Review

Optogenetic insights into striatal function and behavior

Jeffrey D. Lenz, Mary Kay Lobo *

Department of Anatomy and Neurobiology, Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

HIGHLIGHTS

• Optogenetics provides insight into distinct behavioral roles of striatal cell subtypes.
• Selective activation of the two striatal MSNs produces opposite behavioral responses.
• Modulating activity in striatal TANs alters rewarding behaviors and dopamine release.
• Modulating activity in striatal afferents influences reward and reinforcement.
• Temporal control of striatal signaling molecules alters reward.

ABSTRACT

Recent breakthroughs in optogenetic technologies to alter neuronal firing and function with light, combined with cell type-specific transgenic animal lines, has led to important insights into the function of distinct neuronal cell subtypes and afferent connections in the heterogeneously complex striatum. A vital part of the basal ganglia, the striatum is heavily implicated in both motor control and motivation-based behavior; as well as in neurological disorders and psychiatric diseases including Parkinson's Disease, Huntington's Disease, drug addiction, depression, and schizophrenia. Researchers are able to manipulate firing and cell signaling with temporal precision using optogenetics in the two striatal medium spiny neuron (MSN) subpopulations, the striatal interneurons, and striatal afferents. These studies confirmed the classical hypothesis of movement control and reward seeking behavior through direct versus indirect pathway MSNs; illuminated a selective role for TANs in cocaine reward; dissected the roles of glutamatergic and dopaminergic inputs to striatum in reward; and highlighted a role for striatal signaling molecules including an adrenergic C-protein coupled receptor in reward and the rho-GTPase Rac1 in cocaine reward and cocaine induced structural plasticity. This review focuses on how the evolving optogenetic toolbox provides insight into the distinct behavioral roles of striatal cell subpopulations and striatal afferents, which has clinically relevant implications into neurological disorders and psychiatric disease.

© 2013 Elsevier B.V. All rights reserved.

Contents

1. Introduction .......................................................................................................................... 00
2. Medium spiny neuron manipulation .................................................................................... 00
3. Striatal interneurons ........................................................................................................... 00
4. Striatal afferents ................................................................................................................. 00
5. Optogenetic tools to investigate molecular mechanisms of striatal behaviors .................. 00
6. Conclusion and future directions ....................................................................................... 00

References ................................................................................................................................ 00

Abbreviations: Dstr, dorsal striatum; NAc, nucleus accumbens; MSN, medium spiny neuron; TAN, tonically active cholinergic interneuron; FS, fast spiking interneuron; LTS, low threshold spiking interneuron; SN, substantia nigra; VTA, ventral tegmental area.

* Corresponding author. Tel.: +1 410 706 8824; fax: +1 410 706 2043.
E-mail address: mklobo@umaryland.edu (M.K. Lobo).

0166-4328/S – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2013.04.018

Please cite this article in press as: Lenz JD, Lobo MK. Optogenetic insights into striatal function and behavior. Behav Brain Res (2013),
http://dx.doi.org/10.1016/j.bbr.2013.04.018
1. Introduction

Within the last decade, optogenetics has made a profound impact on the field of neuroscience, allowing researchers to probe functioning intact brain circuits in vivo. Optogenetics, a term coined by Deisseroth and Boyden who have been instrumental in developing this technology, involves two components: (1) the genetic targeting of opsins proteins, sensitive to specific wavelengths of light, which can be targeted to specific neuronal populations; (2) the use of light to activate these proteins to modulate cellular functions, including altering membrane potentials in a physiologically relevant time course, as well as G-protein coupled signaling and intracellular signaling with precise temporal precision [1–4] (Fig. 1, Table 1). The most utilized optogenetic tools include the depolarizing channelrhodopsins (ChRs), light gated cation channels to activate neuronal firing and halorhodopsin, a hyperpolarizing light driven chloride pump to silence neurons. The basal ganglia (BG), specifically the striatum, is one system that researchers have begun to explore behavioral functions using optogenetic tools. This review will detail the current findings in striatal function and behavior using optogenetics. More detailed accounts of the optogenetic toolbox or utilization of optogenetics in other neural systems can be found in several recently published reviews [3–5].

The striatum is broadly implicated in action selection including motor behavior, decision-making, and motivational processes [6–10] and has been implicated in neurological disorders and neuropsychiatric diseases such as Huntington’s Disease, Parkinson’s Disease, schizophrenia, depression, and addiction [11,12]. These behaviors and brain diseases are mediated through different striatal regions, the dorsal striatum (dStr) predominantly mediates motor behaviors and decision making while the ventral striatum (a.k.a. nucleus accumbens; NAc) mediates motivational processes such as reward and hedonism [7–10].

Striatal neuronal subtypes are heterogeneously interspersed throughout the striatum and characterized as described below (Fig. 1). GABAergic medium spiny neurons (MSNs) compose 95% of striatal neurons while the remaining 5% are interneurons. The interneurons include large tonically active cholinergic neurons (TANs) that receive glutamatergic inputs from cortex and thalamus, GABAergic inputs from MSNs, and dopaminergic inputs from the SN/VTA. TANs synapse primarily onto MSNs and other TANs [7,13,14]. Another subclass of striatal interneurons are the fast spiking GABAergic (FS) interneurons that express parvalbumin and are similar to the FS interneurons found in the hippocampus and cortex. FS interneurons receive inhibitory inputs from other interneurons, inhibitory inputs from the globus pallidus, as well as excitatory glutamatergic inputs from the thalamus and cortex, with their primary inhibitory synapses occurring on MSNs [12,15–19].

A final distinct group of interneurons are the low threshold spiking (LTS) interneurons, which express somatostatin, neuropeptideY, and nitric oxide synthase [20–24]. LTS neurons are implicated in long-term plasticity, receive glutamatergic inputs from the thalamus and cortex, and synapse directly onto MSNs [16,24–26].

The MSNs are subdivided into two subtypes based on their axonal targets: striatonigral and striatopallidal MSNs. Striatonigral MSNs project to globus pallidus internal (GPI), ventral pallidum (VP) and midbrain regions including substantia nigra (SN) and ventral tegmental area (VTA) while striatopallidal MSNs project to the globus pallidus external (GPe) and VP [27–30]. The two MSN subtypes are further distinguished by their differential expression of various genes; most notably G-protein coupled receptors and neuropeptides. Striatonigral MSNs express dopamine receptor 1 (D1), muscarinic receptor 4 (Chrm4), substance P, and dynorphin while striatopallidal MSNs express dopamine receptor 2 (D2), adenosine receptor 2a (Adora2a), G-protein coupled receptor 6 (Gpr6), and enkephalin [27,31–33]. These two MSN subtypes are heterogeneously distributed in striosomes and matrix compartments in dStr and NAc. The patch vs. matrix compartments receive input from different cortical regions, with differentiated outputs to the SN/VTA, and each region has specialized gene expression [27,34–36]. The dStr is further subdivided into dorsal medial striatum (dMdStr) and dorsal lateral striatum (dLdStr), which have differential inputs and outputs [37–39]. The NAc is divided into core and shell regions, which also have distinct inputs from the cortex and different outputs to BG targets [40]. The projections from the striatum through BG output nuclei are classified into the direct and indirect pathways. The direct pathway, stemming from the striatonigral D1+ MSNs, is implicated in movement initiation, reinforcement,
Table 1
Optogenetic manipulation of striatal neurons.

<table>
<thead>
<tr>
<th>Opsin</th>
<th>Action</th>
<th>Light</th>
<th>Behavioral findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChR2</td>
<td>Depolarizing, non-selective ion channel</td>
<td>Blue light 473 nm</td>
<td>D1+ MSN activation in dorsal striatum increases spontaneous locomotion &amp; rescues 6-OHDA lesion motor deficits.</td>
<td>Kravitz et al. 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unilateral D1+ MSN activation in dStr shifts responses for reward toward the contralateral side of the stimulation.</td>
<td>Tai et al. Nat Neurosci 2012</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D1+ MSN activation in NAc enhances cocaine place preference.</td>
<td>Lobo et al. Science 2010</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D1+ MSN activation in NAc after cocaine exposure induces locomotion.</td>
<td>Lobo et al. Science 2010</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D2+ MSN activation in dStr decreases motor initiations and increases freezing and bradykinesia.</td>
<td>Kravitz et al. Nature 2010</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non selective NAc activation enhances cocaine place preference.</td>
<td>Lobo et al. Science 2010</td>
<td></td>
</tr>
<tr>
<td>eNpHR3.0</td>
<td>Hyperpolarizing, Chloride pump</td>
<td>Yellow light 560 nm</td>
<td>ChAT inhibition in NAC blocks cocaine place preference.</td>
<td>Witten et al. Science 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-selective NAc inhibition suppresses reinstatement after cocaine self administration.</td>
<td>Stefanik et al. Addict Biol 2012</td>
<td></td>
</tr>
<tr>
<td>LOV-Rac1</td>
<td>Activates Rac1</td>
<td>Blue light 473 nm</td>
<td>LOV-Rac1 prevents cocaine place preference and blocks cocaine mediated dendritic plasticity.</td>
<td>Dietz, et al. Nat. Neurosci. 2012</td>
</tr>
</tbody>
</table>

and reward seeking. The indirect pathway, composed of the striatal-pallidal D2+ MSNs, antagonizes the direct pathway by inhibiting movement, promoting punishment, and inhibiting reward seeking [34,41,42]. Despite the known cytoarchitecture of the striatum, the heterogeneity of this structure has posed a major challenge in identification and manipulation of functional and molecular components in specific striatal cell subtypes. This barrier was overcome with the development of fluorescent reporter and Cre-recombinase driver BAC transgenic lines within the last 15 years [27,28,43–48]. Many striatal cell subtype lines, including those expressing fluorescent reporters and Cre-recombinase in the two MSN subpopulations and in striatal interneurons, have become vital tools in elucidating striatal function and behavior [32,49–54]. Many researchers have recently taken advantage of transgenic lines expressing Channelrhodopsin 2 (ChR2) in striatal neurons or used Cre-driver lines coupled with adeno-associated viral (AAV) double inverted open reading frame (DIO) vectors to selectively express optically activated proteins in a specific brain region for optogenetic manipulation. A detailed protocol by Cardin et al. eloquently outlines exactly how to execute this experiment in virtually any brain region [55]. Furthermore, the first research article by Tsai, et al. to utilize the DIO-AAV-ChR2 vector in combination with Tyrosine hydroxylase (TH)-Cre BAC transgenic mice to selectively express ChR2 in VTA dopamine neurons provides an exemplary model of optogenetic manipulation of specific cell types in vivo to examine neuronal correlates of behavioral encoding [56].

2. Medium spiny neuron manipulation

The two MSN subtypes through the direct vs. indirect pathways provide opposing but balanced output through the BG circuit to promote normal function and behavior. This classical BG model was initially proposed to describe balanced movement and coordination through the motor pathways arising from these two MSN subtypes and also provides a framework for the behavioral pathology, including unbalanced movement and coordination of motor disorders [41]. The BG model hypothesizes that activation of the direct pathway facilitates movement while activation of the indirect pathway leads to movement inhibition [41,57,58]. With the advances in optogenetics to alter cell firing or inhibit neurons, it was recently possible to test this classical hypothesis of the function of the direct and indirect pathways in striatal motor function and motor disorders [50,59–61]. Using the Cre recombinase dependent DIO-AAV-ChR2 virus to express ChR2 in each MSN subtype in D1-Cre or D2-Cre BAC transgenic lines, Kravitz et al. were able to selectively activate dorsal striatal direct or indirect pathways MSNs. Light activation of the
D1+ MSNs caused a significant increase in movement and reduced freezing in an open field, while activation of the D2+ MSNs resulted in increased freezing, bradykininesia and decreased motor initiations. Using 6-hydroxydopamine lesions as a model of Parkinson's disease the authors demonstrated that D1+ MSN activation rescued deficits in freezing, bradykininesia and locomotor initiation [50]. These findings demonstrated the ability to selectively activate distinct cell subtypes within the heterogeneous striatum and confirmed that the direct and indirect BG pathways have opposing influences on motor control. Furthermore, this study addressed a need to fully characterize the cell type specific contributions of BG pathways in motor disorders during the progression of the disease. Finally this study highlights the relevance of alternative treatments, aimed at targeting specific neurons and/or circuits, in motor disorders.

The role of the direct vs. indirect pathway in motivation has been poorly understood until recently. Most data implicated a direct role for D1+ MSNs in drug or natural reward behavioral encoding as evidenced by induction of immediate early genes in D1+ MSNs in response to drugs of abuse or natural reward [62–65]. Furthermore, overexpression of the FosB gene isoform, deltaFosB, in D1+ MSNs leads to enhanced drug and natural reward as well as resiliency to social defeat stress [66–70]. Ablating D2+ MSNs in NAc enhanced amphetamine conditioned place preference, which demonstrated a role for D2+ MSNs in inhibiting drug reward [60]. Optogenetic studies have provided direct confirmation for the role of MSN subtypes in motivation. Our group addressed the selective contribution of NAc D1+ and D2+ MSNs in reward seeking behaviors. We selectively expressed ChR2 in D1+ or D2+ MSNs in the NAc using D1-Cre and D2-Cre mouse lines combined with DIO-AAV-ChR2. We then selectively activated either D1+ or D2+ MSNs during cocaine conditioning in a conditioned place preference test, a behavioral paradigm that provides an indirect measure of reward. This study demonstrated that activating D1+ MSNs with 10 Hz frequency stimulation resulted in enhancement in preference for a sub-threshold dose of cocaine (5 mg/kg), a dose that did not elicit a response in D1-Cre mice expressing the control DIO-AAV-EYFP virus. In contrast, the 10 Hz activation of D2+ MSNs attenuated preference for 7.5 mg/kg cocaine [49]. Moreover, we also used a non-selective Herpes Simplex Viral (HSV) ChR2-mCherry vector to express the ChR2 in all NAc neurons. Mice receiving non-selective excitation of the NAC displayed enhanced preference for cocaine (5 mg/kg) suggesting an overriding direct pathway effect. This is consistent with a recent study which used AAV vectors to non-selectively express, in all neurons of the NAC core in rats, either the third generation halorhodopsin chloride pump (eNhHR3.0) or the archaerhodopsin proton pump (ArchT). This study demonstrated that inhibition of NAC core neurons attenuated cocaine seeking during reinstatement, by either cue + drug or drug alone [71]. The role of these two subtypes of MSNs in the core vs. shell of NAc remains largely unanswered due to technical limitations, including the relative difficulty in targeting these discrete areas in mouse. However, with the recent development of rat BAC transgenic lines, studies examining these striatal subtypes in core vs. shell, during complex operant behaviors such as drug self-administration are on the horizon [47,72].

In our D1+ and D2+ NAC MSN cocaine study we also evaluated locomotor responses during activation of each MSN subtype. Unlike the dorsal striatum, we found that activation of either MSN subtype in NAC did not alter locomotor activity. However, mice that received repeated doses of cocaine (15 mg/kg) displayed enhanced locomotor responses when D1+ MSNs where activated with optogenetics, 24 h after the last dose of cocaine [49]. This data suggests that direct pathway NAC neurons are sensitized to activating stimuli after cocaine exposure. Recent studies using alternative methodologies to silence or ablate D1+ or D2+ MSNs [59,60,73] confirmed our optogenetic findings and the hypothesized actions of the direct pathway neurons in promoting drug reward and/or drug locomotor responses, while indirect pathway neurons promoted opposite responses.

Recently, two groups have used selective optogenetic stimulation in D1+ and D2+ dorsomedial striatal MSNs to further examine their roles in reinforcement. Tai et al. used a spatial alternative forced-choice switching task in D1-Cre and D2-Cre mice injected with AAV-Dio-ChR2 in the dorsomedial striatum (dmStr) [74]. Briefly, the task included left or right nose poke triggers that were alternatively assigned as the active trigger, to a reward port for water, within sessions. To inform their choices in the task, mice relied on recent reward history to assess whether water would be delivered from one of two reward ports. Unilateral stimulation (10 and 20 Hz bursts) of D1+ MSNs caused a shift toward the trigger contralateral of the dmStr side stimulated, while D2+ MSN stimulation (3, 10, and 20 Hz burst) shifted animals toward triggers ipsilateral of stimulation. The authors conclude that these spatial selection biases were shifts in action value and suggest that activity in distinct populations of striatal neurons exerts opposing biases on the selection of goal-directed responses [74]. Kravitz et al. also used D1-Cre and D2-Cre mice, transected with AAV-Dio-ChR2, which were placed in operant chambers that contained capacitive touch triggers; one that activated a blue laser and the other inactive [75]. Stimulation of D1+ MSNs in dmStr resulted in persistent increased light paired trigger contacts while D2+ MSN activation caused a transient decrease in light paired trigger contacts. Interestingly, dopamine antagonists did not prevent these behaviors. This study also demonstrated that D1+ dmStr MSN activation promoted place preference for a laser paired chamber, whereas no place preference or aversion was observed after D2+ dmStr MSN activation [75]. Unlike this study, we were unable to drive a place preference for a laser-paired chamber in the absence of rewarding stimuli (i.e. cocaine) [49]. These discrepancies could be accounted for by differences in laser frequency, and laser duration (time on/off), varying opsin expression, or to the targeting of different striatal regions (dmStr vs. NAc) and their differential afferent and efferent connections. The Kravitz, et al. study demonstrated that very low doses of DA antagonists did not alter the operant reinforcing behaviors whereas our study showed a direct modulation of cocaine (which directly blocks DA reuptake from the synapse and strongly increases striatal dopamine) rewarding behaviors. Future studies examining optogenetic manipulation of dStr in the presence of cocaine or other psychostimulants will be necessary for a direct comparison between dStr and NAc.

Other groups have used optogenetics to examine MSN connectivity within the striatum and MSN connectivity to striatal output nuclei. Using a mouse expressing ChR2 non-selectively in both MSN subtypes, Chuhma et al. demonstrated that MSNs connect to other MSNs and TANs but do not directly connect to FS interneurons. Furthermore, this study and another optogenetic MSN study demonstrated that MSNs project predominately to GABAergic compared to dopamine cells in the SN [76,77]. However, a recent study demonstrates that the larger striatal matrix compartments project to GABA midbrain neurons whereas the smaller striosome compartments project mainly to dopamine neurons [36]. Consistent with D1+ MSNs projecting to midbrain the Chuhma, et al. study demonstrated that MSNs connecting to the SN displayed D1 receptor mediated facilitation; whereas MSN connections to globus pallidus display D2 receptor mediated presynaptic inhibition. Finally, we recently used micro positron emission tomography (microPET) with [18F] 2-fluoro-2-deoxy-D-glucose (FDG) to measure changes in regional brain glucose metabolism in response to ChR2 optogenetic stimulation of the nucleus accumbens (NAc) in awake rats. We found regional changes of enhanced or decreased brain glucose metabolism in NAc connected regions and many of these activated regions correlated well with c-Fos induction after...
optogenetic activation of NAc [78]. Future studies using such imaging techniques with selective activation of NAC MSN subtypes or interneurons will be extremely informative to understand the circuit-wide effects of activating selective striatal cell populations. Finally, studies examining MSN specific connections, within and outside of the striatum, during striatal behaviors will be important in defining the striatal connectome in behavioral encoding.

3. Striatal interneurons

The striatal interneurons comprise roughly 5% of all neurons in this region but despite their small number, studies demonstrate a very important role for these interneurons in BG behavioral output and function. Many optogenetic studies have provided insight into the role of the TANs in striatal function and behavioral encoding. These large choline acetyltransferase (ChAT) positive interneurons were previously implicated in reward prediction, task attention, memory, addiction, and aversive behaviors [14,79–82]. Witten et al. used the BAC transgenic ChAT-Cre mice to selectively drive or inhibit TANs by expressing either ChR2 or eNpHR3.0 in NAc during cocaine reward. They demonstrated that inhibiting eNpHR3.0 expressing TANs with continuous yellow light during cocaine place conditioning (20 mg/kg) significantly decreased preference for the cocaine-paired chamber [51]. Interestingly, they demonstrated that yellow light photoinhibition of TANs expressing eNpHR3.0 caused most striatal neurons to be activated while roughly 24% of sites measured displayed inhibition. In contrast activating TANs using ChR2 (10 Hz blue light) predominantly suppressed firing in most NAC MSNs, however a subset of MSNs’ (19%) were activated. Activation of TANs also did not alter cocaine conditioning. This study demonstrated the behavioral contribution of these interneurons in cocaine reward and suggests a role for TANs in mediating MSN firing, which in turn regulates BG output pathways. It will be interesting to determine if TANs preferentially synapse onto D2+ MSNs since activation of these MSNs attenuates cocaine reward and mediates aversive responses [49,83].

Higley et al. found that selective activation of TANs with ChR2 elicited vGluT3 dependent glutamatergic transmission, specifically by observing glutamatergic currents in MSNs [84]. In contrast, English et al. found that activating ChR2 expressing TANs strongly inhibits MSNs via observed optogenetically elicited IPSCs. They further mimicked the pause-excitation sequence previously observed in TANs by temporal inhibition with eNpHR3.0 to demonstrate that the pause-excitation in TANs inhibited MSN activity in freely moving animals [85]. These opposing findings of TAN excitation can be partially explained by the findings from the English et al. study demonstrating optogenetically elicited EPSCs in neuropeptide Y expressing striatal interneurons, which were highly correlated with the observed MSN IPSCs [85].

Future studies manipulating the pause-excitation neural dynamics in vivo during reinforcement would be interesting since previous studies implicate TANs in encoding cue salience information in reinforcing and aversive behaviors [13,86]. Two recent papers further confirmed the function of TANs in processes relevant to motivation by examining phasic dopamine release after ChR2 activation of TANs. Slice voltammetry studies demonstrated dopamine release in the NAc or dStr after ChR2 expressing ChAT cells were selectively excited with a single pulse [87] or at 10 Hz pulse trains [88]. Depolarization of the TANs also resulted in reliable DA spikes in vivo in anesthetized animals [88]. These findings further implicate a role for TANs in reward behavior by their actions on dopamine release. Future studies investigating optogenetic modulation of dStr or NAc TANs in operant behaviors can provide further insight into their function in reinforcement and motivation. Newly generated ChAT-Cre rat BAC transgenic lines will be instrumental in such studies [47].

Optogenetic manipulation has recently been used in the fast spiking (FS) parvalbumin (PV) GABAergic interneurons in whole cell slice physiology. PV-Cre transgenic mice were transfected with DIO-AAV-ChR2 and optical excitation of FS interneurons created robust inhibitory responses onto MSNs. The same optogenetic excitation of FS interneurons displayed minimal inhibitory responses in TANs, with a small proportion of low-threshold spiking LTS interneurons showing inhibitory responses [89]. Future studies using in vivo optogenetic control of FS interneurons will help to parse out the role of these interneurons in striatal dependent behaviors.

4. Striatal afferents

The optogenetic toolbox has been extensively utilized to research striatal afferent connections. Many of these studies focus on how such connections influence reward-related behaviors. Direct dopamine signaling released from varicosities of dopaminergic VTA projections is directly involved in reward prediction and addiction initiation [90,91]. This dopamine release differentially influences the two MSNs due to differential GPCR signaling through D1 vs. D2 receptors, leading to overall enhanced drive of the direct pathway and inhibition of the indirect pathway [92]. Many of the original optogenetic behavioral studies focused on direct manipulation of dopaminergic (DA) VTA neurons, which are the main DA inputs to NAc, by utilizing TH-Cre mouse or rat knock-in lines combined with DIO-AAV-ChR2. These studies examined phasic dopamine neuron stimulation that is characterized by irregular pacemaking single spike activity interspaced with rapid bursts of typically 2–6 spikes in rapid succession [93] in behavioral encoding. Tsai, et al. demonstrated that ChR2 activation induction of phasic firing of VTA DA neurons using ChR2 led to a positive conditioned place preference [56]. Although they did not evaluate VTA-NAc specific projections in this behavior, they demonstrated, using voltammetry, that optogenetic phasic firing of VTA DA neurons enhances dopamine release in NAc. This confirmed the direct effect of VTA dopamine release in the NAc in rewarding behaviors [94]. Furthermore, Adamantidis, et al. showed that phasic optical stimulation of VTA DA neurons in behaving TH-Cre mice expressing the DIO-ChR2 vector led to facilitation of reinforcing food seeking behavior and elicited food seeking reinstatement alone without any other cues [95]. Future work investigating the functional role of projection specific DA neurons to striatal regions and other brain regions in drug and natural reward will be very important in distinguishing the differential behavioral functions of DA neuron subpopulations since aversive or rewarding stimuli alter excitatory synapses in these different DA subpopulations [96].

Koo, et al. recently investigated VTA inputs in NAc in morphine rewarding behaviors. Using non-selective AAVs to express ChR2 in VTA terminals, which predominantly colocalized with TH expression, these authors demonstrated that phasic activation of these terminals in the NAc enhanced morphine conditioned place preference. Additionally, after blocking morphine place preference by injecting BDNF into the VTA, the authors could reverse this blunted morphine reward with ChR2 phasic stimulation of VTA inputs in NAc [97]. Chaudhury et al. used TH-Cre mice combined with DIO-AAV-ChR2 to physiologically stimulate DA cells in the VTA during a social defeat stress paradigm, which can induce persistent depression-like behaviors in mice [98–100]. They demonstrated that phasic activation but not tonic activation of VTA DA neurons induced a susceptible phenotype as observed by decreased social interaction and
sucrose preference after a subthreshold social defeat protocol. Phasic stimulation also rapidly induced the depression-like phenotype in animals previously displaying resilient behaviors after a 10-day social defeat stress protocol. They also examined VTA DA projection specific neurons by using a retrograde viral Cre dependent pseudorabies virus (PRV-Cre), which they injected into the NAc or medial prefrontal cortex (mPFC) to selectively express Cre combined with DIO-AAV-Chr2 or DIO-AAV-eNpHR3.0 in VTA-NAc vs. VTA-mPFC projection DA neurons. They demonstrated that phasic activation of VTA-NAc DA neurons is responsible for the susceptible phenotype. They further demonstrated that inhibition of VTA-NAc DA neurons in mice that were susceptible to the 10-day repeated social defeat stress could shift these mice into a resilient phenotype. In contrast, selective inhibition of VTA-mPFC DA neurons resulted in a susceptible phenotype after subthreshold social defeat stress [100].

Tye et al. demonstrated opposing findings in the role of VTA dopaminergic firing using a different model of depression. First, using TH-Cre mice injected with DIO-AAV-eNpHR3.0 to the VTA, they demonstrated that optogenetic inhibition of VTA DA neurons caused depression-like behaviors as observed by decreased time struggling in the tail suspension test and decreased sucrose preference. They then demonstrated that phasic stimulation of Chr2 expressing VTA DA neurons rescued depression-like phenotypes, induced by a chronic mild stress protocol, as observed by enhanced time spent struggling in tail suspension and enhanced sucrose preference. They showed that the antidepressant responses to phasic stimulation could be reversed by pharmacological blockade of DA receptors in the NAc. Furthermore, using TH-Cre BAC transgenic rats (see discussion below) they were able to perform single unit recordings of NAc neurons during forced swim test coupled with VTA DA phasic stimulation. TH-Cre rats, that underwent chronic mild stress, elicited increased behavioral kicks in the forced swim test during real time phasic firing of VTA DA cells and this was coupled to increased or decreased firing of individual NAc neurons [101]. These opposing findings of phasic VTA DA neuron stimulation in depression-related behaviors in the two studies could likely reflect the different stressors (social defeat vs. chronic mild stress) used in each study as previous work demonstrated differential DA neuron firing to different forms of stress [102]. Future studies will need to address whether the phasic DA firing in both stress conditions have similar or differential effects on the various NAc neuronal subtypes.

Other groups are beginning to use rats in these optogenetic studies. Bass et al. used voltammetry, to demonstrate the ability to drive dopamine release in the NAc of rats by non-selective optogenetic phasic firing of VTA cells [103]. A follow up study compared optical and electrical stimulation of dopamine release in the striatum and found that classical electrical stimulation was able to induce more dopamine release while optogenetic stimulation was more sensitive to stimulation pulse variations [104]. Witten et al. recently developed TH-Cre BAC transgenic rats to selectively stimulate DA cell bodies in the VTA in freely moving animals. TH-Cre rats, selectively expressing Chr2 in VTA DA neurons, were placed in an operant chamber and trained to nose poke for phasic stimulation to VTA DA neurons. The group demonstrated that rats robustly self-stimulate for VTA DA neuron phasic stimulation, which is accompanied by DA release in the NAc [47]. They subsequently used these rats in depression-related studies as discussed above [101].

Two recent studies investigated a role of VTA GABAergic projections to NAc in reward consumption and seeking, van Zessen et al. used vesicular GABA transporter (vGAT)-Cre mice to selectively express Chr2 in the VTA GABA neurons and then activated these neurons at specific time points during a presentation of a reward. Their study demonstrated that stimulation of these inhibitory cells immediately following presentation of the reward led to disruption in reward consumption. However, this study showed that VTA GABAergic projections to the NAc did not play a role in this observed disruption but instead VTA GABAergic inhibition directly onto VTA DA neurons drives this behavior [105]. They did however demonstrate the indirect effects of GABA VTA neurons on NAc by decreased DA release into NAc, after inhibition of GABA VTA neurons. Tan et al. used a similar approach, with GAD-Cre mice to selectively express Chr2 in GABA positive neurons in the VTA. They demonstrated a conditioned place aversion after activation of VTA GABAergic neurons since GAD-Cre animals expressing Chr2 significantly avoided the chamber previously paired with the blue light, compared to control conditions. They suggest this reflects GABAergic inhibition of VTA DA neurons rather than inhibition to NAc since inhibiting VTA DA neurons expressing eNpHR3.0 in TH-Cre mice produces a similar place aversion [106].

Another recent study used optogenetics to manipulate the afferents to the VTA in a conditioned place preference paradigm. Using a retrograde rabies viral vector expressing Chr2 they were able to identify and optogenetically drive VTA afferents from the laterodorsal tegmentum (LDT) and the lateral habenula (LHb). They demonstrated that LDT neurons synapse onto DA neurons in the lateral VTA that send projections to the NAc while LHb neurons synapse on VTA DA neurons in the medial VTA that send projections to the prefrontal cortex (PFC). The authors demonstrated that LDT stimulated mice displayed a place preference for the light paired chamber whereas LHb stimulated mice showed a place aversion. Administration of dopamine antagonists selectively in the NAc was able to block the place preference created by stimulation of LTD projections to the VTA [107]. The findings of the LTD–VTA–NAc circuit in positive reward are consistent with other optogenetic studies examining VTA–NAc reward and function [47, 49, 56, 97], but provide novel insight into more complex circuit levels by investigating these VTA afferents.

Other studies have focused on the role of glutamatergic afferents to the striatum. Stuber et al. used AAV vectors expressing Chr2 under the CAMKIIα promoter to selectively stimulate or inhibit the excitatory projections to NAc. They demonstrated that mice self stimulate for 20Hz light induced excitation of glutamatergic projections from basolateral amygdala (BLA) to the NAc but do not self stimulate for activation of PFC projections to the NAc. The group further demonstrated that inhibition of BLA–NAc fibers expressing eNpHR3.0 blocked reward consumption [108]. In a more recent study, Britt et al. demonstrated enhanced evoked EPSCs and larger peak inward NMDAR mediated currents in the NAc MSNs after optogenetic stimulation of CAMKIIα–Chr2 expressing ventral hippocampus (vHipp) inputs compared to stimulation of the BLA and PFC inputs. They further demonstrated that cocaine exposure selectively strengthens vHipp synapses in the medial NAc shell and that bidirectional optogenetic control of vHipp terminals in the NAc can alter cocaine-induced locomotion. Finally, they demonstrated that a 6Hz optogenetic stimulation of vHipp, BLA, and mPFC glutamatergic inputs to the NAc caused a preference to a laser paired chamber. In addition, Chr2 stimulation of vHipp and BLA (20Hz) and PFC (30Hz) projections in the NAc was sufficient to drive optical self stimulation. Their studies indicate that weaker synapses, including those from the PFC, can drive motivated behaviors when a stronger optical stimulus was elicited indicating that the specific excitatory pathway activated is not as relevant as the amount of glutamate released into the NAc in generating such behaviors [109]. These studies did not examine the combined effects of cocaine and PFC optogenetic activation. However a recent study used Chr2 optogenetic activation of PFC inputs to NAc in animals exposed to cocaine. They show enhanced presynaptic release probability of the PFC-to-NAc synapses after short-term withdrawal (1 d) and long-term (45 d) withdrawal from either
noncontingent (i.p. injection) or contingent (self-administration) exposure to cocaine [110]. It is unknown whether the effects of cocaine on the glutamatergic inputs to the NAc in these previous studies are occurring on a selective NAc MSN subtype. A recent study provides insight into this by demonstrating that optogenetic activation of hippocampal afferents onto striatal MSNs are much weaker onto D2+ MSNs. In contrast, glutamatergic inputs from the PFC and thalamus targeted D1+ and D2+ MSNs equally [111].

Covington et al. investigated PFC activation in mice after social defeat stress and showed that high frequency bursting to PFC neurons expressing HSV-ChR2-mCherry is anti-depressant, as observed by reversal of the social avoidance and anhedonia behaviors normally displayed in mice susceptible to social defeat stress [112]. This study did not distinguish between glutamatergic or GABAergic cell populations in the PFC nor did it examine the effect on PFC inputs to NAC. However, a follow up study used the Thy1-ChR2 transgenic line, which selectively expresses ChR2 in layer V pyramidal neurons of the cortex, to optically stimulate the PFC glutamatergic projection neurons in affective behaviors and then examine neural activity in limbic brain regions with this stimulation. Optogenetic stimulation of layer V pyramidal PFC neurons was able to promote anti-depressant like responses in forced swim test but did not reverse social avoidance after social defeat stress. However the optical stimulation did rescue the anxiety-like behavior exhibited in mice after social defeat stress. The authors further demonstrated with multi unit recordings that activating these layer 5 glutamatergic PFC neurons entrains neural oscillatory activity and drives synchrony across limbic brain regions including the NAc [113]. These studies have relevance to the antidepressant effects of high frequency Deep Brain Stimulation (DBS) to cortical regions in patients with treatment resistant depression [114]. Future studies targeting PFC projections and other glutamatergic afferents to NAC, using CAMKIIx promoter viruses in animal models of depression will be essential for understanding how these striatal glutamatergic afferents encode depression-like behaviors or mediate antidepressive responses.

5. Optogenetic tools to investigate molecular mechanisms of striatal behaviors

Researchers are beginning to examine molecular correlates of behavioral actions after controlling neuronal firing with optogenetics. For instance our group demonstrated that disrupting BDNF signaling in D1+ and D2+ MSNs mimics optogenetic activation of these neurons in cocaine reward. Moreover, we showed that down-stream BDNF signaling molecules, phosphoErk1/2, were decreased following ChR2 activation of D1+ MSNs [49]. Koo, et al. also demonstrated that the blunt morphine rewarding effects of BDNF in VTA are reversed by phasic ChR2 optogenetic stimulation of VTA terminals in NAc [97]. Likely we will see many new studies examining molecular adaptations occurring after optogenetic manipulation of striatal mediated behaviors or the utilization of optogenetics to rescue behaviors altered by genetic manipulation. Such studies are useful in understanding the underlying molecular pathways responsible for brain disease and will aid in the understanding of the adaptive molecular responses that occur in treatments such as DBS.

Additionally, the next generation of optogenetic tools is promising for dissecting the molecular mechanisms, driving behavioral actions, with temporal resolution. The optogenetic toolbox has extended beyond the activating and inhibiting opsins. Researchers are generating light activate G-protein coupled receptors (GPCR’s), cell signaling molecules, and transcription factors [115–119]. Airan, et al. developed and utilized GPCR optical proteins in behavior. They used protein chimerae containing the photoactivatable domains of rhodopsin with the intracellular domains of β2- or α1 adrenergic GPCR’s which they coined OptoXRs [115]. The researchers were able to initiate Gαs adenyl cyclase signaling or the phospholipase C signaling by activating the β2- or α1-adrenergic OptoXR or the α1-adrenergic OptoXR respectively with 473 nm blue light. They expressed these OptoXRs in NAc to demonstrate that activation of opto-α1AR was sufficient to promote a positive place preference for the light paired chamber [115]. This study provided insight into the role of adrenergic signaling in the NAc in reward seeking and demonstrated the utility of OptoXRs in controlling GPCR signaling on a temporal scale during behavioral conditioning.

Other groups are utilizing the LOV (light oxygen voltage) domain, which allosterically inhibits proteins fused to this domain in the dark. Upon blue-light exposure, a helix linking LOV to the protein unwinds and thus allows the protein to perform its normal biological functions. Dietz, et al. examined the role of Rac1, a Rho GTPase, in cocaine structural plasticity and reward by using a LOV domain Rac1 fusion protein [116,117]. The LOV domain binds and blocks the ability of the Rac1 protein to bind to downstream effectors. Upon blue light exposure, the steric hindrance of a constitutively active Rac1 is released, allowing Rac1 to perform downstream cellular functions including altering cytoskeleton remodeling [118]. This study demonstrated that blue light activation of LOV-Rac1 in the NAc during cocaine exposure blocked cocaine induced MSN dendritic plasticity. Furthermore, activation of LOV-Rac1 in NAc only during the cocaine conditioning was sufficient to block cocaine place preference conditioning [116]. This is a powerful display of the use of optogenetic tools to directly manipulate specific cell signaling cascades at precise time points in drug exposure or behavioral paradigms. Given the dynamic progression of drug abuse and other neuropsychiatric diseases over time, which could reflect different molecular mechanisms governing specific time points or symptoms throughout the disease progression; the ability to manipulate molecular functions on a temporal scale can provide novel insights into the molecular correlates of psychiatric disease progression.

6. Conclusion and future directions

In the past five years optogenetic tools have provided extensive insight into the functional roles of striatal neuron subtypes and striatal efferent and afferent connections in complex behaviors. Given the complex heterogeneity of the striatum, the ability to target precise neuronal populations and projections and then manipulate activity in these discrete circuits with light has provided a foundation for future optogenetic striatal studies. Such studies should be aimed at understanding basic behaviors and function in the striatum, as well as complex psychiatric diseases and neurological disorders characterized by dysfunctional striatal circuitry.

With the introduction of OptoXRs, the photoactivatable LOV domain, and other photoactivatable signaling molecules, such as nucleotide cyclases [116,117,120–122] researchers can now discreetly dissect, with temporal resolution, how molecular signaling cascades that are implicated in striatal function and disease can alter complex behaviors and disease progression. This could include possible manipulations of many implicated molecules, including BDNF signaling molecules, D1 receptor mediated adenylyl cyclase signaling, D2 receptor mediated adenylyl cyclase inhibition, and cannabinoid signaling cascades [49,123,124]. For instance, OptoXRs for dopamine receptors and cannabinoid receptors, as well as photoactivatable intracellular signaling proteins implicated in drug addiction could be exploited to control these molecules during specific behavioral time points throughout the induction and expression of addiction behaviors.

Please cite this article in press as: Lenz JD, Lobo MK. Optogenetic insights into striatal function and behavior. Behav Brain Res (2013). http://dx.doi.org/10.1016/j.bbr.2013.04.018
An important caveat in moving forward with in vivo optical stimulation is the possible indirect effects the light itself may be having; as highlighted by the observation of single unit responses after retinal alone laser stimulation [125]. It is also important to note the limitations of ChR2 studies as the supratherapeutic induced neuronal firing by ChR2 may elicit behavioral responses that would not occur under normal physiological conditions, thus such studies must be interpreted with caution. Over-excitation of specific cell types and circuits in the striatum might activate or silence different pathways that are not typically recruited during normal physiological conditions. An example of this could include over exaggerated inhibition of the opposing direct or indirect MSN pathways during selective activation of the other pathway. Indeed a recent study examining MSN subtype activation with in vivo real time Calcium detection shows activation of both direct and indirect MSNs during motor initiation in free moving animals [126]. This study is consistent with basal ganglia models that predict balanced coordination between the two pathways to elicit normal behavioral output. Nonetheless, optogenetic manipulation of each MSN subtype or other striatal cells can be used to model abnormal behavioral output that reflect an imbalance of striatal circuits such as motor disorders [50] or behavioral responses to drugs of abuse for example cocaine which itself is a potent stimulii for selective striatal subtypes [49].

Relevant behavioral paradigms should also be considered for these optogenetic studies in the striatum. Many of the original optogenetic reward studies relied on indirect measures of reward such as conditioned place preference. However, recent studies are examining more complex operant behavioral paradigms including drug self-administration and reinforcement seeking operant behaviors, which are relevant to the human condition of reward seeking and drug taking. Such behavioral tests were originally adapted for rats, although researchers are able to perform these operant behaviors in mice [75,105,108,127–129]. Nonetheless, recent development of BAC transgenic rat lines will be especially useful in such studies [47]. Further optogenetic manipulations of the striatum in non-human primates will be beneficial to understanding striatal function in more complex striatal dependent behaviors [130]. Indeed a recent study demonstrated the feasibility of such an approach by in vivo Chro2 elicited responses in the putamen and other basal ganglia structures in non-human primates [131]. However, molecular tools including promoter based viral vectors will be crucial for selectively targeting striatal cell subtypes in non-human primates or other animal models that lack transgenic models.

A final consideration for these optogenetic tools is the ability to connect to clinical applications relevant to striatal based diseases including depression, drug addiction, Huntington's Disease, Parkinson's Disease, obsessive compulsive disorders, and Tourette's Syndrome. Mimicking the effects of high frequency DBS, which has shown to be an effective treatment in many diseases noted above [132–136] in specific striatal neuron populations or afferent and efferent connections, will be extremely useful for a more conclusive understanding of the mechanisms of DBS, which are currently unclear. Newer Chro2 variants suited for higher frequency activation will be particularly useful [137]. As the optogenetic toolbox continues to expand with newer opsins and molecular signaling tools we will likely see a similar expansion striatal optogenetic behavioral research leading to novel insights in striatal behavior and disease.

References


[27] Gerfen CR. The neostriatal mosaic: multiple levels of compartmental organization. Trends in Neurosciences 1992;15:133–9, http://dx.doi.org/10.1016/0166-2236(92)90355-C.


[29] www.geniat.org


Dureius PF, Schifflmann SN, de Kerckhove d’Exaerde A. Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in the distinct dorsal striatal compartments. EMBO Journal 2011;31:640–53; http://dx.doi.org/10.1038/emboj.2011.400.


