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THE HETEROGENEITY OF VENTRAL TEGMENTAL AREA NEURONS: PROJECTION FUNCTIONS IN A MOOD-RELATED CONTEXT

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Abstract

The ventral tegmental area (VTA) in the brain's reward circuitry is composed of a heterogeneous population of dopamine, GABA, and glutamate neurons that play important roles in mediating mood-related functions including depression. These neurons project to different brain regions, including the nucleus accumbens (NAc), the medial prefrontal cortex (mPFC), and the amygdala. The functional understanding of these projection pathways has been improved since the extensive use of advanced techniques such as viral-mediated gene transfer, cell-type specific neurophysiology and circuit-probing optogenetics. In this article, we will discuss the recent progress in understanding these VTA projection-specific functions, focusing on mood-related disorders.

I. INTRODUCTION OF HETEROGENEITY OF NEURONS IN THE VTA

Midbrain dopamine neurons located in the VTA have been shown to play a key role in several disorders including schizophrenia, drug addiction and mood disorders such as depression (Marinelli and White, 2000, Krishnan et al., 2007, Cao et al., 2010, Valenti et al., 2011, Chaudhury et al., 2013, Friedman et al., 2014). Classically, the VTA was thought to consist of dopamine (DA) neurons characterized by slow firing rate, irregular or burst events, and a broad waveform (Yim and Mogenson, 1980, Grace and Onn, 1989). However, studies have shown that while the majority of cells in the VTA are dopaminergic (~70%), there are also small percentages of both GABA (~30%) and glutamatergic (~2–3%) neurons in this region (Yamaguchi et al., 2007, Nair-Roberts et al., 2008). Additionally, certain subpopulations of neurons have been shown to co-release two transmitters (Sulzer et al., 1998, Stuber et al., 2010, Tritsch et al., 2012). Furthermore, distinct VTA DA neurons project to limbic regions including the nucleus accumbens (NAc), medial prefrontal cortex

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(mPFC), as well as the amygdala are important in mood regulation (Wise and Bozarth, 1985).

The advent of optogenetics, in addition to major advances in viral-mediated gene transfer, has allowed for the dissection of neural circuits in both a cell-type and projection-specific manner (Lobo et al., 2010, Lammel et al., 2011, Chaudhury et al., 2013, Tye et al., 2013). Here, we will focus on studies that have investigated both the functional and anatomically distinct circuits. Specifically, we will focus on studies that investigated the VTA neurons and their projections to the NAc, mPFC, and amygdala and their dysfunction in mood disorders.

II. REWARD CIRCUITRY DYNAMICS/FIRING PROPERTIES OF VTA NEURONS

Classically, midbrain DA neurons have been identified by their broad action potential waveforms and two modes of firing patterns, low-frequency tonic firing (1–5 Hz) and transient high-frequency burst or phasic firing (>15 Hz) (Yim and Mogenson, 1980, Grace and Onn, 1989, Lammel et al., 2008, Tsai et al., 2009, Walsh et al., 2013). Further studies performed in non-human primates suggested that phasic activation of DA neurons was found to serve more in denoting the occurrence in reward related-stimuli than actually mediating the hedonic effects of reward (Schultz, 1998b). More specifically, single unit recordings in non-human primates performing an operant task demonstrated that DA neurons could be activated by conditioned, reward predicting stimuli (Schultz, 1998a). Occurrence of reward in the absence of a conditioned stimulus (CS) induces phasic activation of DA neurons. Further, it was seen that when a CS predicted the occurrence of reward phasic firing was elicited immediately following the CS prior to the onset of the reward. Finally, phasic activation of DA neurons occurs following a CS, however, in the failure of a reward, DA neurons are depressed at the exact expected time of the reward. Further studies in non-human primates showed that with multiple predictive stimuli phasic activation only occurred after the first predictive stimuli (Ljungberg et al., 1992). This suggests that it is the unpredictable occurrence of a reward-related stimulus that results in phasic activation.

Interestingly, studies around that time also showed that a small subpopulation of DA neurons exhibit phasic activation in response to aversive stimuli, such as air puff to the hand in non-human primates (Mirenowicz and Schultz, 1996); however, such stimuli were non-noxious. More recently, studies in C57/BL6 mice have shown that VTA DA neurons have substantial phasic activation to noxious stimuli depending on projection or neurochemical identity (Lammel et al., 2011, Lammel et al., 2012). These studies were done using advanced viral techniques that have allowed us to more accurately parse out the different populations of VTA DA neurons.

Initially, many *in vitro* slice recording experiments, performed both in mice and rats, suggested that VTA DA neurons were a homogenous population (Ungless et al., 2001, Argilli et al., 2008, Chen et al., 2008, Stuber et al., 2008), based on the presence of low-frequency pacemaker activity, a broad action potential, or the presence of an I_h current mediated by hyperpolarization-activated cyclic nucleotide-gated cation channels (HCN channels) (Kitai et al., 1999, Shi, 2009). In fact, some studies performed both in C57/BL6

mice and Sprague-Dawley rats, identified GABA neurons of the VTA as those lacking an I_h , thus possibly including those DA neurons now known to lack this current and skewing the interpretation of results (Ungless et al., 2001, Argilli et al., 2008). Notably, the physiological burst firing that is exhibited in VTA DA neurons is absent in *in vitro* brain slice preparation, suggesting that it is a connectivity property only seen *in vivo* (Grace and Onn, 1989). It is important to note that the criteria for identifying VTA DA neurons have generated some controversy (Ungless and Grace, 2012). Specifically, recent studies performed in both C57/BL6 and DBA/2J mice, as well as in TH-Cre rats, have shown that some VTA DA neurons have either a small or no I_h current that is dependent upon its projection region (Ford et al., 2006, Lammel et al., 2008, Zhang et al., 2010, Witten et al., 2011, Friedman et al., 2014). Other studies in albino rats of the Wistar-derived strain and in C57/BL6 mice have shown that not all VTA DA neurons undergo DA-induced inhibition (Bannon and Roth, 1983, Lammel et al., 2008). These studies suggest that VTA DA neurons are not in fact homogenous, but exhibit varying physiological characteristics (Table 1).

III. NEUROCHEMICAL HETEROGENEITY OF VTA NEURONS

Early *in vitro* electrophysiological studies, performed in Sprague-Dawley rats, classified DA neurons of the VTA as the primary population of neurons (Grace and Onn, 1989, Schultz, 1998a). However, later studies note that other populations of cells also exist within the VTA, GABAergic, as well as glutamatergic neurons (Nair-Roberts et al., 2008). Interestingly, studies performed in guinea-pigs show a small set of VTA neurons, some of which are dopaminergic that distinctly hyperpolarized to serotonin and opioids (Cameron et al., 1997). The neurochemical identities of all of these neurons still remain uncharacterized.

GABAergic neurons within the VTA of Sprague-Dawley rats, exhibit a large amount of heterogeneity with a large range of action potential durations, firing rates, as well as both $I_h(+)$ and $I_h(-)$ cells (Margolis et al., 2012). They constitute approximately 15–20% of the entire neuronal population (Margolis et al., 2012) and synapse onto both DA and non-DA VTA neurons (Bayer and Pickel, 1991, Omelchenko and Sesack, 2009). Similar to DA VTA neurons, GABAergic VTA neurons may also play diverse roles in behavioral responses.

Some neurons in the VTA of both Sprague-Dawley rats and VGLUT1 knockout mice, express vesicular glutamate transporter 2 (VGLUT2), a marker of glutamatergic neurons, and are 2–3% of the total neuronal population, being located primarily in the rostro-medial portion of the VTA (Freneau et al., 2004, Nair-Roberts et al., 2008). All cells contain glutamate for their role in protein synthesis, however, for exocytotic release, the VGLUTs are required (Reimer and Edwards, 2004, Takamori, 2006). Studies of cultured DA neurons express high levels of VGLUT2 (Dal Bo et al., 2004). Post-mortem brain analysis studies have used *in situ* hybridization to show that VTA DA neurons express VGLUT2 mRNA in the intact brain (Dal Bo et al., 2008, Berube-Carriere et al., 2009). Additionally, 25% of DA neurons in the midbrain of a mouse express VGLUT2 at birth as measured by single-cell RT-PCR, with this decreasing to 14% at six-weeks of age (Mendez et al., 2008). However, the exact physiological role these neurons play remains to be elucidated.

IV. CIRCUIT HETEROGENEITY OF VTA NEURONS

The mesolimbic circuits are promising targets for the treatment of depressive disorders considering that one of the major symptoms in depression is anhedonia or lack of motivation for reward (Phelps and LeDoux, 2005, Shin et al., 2005, Krishnan et al., 2007, Yehuda and LeDoux, 2007), in addition to playing a key role in a range of motivational behaviors (Bromberg-Martin et al., 2010). The VTA projects to many regions including the NAc, mPFC, and the amygdala (Wise and Bozarth, 1985). However, until recently, the functional contribution of these specific projections had not been investigated.

While it has been established that the VTA-to-NAc circuit is a crucial element in the pathogenesis of stress-related disorders, other areas, such as the mPFC and amygdala are also known to affect these behaviors. Notably, the mPFC both receives innervations from the VTA and sends projections to the VTA and NAc, forming a regulatory feedback mechanism (Nestler and Carlezon, 2006). Additionally, the amygdala is well known to play a role in fear conditioning and restraint stress and heavily innervates the NAc (Nestler and Carlezon, 2006). Previous studies have shown an increase in spontaneous firing and burst firing, *in vitro* and *in vivo* respectively, of VTA DA neurons, in response to both social defeat stress and chronic restraint stress (Anstrom and Woodward, 2005, Krishnan et al., 2007, Feder et al., 2009, Cao et al., 2010). However, it is important to note that only animals that were susceptible, but not resilient, to the chronic social defeat stress exhibited the increase in VTA DA firing activity (Krishnan et al., 2007, Feder et al., 2009, Cao et al., 2010), which was reversed with chronic administration of the antidepressant fluoxetine (Cao et al., 2010).

a. VTA-to-NAc Projections

Much of the research examining the role of VTA-to-NAc projections in mood disorders has utilized the social defeat mouse model of depression, with C57/BL6 mice as the strain used unless otherwise stated (Berton et al., 2006, Krishnan et al., 2007, Cao et al., 2010, Chaudhury et al., 2013, Friedman et al., 2014, Walsh et al., 2014). Initial work implicated brain derived neurotrophic factor (BDNF) as a key modulator of susceptibility to depressive behaviors in mice, following chronic social defeat (Berton et al., 2006). Floxed BDNF mice, injected with an adeno-associated viral vector expressing Cre recombinase (AAV-Cre) in the VTA, did not exhibit depressive behaviors following social defeat, indicating the presence of the *BDNF* gene in VTA neurons was essential for the onset of the susceptible phenotype. Subsequently, it was shown that BDNF protein was upregulated in the NAc in susceptible animals following defeat, indicating an important target of VTA BDNF release. Additionally, burst firing rates in VTA DA neurons were increased in susceptible individuals (Krishnan et al., 2007). Delivery of genes which either upregulate (Kir2.1) or downregulate (dnK) potassium channels into the VTA, altered VTA neuronal firing rate and resulted in changes in BDNF in the NAc, as well as altered behaviors following defeat. It established a tentative mechanism of action for VTA-to-NAc connections in social defeat depression. Increased firing of VTA DA neurons resulted in BDNF release, which acted on neurons in the NAc. In animals that were resilient to depressive behaviors following social defeat, potassium (K⁺) channels are upregulated in VTA neurons, preventing them from reaching the increased firing rates of the susceptible VTA neurons and, thus, not featuring an

upregulation in BDNF in the NAc. Recently, this K^+ current upregulation has been shown to be dependent upon an extremely larger increase in the I_h current in resilient animals (Friedman et al., 2014).

The development of more targeted neurogenetic tools, including optogenetics, has allowed for greater specificity in investigations of VTA-to-NAc neurons. Studies showed that optogenetic phasic activation of neurons, selectively projecting from the VTA to the NAc, following a subthreshold social defeat, resulted in the onset of a susceptible behavioral phenotype (Chaudhury et al., 2013). This subthreshold stress is a modified version of the chronic defeat protocol, which is unable to induce behavioral changes, but makes the animals more vulnerable to further stress. The phasic, but not tonic, activation of VTA-NAc neurons induced susceptibility, as well as anhedonia, implicating the increased burst firing rates of these neurons as a crucial mediator of social-stressed induced depressive behavior.

Other studies investigating VTA-to-NAc specific projections, using a chronic mild stress model of depression in TH-Cre mice, yielded opposite results (Tye et al., 2013), which is consistent with well-known observations that chronic mild stress downregulates the firing activity of VTA DA neurons (Table 2). Phasic activation of VTA DA neurons in mice that were subjected to an 8–12 week unpredictable chronic mild stress induced an antidepressant like effect. This was measured through an increase in movement of animals exposed to a tail suspension test, as well as an increase in sucrose consumption during a sucrose preference test. It is important to investigate the underlying reasons for the extreme discrepancies between the two papers.

It is possible that in each study, investigators targeted different sub-populations of VTA projecting neurons to the NAc. In fact it has been shown that VTA neurons projecting to the lateral shell have an I_h current and low AMPAR/NMDAR ratio, whereas those projecting to the medial shell do not have an I_h current and have a high AMPAR/NMDAR ratio (Lammel et al., 2011). Studies in both C57/BL6 mice and transgenic mice that express fluorescent proteins in distinct populations of neurons, have shown that VTA DA neurons in the medial posterior VTA project selectively to the NAc medial shell and core, while VTA DA neurons located in the lateral posterior and anterior VTA selectively project to the NAc lateral shell (Lammel et al., 2008, Hnasko et al., 2012).

As previously mentioned, high firing activity of VTA DA neurons has been correlated with increases in BDNF levels in the NAc and plays an important role in determining susceptibility versus resilience (Krishnan et al., 2007). Recent studies have investigated what molecular effector underlies the optogenetically induced susceptibility phenotype that has been seen in the VTA-to-NAc pathway in C57/BL6 mice (Walsh et al., 2014). Utilizing optogenetic and viral-mediated gene transfer approaches, phasic, but not tonic activation of the VTA-to-NAc neurons increased BDNF levels in the NAc, a phenomenon that was blocked by knocking down BDNF in the VTA-to-NAc projection (Walsh et al., 2014). Additionally, the optogenetically induced BDNF was dependent upon the context of stress: phasic activation was not able to increase BDNF in stress-naïve mice, whereas corticotropin-releasing factor (CRF) infusions into the NAc of stress-naïve mice prior to optical stimulation induced an upregulation of BDNF in NAc. These results suggest that CRF, in

addition to optical stimulation, is responsible for the increase in BDNF seen in the NAc. Finally, stress-naïve animals that received CRF infusions into the NAc, but no optical stimulation during behavior, showed no changes in BDNF levels. Together these results show that phasic firing alone of the VTA-to-NAc pathway does not regulate BDNF levels, and is dependent on the context of a stress, which is mediated by CRF acting in the NAc.

More recent studies show that resilient mice have an even larger I_h current when compared to susceptible mice, which occurs in parallel with increased K^+ channel currents. Unexpectedly, further enhancing I_h current in susceptible mice induces resilience-like compensatory K^+ current upregulation, a homeostatic plasticity. Interestingly, this induced homeostatic plasticity occurs only in VTA-to-NAc projecting neurons, but not in the non- I_h VTA-to-mPFC projection (Friedman et al., 2014).

b. VTA-to-mPFC Projections

Studies in C57/BL6 mice have found that DA neurons located in the medial posterior VTA selectively project to the mPFC (Lammel et al., 2008). Additionally, these neurons have been found to have unique functional properties compared to the “classical” functional features previously described. Specifically, they have the ability to fire persistently at high frequencies (>20Hz), have significantly long waveforms, as well as much smaller afterhyperpolarizations (AHPs). Additionally, these projection-specific neurons have very low expression of the plasma membrane dopamine transporter (DAT) and lack functional somatodendritic DA type 2 (D2) receptors. Interestingly, there have been conflicting reports of the magnitude of I_h found in prefrontal projecting VTA neurons, with one study showing a high I_h current (Margolis et al., 2006) and another showing minimal, if any I_h current (Lammel et al., 2011). This may be due to methodological differences with the report of higher I_h being done in 4-week old Sprague-Dawley rats and whereas the other study was done in 3-month old C57/BL6 mice. A recent study shows a small I_h current in this projection pathway, and interestingly, this current is not altered in either susceptible or resilient mice (Friedman et al., 2014). Unlike in VTA-to-NAc neurons, there is no I_h current upregulation when HCN2, a channel isoform that mediates I_h current, selectively expresses in VTA-to-mPFC neurons (Friedman et al., 2014).

Through the use of optogenetics and viral-mediated gene transfer, this projection-specific pathway has been investigated in studies including those investigating the role in depression and in response to aversive stimuli. Specifically, studies showed that C57/BL6 mice subjected to a subthreshold social defeat exhibited social avoidance behavior, but no change in sucrose preference, with halorhodopsin (NpHR) depression of the VTA-to-mPFC pathway (Chaudhury et al., 2013). Furthermore, phasic activation of this projection-specific pathway showed no behavioral changes in addition to no changes in anhedonic-like behaviors.

Studies in C57/BL6 mice that further dissected the VTA-to-mPFC pathway examined the role of phasic stimulation of the laterodorsal tegmentum (LDTg), a region that selectively projects to VTA-to-mPFC neurons (Lammel et al., 2012). Through the use of an intricate optogenetic approach, activation of the LDTg, which excited the VTA-to-mPFC neurons, resulted in a conditioned place aversion. These studies suggest a functional role of the VTA-to-mPFC pathway in both depressive and aversive signaling.

c. VTA-to-Amygdala Projections

Currently, there is a paucity of literature examining those VTA neurons that project to the amygdala. While no studies have directly examined modulation of VTA-to-amygdala neuronal activity, studies using pharmacological agents and viral restoration of dopamine synthesis have established the necessity of dopamine in the basolateral amygdala (BLA) for fear conditioning (Fadok et al., 2010, de Oliveira et al., 2011). Specifically, it was found that D2 antagonism in the BLA reduces the fear-potentiated startle after classical fear conditioning (Greba et al., 2001, de Oliveira et al., 2011). However, it has also been shown that D1 receptor antagonism can similarly impair fear potentiated startle (Lamont and Kokkinidis, 1998). Interestingly, both studies occurred in Wistar rats. This dopamine efflux into the BLA seems to be regulated by mineralocorticoid receptor activation in the VTA (de Oliveira et al., 2014). These studies strongly suggest that VTA dopamine efflux in the BLA regulates fear responses, but more direct investigations modulating the activity of these neurons is necessary. Recent studies in the social defeat mouse model suggest that the firing activity of VTA-to-amygdala neurons may consistently decreased in both susceptible and resilient groups (personal communication), suggesting that this projection could be responsible for anxiety phenotype observed in both groups.

A small number of studies performed examining electrophysiological properties, has conflicting results. One study reports that VTA DA neurons that project to the basolateral amygdala (BLA) reside mostly in the medial posterior portion of the VTA of C57/BL6 mice (Lammel et al., 2008), yet another reports the majority of BLA-projecting VTA DA neurons to reside in the anterior lateral portion of DBA/2J mice (Ford et al., 2006). Both of these studies use retrobeads for the identification of projection specific neurons, however their coordinates for injection of the beads into the BLA substantially differ. Not surprisingly, the electrophysiological properties they report for these two different populations of BLA projecting neurons also differ. While one study reports the absence of an I_h current (Lammel et al., 2008), the other reports an I_h current that is larger than those VTA DA neurons projecting to the NAc, although still much smaller than DA neurons of the substantia nigra pars compacta (SNc) (Ford et al., 2006). Additional electrophysiological properties of these medial posterior BLA-projecting VTA DA neurons were characterized by Lammel et al., including increased firing rate in response to current injection that was greater than 10 Hz, outside the classical defined range for DA neurons, as well as a small AHP (Lammel et al., 2008).

To further complicate the story while one study in DBA/2J mice show minimal response of BLA-projecting neurons to application of the kappa opioid receptor (KOR) agonist U69593 (Ford et al., 2006), another study, in rats, found U69593 application to be hyperpolarizing (Margolis et al., 2008). This same study in rats also show a lack of D₂R autoinhibition upon application of quinpirole and a short action potential (AP) duration (~2ms), while the afore mentioned Lammel study reported autoinhibition upon application of dopamine and a long AP waveform (~6ms). The vast discrepancies between these studies suggest the complicated nature of the VTA-to-amygdala projections and further highlight how different populations of VTA DA neurons, even projecting to the same brain region, can exhibit vastly different properties.

V. CONCLUSIONS

This review aims to illustrate the heterogeneity of the VTA DA neurons, with different subpopulations projecting to the NAc, mPFC, and amygdala. We specifically focused on both classical findings of these specific neurons, as well as recent studies that have investigated the roles of these neurons particularly in depression, as well as aversion.

It is important to take into consideration that many of the studies reported different as well as opposing findings. To address these issues, the technical aspects utilized when conducting experiments particularly the projection-specific neurons must be assessed (Table 3).

Specifically, the opposing results of phasic stimulation of the VTA-to-NAc pathway being both pro- and anti- depressive (Chaudhury et al., 2013) could be due to targeting of specific projections, housing conditions of the animals, or even due to the difference in the types of stressors and timing of stimulation. Additionally, the opposing findings of the existence or lack thereof an I_h current in the VTA-to-mPFC (Margolis et al., 2006, Lammel et al., 2011) pathway could be due to the age of the animals, as well as the specific coordinates. These discrepancies could also be due to the fact that these studies were performed in different species, C57/BL6 mice and Sprague-Dawley rats. While the most promising results among different species are those that are most similar, a direct comparison between transgenic mouse lines that allow for cell type specific manipulations and primates would be difficult. Further studies investigating the contribution of the VTA-to-amygdala pathway also showed several opposing findings that seem in large part to be due to the vast differences in coordinates. An important consideration for future studies is to form a standard protocol, particularly in identification and targeting of particular circuits.

A more targeted approach with increased specificity may also allow for a greater understanding of the neural circuitry underlying many psychiatric disorders. Additionally, it would allow for a greater understanding of which brain regions to target for procedures such as deep brain stimulation. While several studies have used optogenetics in rodents, current research is beginning to use this technique in nonhuman primates. Although it is still in its infancy, using this tool to dissect circuit specific mechanisms in these models will further the translational capacity of basic findings.

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Abbreviations

VTA	ventral tegmental area
NAc	nucleus accumbens
mPFC	medial prefrontal cortex
DA	dopamine
CS	conditioned stimulus

HCN channels	hyperpolarization-activated cyclic nucleotide-gated cation channels
VGLUT2	vesicular glutamate transporter 2
BDNF	brain derived neurotrophic factor
AAV-Cre	adeno-associated viral vector expressing Cre recombinase
CRF	corticotropin-releasing factor
AHPs	afterhyperpolarizations
DAT	dopamine transporter
D2	DA type 2
NpHR	halorhodopsin
LDTg	laterodorsal tegmentum
BLA	basolateral amygdala
SNe	substantia nigra pars compacta
KOR	kappa opioid receptor

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Highlights

- VTA neurons are heterogeneous play an essential role in mood-related disorders
- In addition to dopamine, GABAergic and glutamatergic neurons are also in the VTA
- VTA neurons project the nucleus accumbens, medial prefrontal cortex, and amygdala.

Table 1

The firing activity and ion channel function of projection-specific VTA DA neurons

Projection	Species	Physiological Properties	Reference
VTA-to-NAc Lateral shell Medial shell	C57/BL6	I_h current/low AMPAR/NMDAR ratio No I_h current/high AMPAR/NMDAR ratio	Lammel et al., 2011 Lammel et al., 2011
VTA-to-mPFC	Sprague-Dawley rats C57/BL6	High I_h current Low I_h current	Margolis et al., 2006 Lammel et al., 2011 Friedman et al., 2014
VTA-to-Amygdala	C57/BL6 DBA/2J	No I_h current High I_h current	Lammel et al., 2008 Ford et al., 2006

Table 2

The firing activity and ion channel function of VTA DA neurons.

Stressors	VTA DA Neurons	Reference
Social Defeat Stress	<ul style="list-style-type: none"> • ↑ spontaneous/burst firing, ↔ K^+ channel currents, ↑ I_h current, in susceptible animals. • No change in spontaneous/burst firing, ↑ K^+ channel currents, ↑↑ I_h current, in resilient animals 	Krishnan et al, 2007; Cao et al, 2010; Chaudhury et al, 2013; Friedman et al 2014; Feder et al, 2009
Chronic Restraint Stress	↑ spontaneous/burst firing	Anstrom and Woodward, 2005
Chronic Mild Stress	↓ spontaneous/burst firing	Tye et al, 2013

Table 3

Projection-specific alterations induced by various optogenetic manipulations

Projection	Species	Stimuli	Manipulation	Outcome	Reference
VTA-to-NAc	C57/BL6 mice	Subthreshold Defeat	• Phasic activation	Susceptibility/Anhedonia	Chaudhury et al., 2013
	TH-Cre mice	Chronic Mild Stress	• Phasic activation	Anti-depressant	Tye et al., 2013
	fl/fl BDNF mice	Subthreshold Defeat	• Phasic activation	Blocked susceptibility	Walsh et al., 2014
	C57/BL6 mice	Stress Naïve	• Phasic activation	Blocked ↑ BDNF	Walsh et al., 2014
VTA-to-mPFC			• CRF infusion into NAc with phasic activation	Induced ↑ BDNF	
			• CRF infusion into NAc without phasic activation	Blocked ↑ BDNF	
	C57/BL6 mice	Subthreshold Defeat	• Phasic inhibition	Susceptibility/No change in anhedonia	Chaudhury et al., 2013
VTA-to-Amygdala			• Phasic activation	No change in social behavior or anhedonia	
	C57/BL6	Stress Naïve	• LDTg activation	Conditioned place aversion	Lammel et al., 2012
	Wistar rats	Fear Conditioning	• D2 antagonist infusion into BLA	Decrease fear-potentiated startle	De Oliveira et al., 2011
	Wistar rats	Fear Conditioning	• D1 antagonist infusions into BLA	Decrease fear-potentiated startle	Lamont et al., 1998