Dopamine transport currents are promoted from curiosity to physiology

David Sulzer[1] and Aurelio Galli[2]

[1]Departments of Neurology and Psychiatry, Columbia University, Department of Neuroscience, New York State Psychiatric Institute, Black 305, 650 W 168th Street, New York, NY 10032, USA
[2]Department of Molecular Physiology and Biophysics and Center for Molecular Neuroscience, Vanderbilt University, 465 21st Ave South, Nashville, TN 37232, USA
Corresponding author: David Sulzer (ds43@columbia.edu)

Midbrain dopaminergic neurons maintain low ongoing 'tonic' activity interrupted by high frequency bursts associated with behavioral reward. Dopamine released during bursts feeds back onto D_2 autoreceptors that depress neuronal activity. New findings from Ingram and colleagues suggest that, by contrast, tonic activity excites these neurons by activating an uncoupled Cl^- conductance that is mediated by the dopamine uptake transporter. This response extends the range of states regulated by neurotransmitter feedback, and could contribute importantly to understanding amphetamine action.

The dopamine uptake transporter (DAT) is the primary site for cocaine and amphetamine action, and its activity determines the length of time that dopamine is present in the extracellular milieu after its release from the synapse. Early studies of DAT activity in striatal synaptosomes indicated that, in order to accumulate dopamine cations (DA^+) against a concentration gradient, the cotransport of Na^+ and Cl^- ions were 'coupled' in a fixed stoichiometry of $1Cl^-$:$2Na^+$:DA^+ [1], resulting in net import of two cations per transport cycle.

This coupled transport (also known as 'T-mode') was subsequently confirmed for DAT and other uptake transporters, but evidence arose to suggest additional modes of transporter-associated ion flux. Electrophysiological studies on cell lines and neurons expressing DAT [2], the serotonin transporter (SERT) [3] and glutamate transporters [4] demonstrated the existence of uncoupled ion conductances similar to those recorded in classical ion channels. Most notably, the noradrenaline transporter (NET) [5] possesses a channel-like mode ('C-mode') that could carry an arbitrary number of ions and substrate molecules, apparently dependent on the transporter 'channel' open time.

Although the ability of transporters to maintain a channel-like mode is interesting for biophysicists and could provide a more efficient approach for uptake than the coupled mode [6], uncoupled transporter conductances have remained something of an esoteric curiosity with no identified physiological function. A new study by Susan Ingram, Balakrishna Prasad and Susan Amara [7], however, indicates that neurotransmitter-triggered uncoupled currents might play a physiological role in regulating the ongoing 'tonic' activity and dopamine release by midbrain dopamine neurons.

Uncoupled DAT currents require dopamine and are carried by Cl^-

Midbrain dopaminergic neurons express D_2 autoreceptors that depress neuronal firing by opening K^+ channels and closing Ca^{2+} channels. Because the K^+-channel-induced hyperpolarization overwhelms other dopamine-elicited currents, the authors exposed cultured rat midbrain dopaminergic neurons to the D_2 antagonists raclopride or sulpiride. When D_2 activation was blocked, exposure to low concentrations of dopamine, rather than slowing neuronal firing, increased the firing rate threefold to fourfold. The increased firing was blocked by the DAT antagonists cocaine or GBR12909, providing evidence that, as is the case for NET, the transporter substrate activates an excitatory current. This excitation was confirmed in voltage-clamp experiments to be due to a cocaine-sensitive inward current. The size of this dopamine-induced DAT-mediated current was between one and two orders of magnitude larger than that predicted by multiplying the number of molecules of dopamine transported per second by 2 net charges, and so must have been an uncoupled current (Fig. 1). Replacement of external Cl^- by the highly permeant anion nitrate shifted the reversal potential of the DAT conductance to more negative voltages, whereas substitution of the less permeant anion methanesulfonate shifted the reversal potential in a positive direction. Thus, the authors concluded that uncoupled channel-like DAT currents triggered by dopamine were mainly carried by Cl^-.

A puzzle stemming from these results is why the DAT-mediated Cl^- currents were excitatory. As in the classical Nernst equation, the reversal potential of an ion is determined by its intracellular and extracellular concentration. If the voltage of a neuron is positive relative to the reversal potential of a permeant anion, the net anion flux must be from the extracellular milieu to the cell. The investigators found that the

current induced by muscimol, which elicits classical Cl^- currents through the $GABA_A$ channel, displayed a reversal potential of –57 mV and, thus, at the typical resting potential of –52 mV it slightly hyperpolarized the cell. Surprisingly, the dopamine-stimulated DAT currents reversed at –47 mV, so that at the resting potential of the neuron, dopamine activated an excitatory current. It is not clear why the DAT-mediated Cl^- conductance currents were reversed at a more positive potential than the $GABA_A$ currents. The apparent contradiction of the Nernst equation in Ingram's study is unresolved: perhaps the ability of DAT to co-transport Na^+ provides a coupling mechanism to allow there to be a reversal potential not determined solely by the Cl^- gradient.

Uncoupled DAT currents might filter neuronal activity

Any excitation induced by activation of DAT by dopamine at the cell body would be small, as the reversal potential is close to resting potential and the current would be dwarfed by large currents induced by the D_2 autoreceptors that clamp the neuron near the K^+ reversal potential and block firing. Certainly, these currents would be insufficient to produce depolarization that could underlie neurotoxicity or the 'depolarization inactivation' suggested, albeit controversially, to explain the action of antipsychotic drugs.

Nevertheless, in neuronal culture, the small depolarization by dopamine-induced uncoupled DAT currents increased neuronal firing severalfold. If this also occurs *in vivo*, where dopaminergic neurons switch between two stereotypical firing patterns, it will alter neurotransmitter release. In rodents, the ongoing basal 'tonic' firing frequency is ~4 Hz (i.e. 4 action potentials per second), interrupted by bursts of action potentials of two to six spikes at ~15 Hz [8]. The triggering of bursts is associated with sensory stimuli and reward during learning paradigms [9] and could underlie conditioned learning.

Estimates of the extracellular dopamine levels corresponding to tonic and burst firing have been provided by rapid electrochemical recordings. In the striatum, the basal level of extracellular dopamine during tonic activity is estimated as 5–20 nM [10], in agreement with slower microdialysis measurements [11]. This 'background' level of dopamine does not appear to elicit D_2 autoreceptor feedback depression [12], perhaps because the level is below that required to stimulate the relatively low-affinity D_2 receptors. By contrast, dopmaine released by burst firing induced by extracellular electrodes or NMDA injection in anesthetized rats [13], or by behavioral stimuli in freely moving rats [14], reached a maximum of 0.2–1.0 μM with a half-time of ~25 ms. This high dopamine input depresses subsequent release of dopamine for several seconds via D_2 autoreceptors on dopaminergic terminals [15], most likely by depressing axonal Ca^{2+} currents (Y. Schmitz and D. Sulzer, unpublished).

Ingram found that the uncoupled DAT current was half-maximal at 35 nM dopamine, which is in the range associated with tonic neuronal activity. This suggests a two-component filter model of dopamine-mediated feedback, in which low concentrations associated with tonic firing stimulates release of dopamine via DAT-mediated excitatory currents, whereas higher levels that are capable of activating the D_2 autoreceptor depress release of dopamine (Fig. 2).

A new role for amphetamine?

Although DAT blockers such as cocaine abolish the dopamine-induced uncoupled current, these currents are activated by the DAT substrate amphetamine. Perhaps this could in part explain reports consistent with amphetamine-induced neuronal excitation [16] and exocytic catecholamine release [17,18], even though the drug is otherwise understood to induce non-exocytic neurotransmitter release by reverse transport across the DAT and reuptake inhibition [19]. If low concentrations of amphetamines induce exocytic dopamine release, Ingram's results could have revealed a novel additional mechanism of action by this widely used class of drugs.

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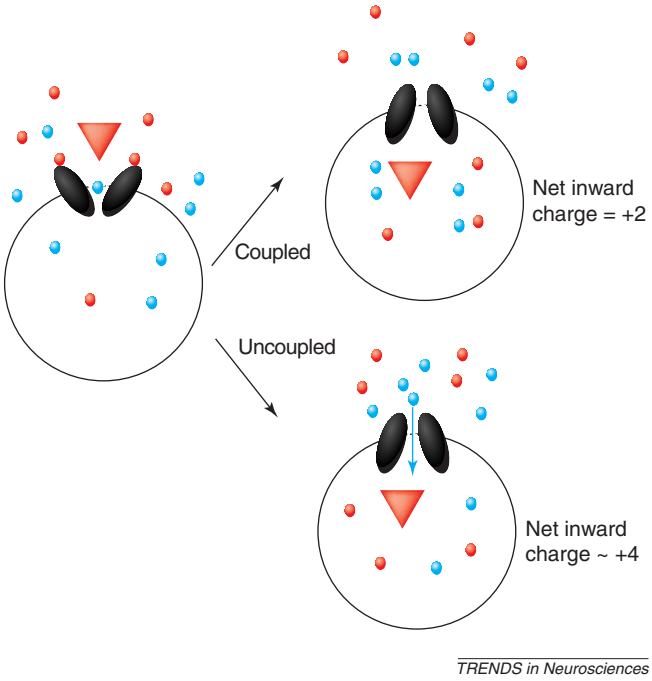

Fig 1. How activation of the dopamine uptake transporter (DAT) might excite neurons. The energy for uptake of dopmaine (red triangle) harnesses transmembrane gradients of Na^+ (red spheres) and Cl^- (blue spheres). The coupled translocation of two extracellular Na^+ and one Cl^- with dopamine provides a net inward charge of two positive charges per transport cycle. If, however, the substrate-activated DAT has channel-like properties that allow uncoupled Cl^- flux, and the DAT reversal potential (~–47 mV) is higher than resting potential (~–52 mV), the current will be far greater. Experimentally, the dopamine-elicited inward excitatory current near resting potential was between one and two orders of magnitude higher than predicted by coupled transport.

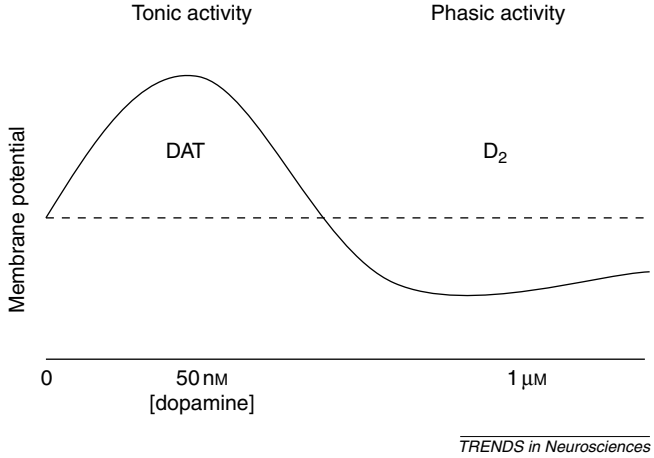

Fig 2. Dopamine release could provide biphasic feedback effects on terminal activity. Low concentrations of striatal extracellular dopamine (~5–20 nM) associated with ongoing 'tonic' firing of midbrain neurons might enhance excitation by activating uncoupled excitatory dopamine uptake transporter (DAT) currents, whereas the 'phasic' firing associated with reward could depress subsequent activity (and dopamine release, not shown) by closing Ca^{2+} channels and opening K^+ channels via activation of D_2 autoreceptors.