

# A Feud that Wasn't: Acetylcholine Evokes Dopamine Release in the Striatum

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In this issue of *Neuron*, Threlfell et al. (2012) report that synchronous activation of cholinergic interneurons evokes striatal dopamine release by activating presynaptic nicotinic acetylcholine receptors. These findings call for a fundamental reevaluation of the long-standing view that dopamine and acetylcholine “feud” over control of striatal circuitry.

Dopamine (DA) and acetylcholine (ACh) have long been thought to be the Hatfields and McCoy's of the striatum—constantly feuding for control. Based upon articles by Threlfell et al. (2012) in this issue of *Neuron* and Cachepe et al. (2012) in *Cell Reports*, it seems that we've misjudged this grudge match.

We've known for a long time that DA and ACh are important to the striatum and to the functions of the basal ganglia in health and disease. Striatal levels of the proteins associated with these two neuromodulators (e.g., synthetic enzymes, receptors) are among the highest of any region in the brain. DA in the striatum is released from the widespread axonal arbors of neurons whose cell bodies reside in the midbrain substantia nigra pars compacta, whereas the ACh comes from the giant striatal cholinergic interneurons (ChIs). Both neuromodulators are critically important for basal-ganglia-based disorders, and both have been strongly implicated in the striatal regulation of ongoing behaviors and learning.

The notion that there is a feud between DA and ACh stretches back decades to clinical observations suggesting that they reciprocally control motor behaviors. In Parkinson's disease, for example, striatal DA levels plummet and ACh levels appear to rise. Anticholinergic drugs, which nominally leveled the playing field, were used as one of the most effective treatments for the motor symptoms of PD early on (before the discovery of levodopa). Based on work by Barbeau, the

metaphor of a child's see-saw was used to capture the apparent antagonism, implying that when the effects of DA fell, those of ACh went up.

But the evidence for the feud has been decidedly one sided. It is very clear from a long parade of biochemical and physiological studies that DA suppresses ACh release. This inhibition is accomplished through G protein-coupled receptors for DA that reduce the spontaneous spiking of ChIs and the terminal release of ACh (Gerfen and Surmeier, 2010). The effects of ACh on DA release have been much more difficult to see clearly. There are acetylcholine receptors on the terminals of dopaminergic axons. Studies mostly suggest that ACh diminishes DA release, creating a symmetry with DA modulation of ACh release, but there is not a clear consensus on this point (Rice et al., 2011).

In situations like this, there is often a technical hurdle that has been difficult to overcome. So it is for ACh-DA interactions. There are multiple cholinergic receptors in the striatum, and those thought to be on DA axon terminals, and hence in a position to control DA release, are predominantly of the nicotinic subtype (Rice et al., 2011). These receptors rapidly desensitize, making it difficult to assess the real effect of ACh on DA transmission by exogenously applying drugs in ways that don't mimic the normal rapid rise and fall of transmitter in the brain.

In vivo, when ChIs are hooked up to their normal inputs, their spontaneous activity is interrupted by episodes of phasic

higher-frequency spiking (bursts) and stretches of silence (pauses) in response to salient events or conditioned stimuli, like the presentation of a sweet. Recordings from behaving monkeys have shown that these activity patterns become synchronous across large regions of the striatum as a result of behavioral learning (Graybiel et al., 1994). This activity of the ChIs was shown to be dependent on DA (Aosaki et al., 1994).

This realization has made it difficult to do the right experiment in vitro, wherein DA concentrations could be tracked quantitatively, because there was no way to get ChIs synchronized. The only hint that phasic activation of a group of ChIs might be doing something unexpected came from recent work using thalamic stimulation to drive ChI activity in brain slices (Ding et al., 2010). Phasic stimulation of thalamic axons that normally control the ChI population triggered a stereotyped burst-pause pattern of spiking in ChIs that strongly resembled the pattern seen in vivo following salient stimuli. Surprisingly, the pause in ChI spiking following the initial burst was dependent on activation of nicotinic receptors and DA release, suggesting that ChIs were evoking release from the terminals of DA axons even though these axons were quiescent. Inducing the same burst of spikes in a single ChI did not reproduce the phenomenon, suggesting that it was the product of a group effort.

Cragg's group recognized that optogenetic techniques could be used to synchronously activate ChIs in brain

slices, in which they could simultaneously monitor DA release with fast-scan voltammetry (Threlfell et al., 2012). Using a virus to deliver a Cre-dependent channelrhodopsin2 (ChR2) construct into the striatum of transgenic mice engineered to express Cre only in cholinergic neurons, they were able to limit ChR2 expression and induce spiking just in ChIs by flashing a blue light on the striatal slice. They found that synchronous activation of ChIs dramatically elevated striatal DA release, increasing it as much as phasic electrical stimulation of DA axons. The DA release didn't involve an intermediary, because it was only dependent on nicotinic receptors and not on glutamate or GABA. The DA release required synchronous activation of a population of ChIs and was insensitive to sustained ChI spiking, just as one might expect of an event that depended upon rapidly desensitizing nicotinic receptors. A major finding is that this intrastriatal, nicotinic receptor-driven DA release could short-circuit DA release evoked by electrical stimulation of DA axons. Threlfell et al. (2012) also demonstrated that optical stimulation of thalamic projections to ChIs could mimic the effect of direct activation of ChIs, raising the remarkable possibility that the thalamic intralaminar nuclei can control DA release—a situation they are well positioned to do, given their sensitivity to salient stimuli (Matsumoto et al., 2001).

The paper in *Cell Reports* by Cheer's group (Cachope et al., 2012) describes a very similar scenario in the ventral striatum-nucleus accumbens (NAc). In addition to showing that optogenetic stimulation of a population of ChIs induces DA release in slices of the NAc, Cachope et al. show that the same thing happens in vivo. One apparent point of divergence with the Threlfell et al. work is the inferred role of glutamate. Threlfell et al. found no effect of glutamate receptor antagonists on the ChI-induced DA release, but Cheer's group did find a partial reduction in release with the antagonism of AMPA receptors. They linked this to the recently described corelease of glutamate and ACh by ChIs (Higley et al., 2011). However, because this release is rapidly lost at the normal spiking rates of ChIs, its enhancement of DA release would be limited to rebound spiking after a long pause.

How does Threlfell et al.'s discovery change our understanding of the striatum? First, their work has vindicated the findings of the French group led by Glowinski, who emphasized intrastriatal control of DA release decades ago but who had few adherents. Clearly, DA release is not driven solely by the substantia nigra. Quite remarkably, even the thalamus can drive DA release in the striatum through the mechanism that Cragg and colleagues have outlined. Moreover, the model that DA and ACh simply oppose one another—as in the feud metaphor—needs to be fundamentally revised. A revision doesn't mean, however, that these two neurotransmitters are bosom buddies. DA does suppress ACh release, even if the converse is not true. Moreover, there is still compelling evidence that DA and ACh can have opposed effects on striatal physiology. For example, the induction of long-term depression at corticostriatal synapses of principal spiny projection neurons (SPNs) is promoted by an elevation in DA and a fall in ACh (Bageeta et al., 2011; Wang et al., 2006). In indirect pathway SPNs that express D<sub>2</sub> DA receptors, DA clearly depresses intrinsic excitability, and ACh increases it through activation of M<sub>1</sub> muscarinic receptors (Gerfen and Surmeier, 2010). In direct pathway SPNs that express D<sub>1</sub> DA receptors, the situation appears to be more nuanced by the coexpression of M<sub>1</sub> and M<sub>4</sub> muscarinic receptors.

In vitro studies of ACh regulation of DA have been plagued by the difficulty in selectively stimulating particular microcircuits. When and where you stimulate matters, as shown by both Threlfell et al. and Cachope et al. At the cellular level in SPNs, the effects of both modulators are mediated by a motley collection of GPCRs that act on different time and spatial scales: D<sub>2</sub> and M<sub>4</sub> receptors rapidly signal within a small region of the plasma membrane, whereas D<sub>1</sub> and M<sub>1</sub> receptors rely upon soluble second messengers that are slow and spread out within the cell. As with the example of pre- and postsynaptic modulation by ACh, it is not difficult to imagine how the sequence of strokes on this GPCR keyboard might matter in the orchestration of SPN spiking.

The finding that synchronous activity of ChIs is essential for the ChI-mediated

release of DA is almost certainly critically important to learning. The activity of ChIs becomes more synchronous as a result of behavioral learning (Graybiel et al., 1994). The mechanisms mediating this change are only beginning to be understood. SNc DA neurons and intralaminar thalamic neurons that innervate ChIs have common inputs (Coizet et al., 2007). This connectivity would suggest that SNc DA neuron and ChI activity would be driven in a temporally coordinated way in response to salient and conditioned stimuli. In fact, as DA neurons spike in phasic bursts, ChIs pause (Graybiel et al., 1994; Morris et al., 2004). The stereotyped pause in ChI activity, seen with or without a leading burst of spikes, was widely viewed as a reflection of this coordination and the release of DA in the striatum by phasic activation of SNc DA neurons. In the early stages of learning, this might very well still be the way it works, in spite of the studies discussed here. However, in the later stages of learning, the phasic modulation of SNc DA neuron activity begins to wane as responding becomes more habitual. The implications of this result for the striatum have always been a bit puzzling. Does the striatum stop needing phasic DA release to respond properly to cortical signals? The data of Threlfell et al. suggest this is not a necessary inference. ChIs continue to respond to salient and conditioned stimuli in this paradigm. The fact that ChIs “stay at the wheel” and continue to respond to sensory signal from the thalamus would allow them to do the job of the dozing SNc DA neurons and keep the striatum working properly (Matsumoto et al., 2001).

These findings have major implications for the interaction between DA and ACh in disease states, including Parkinson's disease, Huntington's disease, and dystonia. For example, nicotine has long been associated with a reduction in the risk of developing Parkinson's disease. This association has been the subject of speculation and debate. The work of Threlfell et al. suggests that by desensitizing presynaptic nAChRs, nicotine might be significantly reducing striatal DA synthesis and turnover, diminishing oxidant stress on terminals and slowing their loss with age (Sulzer, 2007). These studies also have important implications for

transplant studies aimed at restoring striatal DA levels. DA neurons transplanted into the striatum lack the normal innervation of mesencephalic SNc DA neurons; there has consequently been a concern that DA concentrations would not be modulated in the normal way. Maybe this concern is misplaced, at least in part. If ChIs innervate the grafts, they might be able to appropriately modulate DA release. Given the movement of the transplant field toward induced pluripotent stem cells (iPSCs), it also is important that DA neurons derived from iPSCs be pushed far enough toward the terminal phenotype that they express the appropriate complement of nAChRs, enabling ChIs to modulate them.

These studies also point to further questions. One is about the nature of the synchrony requirement. Why is synchronous spiking in a population of ChIs necessary for DA release? The striatal extracellular space is full of acetylcholinesterase (AChE) that rapidly degrades ACh. It could be that synchrony is required to produce a large enough release of ACh so that this enzymatic brake is temporarily overwhelmed, allowing ACh diffusion to DA terminals. Such dynamics would keep the DA release spatially restricted. An important implication is that the effect of ChIs on DA release might not be uniform. AChE density, like choline acetyltransferase activity, is high in the

striatal matrix and low in striosomes. It could be that ChI enhancement of DA release is most prominent in striosomes.

Another question is what sort of nAChR-evoked activity triggers DA release. Cragg and colleagues found that DA release was sensitive to tetrodotoxin (TTX) (Threlfell et al., 2012). The simplest interpretation of this dependence is that propagation of spikes in the axons of ChIs was necessary. However, because the ChI terminals were in the field illuminated by the blue laser and because ChR2 is capable of evoking transmitter release in terminals, it is possible that the TTX-sensitive event is propagation of spikes in the DA axons. This circumstance would allow a relatively focal burst of activity in ChIs to be broadcast to a large region of striatum, because the terminal fields of DA axons are twice as big as those of the ChIs (Matsuda et al., 2009).

There is clearly much still to be done, but what these two beautiful studies make clear is that the interaction between DA and ACh in the striatum is not so much a feud as it is a dance.

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