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Good riddance to dopamine: Roles for the dopamine transporter in synaptic function and dopamine-associated brain disorders

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ABSTRACT

The neurotransmitter dopamine (DA) plays a critical role in CNS circuits that provide for attention, executive function, reward responses, motivation and movement. DA is inactivated by the cocaine- and amphetamine-sensitive DA transporter (DAT), a protein that also provides a pathway for non-vesicular DA release. After a brief review of DAT function and psychostimulant actions, we consider the importance DAT in relation to the distinct firing patterns of DA neurons that permit awareness of novelty and reward. Finally, we review recent efforts to gather direct support for DAT-linked disorders, with a specific focus on DAT mutations recently identified in subjects with ADHD.

1. Introduction

Since the discovery that dopamine (DA) serves as a neurotransmitter in its own right, and not merely as a precursor to norepinephrine (NE) biogenesis (Carlsson et al., 1957, 1958, 1959), the physiology of DA neurons and the actions of secreted DA have been the focus of decades of investigation (Carlsson, 1987). As a result of nearly fifty years of intensive study, we now know that DA exerts powerful, modulatory control of locomotion, cognition, reward and motivation (Robbins, 2003), supported by the action of the neurotransmitter at five G-protein coupled receptors (D1–D5) (Missale et al., 1998). Once DA has been synthesized and packaged into vesicles by the vesicular monoamine transporter 2 (VMAT 2), the catecholamine is released by vesicular fusion mechanisms. The bulk of DA-releasing projections to the forebrain, including major inputs targeted to the nucleus accumbens (NAcc), prefrontal cortex, and striatum, arise from midbrain neurons located in the ventral tegmental area (VTA) and the substantia nigra (SN) (Lindvall and Bjorklund, 1978). DA can also be released by the dendrites of DA neurons (Cheramy et al., 1981; Rice et al., 1997; Morice et al., 2007), acting on somatodendritic D2 receptors (Sesack et al., 1994) to regulate excitability (Lacey et al., 1987). A feature of D2 receptors that distinguishes somatodendritic and presynaptic D2 receptors from postsynaptic D2 receptors is the presence of a shorter intracellular loop 3, generated by alternative mRNA splicing (Guiramand et al., 1995). In the cell body and dendrites, $D2_{short}$ receptors increase the opening of K^+ (GIRK/ Kir 3.2) channels, leading to a dampening of DA neuron excitability, and providing a mechanism of feedback control of DA neuron firing (Lacey et al., 1987; Beckstead et al., 2004). Presynaptic $D2_{short}$ receptors negatively modulate DA biosynthesis (O'Hara et al., 1996) as well as the fusion of DA containing synaptic vesicles (Benoit-Marand et al., 2001), providing two more examples of autoregulatory mechanisms that regulate DA neurons. Finally, as we will see below, presynaptic $D2_{short}$ receptors also regulate the DA transporter (DAT), though as yet many details of this interaction remain to be defined.

Clearance of DA by DAT is the primary mechanism, besides diffusion, for terminating DA signaling (Giros and Caron, 1993). Like $D2_{short}$ receptors, DAT proteins are expressed on both somatodendritic and presynaptic membranes (Nirenberg et al., 1996, 1997a,b). $D2_{short}$ receptors can be isolated in a physical complex with DAT (Lee et al., 2007), though existing data do not preclude populations of uncomplexed receptors and transporters; nor do we understand well whether complexes are transient or constitutive. Cocaine and methylphenidate (Ritalin™) are conventional, competitive DAT antagonists that block DA reuptake (Gether et al., 2006) and thus elevate DA levels in the context of ongoing vesicular DA release. In contrast, amphetamine, (AMPH) is a DAT substrate that is transported into the cell through DAT, though at higher concentrations AMPH can penetrate the membrane in a DAT-independent manner, as illustrated by studies of AMPH-diminished vesicular stores in brain slices from DAT KO mice

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(Jones et al., 1998b). AMPH depletes DA vesicles as a weak base substrate for VMAT2, sequestering protons and diminishing the needed to sustain the vesicular pH gradient that supports neurotransmitter packaging (Sulzer et al., 2005).

Besides being a substrate for DAT, AMPH places DAT in an "efflux-willing state", a process that is supported by a series of steps that involve changes in DAT phosphorylation (Khoshbouei et al., 2004; Fog et al., 2006) and protein associations (Cremona et al., 2011) These changes lead ultimately to the expulsion of cytoplasmic DA stores from the neuron, a process referred to as nonvesicular DA release, DA efflux or DAT-mediated DA release. Evidence also indicates that DAT is trafficked to specialized, cholesterol-rich membrane microdomains, sometimes referred to as "lipid-rafts" (Adkins et al., 2007; Foster et al., 2008). Recent studies by Cremona and colleagues indicate that DAT interactions with the raft-associated protein flotillin-1 is a requirement for AMPH-triggered DA efflux (Cremona et al., 2011; Pizzo et al., 2013), though how this interaction drives efflux-prone conformations remains to be established. Importantly, as AMPH-induced DA efflux is reliant on DAT, but not vesicular DA release, AMPH actions can proceed regardless of the state of DA neuron excitation. Indeed, the action of AMPH can be seen as subverting, rather than amplifying, excitation-coupled vesicular DA release. Prolonged AMPH treatments have been shown to produce DAT internalization (Saunders et al., 2000). Given evidence for physical interactions of $D2_{short}$ receptors with DAT, evidence that the presence or activation of D2 receptors can enhance surface expression of DAT (Bolan et al., 2007; Lee et al., 2007), and evidence that D2 receptors regulate DAT *in vitro* (Dickinson et al., 1999; Mayfield and Zahniser, 2001) and *in vivo* (Benoit-Marand et al., 2011) further efforts are needed to explore the degree to which AMPH-induced DAT trafficking relies on receptor/transporter interactions.

It is somewhat surprising that, despite longstanding evidence of a critical role played by DAT in DA signaling, only recently have brain disorders been directly linked to frank DAT dysfunction. Below, after a review of the patterns of DA neuron activity that dictate basal and elevated DA secretion, we review findings from human genetic studies that provide direct evidence of "DATopathies" and discuss how their functional perturbations, in relation to DA neuron firing patterns, can disrupt DA signaling.

2. DA neuron firing – when does DAT matter?

Both SN and VTA DA neurons exhibit three distinct states of activity – an inactive, hyperpolarized state, a 'tonic', spontaneous, irregular, single spike form of activity and a 'phasic', depolarization-dependent, burst-firing pattern of activity (Grace and Bunney, 1983). Approximately 50% of all DA neurons are not spontaneously active and are held at hyperpolarizing membrane potentials, likely due to GABAergic input from local GABAergic interneurons (Grace and Bunney, 1985) or, in the case of a subpopulation of VTA DA neurons, by afferent input from the ventral pallidum (Floresco et al., 2003). The tonic firing of DA neurons is established via an intrinsic pacemaking mechanism that is dependent on a hyperpolarization-activated cationic conductance (I_h) (Grace and Bunney, 1984). Phasic action of DA neurons is initiated via afferent control by cortical and brainstem nuclei (Charara et al., 1996; Lodge and Grace, 2006a,b). Bursts of activity are often followed by a pause (after-hyperpolarization), and subsequent resumption of spontaneous activity (Overton and Clark, 1997).

The transition from tonic to phasic firing of VTA DA neurons is associated with reward-related cues, reward prediction errors and incentive salience (Berridge and Robinson, 1998; Schultz, 1998). Both tonic and phasic firing can be reduced in response to the absence of expected reward or to aversive stimuli (Ungless et al., 2004). Whereas lesion, pharmacological and transgenic studies have provided evidence for the role of DA signaling in reward circuitry, only recently have studies achieved a level of spatiotemporal precision to support causality. To specifically interrogate the role of the DA neurons in reward-related behavior, Tsai and colleagues (Tsai et al., 2009). utilized animals with channelrhodopsin-2 (ChR2) expressed in the VTA and 1–5 Hz and 20 Hz or higher blue light pulses to drive tonic and phasic DA neuron firing, respectively. Phasic optical stimulation (50 Hz) sufficed to establish place preference for cocaine, a measure of the "liking" aspect of psychostimulant action. Further use of ChR2-based approaches has afforded analysis of the role of DA systems in other components of reward-seeking, as well as the involvement of afferents in inhibiting this behavior (Brown et al., 2010; Adamantidis et al., 2011; van Zessen et al., 2012; Chaudhury et al., 2013; Tye et al., 2013a).

The role of tonic DA neuron firing and DA release has received somewhat less attention. More than two decades ago, Grace (Grace, 1991) proposed that tonic DA signaling at D2 autoreceptors was critical for setting the capacity of DA neurons for phasic firing. More recently, several studies have reported findings that suggest mechanisms by which tonic activity supports distinct features of DA signaling and behavior. Tye and colleagues highlight the importance of a balance between tonic and phasic stimulation of DA release by showing that enhanced basal DA attenuates hindbrain nuclei-mediated increases in transient/phasic DA release (Tye et al., 2013b). Dombrowski and workers also demonstrated that tonic DA plays a key role in the learning of an instrumental avoidance response, via positive prediction errors further underscoring the behavioral importance of tonic DA (Dombrowski et al., 2013). Furthermore, as noted above, VTA neurons are maintained in a hyperpolarized state by ventral pallidal inputs (Floresco et al., 2003). The removal of this inhibition by ventral subiculum projections leads to the tonic firing of VTA DA neurons. This "hippocampal-VTA loop" has been implicated in controlling novelty-dependent information storage (Lisman and Grace, 2005; Grace et al., 2007). In the context of integrating information from limbic and prefrontal cortex (PFC) inputs, Goto and Grace demonstrated that whereas phasic DA release from limbic inputs targets D1Rs, tonic DA fluctuations from the PFC are detected by D2Rs, and that changes in tonic DA are essential in performing switches between an already learned response to a new one (Goto and Grace, 2005). Together, all the above studies illuminate a growing appreciation for the importance of tonic DA in shaping behavioral flexibility (Goto et al., 2007).

The ability of DAT to efficiently clear DA results in a differential contribution of the transporter to tonic vs phasic DA signaling. Burst firing of select dopaminergic afferents produces high-concentration, short-lasting, increases in synaptic DA that act in or around the synapses at which it is released. The activation of burst firing of DA afferents, without a change in the number of actively firing DA neurons, does not elevate extracellular DA in the NAcc, unless DAT is blocked (Floresco et al., 2003). These findings indicate that DAT activity constrains DA signaling to specific synapses and insures a temporal limit on DA availability to more closely match that of presynaptic activation. The DA levels generated by tonic DA neuron firing are thought to be less dependent on DAT. Thus, disinhibition of quiescent DA neurons raises basal extracellular DA levels in NAc, but these levels are not affected by DAT blockade (Floresco et al., 2003). However, *in vivo* studies using fast scan cyclic voltammetry (FSCV) demonstrated that elevations in extracellular DA levels after exposure to a DAT antagonist alters the frequency and amplitude of transient firing (see below for more detailed discussion). Studies using an inducible DAT knock down line that exhibits markedly reduced DA uptake revealed increased

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