors also showed that selectively stimulating excitatory BLA inputs to the CeL exerts feedforward inhibition on the CeM, supported by findings of Knobloch et al. (2012) showing that OTR-expressing CeL neurons inhibit CeM neurons and decreases fear responses.

Another major output of the amygdala is the BNST, which has also been implicated in the expression of anxiety and contextual fear in mice (Sullivan et al., 2004; Walker et al., 2009; Davis et al., 2010) as well as in the pathophysiology of anxiety disorders in humans (Straube et al., 2007; Yassa et al., 2012). The BNST is extensively interconnected with the amygdala–both the BLA and the CeA heavily project to the BNST, which in turn projects back to the CeA (Dong et al., 2001a, 2001b; Alheid, 2003; Dong and Swanson, 2004). Notably, the BNST and the CeA share many of the downstream structures that are known to mediate the expression of fear or anxiety (Alheid et al., 1995). Their shared connectivity, along with their similarities in cell types and neurochemical makeup, have led to the concept of the “extended amygdala”, which refers to a continuum of structures from the CeA to the BNST (Johnston, 1923; Alheid and Heimer, 1988; Alheid et al., 1993). Recently, Li et al. (2012) optogenetically targeted ChR2-expressing GABAergic CeA projections to the BNST. Stimulating CeA terminals in the BNST evoked inhibitory postsynaptic currents (IPSCs) in BNST neurons, and the amplitudes of these IPSCs were reduced by a kappa opioid receptor (KOR) agonist. This suggests that KOR reduces inhibitory synaptic transmission in the CeA-BNST pathway.

6. **Distributed fear circuitry: Looking at the cortex and hippocampus**

Given the critical importance of fear and anxiety in survival, a distributed network mediating negative valence may have provided an evolutionary advantage in the mammalian brain. Indeed, both the prefrontal cortex and the hippocampus have been heavily implicated in mediating several different facets of fear acquisition, expression, and extinction (Morgan and LeDoux, 1995; Maren et al., 1997; Fanselow, 2000; Milad and Quirk, 2002; Sotres-Bayon and Quirk, 2010). While both the prefrontal cortex and the hippocampus are reciprocally connected with the amygdala, they have been noted to have distinct functions (Kim et al., 1993; Garcia et al., 1999, 1999; Frankland et al., 2004; McHugh et al., 2004; Frankland and Bontempo, 2005; Herry et al., 2008).

In the frontal lobe, the mPFC has been implicated in fear expression and extinction (Sotres-Bayon and Quirk, 2010; Milad and Quirk, 2012). Milad and Quirk (2002) showed that infralimbic neurons fire to the CS only when rats are recalling extinction on the following day, suggesting that extinction potentiates infralimbic activity, congruent with data showing that mPFC inputs selectively target extinction neurons in the BA (Herry et al., 2008). Yizhar et al. (2011) showed that sustained depolarization of mPFC pyramidal neurons using the stable step-function opsin (SSFO), a variant of ChR2 that enables prolonged depolarization induced by a pulse of light, impaired the acquisition of both auditory and context-conditioned fear (Fig. 1R), confirming the importance of mPFC in fear learning.
The auditory cortex has been thought to convey information about auditory cues to the LA during auditory fear conditioning (Campeau and Davis, 1995; Boatman and Kim, 2006), and is shown to undergo plastic changes with fear conditioning (Quirk et al., 1997; Suga and Ma, 2003; Weinberger, 2007). More recently, a study using optogenetics dissected the local inhibitory circuitry and demonstrated its necessity for fear learning (Letzkus et al., 2011) (Fig. 1S). Letzkus and colleagues found that footshock activated layer 1 interneurons in the auditory cortex. Layer 1 interneurons inhibit layer 2/3 parvalbumin (PV)-expressing interneurons, which in turn disinhibit layer 2/3 pyramidal neurons. The authors showed that disinhibition of layer 2/3 neurons is critical for fear learning, as optogenetic stimulation of PV+ interneurons during footshock impaired the acquisition of conditioned fear (Letzkus et al., 2011).

Just ventral to the cortex is the hippocampus, which is well-known for its critical role in the processing of spatial memory and the formation of contextual fear memory (Squire, 1992; LeDoux, 2000). The prevalent view, based on lesion and inactivation studies, was that the CA1 region of the hippocampus is required only for the short-term memory, but not for long-term memory (Kim and Fanselow, 1992; Squire and Alvarez, 1995; Mavel et al., 2004; Squire and Bayley, 2007). Goshen and colleagues demonstrated that the CA1 actually contributes to remote memory recall, using fast optogenetic intervention (Fig. 1T). The authors further showed that fast inhibition of the CA1 and prolonged inhibition of the CA1 (for 30 min) differentially influenced network activity during fear recall (Goshen et al., 2011).

The dentate gyrus (DG) of the hippocampus is thought to play an essential role in the discrimination between contexts (McHugh et al., 2007; Nakashiba et al., 2012). In a unique study, Liu et al. (2012) injected an AAV-ChR2 virus with a tetracycline-responsive-element (TRE) into the DG of c-fos-tetracycline transactivator (tTA) transgenic mice. This unique tool allowed for selective labeling of c-Fos-expressing DG neurons with ChR2 following training-induced neuronal activity in the absence of doxycycline (DOX), and importantly prevented labelling in the presence of DOX. Using this technique, the authors were able to show that optogenetic reactivation of distinct DG neurons activated during a fear conditioning task in a different context was able to induce fear-evoked freezing (Fig. 1U). Importantly, activating a subset of DG neurons not active during fear conditioning did not induce freezing. These data demonstrate that reactivating a distinct subset of task-dependent neurons involved in the formation of a memory is sufficient to induce a behavioral response to that memory (Liu et al., 2012).

7. Cautionary note

Despite all the advantages that optogenetic tools offer, we should exercise caution and carefully characterize each new tool, device, and variant to inform our data interpretation. For example, ChR2 is a non-specific cation channel and hence, opening these channels will allow Ca$^{2+}$ influx into the cell. If ChR2 is used to achieve pathway-specific stimulation of pre-synaptic axon terminals, then neurotransmitter release probability can be changed from normal physiological levels due to Ca$^{2+}$ influx (Schoenenberger et al., 2011). Thorough characterization of the effects of ChR2 mediated Ca$^{2+}$ influx into cells is required prior to interpreting mechanisms of synaptic plasticity that will be uncovered using ChR2.

Furthermore, hyperpolarizing opsins must also continue to be characterized with care under each preparation. Two primary classes of hyperpolarizing opsins including a light-activated proton pump, Archaerhodopsin-3 from Halorubrum sodomense (Arch) (Chow et al., 2010; Mattis et al., 2011), and a light-activated chloride pump, Natronomonas pharaonis halorhodopsin (eNpHR3.0 or NpHR), (Zhang et al., 2007; Gradinaru et al., 2010; Mattis et al., 2011), along with their variants, have been commonly used in numerous model organisms and preparations. Since the development of eNpHR3.0, a NpHR variant with reduced toxicity and improved trafficking to the cell membrane (Gradinaru et al., 2010), many groups have adopted this new opsin and use the acronyms of NpHR and eNpHR3.0 interchangeably. One potential caveat of using light-sensitive proton pumps (including variants in the archeaerhodopsin and bacteriorhodopsin families) under continuous illumination parameters is the alterations in intracellular and extracellular pH, due to the efflux of protons (Zhang et al., 2011). A potential caveat for light-sensitive chloride pumps was raised in a recent report regarding the post-photic inhibition spiking with NpHR, likely attributable to the alteration in the GABA$_A$ receptor reversal potential (Raimondo et al., 2012). While optogenetic tools offer a new level of precision and control in neuroscience research, they present new artifacts that must be considered with care.

8. The future of optogenetics: new targets for potential therapies

While the early years of optogenetic research were aimed at developing new opsins, filling the optogenetic toolbox, and conducting proof-of-principle experiments, recent years have seen a maturation of optogenetics, leading to studies utilizing it to produce novel insights about neural circuit, cellular, and synaptic function. Researchers now have a wide selection of well-characterized viruses, promoters, optical equipment, and surgical protocols that facilitate the adoption of optogenetic techniques without significant barriers to entry. We anticipate that one major area of growth for future applications of optogenetics will be in its integration with existing techniques and its use to functionally dissect circuits with causal relationships to behavior.

The body of research we have presented here, detailing how neural circuits are involved in reward, motivation, aggression, feeding, fear, and anxiety in animal models are significant insights that will be applicable to understanding our own processing of emotional valence in health and disease. The recent proliferation of studies applying optogenetic manipulations to the investigation of disease-relevant behaviors has highlighted the complexity of these behaviors, as well as the subtleties of optogenetic manipulations. Beginning with a study from Covington et al. (2010) showing that nonspecific optical stimulation of ChR2-expressing neurons in the mPFC has anti-depressant-like effects, several optogenetic studies have been published recently on
depression. Warden et al. (in press) recently demonstrated that optogenetic stimulation of glutamatergic pyramidal neurons in the mPFC had no effect on depression-like behavior, but specifically stimulating projections to the dorsal raphe (DRN) elicited escape-related behavior in depression assays, while stimulating projections to the LHb had the opposite effect (Fig. 1V and W).

In a pair of recent experiments on the modulation of depression-related behaviors by the mesolimbic dopamine system, Tye et al. (in press); Chaudhury et al. (in press) examined how VTA dopamine neurons respond in distinct mouse models of depression and how optogenetic modulation can reverse these depression-like phenotypes. Although these studies highlight the complexity of depression-related behaviors and the need for deeper investigation, they both demonstrate that optogenetic manipulations of the VTA-NAc pathway are bidirectionally and causally linked to depression-related behaviors. These emerging studies have the potential to reinvigorate systems-level research in the neural underpinnings of psychiatric disease. Optogenetic approaches have opened the floodgates to understanding the distributed, yet evolutionarily well-conserved, neural circuits mediating emotional valence and motivated behaviors in both adaptive and pathological states.

Acknowledgments

We want to acknowledge Lynne D. Tye and Jonathan Leung for their scholarly input and helpful discussion. K.M.T. was supported by the Whitehall Foundation (2012-08-45), the Picower Neurological Disorder Research Fund, the Picower Institute Innovation Fund, and the Wade Award. E.H.N. was supported by the Integrative Neuronal Systems Center Grant (6926328), the Brain and Cognitive Sciences Special Award (1497200), and the National Science Foundation Graduate Research Fellowship. S.-Y.K. was supported by the Samsung Scholarship. P.N. was supported by the Marcus Fellowship to Honor Leventhal (3891441) and the Brain and Cognitive Sciences Special Award (1497200).

References


neurons in the arcuate nucleus shows enhanced desensitiza-

Rymar, V.V., Sasseville, R., Luk, K.C., Sadikot, A.F., 2004. Neuro-
genesis and stereological morphometry of calretinin-
immunoreactive GABAergic interneurons of the neostriatum.

Schilman, E.A., Uylings, H., Graaf, Y.G., Joel, D., Groenewegen, H.J.,
2008. The orbital cortex in rats topographically projects to central

Schoenenberger, P., Schärer, Y.-P.Z., Oertner, T.G., 2011. Channel-
hodopsin as a tool to investigate synaptic transmission and

J. Neurophysiol. 80, 1–27.


Schultz, W., Dayan, P., Montague, P.R., 1997. A neural substrate of
prediction and reward. Science 275, 1593–1599.

Schultz, W., Romo, R., 1987. Responses of nigrostriatal dopamine
neurons to high-intensity somatosensory stimulation in the

Shabel, S.J., Janak, P.H., 2009. Substantial similarity in amygdala
neuronal activity during conditioned appetitive and aversive
emotional arousal. PNAS 106, 15031–15036.


Siegel, A., Skog, D., 1970. Effects of electrical stimulation of the
nucleus accumbens on the nucleus basalis of Meynert. J. Comp.
Neurol. 139, 329–339.


and plasticity in the auditory system. Nat. Rev. Neurosci. 4,
783–794.

Sugrue, L.P., Corrado, G.S., Newsome, W.T., 2005. Choosing the
greater of two goods: neural currencies for valuation and

Ledoux, J.E., 2004. Lesions in the bed nucleus of the stria
terminals disrupt corticosterone and freezing responses elic-
cited by a contextual cue but not by a specific cue-conditioned

Tan, K.R., Brown, M., Labouèbe, G., Yvon, C., Creton, C., Fritschy,

Tan, K.R., Yvon, C., Turiault, M., Mirzabekov, J.J., Doehner, J.,
neurons of the VTA drive conditioned place aversion. Neuron
73, 1173–1183.

Tecuapeta, F., Patel, J.C., Xenias, H., English, D., Tadros, I., Shah,
Glutamatergic signaling by mesolimbic dopamine neurons in

Thomas, K.M., Drevets, W.C., Whalen, P.J., Eccard, C.H., Dahl, R.E.,
Ryan, N.D., Casey, B.J., 2001. Amygdala response to facial expres-

Threlfell, S., Lalic, T., Platt, N.J., Jennings, K.A., Deisseroth, K., Craig,
S.J., 2012. Striatal dopamine release is triggered by synchronized

Tooby, J., Cosmides, L., 1990. The past explains the present::
emotional adaptations and the structure of ancestral environ-

Tritsch, N.X., Ding, J.B., Sabatini, B.L., 2012. Dopaminergic neu-
rions inhibit striatal output through non-canonical release of
GABA. Nature 490, 262–266.

Tsai, H.-C., Zhang, F., Adamantidis, A., Stuber, G.D., Bonci, A., De
neurons is sufficient for behavioral conditioning. Science 324,
1080–1084.

circuits underlying brain disease in animal models.
Nat. Rev. Neurosci. 13, 251–266.

Tye, K.M., Mirzabekov, J.J., Warden, M.R., Tsai, H.-C., Finkelstein,
J., Kim, S.-Y., Ferenczi, E., Adhikari, A., Thompson, K.R.,
Andalman, A.S. Dopamine neurons modulate the neural
encoding and expression of depression-related behavior.
Nature In Press.

Tye, K.M., Prakash, R., Kim, S.Y., Fenn, L.E., Grosenick, L., Zarabi, H.,
Thompson, K.R., Gradinaru, V., Ramakrishnan, C., Deisseroth, K.,
2011. Amygdala circuitry mediating reversible and bidirectional

Rapid strengthening of thalamo-amygdala synapses mediates

Unghes, M.A., Grace, A.A., 2012. Are you or aren’t you? Chal-
lenges associated with physiologically identifying dopamine

dopamine neurons in the ventral tegmental area by aversive

Veening, J.G., Coolen, L.M., de Jong, T.R., Joosten, H.W., de Boer,
S.F., Koolhaas, J.M., Olivier, B., 2005. Do similar neural systems
subserve aggressive and sexual behaviour in male rats?
Insights from c-Fos and pharmacological studies. Eur. J.
Pharmacol. 526, 226–239.

Veinante, P., Freund-Mercier, M.J., 1997. Distribution of oxytocin-
and vasopressin-binding sites in the rat extended amygdala: a


