PNAS COMMENTARY

A detailed look at striatal acetylcholine, dopamine, and their interactions

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How animals flexibly respond to and learn from changes in the environment is an enduring question in neuroscience. An influential theory is that coordinated release of dopamine (DA) and acetylcholine (ACh) in the striatum of the mammalian brain controls when learning should occur and what actions should unfold. Although the discharge of DA neurons has been studied extensively, the activity patterns of striatal ACh-releasing neurons are comparatively less clear. In this issue, Duhne et al. (1) provide a rich, foundational dataset on the spiking activity of cholinergic interneurons (CINs) from multiple subregions of the striatum in rats performing a decision-making task. They then compare this activity to local DA release patterns to reach important conclusions about the dynamics of both modulators, their interactions, and their potential for contributing to striatal physiology and behavior.

Our appreciation for the patterns of ACh release in vivo largely stems from studies recording action potentials from striatal cells whose cholinergic identity could only be inferred (2–5). These studies described cells that continually fire action potentials at low rate (so-called "tonically active neurons," or TANs) and pause their firing when presented with salient cues. Although CINs are intrinsically active (6), the striatum contains other populations of interneurons that fire tonically (7), indicating that TANs are not necessarily cholinergic. Moreover, TAN pauses are occasionally preceded by a burst of action potentials, followed by a burst of action potentials, or both, further adding to concerns that TANs comprise a mix of cell types.

To address this concern, Duhne et al. recorded the electrical activity of striatal neurons, which they positively identified as CINs using a method called "opto-tagging." This technique relies on the expression of a light-gated ion channel in a specific cell type (CINs in this case) using Cre-dependent viral transduction in transgenic rats to identify units that respond to light with short-latency spikes. The authors now report on the activity of 100 confirmed CINs sampled from three different regions of the striatum—the dorsolateral striatum (DLS), the dorsomedial striatum (DMS), and the ventral striatum (VS; more specifically, the nucleus accumbens core)—while rats perform a probabilistic two-arm bandit task.

The first important finding is that the electrophysiological properties used previously to infer CINs are largely valid. In addition, the authors report slight, but significant differences in the baseline firing properties of CINs in different regions of the striatum. Importantly, this study provides a ground-truth dataset of positively identified CINs across a variety of striatal regions and conditions, including sleep–wake cycles to use as a reference for future electrophysiological recordings.

What about phasic pauses, which are central to our understanding of ACh signaling (8)? Here, the picture is more mixed, necessitating a deeper dive into the specific responses of identified CINs, but the main conclusion is that CINs differ in how they respond to rewards and motor events, arguing against a singular role for ACh throughout the striatum.

To characterize this heterogeneity, Dunhe et al. looked in detail at the mean firing rate of CINs at specific moments of the task. Two behavioral events stood out: the moment a salient auditory *Go!* cue sounded to invite rats to visit one of two nose pokes and the moment rats discovered whether or not they would receive a reward (i.e., "click" sound of a sucrose pellet). At the *Go!* cue, a majority of DLS CINs showed the characteristic short-latency burst–pause–burst sequence originally described in TANs (2, 3). In VS, however, the *Go!* cue triggered a more sluggish increase in firing followed by a less well-defined pause, while in DMS, only a pause was observed.

Region-specific responses were also observed to reward "clicks": DLS CINs again showed a burst–pause–burst response, but CINs in the DMS and VS slowly increased their firing without any discernible pause. Thus, neither bursts nor pauses are universal features of striatal CIN responses to reward-predictive stimuli, forcing a reconsideration of striatal-wide models positing their involvement for reward-based learning and action.

Duhne et al. also gained insights into the factors that shape the activity of CINs. For example, the authors observed that the activity patterns of CINs are largely insensitive to changes in motivation or reward expectation. These results indicate that the circuit mechanisms driving CIN spiking differ from those shaping DA release since the amplitude of DA transients is strongly sensitive to errors in reward prediction. The authors also discovered that short-latency CIN bursts and pauses are driven by sensory cues, while postpause bursts track body movements and their direction. The latter are therefore more likely to reflect efference copy or proprioceptive feedback as opposed to cell-intrinsic "rebound" bursts, as suggested previously (4).

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DA release is also modulated by salient and reward-predicting sensory stimuli (9–11), and DA and ACh are both capable of influencing each other's release (12, 13). Experiments in brain slices showed, for example, that CINs can drive DA release via nicotinic ACh receptors on DA axons (14, 15). DA neurons can in turn evoke pauses and bursts in CINs via corelease of DA and glutamate, respectively (16, 17). Duhne et al. therefore wondered whether the variable CIN responses they observed might reflect interactions between DA and ACh. To this end, they monitored fluctuations in extracellular DA from the same striatal regions in another cohort of rats using fiber photometry of the genetically encoded DA sensor dLight 1.3b.

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As expected, *Go!* and reward cues evoked phasic elevations in DA across the striatum that scaled in amplitude with reward-prediction errors (RPE). Contrary to expectations, however, the authors did not obtain evidence for any strong or systematic relationship between DA and CIN firing across the striatum. In DMS, for instance, CIN pauses often arose before *Go!* cue-evoked DA increases, and DA bursts were not followed by RPE-scaled CIN pauses, casting doubt on DA's ability to phasically silence CIN firing. In DLS, postpause bursts were not time-locked to DA neuron activation, suggesting that phasic corelease of glutamate is not sufficient to drive delayed CIN bursts either. Finally, CINs' capacity for dissociating DA release from DA neuron discharge in the midbrain (18) appeared equally elusive. These observations indicate that the strong intrastriatal interactions reported in slice are not readily evident in vivo, perhaps because they unfold over longer time courses or because they take place on smaller spatial scales that are not resolved by fiber photometry.

Overall, this study provides needed clarity on the dynamics of the neuromodulatory milieu in which striatal circuits exist during motivated behavior. It makes a convincing argument that many patterns of CIN activity and DA release exist across the striatum. In other words, the dynamics of DA and ACh cannot be boiled down to a set of striatal-wide rules or relationships. Instead, the conditions necessary for learning and moving may be tailored to individual striatal microcircuits. This study also reminds us of the challenges associated with applying mechanistic insights gained ex vivo to dynamical systems in vivo. Although the molecular and cellular substrates for DA–ACh interactions clearly exist, exactly when, where, and how DA and ACh influence each other in vivo remain to be established (5, 19, 20). In many ways, we are still in the early days of understanding how DA and ACh signals combine to modify behavior.

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