

Chapter 18

Optogenetic Analysis of Striatal Connections to Determine Functional Connectomes

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Abstract Neural circuit function is determined not only by anatomical connections but also by the strength and nature of the connections, that is functional connectivity. To elucidate functional connectivity, selective stimulation of presynaptic terminals of an identified neuronal population is crucial. However in the central nervous system (CNS), intermingled input fibers make selective electrical stimulation impossible. With optogenetics this becomes possible, and enables the comprehensive study of functional synaptic connections between an identified population of neurons and defined postsynaptic targets to determine the functional connectome. By stimulating convergent synaptic inputs impinging on individual postsynaptic neurons, low frequency and small amplitude synaptic connections can be detected. Further, the optogenetic approach enables measurement of cotransmission and its relative strength. In this chapter, optogenetic studies in the striatum (Str) are introduced to demonstrate the functional connectome approach. For spiny projection neurons, this has revealed cell-type specific intra-striatal connections as well as striatal output connections. Cholinergic interneurons in the ventral striatum have been shown to use glutamate as a cotransmitter. Examining striatal afferents from the ventral midbrain has identified fast, direct dopaminergic connection onto cholinergic interneurons and preferential connections of γ -aminobutyric acid (GABA) neurons to cholinergic interneurons in the nucleus accumbens. Further, it has revealed regionally heterogeneous glutamate and GABA cotransmission of dopamine neurons in the Str. These connections can be quite plastic, revealing a new vista of connectivity that is likely to be important in understanding the circuitry of neuropsychiatric disorders.

Keywords Nucleus accumbens • Spiny projection neurons • Channelrhodopsin • Dopamine neurons • Ventral tegmental area • Substantia nigra • Cholinergic interneurons

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18.1 Introduction

The mammalian brain is made from billions of cells and the majority of its functions are determined by connectivity of neurons. Since the identification of synapses as the point of communication between neurons by Sherrington (Shepherd 1994), determining the wiring diagram of a brain has been a central interest of neuroscientists in order to understand brain functions. Actual neuronal connectivity includes two levels: the physical wiring diagram (anatomical connectivity) and the strength and nature of the connection (physiological or functional connectivity). Anatomical connectivity is typically determined using retrograde and anterograde axonal tracers, including viruses with reporter proteins. Though these tracers are powerful tools to elucidate the wiring diagram, they are not suitable to examine synaptic connections. Synaptic connections can be identified by trans-synaptic tracers (e.g., pseudo-rabies virus), green fluorescent protein (GFP) reconstitution across synaptic partners (GRASP) (Feinberg et al. 2008) or observation with electron microscopy. However, the functional strength of a particular synaptic connection is difficult to discern with these methods. Thus, direct measurement of synaptic response between identified populations is crucial to define physiological or functional connectivity.

18.1.1 *Functional Connectome*

We introduced the concept of the *functional connectome* to describe comprehensive studies of functional connectivity (Chuhma et al. 2011). The word *connectome* was used by Sporns in 2005 to describe the wiring diagram of the human brain (Sporns et al. 2005). The current definition of *connectome* is the comprehensive mapping of wiring in a brain (not limited to human) or a region of brain (Lichtman and Sanes 2008; Seung 2009). In contrast to *connectome*, defined as the comprehensive study of anatomical connections of neurons, *functional connectome* can be defined as the comprehensive study of the physiological strength and nature (e.g., kinds of transmitters) of monosynaptic connections between two identified populations of neurons. It should be noted that the concept of functional connectivity used in the *functional connectome* approach is different from the *functional connectivity* used in brain imaging, which refers to temporally associated activities of two or more brain regions during a certain brain state (Friston et al. 1993). Functional connectivity in brain imaging is not necessarily relevant to direct synaptic connections among recorded regions, while *functional connectome* is restricted to *direct synaptic connections* from an identified population of neurons to their defined postsynaptic targets.

18.1.2 Why the Optogenetic Approach Is Suitable for Functional Connectome Studies

For a functional connectome study, selective and reliable stimulation of an identified population of neurons and recordings from defined neurons are crucial. Although identification of postsynaptic neurons is possible in vivo, intracellular recording from brain slices has advantages. In contrast to relatively easy identification of postsynaptic neurons, selective stimulation of identified presynaptic neurons has been hard to achieve. Particularly in the basal ganglia, where cell architecture is not layered and input fibers from multiple structures are intermingled, population-selective stimulation with electrical stimulation is almost impossible. The advent of optogenetics has enabled selective activation of identified neuronal populations by genetically limited expression of photoactivatable proteins. Optogenetic activation causes convergent input stimulation to a single neuron and is suitable to detect low-frequency small-amplitude responses. The optogenetic approach also reveals existence and strength of cotransmission. Immunohistochemistry may indicate the potential for cotransmission; however, the functional contribution of any cotransmitter is difficult to discern without physiological measurement.

For functional connectome studies, fast-on fast-off excitation current generation is necessary. The suitable photoactivatable proteins are non-selective cation channel channelrhodopsin (ChR)-2 and its mutants with faster kinetics and/or higher light sensitivity (Yizhar et al. 2011). Because of its membrane-targeted nature, ChR2 can also be used as a genetically controlled axonal tracer. Indeed, a study using ChR2 as an axonal tracer revealed novel projections from pontine tegmental nuclei to the striatum (Str) (Dautan et al. 2014). Although this makes coordinated anatomical studies feasible, this chapter focuses on excitation of genetically identified axon terminals to reveal functional connectivity. In the following section, I focus on the Str and its intrinsic and output connectivity. The Str is one of the most suitable locations to apply the optogenetic approach to the functional connectome, because of intermingled inputs, existence of genetic markers suitable for conditional expression of ChR2, and lack of thorough studies of functional strength of synaptic connections in spite of its importance both in normal functions of a brain and in the pathophysiology of neuropsychiatric disorders.

18.2 Functional Connectome of the Striatum with Optogenetics

18.2.1 Striatal Make-up and Inputs

The rodent Str comprises the nucleus accumbens (NAc) and the dorsal or neostriatum (dStr). The NAc corresponds to the ventral Str in primates. Although the striatal region corresponding to the primate caudate or putamen is not clear in

rodents, the ventromedial dStr is regarded as roughly homologous to the caudate and the dorsolateral dStr as roughly homologous to the putamen (Graybiel 2008). Both the NAc and the dStr share similar cytoarchitectures; about 95 % γ -aminobutyric acid (GABA)-ergic spiny projection neurons (SPNs) and about 5 % interneurons (Wilson 2004). SPNs are the sole population of output neurons, and their major projection sites are the globus pallidus (GP)/ventral pallidum (VP) and the ventral midbrain (Wilson 2004). Striatal interneurons comprise cholinergic and GABAergic interneurons. Cholinergic interneurons (ChIs) are a single population and make up about 1 % of striatal neurons, while GABAergic interneurons comprise several different types, namely fast spiking interneurons (FSIs), low-threshold spike interneurons (LTSIs), and neurogliaform interneurons (Tepper et al. 2010; Kreitzer 2009). Although interneurons make up small minority populations, they exert strong control of SPN excitability and axo-axonal modulation of dopamine neuron afferents (Tepper et al. 2004; Oldenburg and Ding 2011; Exley and Cragg 2008).

Both the Str and the NAc receive inputs from the prefrontal cortex (PFC), the thalamus (Thal) and the ventral midbrain (Yin and Knowlton 2006). The NAc is distinguished by inputs from limbic structures: the hippocampus and the basolateral amygdala (BLA) (Pennartz et al. 2009; Belujon and Grace 2011). Those inputs show a topographic projection pattern. Ventral midbrain dopamine (DA) neuron fibers from the medial ventral tegmental area (VTA) project to the NAc medial shell, namely the most ventromedial subregion of the striatal complex, and more lateral DA neurons in the substantia nigra pars compacta (SNc) project to dorsolateral subregion of the Str (Haber et al. 2000; Ikemoto 2007). These connections modulate cortex-basal ganglia-thalamus loops that run in parallel from the more ventral ‘limbic loop’ to the dorsal ‘sensorimotor loop’ (Pennartz et al. 2009; Yin and Knowlton 2006).

18.2.2 Output from the Striatum/Accumbens

SPNs are divided into two populations based on their projections to the output nuclei of the basal ganglia directly (direct pathway) or via the GP/VP (indirect pathway). The output nuclei comprise the VTA/SN and the entopeduncular nucleus (the rodent counterpart of the internal segment of the GP in primates) and send projections to the Thal (Gerfen and Surmeier 2011). Direct pathway SPNs (dSPNs) express DA D1 receptors and enkephalin, while indirect pathway SPNs (iSPNs) express DA D2 receptors and substance P (Gerfen and Surmeier 2011). Those two populations of SPNs are segregated almost completely in the dStr and NAc core in adult rodents (Bertran-Gonzalez et al. 2008). In the NAc shell, 17–38 % of SPNs express both D1 and D2 receptors (Bertran-Gonzalez et al. 2008; Gangarossa et al. 2013), although it is not clear whether SPNs co-expressing both receptors belong to dSPNs or iSPNs.

18.2.2.1 Direct Pathway

Retrograde tracer studies have shown that dSPNs send axons to both DA neurons in the SNc and to GABA neurons in the SN pars reticulata (SNr) (Gerfen 1985; Fujiyama et al. 2011). Synaptic connections of dSPNs with both populations in the SN were confirmed by pseudo-rabies virus tracer (trans-synaptic retrograde tracer) injected into the ventral midbrain (Watabe-Uchida et al. 2012). However, these anatomical studies revealed neither the strength of the connections nor the responsible transmitters.

The first functional connectome study with optogenetics used transgenic mice expressing ChR2 selectively in SPNs with a tetracycline transactivator (tTA)-tetracycline operator (tetO) strategy (Chuhma et al. 2011). In these mice, tTA is expressed selectively in SPNs driven by the alpha-calcium-calmodulin-kinase II (α CaMKII) promoter (Mayford et al. 1996). tTA binds and activates tetO driving ChR2 expression. Whole cell recording from SN neurons and activating SPN terminals with wide-field photostimulation revealed that dSPNs make strong connections to SNr GABA neurons via GABA_A receptors, but made no detectable connections to SNc DA neurons (Chuhma et al. 2011) (Fig. 18.1a). However, only 10 % of SPNs express ChR2 in α CaMKII-tTA::tetO-ChR2 mice. Although the distribution of ChR2 expression in SPNs appears to be random, as it does not distinguish between SPNs in patch and matrix striatal regions or between direct or indirect pathway SPNs, it is possible that SPN inputs to SNc DA neurons were too weak to be detected or that the SPNs expressing ChR2 did not include SPNs connecting to DA neurons. Similarly, a functional connectome study of accumbal SPNs with ChR2-viral vector injection found only photo-evoked GABA_A responses in non-DA neurons, but not DA neurons in the VTA (Xia et al. 2011). In a subsequent study using viral transfection, weak SPN GABAergic connections to VTA DA neurons were seen; however, the GABAergic input to VTA GABA neurons was overwhelmingly stronger (Bocklisch et al. 2013). Taken together, these optogenetic functional connectome studies reveal that dSPNs make preferential connections to non-DA neurons in the VTA/SN and that connections to DA neurons are extremely weak (Fig. 18.1a).

Most dSPNs also send collaterals to the GP (Fujiyama et al. 2011), which account for about one-third of striatal inputs to the region (Kita 2007). In vivo activation of the dSPN collaterals in the GP inhibits GP neuron firing (Cazorla et al. 2014). Although the inhibition is likely mediated by direct collateral connections activating GABA_A receptors, inhibitory synaptic responses were not evident in GP neurons (N. Chuhma, unpublished observations). The functional connectome of dSPNs in the GP remains to be determined (Fig. 18.1a, dashed line).

18.2.2.2 Indirect Pathway

The major projections of iSPNs are to the GP/VP. In α CaMKII-tTA::tetO-ChR2 mice, photo-activation of striatal inputs in the GP evokes GABA_A responses (Chuhma et al. 2011). Almost all GP neurons are GABAergic projection neurons;

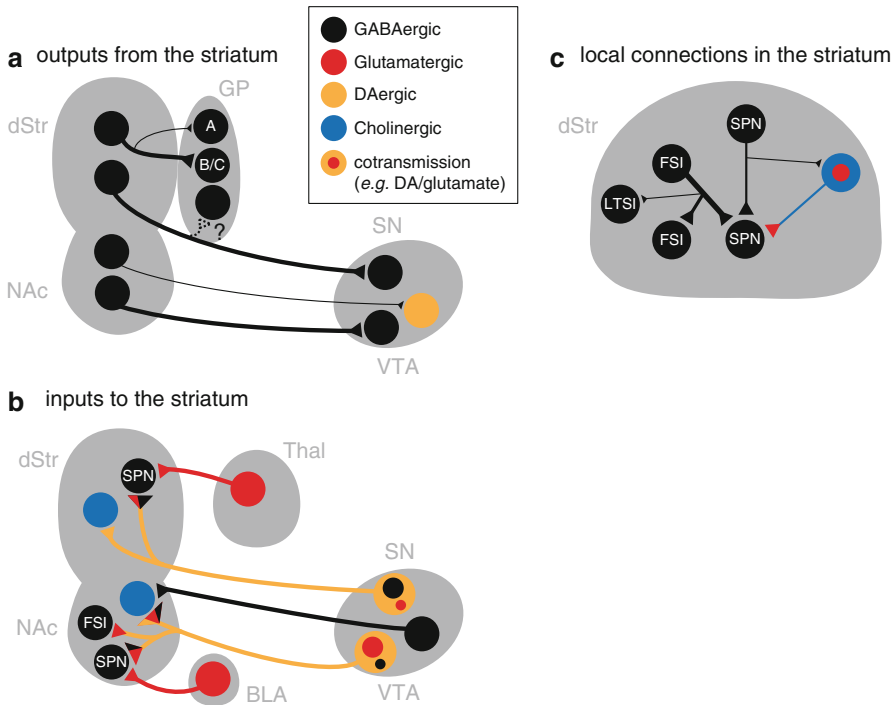


Fig. 18.1 Optogenetically identified fast direct synaptic connections are shown for striatal outputs (a), inputs (b), and local connectivity (c). Within cells, transmitters are color coded. Cotransmission is indicated by smaller circles in cells using the cotransmitters. Axon terminals are color coded accordingly. Significant differences in the strength of connections are indicated by the thickness of axons. In *panel A*, the dashed line indicates a connection suggested by *in vivo* recording, but the direct synaptic connection has not been confirmed. In *panel B*, observations in NAc shell and core are merged. *dStr* dorsal striatum, *NAc* nucleus accumbens, *GP* globus pallidus, *SN* substantia nigra, *VTA* ventral tegmental area, *Thal* thalamus, *BLA* basolateral amygdala, *A* Type A-like neurons, *B/C* Type B/C-like neurons, *SPN* spiny projection neuron, *FSI* fast spiking interneuron, *LTSI* low-threshold spiking interneuron

however, there has not been a clear consensus about classification of these neurons. We observed two types of electrophysiologically distinguishable populations; slower-firing high-input impedance neurons and faster-firing low-input impedance neurons (Chuhma et al. 2011). The former population shared the nature of Cooper-Stanford type A neurons (Cooper and Stanford 2000) and Lim homeobox 6 (*Lhx6*) positive neurons (Mastro et al. 2014). The latter shared some characteristics of Cooper-Stanford type B and C neurons (Cooper and Stanford 2000) and parvalbumin (PV)-positive neurons (Mastro et al. 2014). Striatal GABA_A input was stronger in type B/C-like neurons and very weak in type A-like neurons (Chuhma et al. 2011) (Fig. 18.1a). Since ChR2 was expressed in both dSPNs and iSPNs in α CaMKII::tetO-ChR2 mice, the GABA_A responses were combined responses of iSPN

inputs and dSPN collateral inputs. However, when ChR2 was selectively expressed in dSPNs by viral vector injection, small GABA_A response was evoked and the relative connectivity strength to the two populations was the same (Chuhma, unpublished observation), suggesting that the preferential connectivity to type B/C-like neurons was mostly determined by iSPNs.

18.2.3 Inputs to the Striatum/Accumbens

The Str receives spatially segregated subcortical and cortical inputs. Intermingled subcortical inputs enter the Str from the caudal side, so electrical stimulation activates multiple inputs, while cortical inputs enter the Str from the rostral side, so electrical stimulation activates these inputs selectively. Thus, functional connectome studies have focused on the subcortical inputs.

18.2.3.1 Ventral Midbrain Dopamine Neurons

The most characteristic projection to the Str is the one from DA neurons in the ventral midbrain. This is the densest DA projection in the brain, and it has a range of functions depending on projection sites in the Str, e.g., motivation and instrumental learning in the NAc shell, cognitive learning in the ventromedial Str, and motor control and habit formation in the dorsolateral Str (Haber et al. 2000; Graybiel 2008). Ventral midbrain DA neuron inputs are particularly suitable for optogenetic studies, because of intermingled inputs, expression of selective genetic markers, and controversial cotransmission. Optogenetic studies have revealed two interesting features of this DA neuron transmission besides DA release and its modulatory action: cotransmission involving two fast transmitters and fast direct DA transmission through D2 receptors.

Glutamate cotransmission by ventral midbrain DA neurons is an excellent example to show how powerful optogenetics is in functional connectome studies. DA neuron glutamate cotransmission was reported in DA neuron culture (Sulzer et al. 1998) and in slices (Chuhma et al. 2004). However, its existence in a mature intact brain was questioned, because the stimulation in slice preparation was not selective to DA neurons, and there are purely glutamatergic neurons in the VTA that possibly project to the NAc (Yamaguchi et al. 2007). The existence of functional cotransmission and its strength is hard to discern with anatomical methods. Selective stimulation of DA neuron terminals in the NAc with ChR2 solves these problems. Optogenetic stimulation of DA neuron terminals generated fast glutamatergic responses in all recorded SPNs in the NAc medial shell (Stuber et al. 2010; Tecuapetla et al. 2010; Mingote et al. 2012; Chuhma et al. 2014), and most recorded SPNs in the NAc core (Mingote et al. 2012). In the NAc medial shell, photo-evoked glutamate responses were also observed in all recorded ChIs and FSIs. Responses in ChIs were significantly larger (Chuhma et al. 2014). Therefore, it can be concluded

that all the NAc medial shell neurons receive glutamate cotransmission from VTA DA neurons and preferential connections are to ChIs (Fig 18.1b). In the dStr, glutamate cotransmission to SPNs was observed by one group (Tritsch et al. 2012), but not by others (Stuber et al. 2010; Mingote et al. 2012). This discrepancy could be due to finer regional heterogeneity of DA neuron input in the dStr and bears further study.

Finding GABA cotransmission with VTA/SNc DA neurons is another triumph of optogenetic studies of synaptic transmission in the basal ganglia (Tritsch et al. 2012). This GABAergic cotransmission has a very unique nature, as it does not require glutamic acid decarboxylase (GAD) or vesicular GABA transporters, which have been thought to be critical for GABAergic transmission. Therefore, anatomical identification of cotransmission (e.g., co-localization of DA and GABA markers) may not detect this non-canonical cotransmission. The DA neuron GABA cotransmission was observed in both dSPNs and iSPNs in the dStr (Tritsch et al. 2012), both dSPNs and iSPNs in the NAc (Tritsch et al. 2014), and ChIs in the NAc core (Chuhma et al. 2014), indicating that GABA cotransmission is not limited to nigro-striatal projections in the dStr (Fig. 18.1b). However, DA neurons do not make significant GABA connections to ChIs in the most medial part of the NAc shell (Chuhma et al. 2014), suggesting that there is a regional difference in the strength of GABAergic cotransmission within the NAc.

Selective activation of DA neuron terminals with ChR2 revealed fast D2-mediated DA transmission in ChIs in the medial dStr (Chuhma et al. 2014) (Fig. 18.1b). Fast D2-mediated responses are observed in ventral midbrain DA neuron through dendritic release (Beckstead et al. 2004; Gantz et al. 2013); however it was not believed that DA has fast direct transmission in projection areas. These D2-mediated fast inhibitory responses were observed in all recorded ChIs in medial dStr (Chuhma et al. 2014). Since D2-mediated fast response has not been reported in iSPNs in the dStr, this direct DA transmission appears to be specific to ChIs.

18.2.3.2 Ventral Tegmental Area GABA Neurons

The VTA contains GABA neurons that project to the NAc (Van Bockstaele and Pickel 1995). However, the direct synaptic connectivity was not studied. Since the basal ganglia have GABA-dominant circuits, it has been difficult to stimulate only a particular subset of GABAergic inputs. ChR2 virus injection to the ventral midbrain of GAD-Cre mice enabled selective activation of VTA-GABA neurons (Brown et al. 2013). Large-amplitude photoactivated GABAergic responses were observed in all recorded ChIs, while modest connections were seen in SPNs in the NAc (Brown et al. 2013) (Fig. 18.1b). In NAc FSIs, 20 % of recorded neurons showed responses but the amplitude of the response was extremely small (Brown et al. 2013). This study revealed another preferential ventral midbrain input to ChIs.

18.2.3.3 Other Inputs

Compared with projections from the ventral midbrain, functional connectome studies of other inputs have not been conducted in detail. Thalamic inputs were stimulated with a viral vector of ChR2 injected to the intralaminar nuclei (Ellender et al. 2013) or the parafascicular nucleus (Ding et al. 2012), and strong glutamatergic connections were observed both in dSPNs and iSPNs in the dStr (Fig. 18.1b). Photoactivation of ChR2-expressing BLA terminals generated strong glutamatergic responses in SPNs in the NAc (Stuber et al. 2011) (Fig. 18.1b). However, these studies were conducted to confirm proper activation of synaptic connections with ChR2, and a functional connectome study has not yet been carried out.

18.2.4 Intrinsic Striatal Connections

In addition to synaptic inputs from other structures, striatal neurons make synaptic connections with each other; there are not only connections from interneurons to SPNs, but also connections amongst SPNs (Tepper et al. 2004). Because of physical proximity, paired recordings are possible to study synaptic connections between identified neurons. However, paired recordings will miss weak or lower-probability connections. Optogenetic stimulation of an identified neuronal population activates convergent inputs, and is more likely to detect low-probability synaptic connections.

18.2.4.1 SPN Connections to Striatal Neurons

Inter-SPN connections have been studied with paired whole-cell recordings (Taverna et al. 2008). However, the probability of connections is low (10–37 %), response amplitude is small (Tepper et al. 2004), and connections are difficult to detect. Introducing ChR2 to a subset of SPNs with the tetO-tTA strategy (Chuhma et al. 2011) and recording from non-ChR2-expressing SPNs provides information about convergent inputs from other SPNs. Under these conditions, about 60 % of recorded SPNs show GABAergic connections (Chuhma et al. 2011). Considering that only 10 % of SPNs expressed ChR2 in these mice, inter-SPN connections are stronger and more frequent than was estimated from paired recordings.

SPNs also make synaptic connections to ChIs, though the frequency is lower than connections to other SPNs (Chuhma et al. 2011) (Fig. 18.1c). Since the frequency and amplitude of this SPN–ChI connection is low, it is hard to identify this connection without optogenetics. On the other hand, SPNs do not make recognizable connections to FSIs (Chuhma et al. 2011). Considering the strong input from FSI to SPN (Tepper et al. 2004; Szydlowski et al. 2013), the connection between FSI and SPN is unidirectional (Fig. 18.1c).

18.2.4.2 Interneuron Connection

Most interneuron-to-SPN connection studies were conducted with simultaneous whole-cell recording, since interneurons have relatively strong and high-incidence connections to SPNs. However, for a comprehensive connectivity study, optogenetic stimulation of interneurons is necessary. ChR2 expression in PV-positive FSIs revealed inter-FSI connections, strong connections to SPNs, and weak and low-frequency connections to LTSIs, while there were no detectable connections to ChIs (Szydlowski et al. 2013) (Fig. 18.1c).

Striatal ChIs express vesicular glutamate transporter 3 (VGLUT3) and have been thought to be capable of glutamatergic transmission (Fremeau et al. 2002). However, it has been difficult to detect physiologically, since the response is not strong enough to be detected by simultaneous recording. Population activation of ChIs with ChR2 made the recording of convergent synaptic inputs possible, and fast glutamate cotransmission onto SPNs was revealed (Higley et al. 2011) (Fig. 18.1c). This shows two advantages of optogenetic approaches: stimulation of convergent inputs and identifying cotransmission.

18.3 Discussion

The advent of optogenetics has made possible selective stimulation of identified neuronal populations and has enabled functional connectome studies in the Str. Optogenetic activation of presynaptic terminals stimulates convergent inputs to single postsynaptic neurons. This enables detection of low-probability and small-amplitude synaptic connections. The optogenetic approach is also a powerful tool to identify cotransmission and its relative strength. A functional connectome approach revealed cell-type-specific connections of striatal output and preferential connections among different cell types in the local intra-striatal circuits. A regionally heterogeneous blend of cotransmitters of DA neuron projection and its preferential connection to particular cell types in the Str were also shown. The optogenetic approach also revealed new synaptic connections: VTA GABA neuron connection to ChIs and fast D2 response in ChIs.

ChR2 can be introduced to identified populations of neurons in two ways: conditional viral vector injection and transgenic mice. Viral vector injection provides strong expression with multiple copies of ChR2 and expression selectivity by both conditional expression and injection location and timing. However, ChR2 expression varies among animals and may not reach all cells when the targeted structure is large, e.g., the dStr, where this variation cannot be ignored in functional connectome studies. The virus used to deliver the ChR2 may also have an impact on synaptic properties (Jackman et al. 2014). The transgenic strategy gives reliable expression among animals, while expression is weaker than viral injection even using enhanced-expression techniques. This implies that there is a possibility of false-negative findings of synaptic connectivity in transgenic mice. These two

approaches are complementary. Since there is no one method combining the advantages of both approaches, it will be important to compare the results obtained by these two approaches to achieve a more comprehensive functional connectome.

Several issues exist in assessing the fidelity of functional connectome studies with optogenetics. Because of penetration of blue light in tissue (Al-Juboori et al. 2013), it is not clear how much of the total synaptic inputs are activated with illumination. Light penetration can be improved by mutants of ChR2 with red-shifted excitation (Lin et al. 2013), since longer wavelength light penetrates further in brain tissue. Another possible issue is synchronous activation of terminals. Since ChR2 is activated by blue laser or high-power LED, it may activate terminals more synchronously than occurs in vivo under normal physiological conditions. However, in functional connectome studies, the aim is to obtain relative strength and capacity of transmission in different populations of postsynaptic neurons, and synchronous activation is preferable for estimation of relative strength. This information of basic functional connectivity is necessary for further studies of regional or synapse specific plasticity, which is likely to happen in vivo under both physiological and pathological conditions.

Though the anatomical connectome is important, anatomical information alone is not sufficient to elucidate brain function. Determining the connectivity of identified populations of neurons will be crucial for understanding how neuronal wiring diagrams correlate with function. Comprehensive mapping of the functional connectome is one of the ways to explore how our brain works, and optogenetics shines light to show the direction.

References

- Al-Juboori SI, Dondzillo A, Stubblefield EA et al (2013) Light scattering properties vary across different regions of the adult mouse brain. *PLoS One* 8(7):e67626. doi:10.1371/journal.pone.0067626
- Beckstead MJ, Grandy DK, Wickman K et al (2004) Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. *Neuron* 42(6):939–946
- Belujon P, Grace AA (2011) Hippocampus, amygdala, and stress: Interacting systems that affect susceptibility to addiction. *Ann NY Acad Sci* 1216:114–121
- Bertran-Gonzalez J, Bosch C, Maroteaux M et al (2008) Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J Neurosci* 28(22):5671–5685
- Bocklisch C, Pascoli V, Wong JCY et al (2013) Cocaine disinhibits dopamine neurons by potentiation of GABA transmission in the ventral tegmental area. *Science* 341(6153):1521–1525
- Brown MTC, Tan KR, O'Connor EC et al (2013) Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature* 492(7429):452–456
- Cazorla M, de Carvalho FD, Chohan MO et al (2014) Dopamine D2 receptors regulate the anatomical and functional balance of basal ganglia circuitry. *Neuron* 81(1):153–164
- Chuhma N, Zhang H, Masson J et al (2004) Dopamine neurons mediate a fast excitatory signal via their glutamatergic synapses. *J Neurosci* 24(4):972–981
- Chuhma N, Tanaka KF, Hen R et al (2011) Functional connectome of the striatal medium spiny neuron. *J Neurosci* 31(4):1183–1192

- Chuhma N, Mingote S, Moore H et al (2014) Dopamine neurons control striatal cholinergic neurons via regionally heterogeneous dopamine and glutamate signaling. *Neuron* 81(4):901–912
- Cooper AJ, Stanford IM (2000) Electrophysiological and morphological characteristics of three subtypes of rat globus pallidus neurone in vitro. *J Physiol* 527(2):291–304
- Dautan D, Huerta-Ocampo I, Witten IB et al (2014) A major external source of cholinergic innervation of the striatum and nucleus accumbens originates in the brainstem. *J Neurosci* 34(13):4509–4518
- Ding JB, Oh W-J, Sabatini BL et al (2012) Semaphorin 3E-plexin-D1 signaling controls pathway-specific synapse formation in the striatum. *Nature Neurosci* 15(2):215–223
- Ellender TJ, Harwood J, Kosillo P et al (2013) Heterogeneous properties of central lateral and parafascicular thalamic synapses in the striatum. *J Physiol* 591(1):257–272
- Exley R, Cragg SJ (2008) Presynaptic nicotinic receptors: A dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. *Brit J Pharmacol* 153(Suppl 1):S283–S297
- Feinberg EH, Vanhoven MK, Bendesky A et al (2008) GFP reconstitution across synaptic partners (GRASP) defines cell contacts and synapses in living nervous systems. *Neuron* 57(3):353–363
- Fremeau RT, Burman J, Qureshi T et al (2002) The identification of vesicular glutamate transporter 3 suggests novel modes of signaling by glutamate. *Proc Nat Acad Sci* 99(22):14488–14493
- Friston KJ, Frith CD, Liddle PF et al (1993) Functional connectivity: The principal-component analysis of large (PET) data sets. *J Cereb Blood Flow Metab* 13(1):5–14
- Fujiyama F, Sohn J, Nakano T et al (2011) Exclusive and common targets of neostriatofugal projections of rat striosome neurons: A single neuron-tracing study using a viral vector. *Eur J Neurosci* 33(4):668–677
- Gangarossa G, Espallergues J, de Kerchove d'Exaerde A et al. (2013) Distribution and compartmental organization of gabaergic medium-sized spiny neurons in the mouse nucleus accumbens. *Front Neural Circuits* 7:22. doi: [10.3389/fncir.2013.00022](https://doi.org/10.3389/fncir.2013.00022)
- Gantz SC, Bunzow JR, Williams JT (2013) Spontaneous inhibitory synaptic currents mediated by a G protein-coupled receptor. *Neuron* 78(5):807–812
- Gerfen CR (1985) The neostriatal mosaic. I Compartmental organization of projections from the striatum to the substantia nigra in the rat. *J Comp Neurol* 236(4):454–476
- Gerfen CR, Surmeier DJ (2011) Modulation of striatal projection systems by dopamine. *Ann Rev Neurosci* 34:441–466
- Graybiel AM (2008) Habits, rituals, and the evaluative brain. *Ann Rev Neurosci* 31:359–387
- Haber SN, Fudge JL, McFarland NR (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *J Neurosci* 20(6):2369–2382
- Higley MJ, Gittis AH, Oldenburg IA et al (2011) Cholinergic interneurons mediate fast VGLUT3-dependent glutamatergic transmission in the striatum. *PLoS One* 6(4):e19155. doi:[10.1371/journal.pone.0019155](https://doi.org/10.1371/journal.pone.0019155)
- Ikemoto S (2007) Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev* 56(1):27–78
- Jackman SL, Beneduce BM, Drew IR et al (2014) Achieving high-frequency optical control of synaptic transmission. *J Neurosci* 34(22):7704–7714
- Kita H (2007) Globus pallidus external segment. *Prog Brain Res* 160:111–133
- Kreitzer AC (2009) Physiology and pharmacology of striatal neurons. *Ann Rev Neurosci* 32:127–147
- Lichtman JW, Sanes JR (2008) Ome sweet ome: What can the genome tell us about the connectome? *Curr Opin Neurobiol* 18(3):346–353
- Lin JY, Knutsen PM, Muller A et al (2013) ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. *Nat Neurosci* 16(10):1499–1508
- Mastro KJ, Bouchard RS, Holt HAK et al (2014) Transgenic mouse lines subdivide external segment of the globus pallidus (GPe) neurons and reveal distinct gpe output pathways. *J Neurosci* 34(6):2087–2099
- Mayford M, Bach ME, Huang YY et al (1996) Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678–1683

- Mingote S, Chuhma N, Kusnoor SV et al (2012) The VTA dopamine neuron excitatory functional connectome. In: Champalimaud Neuroscience Symposium 2012, Champalimaud Centre for the Unknown, Lisbon, 30 Sep–3 Oct 2012
- Oldenburg IA, Ding JB (2011) Cholinergic modulation of synaptic integration and dendritic excitability in the striatum. *Curr Opin Neurobiol* 21(3):425–432
- Pennartz CMA, Berke JD, Graybiel AM et al (2009) Corticostriatal interactions during learning, memory processing, and decision making. *J Neurosci* 29(41):12831–12838
- Seung HS (2009) Reading the book of memory: Sparse sampling versus dense mapping of connectomes. *Neuron* 62(1):17–29
- Shepherd GM (1994) *Neurobiology*, 3rd edn. Oxford University Press, Oxford
- Sporns O, Tononi G, Kötter R (2005) The human connectome: a structural description of the human brain. *Plos Comput Biol* 1(4):e42. doi:[10.1371/journal.pcbi.0010042](https://doi.org/10.1371/journal.pcbi.0010042)
- Stuber GD, Hnasko TS, Britt JP et al (2010) Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J Neurosci* 30(24):8229–8233
- Stuber GD, Sparta DR, Stamatakis AM et al (2011) Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature* 475(7356):377–380
- Sulzer D, Joyce MP, Lin L et al (1998) Dopamine neurons make glutamatergic synapses in vitro. *J Neurosci* 18(12):4588–4602
- Szydlowski SN, Pollak Dorocic I, Planert H et al (2013) Target selectivity of feedforward inhibition by striatal fast-spiking interneurons. *J Neurosci* 33(4):1678–1683
- Taverna S, Ilijic E, Surmeier DJ (2008) Recurrent collateral connections of striatal medium spiny neurons are disrupted in models of Parkinson's disease. *J Neurosci* 28(21):5504–5512
- Tecuapetla F, Patel JC, Xenias H et al (2010) Glutamatergic signaling by mesolimbic dopamine neurons in the nucleus accumbens. *J Neurosci* 30(20):7105–7110
- Tepper JM, Koós T, Wilson CJ (2004) Gabaergic microcircuits in the neostriatum. *Trend Neurosci* 27(11):662–669
- Tepper JM, Tecuapetla F, Koos T et al (2010) Heterogeneity and diversity of striatal gabaergic interneurons. *Front Neuroanat* 4:150. doi:[10.3389/fnana.2010.00150](https://doi.org/10.3389/fnana.2010.00150)
- Tritsch NX, Ding JB, Sabatini BL (2012) Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. *Nature* 490(7419):262–266
- Tritsch NX, Oh WJ, Gu C et al (2014) Midbrain dopamine neurons sustain inhibitory transmission using plasma membrane uptake of GABA, not synthesis. *eLife* 3:e01936. doi:[10.7554/eLife.01936](https://doi.org/10.7554/eLife.01936)
- Van Bockstaele EJ, Pickel VM (1995) GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res* 682(1–2):215–221
- Watabe-Uchida M, Zhu L, Ogawa SK et al (2012) Whole-brain mapping of direct inputs to mid-brain dopamine neurons. *Neuron* 74(5):858–873
- Wilson CJ (2004) The basal ganglia. In: Shepherd GM (ed) *The synaptic organization of the brain*, 5th edn. Oxford University Press, Oxford, pp 361–413
- Xia Y, Driscoll JR, Wilbrecht L et al (2011) Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area. *J Neurosci* 31(21):7811–7816
- Yamaguchi T, Sheen W, Morales M (2007) Glutamatergic neurons are present in the rat ventral tegmental area. *Eur J Neurosci* 25(1):106–118
- Yin HH, Knowlton BJ (2006) The role of the basal ganglia in habit formation. *Nat Rev Neurosci* 7(6):464–476
- Yizhar O, Fenno LE, Davidson TJ et al (2011) Optogenetics in neural systems. *Neuron* 71(1):9–34