Published in final edited form as:

Curr Opin Neurobiol. 2011 June ; 21(3): 425–432. doi:10.1016/j.conb.2011.04.004.

Cholinergic modulation of synaptic integration and dendritic excitability in the striatum

Ian Antón Oldenburg¹ and Jun B. Ding^{1,*}

¹Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Abstract

Modulatory interneurons such as the cholinergic interneuron are always a perplexing subject to study. Far from clear-cut distinctions such as excitatory or inhibitory, modulating interneurons can have many, often contradictory effects. The striatum is one of the most densely expressing brain areas for cholinergic markers, and acetylcholine (ACh) plays an important role in regulating synaptic transmission and cellular excitability. Every cell type in the striatum has receptors for ACh. Yet, even for a given cell type, ACh affecting different receptors can have seemingly opposing roles. This review highlights relevant effects of ACh on medium spiny neurons (MSNs) of the striatum and suggests how its many effects may work in concert to change MSN firing properties.

Keywords

Striatum; medium spiny neuron; acetylcholine; cholinergic interneuron; muscarinic receptor; synaptic integration; synaptic plasticity

Introduction

Although comprising only 1–3% of all striatal neurons, cholinergic interneurons (thought to correspond to the tonically active neurons, TANs) have widespread connections throughout the striatum and provide the sole source of acetylcholine (ACh) to striatum [1]. The striatum contains some of the highest levels of cholinergic biomarkers in the brain, including, muscarinic receptors, cholinesterase and others [2]. As reviewed by Cragg, 2006 and Exley, 2008, [3,4], modulation of ACh and its interaction with dopamine (DA) are critical for normal striatal function [3,4]. Additionally, in a 2007 review, Pisani discussed how dysfunction of cholinergic signaling is associated with pathophysiological changes in movement disorders, such as Parkinson's disease (PD), Huntington's disease (HD) and dystonia [5,6].

This review will focus on the pharmacological targets of ACh and the role of ACh modulation in dendritic integration and synaptic plasticity in the striatum; in particular, the role of ACh-releasing cholinergic interneurons in regulating striatal output neurons.

Cholinergic Interneurons

The output cells of the striatum are the medium spiny neurons (MSNs). Their firing is affected by the three major groups of interneurons: (1) fast spiking, parvalbumin (PV)

*Correspondence should be addressed to Jun B. Ding (Jun_Ding@hms.harvard.edu), Department of Neurobiology, Harvard Medical School, Boston, MA 02115, (Ph) 617 432-5671, (Fax) 617 734-7557.

expressing GABAergic interneurons, (2) burst firing, somatostatin/neuropeptide-Y (NPY)-releasing GABAergic interneurons and (3) slow tonically firing large aspiny cholinergic interneurons [1,7]. While the PV and NPY-expressing GABAergic interneurons exert a powerful inhibitory influence on MSNs [8,9], the function of cholinergic neurons is primarily modulatory and cannot be simply characterized as excitatory or inhibitory. The cholinergic interneurons in the striatum help regulate the duration, strength, and spatial pattern of striatal MSNs output.

Each of these interneurons comprises only 1–3% of all striatal neurons, impeding their thorough examination. Nonetheless, cholinergic interneurons are well studied due to their large size aiding identification [10]. Cholinergic interneurons are tonically active pacemaking neurons, even in the absence of synaptic inputs [11]. In primates, cholinergic interneurons demonstrate distinct pauses in their tonic firing during motor learning and reward-related behaviors [12,13]. These dopamine-dependent-pauses are hypothesized to serve a “teaching” role in associative and motor learning [14], presumably by altering the strength of corticostriatal glutamatergic synapses. Thalamic activities, from the intralaminar thalamic neurons, give rise to the burst-and-pause firing of cholinergic interneurons [15,16]. This pattern of cholinergic activity produces dichotomic modulation of corticostriatal synaptic transmission through pre- and postsynaptic mechanisms, providing a neural substrate for attentional shifts with appearance of salient environmental stimuli [15]. Previous studies also suggest that sodium currents [13], slow afterhyperpolarization (sAHP) [17] and Ih [18] are involved in the decrease in firing rate or pause in tonic spiking.

ACh and its pharmacological targets

In the striatum, instead of evoking fast synaptic transmission through ionotropic receptors, ACh primarily acts on G-protein coupled muscarinic receptors. Therefore, it is through a modulatory role that ACh is critical in determining the final activity of striatal neurons that project to the output structures of the basal ganglia.

The five muscarinic receptors that have been identified can be grouped into two families. The M1-like receptors (M1 and M5) are coupled to Gq/11. Their activation will increase intracellular Ca^{2+} mobilization and activate phospholipase C (PLC) and protein kinase C (PKC). M2-like receptors (M2, M3 and M4) activate Gi/o proteins, which reduce c-AMP concentration and inhibit Ca^{2+} channels. Within the striatum, M1 and M4 are the major muscarinic receptors expressed in MSNs and NPY releasing interneurons, with M4 being overexpressed in striatonigral MSNs [19,20]. Furthermore, M2 and M3 receptors are located on presynaptic glutamatergic terminals. Additionally M2 and M4 receptors exist on cholinergic neurons acting as autoreceptors [5, 19]. Ionotropic nicotinic ACh receptors are expressed on glutamatergic and dopaminergic terminals as well as PV expressing interneurons but are absent in MSNs [21]. Therefore, in this review, we only discuss the muscarinic signalling in the striatum.

Modulation of intrinsic excitability and glutamatergic signaling by M₁ receptors

M1 receptors are highly expressed in both striatonigral and striatopallidal MSNs [20]. Unlike D1 and D2 dopamine receptors, the prevailing view is that M1 receptor activation does not directly regulate glutamatergic synapse function from the postsynaptic side. Studies of voltage-gated channels suggest M1 receptor activation excites MSNs by modulating potassium channels [22,23]. M1 receptor activation reduces opening of Kv4 channels (A-type potassium channels) and shifts their activation and inactivation voltage dependence [24] in a PKC dependent process [25]. In addition, M1 receptor activation coupled to PLC β

and PKC leads to membrane depletion of PIP₂, which modulates subthreshold potassium conductances of Kv7 (M-channel, KCNQ) and Kir2 (inward-rectifying potassium channel) channels [26,27] all contributing to MSN depolarization.

M1 receptor activation also regulates MSNs by modulating Cav channels. M1 receptor activation negatively regulates Cav1.3 by increased Ca²⁺ mobilization via phospholipase C (PLC) and phosphatase 2B (PP-2B) pathway [28,29,30]. While reducing currents through Cav2 channels inhibiting the AHP in MSNs in a pertussis-toxin-sensitive, Gβγ-mediated membrane delimited pathway [31,32]. Consistent with its effect on potassium channels, the inhibition of the AHP can increase firing frequency. Therefore, by coordinated modulation of these potassium and calcium channels, ACh can shape the synaptic integration and spiking activity in MSNs.

Modulation of intrinsic excitability and glutamatergic signaling by M₂ receptors

M2-like receptors are located both presynaptically and postsynaptically. M2/3 receptors are expressed on presynaptic glutamatergic terminals [33], whereas M4 receptors are expressed postsynaptically in MSNs and have higher expression levels in striatonigral neurons than those in striatopallidal neurons [20]. Presynaptically, M2/3 receptor activation can control the excitatory inputs by reducing glutamatergic transmission [33,34,35,36,37]. This presynaptic inhibition is mediated by inhibition of presynaptic Cav2 channels and decrease in probability of release, similar to mGluR2 and GABA_B receptor signaling. This modulation occurs rapidly and causes a reduction in glutamate release at corticostriatal and thalamostriatal terminals on both striatonigral and striatopallidal MSNs [15].

On the postsynaptic side, M4 receptor activation inhibits Cav2 channels and therefore shapes the spiking and up-state transitions in MSNs [5,31,32]. Although M4 receptor modulation is readily seen in nearly all MSNs, M4 receptors are expressed at higher levels in striatonigral MSNs [20]. The function of this imbalance is still not understood. It is likely that M4 receptor activation, together with DA signaling, produces differential modulations in striatonigral and striatopallidal MSNs. The muscarinic receptor signaling mechanisms outlined here are summarized in Figure 1.

Modulation of dendritic excitability

ACh activation of M1 receptors elevates MSN excitability by promoting the closure of several potassium channels, including Kv7 (KCNQ) [38], and Kir2 [27], on the postsynaptic side. Unlike Kv7 and SK channels, which are active only near spike threshold, Kir2 channels are constitutively active. Kir2 channels are dendritically positioned in MSNs as in many other types of neurons [27]. Three Kir2 channel subunits are abundant in MSNs. Kir2.1 subunits in MSNs are largely restricted along dendritic shafts. By contrast, Kir2.3 subunits are found primarily in spines. Kir2 channels mediate the hyperpolarization common to MSNs at rest. ACh modulates Kir2 conductance, by reducing its opening [27], profoundly altering dendritic input resistance - a key factor controlling dendritic excitability and synaptic integration.

The proximity of Kir2.3 to synaptic inputs suggests that they hold the dendritic membrane potential near the potassium equilibrium potential, dampening responsiveness to excitatory inputs. Closure of these channels by M1 receptor activation enhances integration of EPSPs in striatopallidal neurons [27]. The opening of Kir2 channels depends on membrane lipid phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂). M1 receptor activation reduces channel opening by activating PLC and lowering membrane PtdIns(4,5)P₂ (PIP₂) levels.

Interestingly, this modulation is almost exclusively in striatopallidal MSNs due to differential Kir2 subunit expression in striatonigral and striatopallidal neurons. Kir2 channels are constructed from a family of at least four subunits (Kir2.1–2.4). Kir2.1 subunits have a higher affinity for binding PIP2, whereas Kir2.3 subunits have a significantly lower affinity. Using a serial dilution scRT-PCR strategy, Shen et al. showed that the Kir2.3 mRNA is roughly two-fold higher in striatopallidal than in striatonigral MSNs [27]. The consequence of this imbalance is that channels with Kir2.3 subunits, such as in striatopallidal MSNs, are much more potently modulated by receptors coupled to PLC.

Thus, burst-pause firing activity in cholinergic interneurons creates a temporal window in which a transient presynaptic inhibition, mediated by M2, is followed by a period of enhanced postsynaptic excitability triggered by M1 receptors in striatopallidal MSNs. During this period, the striatal network is strongly biased toward cortical activation of striatopallidal MSN ensembles [15]. The indirect pathway, anchored by striatopallidal MSNs is widely thought to be responsible for creating a “no-go” signal to the motor thalamus [39]. Indeed, recording in behaving monkeys suggest that strongest responses of TANs were self-timed No-Go responses [40].

Excitatory synaptic transmissions to MSNs are both directly and indirectly regulated by muscarinic receptor signaling. First, glutamatergic EPSCs are presynaptically suppressed by cholinergic agonists [41] through direct activation of mAChRs on presynaptic terminals [42]. Second, postsynaptic activation of M1 receptor can enhance responsiveness of MSNs when they do receive excitatory input. It is hypothesized that the cholinergic system highlights activated excitatory inputs but suppresses background excitation to improve the signal-to-noise ratio of information carried by glutamatergic synapses in the striatum [43].

Indirect modulation of inhibitory synaptic transmission by ACh

In addition to its direct modulation on voltage-gated and ligand-gated channels, ACh also exerts its modulatory effect through the endocannabinoid (eCB) system, which may influence synaptic transmission and synaptic plasticity in the striatum (figure2).

Wang et al. revealed an indirect mechanism where M1 receptor signaling reversibly enhances glutamatergic synaptic transmission[46]. The modulation is dependent on Cav1.3 channels and CB1 receptors. M1 receptor promotes excitatory glutamatergic transmission by reducing opening of postsynaptic Cav1.3 channels, which in turn diminishes endocannabinoid production and presynaptic CB1 receptor activation.

M1 receptor activation also suppresses inhibitory synaptic transmission in MSNs through modulating the endocannabinoid system [47]. Tonic ACh from cholinergic interneurons constitutively enhances depolarization-induced release of endocannabinoids from MSNs. The retrogradely released endocannabinoids cause suppression of inhibitory synaptic currents in MSNs through presynaptic CB1 receptors. Muscarinic receptor activation also significantly enhances depolarization-induced suppression of inhibition (DSI) in MSNs [47]. Pharmacological manipulation that elevates ambient ACh level or suppresses spontaneous firing of cholinergic interneurons can enhance or reduce DSI, respectively.

Interestingly, M1 activation exerts opposite effects on endocannabinoid release at excitatory and inhibitory synapses. At glutamatergic synapses, M1 activation tonically inhibits endocannabinoid release through suppressing the opening of Cav1.3 channels, whereas at inhibitory synapses, M1 receptor activation promotes endocannabinoid release and thus suppresses inhibitory synaptic transmission. This difference may be attributed to differences in subcellular localization of M1 receptor. Excitatory synapses in MSNs are formed mostly on spines and inhibitory synapses are primarily located on dendritic shafts and soma

[47,48,49]. The M1-CB1 mediated excitatory effect requires functional interaction between Cav1.3 and scaffolding proteins Shank and Homer, which are enriched in spines [28]. However, M1-mediated suppression of inhibitory transmission more likely is caused by direct enhancement of production of endocannabinoids at inhibitory synapses through Gq, PLC β signaling by M1 receptor activation.

Recent study also suggested that cholinergic activation exerts strong di-synaptic GABA_A-mediated inhibition onto striatal MSNs, whereas cholinergic neuron inactivation increases firing of these MSNs *in vivo* [44]. While, the underlying circuit mechanism remains unknown, it is likely that fast-spiking interneurons are recruited by cholinergic activation. Fast-spiking parvalbumin expressing inhibitory interneurons are strongly excited by acetylcholine through nAChRs, which are widely expressed on presynaptic terminals of these neurons [45]. Activation or inactivation of cholinergic interneurons can directly excite or suppress fast-spiking interneurons firing. These GABAergic interneurons in turn provide strong corticostriatal feed-forward inhibition [50,51].

Taken together, ACh modulation of inhibition consists of two opposing components, simultaneously enhancing di-synaptic inhibition by nAChRs while suppressing GABAergic IPSCs through CB1 signaling. These two mechanisms may work in concert to provide two independent avenues of modulation. Nicotinic receptors exist in fast spiking interneurons of striatum and not in MSNs. Conversely, CB1 receptors are richly expressed in MSNs, but fast spiking interneurons have been shown to be insensitive to CB1 signaling in cortex and hippocampus [52]. However, some fast-spiking interneurons in the striatum are shown to be modulated by CB1 signaling [53]. Therefore, modulation of inhibition by nAChR may only contribute to direct regulation in feed-forward inhibition, while modulation by mAChR/CB1 signaling provide a feed-back component that target both FS-MSN feedforward and MSN-MSN collateral inhibition. These two very different forms of modulation differ in both their temporal scale and their cell type specificity and hence generate different epochs of inhibition that govern the spiking output of MSNs.

Concluding remarks

In the last few years, our understanding of the signaling mechanism controlling synaptic plasticity in the corticostriatal circuits by ACh has expanded significantly. The muscarinic receptor signaling cascades outlined here are summarized in Figure 3. Several lines of evidence converge to suggest that muscarinic M1-like receptor signaling enhances dendritic excitability and spiking of striatal MSNs, whereas M2-like receptor exerts the opposite effect in shaping excitability. In line with this principle, recently, a class of mutated muscarinic receptors that can be activated non-invasively by synthetic ligands have been engineered [54]. Among these, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) [55] are modified muscarinic receptors that can no longer be activated by acetylcholine and can be activated instead by clozapine-N-oxide (CNO). The hM3Dq, modified M3 receptor is Gq coupled and is excitatory [56], whereas the modified M4, hM4D, is Gi coupled and inhibits firing [55,57].

How a relatively sparse interneuron population, such as striatal cholinergic interneurons, contributes to *in vivo* function remains to be elucidated. One challenge we face is that, using conventional techniques it is difficult to achieve selective control of interneuron activities with temporal and spatial precision. Cholinergic interneurons are physically interspersed with the other neurons in the striatum preventing conventional manipulations. However, recently developed optogenetic tools, such as Channelrhodopsin-2 (ChR2) and Halorhodopsin (eNpHr3.0), can be expressed in mammalian neurons and used to enhance or suppress their firing with millisecond precision [58,59,60,61]. The development of

transgenic mice expressing ChR2 or Cre specifically in cholinergic interneurons allows selective expression of these optogenetic tools [44,67]. New devices, such as optrodes, have been designed to simultaneously record from cells while delivering light through a fiberoptic *in vivo* allowing new means of controlling cholinergic interneurons in freely moving behaving mice [63].

These new approaches, in combination with conventional physiology and behavioral analysis should advance our knowledge of the contribution of specific neuronal population to circuit function. For example, there is a growing appreciation that many “non-glutamatergic” neurons that release neuromodulators also co-release glutamate. Using optogenetic tools, recent studies provided convincing evidence for this type of phenomenon in dopaminergic [64,65], serotonergic [66] and cholinergic neurons [67]. Striatal cholinergic interneurons express vesicular glutamate transporter 3 (vGluT3) [68], it is likely glutamate is co-released at interneuron terminals. However, it remains unclear under what circumstance glutamate is co-released and what the functional significance of this would be. Using a similar approach, recent studies suggest that cholinergic activation exerts strong di-synaptic inhibitory action onto striatal MSNs (possibly by co-release of glutamate and activation of nAChRs) [44]. This new finding adds a novel component to striatal microcircuits - feed-forward inhibition controlled by cholinergic interneurons. In the coming years, a molecular dissection of cholinergic function should be further propelled by optogenetic tools. Application of these new approaches should allow us to gain a better understanding of cholinergic function in the cortico-thalamo-basal ganglia circuitry, potentially accelerating the development of new therapeutic strategies for psychomotor disorders.

Acknowledgments

The authors thank Dr. Meron Gurkiewicz, Dr. Yevgenia Kozorovitskiy and members of Sabatini laboratory for comments and helpful discussions. This work was funded by NINDS postdoctoral training grant (T32-NS007484, JBD).

References

1. Woolf NJ, Butcher LL. Cholinergic neurons in the caudate-putamen complex proper are intrinsically organized: a combined Evans blue and acetylcholinesterase analysis. *Brain Res Bull.* 1981; 7:487–507. [PubMed: 7317794]
2. Weiner DM, Levey AI, Brann MR. Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. *Proc Natl Acad Sci U S A.* 1990; 87:7050–7054. [PubMed: 2402490]
3. Cragg SJ. Meaningful silences: how dopamine listens to the ACh pause. *Trends Neurosci.* 2006; 29:125–131. [PubMed: 16443285]
4. Exley R, Cragg SJ. Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. *Br J Pharmacol.* 2008; 153 Suppl 1:S283–S297. [PubMed: 18037926]
5. Ding J, Guzman JN, Tkatch T, Chen S, Goldberg JA, et al. RGS4-dependent attenuation of M4 autoreceptor function in striatal cholinergic interneurons following dopamine depletion. *Nat Neurosci.* 2006; 9:832–842. [PubMed: 16699510]
6. Pisani A, Bernardi G, Ding J, Surmeier DJ. Re-emergence of striatal cholinergic interneurons in movement disorders. *Trends Neurosci.* 2007; 30:545–553. [PubMed: 17904652]
7. Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC. Striatal interneurons: chemical, physiological and morphological characterization. *Trends Neurosci.* 1995; 18:527–535. [PubMed: 8638293]
8. Koos T, Tepper JM. Inhibitory control of neostriatal projection neurons by GABAergic interneurons. *Nat Neurosci.* 1999; 2:467–472. [PubMed: 10321252]
9. Tepper JM, Koos T, Wilson CJ. GABAergic microcircuits in the neostriatum. *Trends Neurosci.* 2004; 27:662–669. [PubMed: 15474166]

10. Kawaguchi Y. Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. *J Neurosci.* 1993; 13:4908–4923. [PubMed: 7693897]
11. Bennett BD, Wilson CJ. Spontaneous activity of neostriatal cholinergic interneurons in vitro. *J Neurosci.* 1999; 19:5586–5596. [PubMed: 10377365]
12. Aosaki T, Graybiel AM, Kimura M. Effect of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys. *Science.* 1994; 265:412–415. [PubMed: 8023166]
 ●●This classic paper describes several aspects of tonically active neurons, TANs, now thought to be cholinergic interneurons. Specific attention is given to the dopamine dependent pause in TAN firing and how it is acquired in behaving monkeys. The paper proposes that TAN synchronous activity may be responsible for issues of binding information across many striatal regions, and that TANs may provide a teaching role in striatum.
13. Maurice N, Mercer J, Chan CS, Hernandez-Lopez S, Held J, et al. D2 dopamine receptor-mediated modulation of voltage-dependent Na⁺ channels reduces autonomous activity in striatal cholinergic interneurons. *J Neurosci.* 2004; 24:10289–10301. [PubMed: 15548642]
14. Graybiel AM, Aosaki T, Flaherty AW, Kimura M. The basal ganglia and adaptive motor control. *Science.* 1994; 265:1826–1831. [PubMed: 8091209]
15. Ding JB, Guzman JN, Peterson JD, Goldberg JA, Surmeier DJ. Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron.* 2010; 67:294–307. [PubMed: 20670836]
 ●Stimulation of thalamic nuclei can induce a burst-pause pattern in the spiking of striatal cholinergic neurons similar to that observed in behaving animals. This dopamine dependent pattern of activation triggered brief presynaptic inhibition of corticostriatal transmission followed by a prolonged enhancement of excitability in striatopallidal MSNs. This thalamic-gated change in MSN behavior may be the neural underpinnings of complicated phenomena such as the changing of ones attention.
16. Thorn CA, Graybiel AM. Pausing to regroup: thalamic gating of cortico-basal ganglia networks. *Neuron.* 2010; 67:175–178. [PubMed: 20670826]
17. Reynolds JN, Wickens JR. The corticostriatal input to giant aspiny interneurons in the rat: a candidate pathway for synchronising the response to reward-related cues. *Brain Res.* 2004; 1011:115–128. [PubMed: 15140651]
18. Deng P, Zhang Y, Xu ZC. Involvement of I(h) in dopamine modulation of tonic firing in striatal cholinergic interneurons. *J Neurosci.* 2007; 27:3148–3156. [PubMed: 17376976]
19. Bernard V, Normand E, Bloch B. Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. *J Neurosci.* 1992; 12:3591–3600. [PubMed: 1527598]
20. Yan Z, Flores-Hernandez J, Surmeier DJ. Coordinated expression of muscarinic receptor messenger RNAs in striatal medium spiny neurons. *Neuroscience.* 2001; 103:1017–1024. [PubMed: 11301208]
21. Zhou FM, Wilson CJ, Dani JA. Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J Neurobiol.* 2002; 53:590–605. [PubMed: 12436423]
22. Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G. Endogenous ACh enhances striatal NMDA-responses via M1-like muscarinic receptors and PKC activation. *Eur J Neurosci.* 1998; 10:2887–2895. [PubMed: 9758158]
23. Ben-Ari Y, Aniksztejn L, Bregestovski P. Protein kinase C modulation of NMDA currents: an important link for LTP induction. *Trends Neurosci.* 1992; 15:333–339. [PubMed: 1382331]
24. Akins PT, Surmeier DJ, Kitai ST. M1 muscarinic acetylcholine receptor in cultured rat neostriatum regulates phosphoinositide hydrolysis. *J Neurochem.* 1990; 54:266–273. [PubMed: 2152795]
25. Nakamura TY, Coetzee WA, Vega-Saenz De Miera E, Artman M, Rudy B. Modulation of Kv4 channels, key components of rat ventricular transient outward K⁺ current, by PKC. *Am J Physiol.* 1997; 273:H1775–H1786. [PubMed: 9362243]
26. Shen W, Hamilton SE, Nathanson NM, Surmeier DJ. Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. *J Neurosci.* 2005; 25:7449–7458. [PubMed: 16093396]
27. Shen W, Tian X, Day M, Ulrich S, Tkatch T, et al. Cholinergic modulation of Kir2 channels selectively elevates dendritic excitability in striatopallidal neurons. *Nat Neurosci.* 2007; 10:1458–1466. [PubMed: 17906621] ●●In this paper through careful experiments activation of M1 mAChR

was able to reduce the opening of Kir2 in MSN, increasing the excitability of these neurons. Interestingly this modulation was only seen in striatopallidal neurons, indicating that cholinergic activation has differential effects on the two striatal pathways.

28. Olson PA, Tkatch T, Hernandez-Lopez S, Ulrich S, Ilijic E, et al. G-protein-coupled receptor modulation of striatal CaV1.3 L-type Ca²⁺ channels is dependent on a Shank-binding domain. *J Neurosci.* 2005; 25:1050–1062. [PubMed: 15689540]
29. Perez-Burgos A, Perez-Rosello T, Salgado H, Flores-Barrera E, Prieto GA, et al. Muscarinic M(1) modulation of N and L types of calcium channels is mediated by protein kinase C in neostriatal neurons. *Neuroscience.* 2008; 155:1079–1097. [PubMed: 18644425]
30. Perez-Burgos A, Prieto GA, Galarraga E, Bargas J. CaV2.1 channels are modulated by muscarinic M1 receptors through phosphoinositide hydrolysis in neostriatal neurons. *Neuroscience.* 2010; 165:293–299. [PubMed: 19883739]
31. Howe AR, Surmeier DJ. Muscarinic receptors modulate N-, P-, and L-type Ca²⁺ currents in rat striatal neurons through parallel pathways. *J Neurosci.* 1995; 15:458–469. [PubMed: 7823150]
32. Perez-Rosello T, Figueroa A, Salgado H, Vilchis C, Tecuapetla F, et al. Cholinergic control of firing pattern and neurotransmission in rat neostriatal projection neurons: role of CaV2.1 and CaV2.2 Ca²⁺ channels. *J Neurophysiol.* 2005; 93:2507–2519. [PubMed: 15615835]
33. Alcantara AA, Mrzljak L, Jakab RL, Levey AI, Hersch SM, et al. Muscarinic m1 and m2 receptor proteins in local circuit and projection neurons of the primate striatum: anatomical evidence for cholinergic modulation of glutamatergic prefronto-striatal pathways. *J Comp Neurol.* 2001; 434:445–460. [PubMed: 11343292]
34. Barral J, Galarraga E, Bargas J. Muscarinic presynaptic inhibition of neostriatal glutamatergic afferents is mediated by Q-type Ca²⁺ channels. *Brain Res Bull.* 1999; 49:285–289. [PubMed: 10424849]
35. Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G. Blockade of M2-like muscarinic receptors enhances long-term potentiation at corticostriatal synapses. *Eur J Neurosci.* 1998; 10:3020–3023. [PubMed: 9758172]
36. Pakhotin P, Bracci E. Cholinergic interneurons control the excitatory input to the striatum. *J Neurosci.* 2007; 27:391–400. [PubMed: 17215400] ● This paper provided detailed evidence that cholinergic activation, rather than pharmacological manipulation, can effectively regulate corticostriatal synaptic transmission. Through double patch clamp recordings of cholinergic neurons and MSNs they observed single cholinergic spikes could reduce the amplitude of cortically evoked EPSCs, indicating timing of cholinergic activity may play an important role in the gating of MSN inputs.
37. Higley MJ, Soler-Llavina GJ, Sabatini BL. Cholinergic modulation of multivesicular release regulates striatal synaptic potency and integration. *Nat Neurosci.* 2009; 12:1121–1128. [PubMed: 19668198] ●● This paper demonstrated that glutamatergic synapses in striatum exhibit a high degree of multivesicular release and that their probability of release at these synapses is decreased by muscarinic activation. This provided a detailed description of the mechanism on presynaptic inhibition by cholinergic activation.
38. Shen W, Hernandez-Lopez S, Tkatch T, Held JE, Surmeier DJ. Kv1.2-containing K⁺ channels regulate subthreshold excitability of striatal medium spiny neurons. *J Neurophysiol.* 2004; 91:1337–1349. [PubMed: 13679409]
39. Kravitz AV, Freeze BS, Parker PR, Kay K, Thwin MT, et al. Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature.* 2010; 466:622–626. [PubMed: 20613723] ●● The authors used optogenetic tools to dissect striatal neural circuit in an in vivo context. Activation of either direct or indirect pathways of striatal neurons can effectively modulate the movement behavior of mice, demonstrating a bidirectional regulation of motor behavior by striatal circuits.
40. Lee IH, Seitz AR, Assad JA. Activity of tonically active neurons in the monkey putamen during initiation and withholding of movement. *J Neurophysiol.* 2006; 95:2391–2403. [PubMed: 16407429]
41. Hernandez-Echeagaray E, Starling AJ, Cepeda C, Levine MS. Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? *Eur J Neurosci.* 2004; 19:2455–2463. [PubMed: 15128399]

42. Narushima M, Hashimoto K, Kano M. Endocannabinoid-mediated short-term suppression of excitatory synaptic transmission to medium spiny neurons in the striatum. *Neurosci Res.* 2006; 54:159–164. [PubMed: 16413076]
43. Hashimoto-dani Y, Ohno-Shosaku T, Kano M. Mechanisms of triggering endocannabinoid release. *Seikagaku.* 2006; 78:126–130. [PubMed: 16541803]
44. Witten IB, Lin SC, Brodsky M, Prakash R, Diester I, et al. Cholinergic interneurons control local circuit activity and cocaine conditioning. *Science.* 2010; 330:1677–1681. [PubMed: 21164015] ●●
The authors express optogenetic tools in cholinergic neurons in nucleus accumbens and observe cholinergic activity inhibits the firing of other neurons in vivo. They demonstrated that cholinergic interneuron activity is altered during cocaine exposure. Silencing cholinergic interneurons using optogenetic tools during cocaine exposure can block cocaine conditioning in freely moving animals.
45. Koos T, Tepper JM. Dual cholinergic control of fast-spiking interneurons in the neostriatum. *J Neurosci.* 2002; 22:529–535. [PubMed: 11784799]
46. Wang Z, Kai L, Day M, Ronesi J, Yin HH, et al. Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. *Neuron.* 2006; 50:443–452. [PubMed: 16675398]
47. Narushima M, Uchigashima M, Fukaya M, Matsui M, Manabe T, et al. Tonic enhancement of endocannabinoid-mediated retrograde suppression of inhibition by cholinergic interneuron activity in the striatum. *J Neurosci.* 2007; 27:496–506. [PubMed: 17234582] ●This paper demonstrated that tonic Ach released from cholinergic neurons upregulates the release of endocannabinoids in a manner dependent on M1 mAChR. This signaling suppresses inhibitory connections to MSNs and provides another means that cholinergic neurons can control cells in the striatum.
48. Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, et al. Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. *J Neurosci.* 2007; 27:3663–3676. [PubMed: 17409230]
49. Hashimoto-dani Y, Ohno-Shosaku T, Watanabe M, Kano M. Roles of phospholipase C β and NMDA receptor in activity-dependent endocannabinoid release. *J Physiol.* 2007; 584:373–380. [PubMed: 17615097]
50. Gittis AH, Nelson AB, Thwin MT, Palop JJ, Kreitzer AC. Distinct roles of GABAergic interneurons in the regulation of striatal output pathways. *J Neurosci.* 2010; 30:2223–2234. [PubMed: 20147549]
51. Planert H, Szydlowski SN, Hjorth JJ, Grillner S, Silberberg G. Dynamics of synaptic transmission between fast-spiking interneurons and striatal projection neurons of the direct and indirect pathways. *J Neurosci.* 2010; 30:3499–3507. [PubMed: 20203210]
52. Glickfeld LL, Scanziani M. Distinct timing in the activity of cannabinoid-sensitive and cannabinoid-insensitive basket cells. *Nat Neurosci.* 2006; 9:807–815. [PubMed: 16648849]
53. Narushima M, Uchigashima M, Hashimoto K, Watanabe M, Kano M. Depolarization-induced suppression of inhibition mediated by endocannabinoids at synapses from fast-spiking interneurons to medium spiny neurons in the striatum. *Eur J Neurosci.* 2006; 24:2246–2252. [PubMed: 17042791]
54. Conklin BR, Hsiao EC, Claeyssen S, Dumuis A, Srinivasan S, et al. Engineering GPCR signaling pathways with RASSLs. *Nat Methods.* 2008; 5:673–678. [PubMed: 18668035]
55. Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL. Evolving the lock to fit the key to create a family of G protein-coupled receptors potentially activated by an inert ligand. *Proc Natl Acad Sci U S A.* 2007; 104:5163–5168. [PubMed: 17360345]
56. Alexander GM, Rogan SC, Abbas AI, Armbruster BN, Pei Y, et al. Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors. *Neuron.* 2009; 63:27–39. [PubMed: 19607790]
57. Ferguson SM, Eskenazi D, Ishikawa M, Wanat MJ, Phillips PE, et al. Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nat Neurosci.* 2010
58. Zhang F, Wang LP, Boyden ES, Deisseroth K. Channelrhodopsin-2 and optical control of excitable cells. *Nat Methods.* 2006; 3:785–792. [PubMed: 16990810]

59. Zhang F, Wang LP, Brauner M, Liewald JF, Kay K, et al. Multimodal fast optical interrogation of neural circuitry. *Nature*. 2007; 446:633–639. [PubMed: 17410168]
60. Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci*. 2005; 8:1263–1268. [PubMed: 16116447]
61. Chow BY, Han X, Dobry AS, Qian X, Chuong AS, et al. High-performance genetically targetable optical neural silencing by light-driven proton pumps. *Nature*. 2010; 463:98–102. [PubMed: 20054397]
62. Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. Optical Deconstruction of Parkinsonian Neural Circuitry. *Science*. 2009; 324:354–359. [PubMed: 19299587]
63. Aravanis AM, Wang L-P, Zhang F, Meltzer LA, Mogri MZ, et al. An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. *J Neural Eng*. 2007; 4:S143–S156. [PubMed: 17873414]
64. Tecuapetla F, Patel JC, Xenias H, English D, Tadros I, et al. Glutamatergic signaling by mesolimbic dopamine neurons in the nucleus accumbens. *J Neurosci*. 2010; 30:7105–7110. [PubMed: 20484653]
65. Stuber GD, Hnasko TS, Britt JP, Edwards RH, Bonci A. Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J Neurosci*. 2010; 30:8229–8233. [PubMed: 20554874]
66. Varga V, Losonczy A, Zemelman BV, Borhegyi Z, Nyiri G, et al. Fast synaptic subcortical control of hippocampal circuits. *Science*. 2009; 326:449–453. [PubMed: 19833972]
67. Ren J, Qin C, Hu F, Tan J, Qiu L, et al. Habenula "Cholinergic" Neurons Corelease Glutamate and Acetylcholine and Activate Postsynaptic Neurons via Distinct Transmission Modes. *Neuron*. 2011; 69:445–452. [PubMed: 21315256]
68. Fremeau RT Jr, Burman J, Qureshi T, Tran CH, Proctor J, et al. The identification of vesicular glutamate transporter 3 suggests novel modes of signaling by glutamate. *Proc Natl Acad Sci U S A*. 2002; 99:14488–14493. [PubMed: 12388773]

Figure 1. Muscarinic signaling affecting the integration of glutamatergic signaling in MSNs
Schematic of a striatopallidal MSN dendrite and spine. Muscarinic receptor activation modulates glutamate release and intrinsic excitability of MSNs by altering the gating of Ca^{2+} and K^+ channels.

Figure 2. Interactions between M1 and CB1 signalling

In the striatal medium spiny neuron, M1 receptor activation promotes PLC β 1/DAGL α -mediated production of 2-AG to induce retrograde suppression of inhibitory synaptic transmission. At excitatory synapses, lowering ACh release reduces the activity of M1 muscarinic receptors, which leads to enhanced opening of Cav1.3 channels in response to synaptic depolarization. The elevated Ca²⁺ influx results in enhanced production of endocannabinoid and activation of presynaptic CB-1 receptors that reduce glutamate release, which is critical for LTD induction in the striatum. Abbreviations: PLC, phospholipase C; DAG, 1,2-diacylglycerol.

Figure 3. Signal transduction pathways mediating the effects of muscarinic receptors in MSNs
 Abbreviations: ACh, acetylcholine; DA, dopamine; DAG, 1,2-diacylglycerol; M1R, Muscarinic M1 receptor; M2R, Muscarinic M2 receptor; IP3, inositol 1,4,5 trisphosphate; NMDAR, NMDA receptor; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PP-2B, protein phosphatase 2B; RCS, regulator of calmodulin signaling.