Opposing regulation of striatal dopamine release and exploratory motor behavior by forebrain and brainstem cholinergic circuits

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Introduction

Dopamine (DA) transmission is implicated in a variety of motor, emotional, and cognitive functions [1-3]. Midbrain DA neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) provide important modulatory input to the striatal complex, including the caudate putamen (CPu) and nucleus accumbens (NAc). Acetylcholine (ACh) signaling pathways regulate DA efflux within the striatum at two fundamental levels of the circuit [4] (Figure 1A). First, the burst firing of DA neurons in the SNc and VTA is stimulated perisomatically by cholinergic and glutamatergic inputs from the pedunculopontine tegmental (PPTg) and laterodorsal tegmental (LDTg) nuclei in the rostral brainstem [5-7], resulting in increased striatal DA efflux [6] and locomotor activity [8]. In particular, cholinergic inputs from the LDTg appear to act as a gate for the transition from tonic to burst firing modes, thereby regulating the responsiveness of DA neurons to glutamatergic input [9]. Second, ACh release from striatal cholinergic interneurons can trigger DA release via presynaptic nicotinic ACh receptors (nAChRs) on DA axons [10-11].

We hypothesized that broad removal of ACh within the forebrain would result in exaggerated striatal DA signaling and altered motor behaviors in a manner dependent on the stimulation of burst firing of midbrain DA neurons by brainstem-derived ACh. To test the relative contributions of forebrain and brainstem cholinergic input to the regulation of DA signaling, we took advantage of genetic ablation strategies in mice to eliminate ACh synthesis selectively in the forebrain, brainstem, or both regions simultaneously. We then assessed the effects on evoked DA release in CPu and NAc core and shell in vitro, and the behavioral propensity for open field exploration in a novel environment, which is highly responsive to changes in striatal DA efflux.

Methods

The enzyme choline acetyltransferase (ChAT) is essential for ACh synthesis. To generate animals with a forebrain-restricted ablation of ACh production, we combined a conditional floxed allele of ChAT (ChATfloxed) with an Nkx2.1Cre transgenic line in which cumulative recombination is restricted to the forebrain and hypothalamus to generate ChAT knock-out (KO) mice [12]. Using a similar genetic approach, we then generated rostral brainstem ChAT KO animals using the En1Cre driver, whose cumulative recombination is restricted to the mes/r1 embryonic territory, and as such, primarily affects the PPTg and LDTg but not
brainstem motor nuclei that arise from more caudal regions [12]. To assess striatal DA release, coronal forebrain slices (350 µm) were prepared from transgenic mice and their non-mutant control littermates (3-5 mo) in accordance with NIH guidelines and with IACUC approval. Methods for slice preparation and DA release detection using fast-scan cyclic voltammetry and carbon-fiber microelectrodes were as described elsewhere [12-14]. All measurements were made in a submersion chamber at 32°C in ACSF that contained (in mM): NaCl (124); KCl (3.7); NaHCO₃ (26); CaCl₂ (2.4); MgSO₄ (1.3); KH₂PO₄ (1.3); and glucose (10), saturated with 95% O₂/5% CO₂. Single stimulus pulses or 5-pulse trains at varying frequency were used to evoke DA release in CPu, NAc shell and NAc core. Locomotor activity was monitored during the first 10 min in an open field arena; raclopride was administered at 0.25 or 0.5 mg/kg before testing [12].

Results and Discussion

As predicted from previous pharmacological studies [15-17], mice lacking forebrain ACh (forebrain KO) showed enhanced phasic-to-tonic evoked DA release throughout the striatal complex, including CPu (Figure 1B), NAc shell and NAc core. In contrast to the frequency-insensitive response in slices from wild-type mice, DA release in slices from forebrain KO mice was insensitive to nAChR antagonism by mecamylamine or by a desensitizing concentration of nicotine. When placed in a novel environment, forebrain KO mice also showed locomotor hyperactivity compared to wild-type mice. Consistent with our in vitro evidence for exaggerated striatal DA signaling in forebrain KO mice, suppression of novelty induced locomotor activity in forebrain KO mice required a higher dose of a D2 DA receptor antagonist raclopride than in wild-types.

In contrast to forebrain ACh KO mice, mice with selective deletion of brainstem ACh (brainstem KO) showed patterns of striatal DA release that were unaltered from those in wild-type and were sensitive to nAChR antagonism. Nevertheless, brainstem KO mice were hypoactive in a novel environment compared to wild-type mice, consistent with a predicted decrease in DA neuron burst firing. Unsurprisingly, therefore, this locomotor behavior was insensitive to raclopride.

Figure 1. Patterns of evoked extracellular DA concentration ([DA]₀) in CPu from ChAT KO mice. A) Model for coordinate regulation of striatal DA release by striatal ACh interneurons (green territory) and by midbrain DA neuron activity regulated by PTg/LDTg ACh projection neurons (red). B) Evoked [DA]₀ with single pulse (1 p) and 5-pulses (5 p) in slices from wild-type and forebrain ACh KO mice. C) Evoked [DA]₀ in slices from brainstem KO and forebrain/brainstem KO mice (**p<0.01; ***p<0.001 vs. wild-type; means ± SEM).
Remarkably, motor behavior in a novel environment was normalized in mice lacking both forebrain and brainstem ACh. This behavior was also raclopride insensitive suggesting that enhanced phasic-to-tonic striatal DA release in double KOs may amplify motor signaling from the diminished burst output of midbrain DA neurons after loss of brainstem ACh inputs to maintain normal motor output.

Together, these findings support a key role for burst-dependent DA release in locomotor activity in a novel environment. Moreover, they refocus attention back on cholinergic regulation of striatal DA release as a component of motivated behavior. Our results from forebrain ACh KO mice imply that only in the absence of local DA release regulation by ACh will striatal DA signaling reflect DA neuron activity. Overall, the distinct differences in DA-dependent behaviors between forebrain and brainstem ACh KO mice and the normalization of motor behavior in double KOs provide novel insights into ACh/DA interactions and the balance of ACh activities throughout the brain that is required for proper motor function.

References


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