Synchronous activation of cholinergic interneurons triggers striatal dopamine release

Threlfell S\textsuperscript{1,2}, Lalic T\textsuperscript{1}, Platt NP\textsuperscript{1}, Jennings KA\textsuperscript{1}, Cragg SJ\textsuperscript{1,2}

\textsuperscript{1}Department of Physiology, Anatomy and Genetics, Sherrington Building, University of Oxford, OX1 3PT, UK
\textsuperscript{2}Oxford Parkinson’s Disease Centre, University of Oxford, Oxford, OX1 3PT, UK

(*sarah.threlfell@dpag.ox.ac.uk)

Introduction

Striatal dopamine (DA) plays key roles in our normal and pathological goal-directed actions. To understand DA function, much attention has centred on how midbrain DA neurons modulate their firing patterns [1]. Action potentials originating in DA neurons have been assumed to be the principal trigger for DA transmission from striatal axons. How DA firing rates are translated into striatal DA release has also been shown to be modulated by presynaptic filters in DA axons [2,3]. However, here we identify a presynaptic mechanism that triggers dopamine release directly from axons, bypassing dopamine neurons [4].

Methods

We incorporated the light-activated ion channel channelrhodopsin2 (ChR2) into striatal cholinergic interneurons by injecting a cre-dependent AAV into the striatum of mice expressing cre-recombinase under the control of the promoter for choline acetyltransferase (ChAT-Cre). We paired electrophysiological recordings of ChR2-expressing striatal cholinergic interneurons with simultaneous detection of dopamine release using fast-scan cyclic voltammetry (FCV) at carbon-fibre microelectrodes in striatal slices. To investigate the activity-dependence of striatal DA signaling and the inputs able to drive cholinergic interneurons we also performed experiments in mice expressing ChR2 in DA neurons or thalamostriatal terminals. We injected cre-dependent AAV into the midbrain or thalamus of mice expressing cre-recombinase under the control of the promoter for the dopamine uptake transporter (DAT-Cre) or Ca\textsuperscript{2+}-calmodulin-dependent kinase II (CaMKII-Cre). To determine the specificity of ChR2 expression in ChAT- or DAT-Cre mice, we fixed acute striatal or midbrain slices containing ChR2-eYFP neurons following recordings and processed them for ChAT or TH immunoreactivity.

Results and Discussion

We found that light-activation of cholinergic interneurons triggers striatal DA release (Figure 1) in both dorsal and ventral striatum. Extracellular DA concentrations evoked following light-activation of cholinergic interneurons were similar to those evoked by local electrical stimuli (Figure 1) and reproducible for several hours (sampling interval 2.5 mins), indicating DA release from a
population of axons. This acetylcholine- (ACh) evoked DA release required activation of nicotinic acetylcholine receptors (nAChRs) not muscarinic acetylcholine receptors (mAChRs), glutamate receptors or GABA receptors. ACh-evoked DA release was prevented by manipulations which normally limit ACh or DA exocytosis including blockade of voltage-gated Na⁺-channels, removal of extracellular Ca²⁺ from recording aCSF, activation of D₂ receptors or mAChRs.

**Figure 1. Activation of cholinergic interneurons drives striatal dopamine release.** (A) Delivery of Cre-dependent AAV ChR2(H134R)-eYFP into striatum of ChAT-Cre mice results in ChR2-eYFP expression (green) in a population of cholinergic interneurons (ChAT; red). (B) A local laser pulse (473 nm, 2 ms) in striatal slices from these mice evokes release of dopamine in striatum, which is comparable in magnitude to that evoked by local electrical stimulation (0.6 mA, 200 µs). Inset; Cyclic voltammograms identify DA.

We paired recording of laser-evoked DA using FCV with whole-cell patch-clamp recording of ChR2-expressing cholinergic interneurons to understand the neuronal events required for ACh-evoked DA release. Short laser pulses that generated only a single action potential in any recorded cholinergic interneuron were sufficient to evoke DA release; however when we used current injection through the patch pipette to stimulate those same neurons individually, DA release was not evoked, suggesting activity in more than one cholinergic interneuron is required. Ramping the intensity of the laser until the threshold for spiking in a given cholinergic interneuron was reached also did not evoke DA release – this approach will minimize synchrony between cholinergic interneurons with individual neurons becoming activated at different points, suggesting that synchronous activation of multiple cholinergic interneurons is required to drive DA release.

ACh-evoked DA release was independent of the frequency of cholinergic interneuron firing. In contrast, DA release evoked following direct activation of DA
axons in striatal slices expressing ChR2 in DA terminals was frequency-dependent. When both DA axons and ACh axons are co-activated following electrical stimuli resulting DA signals are insensitive to frequency of activation [5] suggesting that ACh-evoked DA release overrides ascending activity from dopamine neurons. Furthermore, ACh-evoked DA release was reproduced by activating ChR2-expressing thalamostriatal inputs, which synchronize cholinergic interneurons in vivo [6,7,8]. Activation of thalamostriatal terminals evoked DA release that was dependent on nAChRs and AMPA receptors. DA release following thalamostriatal terminal activation was also insensitive to frequency of activation.

These findings indicate that synchronized activity in cholinergic interneurons directly generates striatal dopamine signals whose functions will extend beyond those encoded by dopamine neuron activity. Our findings have several implications. Activity in DA cell bodies is not an exclusive trigger for striatal DA release; striatal ACh acting at nAChRs on DA axons bypasses midbrain neurons to trigger DA release directly. These data also indicate that circuits that drive synchronous activity in striatal cholinergic interneurons have privileged roles as triggers of DA signals. ACh-evoked DA release will also have profound outcomes for DA functions encoded by dynamic patterns of activity in DA neurons, this outcome will be entirely dependent on the timing of activity in DA neurons relative to cholinergic interneurons. These data may also provide unique insights into Parkinson’s disease neuroprotective strategies. For example, nicotine has long been associated with reduced risk of Parkinson’s disease, by desensitizing nAChRs, nicotine may reduce the stress placed on the DA terminal processing ascending activity from DA neurons as well as from ACh acting at nAChRs.

References