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Current Opinion in Neurobiology

Glutamatergic signaling by midbrain dopaminergic neurons: recent insights from optogenetic, molecular and behavioral studies

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Although the notion that dopaminergic neurons utilize glutamate as a co-transmitter has long been supported by tantalizing molecular, immunocytochemical and electrophysiological evidence it has only been with the recent addition of optogenetic and other approaches that the existence and functional relevance of this mechanism could be unambiguously demonstrated. Here we discuss the possible mechanisms of action of glutamate released from mesoaccumbens dopaminergic neurons based on recently accumulated evidence. Surprisingly, rather then to confirm a role in conventional fast excitatory transmission, the latest evidence suggests that glutamate released from dopaminergic neurons may primarily act through different unconventional presynaptic and postsynaptic mechanisms.

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Current Opinion in Neurobiology 2011, **21**:1–9

This review comes from a themed issue on
Behavioral and Cognitive Neuroscience
Edited by Ann Graybiel and Richard Morris

0959-4388/$ – see front matter
Published by Elsevier Ltd.

DOI 10.1016/j.conb.2011.05.010

Introduction

Although the precise role(s) of the midbrain dopaminergic system in learning are not yet completely understood, there is compelling neurophysiological evidence that these neurons encode information about momentary changes in the expectation of reinforcement [1]. This information is represented in brief firing rate changes which conform with remarkable precision to the theoretically predicted properties of a supervisory signal required for the adaptive modification of sensory representation and behavior in temporal difference models of conditioning [1–3]. In order to fulfill this role, dopaminergic neurons need to activate postsynaptic signaling mechanisms that exhibit sufficiently fast kinetics to preserve the temporally encoded information about reinforcement. Although dopaminergic mechanisms may themselves serve such a function [3,4], recent evidence of glutamatergic signaling by midbrain dopaminergic neurons suggests the exciting possibility that this novel glutamatergic mechanism may also be involved in the transmission of reward information. Here we review the currently available information about glutamatergic signaling by mesencephalic dopaminergic neurons and examine the implications of this phenomenon for the functioning of the mesoaccumbens dopaminergic system.

Controversy about functional glutamatergic signaling by mesencephalic dopaminergic neurons

The possibility of glutamate release by dopaminergic neurons was first suggested by the detection of phosphate activated glutaminase, a presumed marker of a glutamatergic phenotype, in many monoaminergic neurons including mesencephalic dopaminergic neurons [5]. Further molecular evidence was obtained with the detection of an isoform of the vesicular glutamate transporter, VGluT2, in dopaminergic neurons under various conditions and using an array of techniques [6–16]. The first functional evidence for *co-release* of glutamate by dopaminergic neurons was obtained from recordings in primary cell cultures of mesencephalic dopaminergic neurons in which autaptic and synaptic glutamatergic responses were observed [17] – a finding that was later replicated and extended [7,10,12,18].

Despite these lines of evidence it remained controversial whether there was glutamate co-release from dopaminergic neurons in the normal adult brain and if so, was sufficient to support functionally significant glutamatergic communication with postsynaptic targets. The controversy arose primarily from the conflicting findings and functional interpretations of comparable experiments that in most cases resulted from inherent limitations of available methods. For example, the electrophysiological demonstration of glutamatergic transmission by dopaminergic neurons in cell culture was weakened by evidence showing that VGluT2 expression was strongly up regulated in dopaminergic neurons in culture preparations partly owing to the loss of contact mediated inhibition by postsynaptic targets [7,14]. Similarly, it was shown that VGluT2 was expressed at low levels *in vivo* and that its expression decreased over development [6,13,14] so that it could be reliably detected only with

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RT-PCR based methods or indirect reporters [6,14]. These findings raised the possibility that the releasable glutamate content of adult dopaminergic neurons may be insufficient to support a functional glutamatergic phenotype and pointed to alternative developmental functions or an involvement in neuronal plasticity and repair [6,15,16,19].

In parallel with these studies, in a series of technically challenging and creative experiments the existence of glutamatergic signaling in the forebrain by mesolimbic dopaminergic neurons was also examined in acute slice preparations [8,20,21]. The important advantages of this approach include the possibility of providing direct functional evidence for glutamatergic synaptic transmission and avoiding the potential problems and potential associated with cell culture methods. Although these experiments were able to demonstrate that electrical or chemical stimulation of neurons in the VTA can elicit glutamatergic responses in spiny projection neurons (SPN) of the nucleus accumbens that exhibit the conduction latency and D_2 receptor (D_2R) mediated regulation expected for responses mediated by mesolimbic dopaminergic axons [8,20,21], the complex and poorly understood organization of the VTA and surrounding areas, and in particular, the documented existence of non-dopaminergic glutamatergic neurons in the VTA[22] and the expression of D_2Rs by non-dopaminergic VTA neurons [23] left open the possibility that an unknown, possibly significant fraction of the responses recorded in the accumbens following stimulation of the VTA originated from non-dopaminergic projections.

Optogenetic disambiguation of the glutamatergic phenotype of dopaminergic neurons

The controversy about the glutamatergic nature of dopaminergic neurons has recently been resolved in 2 studies in which postsynaptic responses of SPNs in the nucleus accumbens were examined using channelrhodopsin-2 (ChR2) mediated optogenetic activation of dopaminergic axons [24,25]. In these experiments ChR2 was expressed in dopaminergic neurons using viral mediated transfer of a Cre-lox controlled transgene [26–29] in transgenic mice that express Cre recombinase in neurons expressing the dopamine transporter (DAT; DAT-IRES-Cre) or tyrosine hydroxylase (TH; BAC TH-Cre). This approach ensures the specific and selective activation of dopaminergic axons because the expression of ChR2 in the absence of Cre recombinase is completely prevented by the inverted orientation of the ChR2 coding sequence and because the expression of Cre itself is specific in these mice to neurons that contain TH or DAT and which are therefore conclusively dopaminergic [26–29].

These experiments demonstrated unambiguously that selective activation of dopaminergic axons elicits small amplitude excitatory postsynaptic glutamatergic currents/potentials (EPSC/Ps) in all SPNs in the nucleus accumbens (Figure 1). These responses comprised activation of both AMPA and NMDA glutamate receptors (Figure 1b) and were accompanied by simultaneous dopamine release detected using fast-scan cyclic voltammetry (FSCV) [24,25]. The possibility that the glutamatergic EPSCs were artifacts of abnormalities of optogenetic stimulation such as burst firing or plateau depolarizations in axons [30] or abnormal facilitation of release by local depolarization and Ca^{2+} influx in axon terminals through ChR2 channels was excluded using cell attached axonal recordings (Figure 1a) and focal laser stimulation of distal segments of axons (Figure 1c), and by estimating the release probability during electrical or optical stimulation of dopaminergic axons based on voltammetric measurement of the rate of extracellular dopamine accumulation in response to train stimuli [25]. The glutamatergic responses were not secondary to dopaminergic signaling because they could not be blocked by D_1R and D_2R antagonists [24,25] or by complete pharmacological depletion of the vesicular dopamine pool [25]. Importantly, Stuber *et al.* (2010) was able to demonstrate that EPSPs in SPNs resulted from VGluT2 mediated vesicular glutamate sequestration using an innovative combination of Cre-mediated cell type specific conditional knockout of the VGluT2 gene and Cre/lox controlled targeting of ChR2 in DAT^+ neurons with viral delivery. Similar evidence was also obtained with electrical stimulation of the VTA in hemizygotic conditional VGluT2 knockouts [31]. Interestingly, Stuber *et al.* (2010) also demonstrated the absence of comparable glutamatergic responses in the dorsal neostriatum. More ventrally, however, lateral to the accumbens, at the level of the anterior limb of the anterior commissure, glutamatergic EPSPs that were similar to the responses recorded in the accumbens could be elicited with optical stimulation of DA axons in a subset (3 of 10) of SPNs (Tecuapetla *et al.*, unpublished observations). These responses may be supported by a dopaminergic projection from the parabrachial pigmented, retrorubral and/or possibly other dopaminergic nuclei that (in addition to their projections to the nucleus accumbens) provide less substantial innervation to the neostriatum [32]. These findings suggest that the role of glutamatergic signaling by dopaminergic neurons may not be constrained to the nucleus accumbens.

Behavioral significance of VGluT2 expression in dopaminergic neurons

While the evidence reviewed above clearly demonstrates that dopaminergic neurons of the VTA accumulate and release glutamate in an action potential-dependent manner and that the activity-dependent glutamate release can activate ionotropic glutamate receptors, they do not provide direct guidance regarding the *function* of glutamate accumulation and/or release by these neurons. Significant

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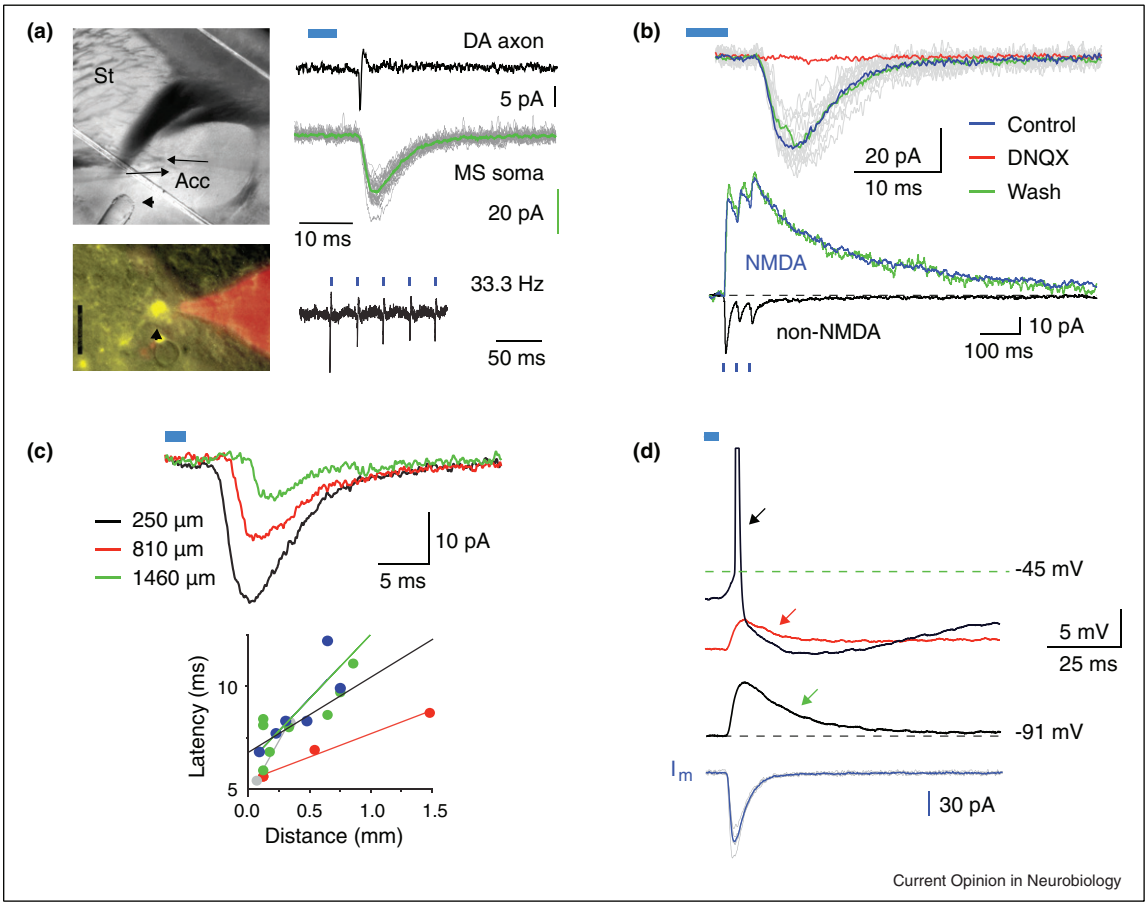
Figure 1



Biophysical properties of glutamatergic responses elicited with selective stimulation of dopaminergic afferents to projection neurons in the nucleus accumbens. **(a)** Optogenetic activation of dopaminergic axons in the nucleus accumbens. A DIC photomicrograph (top left panel) illustrates the localization of a typical recording site in the accumbens (note the position of 2 patch pipettes used for simultaneous cell-attached axonal, and whole cell recordings (arrows) and the relative position of the optical fiber (arrowhead), used for focal stimulation near the recording site). An epifluorescence composite micrograph obtained with YFP (yellow) and Texas-Red (red) filter sets illustrates a typical axon bleb formed by the severing of a dopaminergic axon at the surface of the slice that expresses ChR2-EYFP (yellow structure, arrow) and the cell attached recording pipette (red) used for obtained recordings from this axon. Right panel, top trace is a typical response of a ChR2-EYFP expressing dopaminergic axon exhibiting a single optically elicited action potential. Middle traces are simultaneously recorded postsynaptic responses in a nearby SPN ('MS soma', green trace is the average response, gray traces are individual responses). Bottom trace illustrates reliable firing of action potentials recorded in cell-attached mode in a dopaminergic axon in response to a 33.3 Hz train of light pulses. Blue bars indicate light pulses in all panels. **(b)** Postsynaptic AMPA and NMDA receptor mediated glutamatergic EPSCs recorded in 2 SPNs in response to optical stimulation of dopaminergic axons (blue bars). Top panel, a single light pulse elicits EPSCs (individual EPSCs are in gray, the average EPSC is in green) that are reversibly and completely blocked of the selective AMPA receptor antagonist DNQX. Bottom panel, shows postsynaptic responses in an SPN to optical train stimuli designed to approximate the pattern of reward-related dopaminergic activity. Responses recorded at the resting membrane potential (black trace, 'non-NMDA') and at +50 mV (blue and green traces) are shown. The NMDA receptor mediated component ('NMDA', blue trace) was isolated with pharmacological block of AMPA receptors (CNQX, 10 µM) and exhibits significant temporal summation of depressing EPSC components. Note also that most SPNs exhibit NMDA receptor mediated EPSCs with significantly smaller amplitudes than seen in this particular neuron [24]. **(c)** Distal optogenetic laser stimulation of dopaminergic axons elicits postsynaptic EPSCs in SPNs that are triggered by propagating action potentials, demonstrating that the EPSCs are not artifacts of direct CHR2 induced release from axon terminals. Top panel shows 3 average EPSCs elicited by stimulating at increasing distances from the neuron. Corresponding distances are indicated in the panel. Bottom panel shows fitted linear correlations of latencies of EPSCs and simulation distances in 3 experiments. Note the slow conduction velocity indicated by the slope of the linear fits. **(d)** Depolarizing EPSPs and their effects on firing in an SPN. Bottom trace is the average EPSC recorded in voltage clamp at the resting membrane potential of this neuron (–91 mV). Note the unusually large peak amplitude of the EPSC [24,25]. Top traces are voltage recordings with different levels of depolarizing current injected into the neuron. Note that an action potential (black arrow) could be elicited only when the neuron was depolarized to ~5 mV below action potential threshold but not at lower levels of depolarization (red and green arrows.

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progress in this regard has been made in 2 recent studies in which the behavioral effects of a cell type-specific conditional knockout of VGluT2 in DA neurons were investigated. First, these experiments showed that conditional VGluT2 knockout did not affect locomotor behavior [31,33], coarse or fine motor coordination or learning (accelerated rotarod and beam walking tests, respectively) [31,33]. Nor did the knockout affect the acquisition of conditioned place preference in response to pairing with cocaine [31] or induce depression (forced swim test) [33] or cause cognitive deficits (radial maze) [33]. Importantly, however, the VGluT2 knockout *did* cause a large reduction in the locomotor response to both amphetamine [33] and cocaine [31]. In addition, a complex effect on behaviors related to risk taking and anxiety has also been demonstrated [33]. Together these observations demonstrate that adult and/or developmental expression of VGluT2 in dopaminergic neurons is necessary for normal responses to psychostimulants and as well as some more natural behavioral functions of the dopaminergic system. Moreover, the normal conditioned place preference learning in VGluT2 knockouts raises the interesting possibility that the locomotor and rewarding effects of cocaine are mediated by different mechanisms with the former involving, perhaps preferentially, glutamatergic transmission. However, it is also possible that this difference simply reflects different dose-response relations in the different tasks.

A novel effect of VGluT2 mediated glutamate sequestration may be sufficient to explain the behavioral abnormalities of the cell type specific VGluT2 knockouts

Although the mechanism of these behavioral effects has not been clarified, Hnasko *et al.* (2010) have shown that the knockout of VGluT2 is associated with an impaired release of dopamine as shown by a 30–40% reduction in striatal total tissue content of dopamine and the dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Moreover, the same authors have also shown that vesicular accumulation of dopamine can be facilitated by VGluT2, because vesicular accumulation of negatively charged glutamate molecules reduces the electromotive force across the vesicular membrane and therefore increases the transmembrane proton gradient driving VMaT-mediated dopamine transport. Thus, the knockout of VGluT2, which reduces glutamate vesicular uptake can be expected to reduce the vesicular sequestration of dopamine and therefore may directly account for the reduced dopamine content and release observed in these animals [31]. Surprisingly, these observations suggest that the primary mechanism through which VGluT2-mediated vesicular glutamate sequestration contributes to behaviorally manifest functions of dopaminergic neurons may be a *presynaptic* modulation of dopamine release itself and not necessarily the postsynaptic effect dependent on the co-release of glutamate. It is important to note however that, although the direct effect of cocaine and amphetamine is to increase extracellular dopamine concentration owing to block and/or reversal of the dopamine transporter, these drugs also elicit a significant increase in NMDA receptor-mediated burst firing in VTA dopaminergic neurons through indirect circuit mechanisms [34] and therefore it is possible that some of the behavioral effects of dopamine revealed by cocaine and amphetamine administration are mediated by altered postsynaptic glutamatergic signaling secondary to firing rate changes in dopaminergic neurons.

Functional considerations of glutamatergic signaling in the nucleus accumbens

As indicated in the previous discussion it remains uncertain if the postsynaptic glutamatergic signals elicited by dopaminergic axons in the nucleus accumbens play a significant role in the functioning of the dopaminergic system. One approach to evaluate such potential functions is to consider what type of cellular and network effects may be supported by the glutamatergic signals of dopaminergic neurons based on the biophysical characteristics of the elicited postsynaptic responses.

It is important to note that that the quantitative aspects of the following discussion rests on a key assumption whose validity has not been directly demonstrated. Specifically, we assume that in optogenetic experiments, locally delivered wide field high intensity light pulses activate release from all dopaminergic synapses that are associated with ChR2 expressing presynaptic axons in the slice preparation. Although this assumption seems highly plausible, and is supported by our cell-attached axonal recordings (Figure 1a) and other considerations we would like to caution that it has not been proven experimentally.

The glutamatergic EPSCs elicited in SPNs neurons by dopaminergic neurons are mediated either by glutamatergic receptors that reside inside dopaminergic synapses or by receptors that are extrasynaptic or are associated with asymmetrical synapses formed by the main (cortical, thalamic, hippocampal and amygdalar) glutamatergic inputs to the accumbens. In either case it is instructive to estimate the number of AMPA and NMDA receptors that are activated by glutamate release from dopaminergic axons. Remarkably, even with the assumption of a 5–10 fold mean electrotonic attenuation [35–37] and a ~10 pS single channel conductance [38], the EPSCs observed in SPNs corresponds to activation of as few as ~200–400 AMPA receptors. If these receptors were evenly distributed among the approximately 900 dopaminergic synapses made onto each SPN [39–43], each dopaminergic synapse would lead to the stimulation, on average, of less then a single (~0.2–0.4) AMPA receptor. Although the saturation of synaptic AMPA receptors depends on the poorly understood parameters of vesicular

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glutamate concentration and the number of vesicles released, if these synapses were similar to other glutamatergic synapses with respect to these properties [44], each would still contain only about 3–4 AMPA receptors (assuming the presence of a fraction of 'silent' synapses, the minimal open receptor probability of 0.5 [38] and that 20% of the synaptic AMPA receptors are fully occupied [44]). Similar calculations can be made for NMDA receptors using the maximal NMDA current amplitudes measured by Tecuapetla *et al.* (2010), and biophysical properties of these receptors [38], yielding approximately 0.2–0.5 stimulated NMDA receptors/synapse. Although these estimates are liable to significant errors owing to the uncertainty of numerous parameters, the estimated number of receptors activated by glutamate released from dopaminergic synapses is still very small compared to other (non-silent) glutamatergic synapses [44–46]. Consequently if the dopaminergic synapses represent a functionally homogenous population (with the possible exception of a modest fraction of silent synapses) they would activate so few glutamatergic receptors as to question whether these synapses can be considered genuinely glutamatergic in the sense of being specialized for glutamatergic transmission by localized targeting of ionotropic receptors to the postsynaptic membrane.

An interesting alternative possibility is that dopaminergic synapses may be highly heterogeneous so that a small minority is responsible for the bulk of the glutamatergic responses in SPNs. Consistent with this idea, immunocytochemical data suggest that, at least in primary cultures [6], only a subset of presynaptic terminals of dopaminergic neurons contain VGluT2 [6,10]. Moreover, while dopaminergic synapses in the dorsal striatum have been found to be exclusively symmetrical using TH immunocytochemistry [40], there is some evidence that a fraction of dopaminergic synapses may exhibit an asymmetrical morphology resembling excitatory synapses [41] (but see [43]). These considerations lend some support to the notion that the EPSCs elicited in SPNs by dopaminergic axons may originate from a subset of specialized synaptic contacts that contain a postsynaptic density enriched with glutamatergic receptors. This conclusion may also provide a simple explanation for the observation that the peak amplitude ratios of AMPA to NMDA receptor-mediated currents (the AMPA/NMDA ratio) elicited by dopaminergic axons and the main glutamatergic inputs to SPNs are similar (0.85 vs. 0.71, respectively [47]. If the synapses responsible for the glutamatergic EPSCs elicited from dopaminergic axons did not comprise a specialized subset, in the absence of compensatory factors, the relative activation of AMPA and NMDA receptors at synaptic and extrasynaptic sites should be significantly different and favor the activation of NMDA receptors owing to the difference in the affinities of these receptors for glutamate and the severe spatial restriction of glutamate diffusion [48–54].

In summary, glutamatergic transmission between dopaminergic axons and SPNs in the nucleus accumbens is mediated either by a small subset of specialized synapses containing postsynaptic glutamate receptors or by extrasynaptic or heterosynaptic receptor activation through glutamate diffusion. In the latter case however, the subtype, density and/or distribution of extrasynaptic or heterosynaptic AMPA and NMDA receptors would have to be favorable for increased relative access or activation of AMPA receptors [48–54].

What are the operational roles of glutamatergic signaling in the nucleus accumbens?

From a functional point of view the glutamatergic signaling originating from mesotelencephalic dopaminergic axons within nucleus accumbens neurons may serve at least 3 different functions. First, these signals may function primarily by eliciting postsynaptic depolarization and consequently increasing the firing rate, influencing spike timing, or regulating dendritic excitability and action potential back-propagation in SPNs. This possibility will be discussed in detail in below. Second, glutamatergic signaling may regulate the development or maintenance of dopaminergic synapses. Finally, the glutamate release form these terminals may function as a heterosynaptic modulator of other inputs through ionotropic and/or metabotropic mechanisms.

If a specialized subset of glutamatergic synapses of dopaminergic neurons indeed exist, these contacts may represent a specific stage of synaptogenesis or axon growth that is predominant early in development but remains observable in adult animals reflecting potentially continuing homeostatic or adaptive remodeling of the neuronal circuitry [6,15,16,19]. This hypothesis is at least consistent with the demonstration that dopaminergic axon growth is controlled by glutamate through ionotropic receptors [19] and may explain the gradual developmental downregulation of VGluT2 expression that approximately parallels the delayed postnatal development of the dopaminergic system [6,14,55,56]. If this were the case, glutamatergic signaling by dopaminergic axons might be utilizing the molecular mechanisms that evolved to control the long-term functional and structural plasticity of other glutamatergic synapses in the nervous system and aid target selection and the establishment or reorganization of dopaminergic synaptic contacts.

In addition or alternatively, activation of extrasynaptic or heterosynaptic glutamate receptors may, in principle, regulate the functioning of other synapses. This concept was first proposed in the context of a regulatory dopaminergic cross-talk mechanism of cortico-striatal and cortico-cortical synapses with dopaminergic contacts on dendritic spines within synaptic 'triads' [40,57], and may be related to the regulation of synaptic efficacy

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or long-term plasticity through extrasynaptic [58] heterosynaptic [51,53] or metabotropic glutamate receptor activation [59–63]. While it is not possible to exclude that glutamate release from dopaminergic terminals regulates other synapses, the quantitative analysis presented above indicates that the EPSCs recorded in SPN are mediated by the activation of very few glutamate receptors. Sine the receptors and their signaling effects are necessarily distributed over large dendritic areas and among many synaptic contacts, it is unclear if their local impact can be sufficient for an efficient neuromodulatory role. We want to emphasize however, the tentative nature of this conclusion since the limited activation of ionotropic receptors in the optogenetic *in vitro* experiments may not correctly represent the physiological magnitude of glutamate release owing to currently unappreciated factors and furthermore, may be misleading when attempting to estimate the possible functional significance of the activation of metabotropic receptors.

Thirdly, the most obvious possible function of glutamatergic transmission by dopaminergic axons may be the regulation of the firing rate or action potential timing of accumbens SPNs. The possible functional significance of this effect is perhaps best put into perspective by comparison of the peak EPSC amplitudes with somatically recorded amplitude of miniature EPSCs originating from glutamatergic synapses formed by the major excitatory afferents of SPNs in the accumbens. The amplitude of AMPA receptor mediated EPSCs elicited with synchronous activation of approximately 70% or 99.5% of dopaminergic axons (defined by the fraction of neurons expressing ChR2) was reported to be 24 ± 3 pA (6–87 pA) [25] and approximately 38 pA (SD $\cong$ 14 pA) [25], respectively. Using various methods, miniature AMPA EPSCs in the accumbens and the dorsolateral neostriatum have been estimated to be 12–15 pA [47,64]. Since these EPSCs are likely to be subject to the same average degree of electrotonic attenuation as the EPSCs from dopaminergic axons, the entire dopaminergic innervation activates the equivalent of 2–4, and at most 10, glutamatergic synapses. Although the precise number of synchronously active synapses required for a transition from the down to up state of SPN and for eliciting action potentials is not known, an estimate of ~140 synapses was made by Wilson (1995) using a compartmental model [37]. A similar number (~100) can be inferred from an another study [65] in which the number of miniature EPSCs required for maintaining a stable up-state in a dendritic cylinder containing realistic membrane properties and active conductances was estimated, if the results are adjusted by scaling up the estimated number of synapses (~30) needed to be active within the time window of the membrane time constant of the simulated 500 μm long cylinder (25 ms) to be applicable to the 1.2–2 mm total dendritic length of SPNs [66]. We note that this number is significantly higher than would be suggested by dividing the rheobase measured for adult SPNs *in vitro* [66] by the unitary EPSC amplitude, because of the short duration of the EPSCs and the complex nonlinear electrotonic integration of synaptic inputs [36,37,66]. Consequently, the maximal glutamatergic input to SPNs from the dopaminergic projection constitutes, about ~2–3% (at the maximum ~15%) of the excitatory drive required for minimal activation of a SPN. Furthermore, during the reward-associated burst firing of dopaminergic neurons *in vivo*, the action potentials of individual neurons are significantly more dispersed in time than what was induced in slice experiments [1,67,68]. Considering the decay time constant of the AMPA receptor mediated EPSC elicited by dopaminergic neurons (~6 ms, Figure 1) [25] the apparent strong use-dependent depression of the EPSC during train stimulation (Figure 1c) [25], (but see [20] for other possible use-dependent effects), and the temporal distribution of action potentials during reinforcement related burst firing of dopaminergic neurons (~60 ms) [68], the AMPA mediated current during the dopaminergic burst response is probably much smaller in amplitude then the synchronously elicited EPSC recorded *in vitro*. Taken together, these considerations suggest that semi-synchronous activity of dopaminergic neurons can be expected to elicit timed action potentials from SPNs only when these neurons are depolarized by other inputs to levels approaching action potential threshold and to result only in a small increase in the firing rate of these neurons. This conclusion is also supported by the observation that *in vitro*, synchronous activation of dopaminergic axons was capable of eliciting action potentials in MS neurons only when the neurons were depolarized to a level only a few millivolts below action potential threshold with current injection and that this effect was observable only in neurons that exhibited the largest peak synaptic currents (Figure 1d) [25]. Recordings from SPNs in the nucleus accumbens in behaving animals also suggest that this input is not a main determinant of the firing activity of these neurons [69–75]. Although in a minority of neurons in the nucleus accumbens a biphasic excitatory response is observed following the predicted or electrochemically detected phasic activation of dopaminergic neurons [70,71,74,76–79], this excitatory response comprises 2 different components of which only the early one may be driven by the excitatory effect of the dopaminergic projection while the second is too long in duration to be supported by this mechanism [70,71,74,76–79]. Moreover, the majority of accumbens neurons respond with a short latency inhibition, which is not preceded by a brief excitation that might be expected if these neurons were effectively excited by the dopaminergic input [70,71,74,76–79]. Finally it is also difficult to reconcile the idea of a significant excitatory effect of dopaminergic neurons with the observation that accumbens neurons change their response characteristics

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between excitation and inhibition as a function of the nature of reinforcement [80].

A promising possibility is that dopaminergic neurons may exert a significant influence on the excitability of specific dendritic areas, through local depolarization. Since very little is known about the active properties of the dendrites of SPNs it is not possible to make predictions about the efficacy of this mechanism [36,37]. Regulation of dendritic excitability however would provide a potentially effective mechanism for controlling spike-timing dependent plasticity of excitatory synapses of SPN and therefore contribute to the regulation of learning in the basal ganglia by the dopaminergic projection.

Conclusion and future perspectives

It is evident from the data reviewed here that the expression of VGluT2 in dopaminergic neurons shapes significantly the functioning of these neurons and possibly endows them with the ability to exert a functionally significant glutamatergic influence on postsynaptic neurons and circuits. Interestingly, the currently available evidence leaves open the questions if and how the postsynaptic glutamatergic effects contribute to the functioning of the mesotelencephalic dopaminergic system. There are several highly intriguing issues that may be relatively straightforward to address with new technologies and which may in the near future provide a better insight into the role of glutamatergic signaling by dopaminergic neurons. First, imaging action potential-associated dendritic Ca^{2+} transients of SPNs in combination with optogenetic stimulation of dopaminergic axons should allow direct examination of the possible glutamatergic effects on dendritic excitability. Furthermore, in appropriately designed slice preparations selective stimulation of dopaminergic and other glutamatergic inputs may provide an opportunity to directly examine the effects of phasic activation of the dopaminergic projection on the induction or maintenance of spike-timing dependent synaptic plasticity in SPNs. Finally, optogenetic stimulation of dopaminergic axons should be useful to examine the glutamatergic responses of various GABAergic interneurons in the nucleus accumbens as well as the responses of neurons in the prefrontal cortex where significant glutamatergic responses may be expected based on previous observations [81] and the preferential expression of VGlut2 in prefrontally projecting midline nuclei of the VTA[13].

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