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Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission

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The mesolimbic dopamine system is centrally involved in reward and goal-directed behavior, and it has been implicated in multiple psychiatric disorders. Understanding the mechanism by which dopamine participates in these activities requires comprehension of the dynamics of dopamine release. Here we report dissociable regulation of dopamine neuron discharge by two separate afferent systems in rats; inhibition of pallidal afferents selectively increased the population activity of dopamine neurons, whereas activation of pedunculopontine inputs increased burst firing. Only the increase in population activity increased ventral striatal dopamine efflux. After blockade of dopamine reuptake, however, enhanced bursting increased dopamine efflux three times more than did enhanced population activity. These results provide insight into multiple regulatory systems that modulate dopamine system function: burst firing induces massive synaptic dopamine release, which is rapidly removed by reuptake before escaping the synaptic cleft, whereas increased population activity modulates tonic extrasynaptic dopamine levels that are less influenced by reuptake.

The release of mesolimbic dopamine is regulated by a number of factors. Synaptic or phasic levels of dopamine are mediated primarily by bursting events at the level of the cell body[1,2] and are believed to lead to a much larger dopamine release than when these neurons fire in a slow, irregular single spike mode[3,4]. The time course (seconds) and localization of dopamine released by burst firing is restricted by a high-affinity and rapid reuptake system that exists in the dopamine synapse. Bursting activity of dopamine neurons is thought to represent a key component of reward circuitry; brief (~300 ms) periods of bursting activity of dopamine neurons in primates are associated with conditioned stimuli that predict the delivery of reward[5] and are believed to be modulated by glutamatergic and cholinergic inputs[6,7]. In contrast, extrasynaptic or 'tonic' levels of dopamine are also dependent on dopamine neuron firing, but in addition are modulated by presynaptic limbic and cortical glutamatergic inputs[1,8]. Moreover, it has been proposed that changes in the total number of dopamine neurons within the ventral tegmental area (VTA) that are active (*i.e.*, population activity) may also affect tonic dopamine levels[1]. Alterations in tonic levels of dopamine efflux occur on a much slower timescale than changes in phasic levels and enable a wide variety of motor, cognitive and motivational functions[5].

Research efforts to elucidate the mechanisms by which dopamine neuron activity can regulate dopamine release in terminal field regions such as the nucleus accumbens (NAc) have typically used electrical or chemical stimulation of the dopamine cell bodies to determine how dopamine neuron firing rate and patterns correlate with dopamine release. An inherent confounding factor in these procedures, however, is that stimulation would be expected to activate all dopamine fibers synchronously, leading to an increase in the overall population activity of the dopamine axons (in addition to mimicking natural bursting activity), thereby altering the dynamics of dopamine regulation at the nerve terminal. A more effective way of investigating how changes in firing parameters of dopamine neurons can effect changes of mesolimbic dopamine release is to manipulate endogenous afferent systems. For example, in a previous study[9], we showed that activation of the ventral subiculum region of the hippocampus causes a selective increase in the population of spontaneously active dopamine neurons without affecting burst firing; this effect is mediated by excitatory hippocampal inputs to the NAc. In the present study, we examined the regulation of dopamine cell activity and dopamine release in the NAc by manipulating GABA transmission in two distinct afferent pathways to the VTA: (i) the ventral pallidum (VP), a subcortical region that receives GABAegic inputs from the NAc and in turn provides a tonically active inhibitory GABAergic input to the VTA[10,11] and (ii) the pedunculopontine tegmental nucleus (PPTg), a region of the hindbrain that sends glutamatergic and cholinergic projections to dopamine cell bodies[12,13]. We observed that pallidal and pedunculopontine afferents selectively regulated population activity and burst firing, respectively, of VTA dopamine neurons. These manipulations caused corresponding changes to tonic and phasic dopamine release in the NAc.

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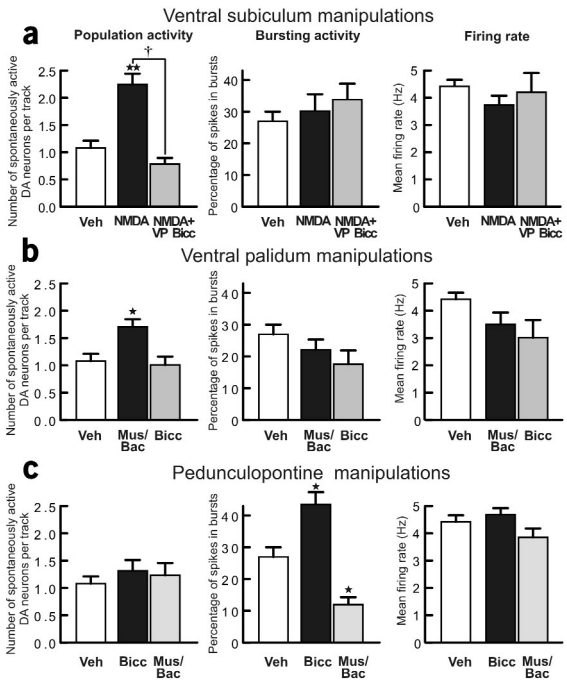

Figure 1 Dopamine neuron activity states can be differentially modulated by subcortical nuclei. (**a**) Left, activation of the ventral subiculum with NMDA (black bars) selectively increased dopamine neuron population activity that was abolished by a co-infusion of the $GABA_A$ antagonist bicuculline (gray bar) into the ventral pallidum (VP). Neither manipulation altered burst firing (middle) or firing rate (right) of dopamine neurons. $^{**}P < 0.01$ compared with vehicle treatments (Dunnett's test); $\dagger P < 0.05$ between subiculum NMDA and NMDA + VP bicuculline treatment groups. (**b**) Similar effects were observed following inactivation of the VP with muscimol and baclofen (black bars), whereas activation of this nucleus with bicuculline (gray bars) had no significant effect. (**c**) In contrast, activation of the PPTg (black bars) had no effect on population activity (left) or firing rate (right), but significantly increased burst firing of dopamine neurons (middle). PPTg inactivation (gray bars) had the opposite effect, decreasing bursting. Analysis of these data revealed a significant group difference for population activity ($F_{6,42} = 7.6$, $P < 0.001$) and burst firing ($F_{6,42} = 7.2$, $P < 0.001$), but not for firing rate ($F_{6,42} = 1.9$, n.s.). (**b**,**c**) $^{*}P < 0.05$ compared with vehicle treatments (Dunnett's test).

RESULTS

Dissociable afferent regulation of dopamine neuron firing

Rats that received control vehicle infusions (n = 8 rats, 74 neurons) exhibited an average of 1.08 ± 0.1 spontaneously active dopamine neurons per electrode track (a standard measure of population activity), which fired at an average rate of 4.4 ± 0.2 Hz with 27 ± 3% of action potentials fired in bursts, consistent with previous findings[9] (**Fig. 1 a–c**, white bars). Activation of the hippocampal-NAc pathway by an intra-subiculum infusion of NMDA (n = 6 rats, 107 neurons) resulted in a significant (>50%, $P < 0.05$) increase in dopamine neuron population activity relative to control treatments. This is reflected as an increase in the number of spontaneously active dopamine neurons observed per electrode track (**Fig. 1a**, black bars). The effect of ventral subicular stimulation was abolished either by a co-infusion of the $GABA_A$ antagonist bicuculline into the VP (**Fig. 1a**, gray bar; n = 6 rats, 40 neurons) or by infusion of the glutamate receptor antagonist kynurenate into the NAc[9], indicating that a hippocampal-NAc-VP pathway mediates this effect. Similarly, inactivation of the VP with a direct infusion of the $GABA_A$ and $GABA_B$ agonists muscimol and baclofen, respectively (n = 8 rats, 102 neurons) also increased dopamine neuron population activity (**Fig. 1b**, black bar). Infusions of bicuculline into the VP alone had no effect on the number of spontaneously active dopamine neurons, but did cause a non-significant decrease in the mean firing rate of these cells (**Fig. 1b**, gray bar; n = 7 rats, 58 neurons), presumably due to an increase in GABAergic input from the VP-VTA pathway. Importantly, neither inactivation of the VP nor stimulation of the ventral hippocampus produced a significant change in the mean bursting activity of dopamine neurons (**Fig. 1a,b**). Given that a subpopulation of VP GABAergic projection neurons show high rates of spontaneous activity[14,15], the increase in dopamine neuron population activity following inactivation of the VP was probably due to disinhibition of silent dopamine neurons that now displayed activity at substantially lower firing rates, as suggested by the non-significant reduction in the average firing rate (**Fig. 1b**) and differences in the firing rate distribution (Kolmogorov-Smirnov test, $P < 0.01$; **Fig. 2a,b**). Thus, when the inhibitory VP-GABAergic input to VTA is inactivated, either by activation of NAc afferents to the VP or by direct infusion of GABAergic agonists into the VP, there is a resultant disinhibitory increase in the overall population activity of dopamine neurons, which activates a 'reserve pool' of quiescent dopamine neurons. However, this manipulation has no effect on the bursting activity of these cells.

In contrast to the effects of VP manipulations, activation of excitatory glutamatergic and cholinergic afferents to the VTA by infusion of bicuculline into the PPTg (n = 7 rats, 78 neurons) produced a ~50% increase in the bursting activity of dopamine neurons relative to

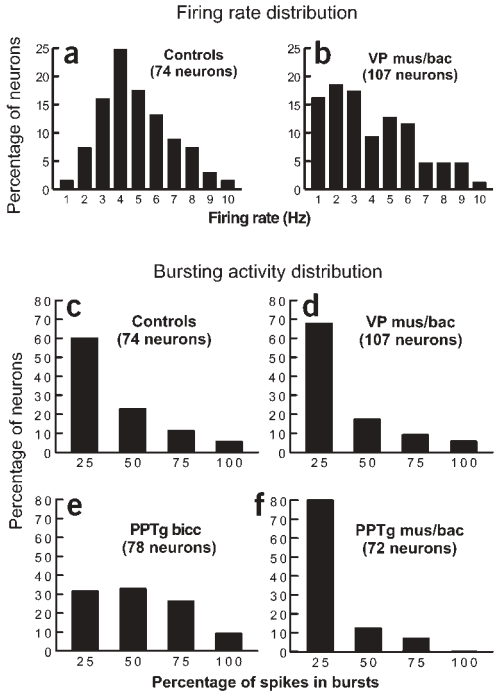

Figure 2 Analysis of the firing rate distributions of dopamine neurons following manipulation of VP and PPTg afferents to the VTA. Relative to control treatments (**a**,**c**), inactivation of the VP increased the proportion of slow-firing dopamine neurons (**b**), but had no effect on the distribution of bursting activity (**d**). PPTg activation increased the number of dopamine neurons with >50% of spikes in bursts (**e**), whereas inactivation increased the proportion of neurons with <25% of their spikes in bursts (**f**).

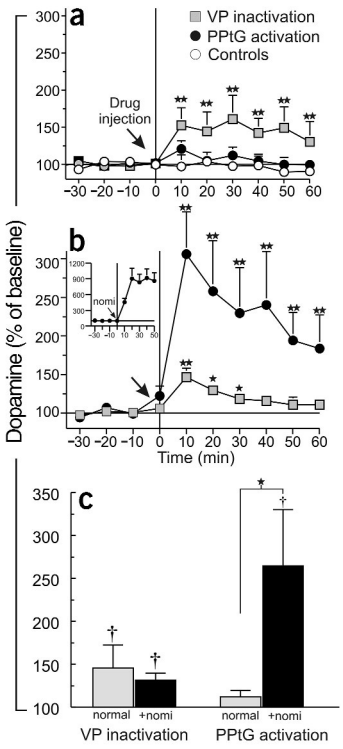

Figure 3 Modulation of dopamine release in the nucleus accumbens by manipulations which selectively increase dopamine neuron population activity or burst firing. (**a**) Inactivation of the VP increases extracellular dopamine levels in the NAc, measured with microdialysis (■), but activation of the PPTg (■) or control treatments (○) were without effect. (**b**) Local application of the dopamine uptake blocker nomifensine (nomi) increased baseline dopamine levels ~9-fold (inset). Inactivation of the VP produced a comparable increase in dopamine release relative to **a**, but in stark contrast, activation of the PPTg now produced a 3-fold increase in dopamine release. Analysis of these data revealed a significant Sample × Drug Treatment interaction ($F_{36,180} = 3.6$, $P < 0.01$). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to baseline (Dunnett's test). (**c**) Average change in dopamine release over the first 30 min of sampling after inactivation of the VP or activation of the PPTg under normal conditions (gray bars) or in the presence of dopamine reuptake blockade (black bars). † denotes significant increase from baseline at $P < 0.05$ and star denotes significant difference between PPTg activation groups with or without nomifensine.

control treatments ($P < 0.05$) while having no effect on the overall population activity or mean firing rate of these cells (**Fig. 1c**, black bars). Accordingly, inactivation of the PPTg via infusion of muscimol/baclofen ($n = 7$ rats, 72 neurons) had the opposite effect, significantly decreasing ($P < 0.01$) bursting activity, while again having no effect on the number of spontaneously active dopamine neurons observed or their mean firing rate (**Fig. 1c**, gray bars). Analysis of the distribution of dopamine neurons as a function of the percentage of spikes fired in bursts revealed that activation of the PPTg increased the proportion of dopamine neurons that fired >50% of their action potentials in a burst mode, whereas inactivation of this nucleus caused a pronounced increase in the number of dopamine neurons that showed bursts <25% of the time (**Fig. 2c,e,f**). Thus, unlike manipulations that disinhibited the VTA (which lead to an overall increase in the recruitment of non-firing VTA dopamine neurons; **Fig. 2b**), activation of excitatory input from the PPTg causes a robust increase in the bursting activity of already-firing dopamine neurons, while leaving population activity intact. The observation that PPTg activation only increased bursting in spontaneously active neurons may be due to the proposed VP-mediated hyperpolarization of a population of 'silent' dopamine neurons[9]. Thus, in those dopamine neurons that are inactive due to a hyperpolarization by VP afferents, one would expect little effect of glutamate, as glutamate acts primarily on NMDA receptors on dopamine neurons[16]; dopamine neurons hyperpolarized by VP GABAergic afferents would be unresponsive to NMDA receptor stimulation due to Mg^{2+} blockade[17]. Therefore, PPTg afferents would only be capable of increasing burst firing of dopamine neurons that were already spontaneously active. Collectively, these data imply that these distinct afferent pathways to the VTA exert a dissociable regulation of dopamine neuron population activity and burst firing.

Effects on extrasynaptic dopamine release

In light of this dissociation of dopamine neuron activity exerted by inhibitory and excitatory afferents to the VTA, we assessed how manipulations that selectively increase population activity or burst firing would influence dopamine release in a dopamine neuron terminal region, the NAc. Using *in vivo* microdialysis, we observed that inactivation of the VP (which increased population activity but not bursting) caused a significant ($P < 0.05$) increase in extracellular dopamine levels in the NAc, which lasted the entire 1-h recording period (peak level, 52.5 ± 23%; **Fig. 3a**, gray squares). However, when the PPTg was activated by infusion of bicuculline ($n = 5$), which increases bursting activity of dopamine neurons but leaves population activity intact, we observed no reliable change in the release of extracellular dopamine in the NAc (**Fig. 3a**, black circles), similar to that observed after vehicle infusions (**Fig. 3a**, white circles).

The observation that activation of the PPTg, which increases burst firing of dopamine neurons, produced no discernable increase in dopamine release in the NAc was surprising because bursting is generally thought to be the primary mechanism by which dopamine efflux is facilitated[3,4]. Nevertheless, the tonic/phasic model of dopamine release states that a switch to a burst-firing pattern of dopamine neurons would result in a massive (two- to three-fold) increase in dopamine release. However, this efflux would be highly localized to the synaptic cleft, due to the rapid reuptake by the dopamine transporter localized at the borders of the synaptic junction where they could most effectively allow large intrasynaptic transients (estimated in the micromolar range), while limiting diffusion from the synaptic space[18]. Thus, discharge of dopamine neurons in a bursting mode would be expected to dramatically increase synaptic (phasic) levels of dopamine, but the escape of dopamine from the synapse would be curtailed by the high-affinity rapid reuptake system localized in the dopamine nerve terminal.

To directly test this hypothesis, we conducted another series of experiments in which the dopamine reuptake mechanism in the NAc was blocked via local application of nomifensine through the microdialysis probe. Continuous perfusion of the dopamine reuptake blocker nomifensine (10 μM) caused a nine-fold increase in the basal extracellular levels of dopamine, which peaked at 20 min after the onset of application and remained stable for 40 min (**Fig. 3b**, inset). Once a new, elevated baseline was established, we administered the pathway manipulations that were shown to selectively increase population activity or burst firing of dopamine neurons. On top of this nomifensine-enhanced baseline, inactivation of the VP ($n = 5$) produced a significant ($P < 0.05$) increase in mesoaccumbens dopamine release that was proportional to the percentage increase observed under conditions where dopamine reuptake was unperturbed (46.5 ± 11%; **Fig. 3b**, gray squares). In con-

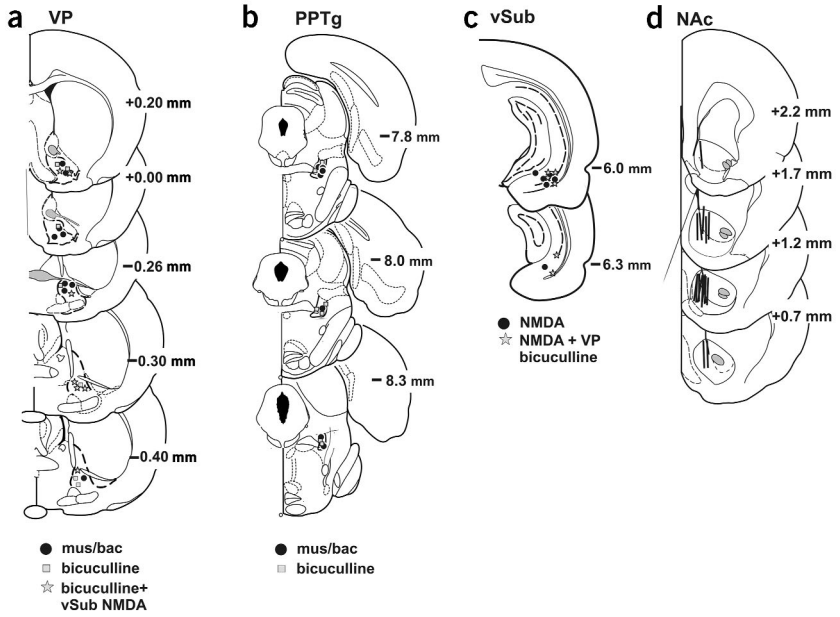

Figure 4 Histology. Location of infusions for all rats receiving infusions of (**a**) bicuculline (■ and ★) or baclofen/muscimol (●) into the VP (**b**) baclofen/muscimol (●) or bicuculline (■) into the PPTg or (**c**) NMDA (● and ★) into the ventral subiculum (vSub). (**d**) Location of microdialysis probes in the NAc. Numbers beside each plate represent distance from bregma.

trast to the effects observed in the absence of nomifensine or following inactivation of the VP, activation of the PPTg ($n = 5$) produced a massive increase in extracellular dopamine in the presence of dopamine reuptake blockade in the NAc (**Fig. 3b**, black circles; **Fig. 3c**) that was significantly ($P < 0.01$) greater than the change seen after activation of the PPTg without nomifensine (**Fig. 3c**). This effect peaked 10 min after the infusion of bicuculline into the PPTg (306.2 ± 55% increase) and remained significantly elevated for the entire 1-h recording period. From these data, it is apparent that a selective increase in burst firing of dopamine neurons does cause a multi-fold increase in dopamine release at the terminal level. Under normal conditions, however, dopamine reuptake mechanisms severely limit the amount of dopamine that escapes from the synaptic cleft, thereby occluding detection of a sustained and measurable increase in extracellular dopamine.

DISCUSSION

Here we report that separate afferent pathways to the VTA can exert a differential control over firing properties of dopamine neurons, which in turn can influence differentially tonic and phasic levels of dopamine in the NAc. A reduction of GABAergic input from the VP produces a selective increase in dopamine neuron population activity which is associated with an increase in mesoaccumbens extracellular dopamine levels. In contrast, stimulation of excitatory inputs from the PPTg increases burst firing of dopamine neurons but has no effect on either their population activity or dopamine efflux in the NAc under normal conditions. In the presence of dopamine reuptake blockade, however, enhancement of burst firing by stimulation of the PPTg caused a 3–4-fold increase in dopamine release. This latter finding suggests that enhanced bursting in a subpopulation of spontaneously active dopamine neurons causes a spatially-restricted increase in dopamine at best; one that is highly localized to the dopamine synapse, curtailed by dopamine reuptake and unlikely to exert a dramatic influence on extrasynaptic levels of dopamine. With respect to the ultrastructural synaptic arrangement of dopamine projections, it is known that the dopamine innervation of the NAc consists both of classical synaptic junctions with postsynaptic neurons, as well as at non-synaptic varicosities where no synaptic cleft is evident. However, the functional impact and mode of regulation of non-synaptic dopamine varicosities is not clear; indeed, the current state of knowledge is not consistent in supporting these varicosities as functional dopamine release sites[19]. In the context of the present data, the fact that the increase in burst firing of dopamine neurons did not affect extracellular dopamine levels suggest that if dopamine is released by burst firing from these non-synaptic vesicular sites, then it must still be subjected to the same types of uptake-dependent regulation as terminals forming classic synaptic junctions.

It is well established that the release of dopamine from nerve terminals in the forebrain is more pronounced when dopamine neurons are electrically stimulated using a bursting pattern, when compared to protocols that deliver impulses in a single-spike mode[3,4]. It is important to note that in the present study, we were able to selectively enhance either the natural asynchronous bursting activity or the population activity of VTA dopamine neurons by manipulations of endogenous brain pathways. This is in contrast to previous studies that used electrical stimulation of the dopamine cell bodies with stimulation frequencies that are substantially higher than normally observed *in vivo*, which would increase both bursting and population activity and do so synchronously and simultaneously across the entire population of axons stimulated. The fact that only manipulations that increased dopamine neuron population activity (and not burst firing) were able to produce a reliable increase in dopamine efflux suggests that increases in extracellular dopamine observed in these previous studies may be attributable primarily to the increase in the overall activity of dopamine neurons induced by electrical stimulation of the dopamine cell bodies, and that the burst pattern of stimulation made only a secondary contribution. One possibility is that activation of the PPTg may have caused a transient and more spatially-restricted increase of dopamine levels in the extracellular space immediately surrounding the synapse, but this would not have impacted on tonic extracellular dopamine in our protocol because of the spatial and temporal resolution of our microdialysis probe, relative to voltametric probes used in previous studies[3,4]. However, given that burst firing was increased over the entire time period of the microdialysis sampling procedure, this explanation would not be able to account for all of our findings. On the other hand, the increase in synaptic levels of dopamine due to enhanced burst firing would also be expected to increase activation of D_2 receptors in the synaptic cleft, which could in turn cause a compensatory increase in dopamine reuptake kinetics[20] and further limit diffusion out of the synapse. Accordingly, in the presence of nomifensine, activation of the PPTg caused a massive increase in mesoaccumbens dopamine.

The present findings have important implications regarding our understanding of the mechanisms by which changes in activity at the level of the dopamine cell body can influence dopamine terminal release. Burst discharge of dopamine neurons represents a mechanism

by which this system communicates phasic information, triggering a high-amplitude release of dopamine into the synaptic cleft that is removed shortly after release. In contrast, the tonic extracellular dopamine levels exist at much lower, steady-state levels (~5–40 nM)[21], regulated by multiple feedback systems[1,2]. Our finding that manipulations that increase the total number of spontaneously active dopamine neurons were associated with an increase in extrasynaptic levels of dopamine implies that regulation of tonic dopamine is depends on the spontaneous discharge of the population of VTA dopamine neurons. In light of the present data, it is apparent that extracellular (tonic) levels of dopamine measured with traditional *in vivo* neurochemical assays are not altered dramatically by increases in burst firing at the level of the dopamine cell bodies. Rather, these data suggest that the increase in the overall population of dopamine neurons firing is the primary determinant of tonic extrasynaptic levels of dopamine. In addition, other studies have shown that the glutamatergic afferents to the NAc are also important for modulating tonic dopamine release[8]. Although such tonic levels are clearly too low to trigger intrasynaptic receptors, they are sufficient to stimulate presynaptic D_2 receptors to downregulate neurotransmitter release, as has been shown for glutamate[22]. In particular, tonic extracellular dopamine is known to provide a potent downregulation of dopamine terminals via stimulation of sensitive presynaptic autoreceptors[23]. As such, alterations in tonic levels of dopamine (as would be achieved by changes in either the population activity of dopamine neurons or increased activity in glutamatergic afferents[8]) could directly influence the magnitude of the phasic dopamine response mediated by bursting activity at the dopamine cell bodies.

It has been proposed that the dopamine system can communicate two signals: a fast phasic dopamine response that is limited to the synapse and a slower tonic dopamine response that is more spatially diffuse. In addition to regulating synaptic levels of dopamine, each of these signals can also serve distinct neurophysiological and behavioral functions. For example, recordings from dopamine neurons in behaving primates show that bursting events, produced on a timescale of hundreds of milliseconds, occur in response to reward-related events. These events are thought to serve as an important teaching signal involved in error detection[5] or reorganization of behavior in response to changes in the environment[24] by altering synaptic strengths of selected inputs to particular ensembles of striatal neurons, leading to a facilitation of learning[25,26]. One of the possible functions of the slower changes in tonic levels of dopamine may be to play a permissive role in preparing an organism to respond appropriately in response to environmental cues that have been previously associated with reward[27,28]. The dissociable regulation of dopamine release described here could account for the discrepancy observed in dopamine system responses, whereby conditioned stimuli elicit brief (<1 s) increases in dopamine neuron activity, but neurochemical studies show that dopamine efflux is enhanced over a prolonged period (minutes) under similar conditions. Interpreted in relation to our data, increased bursting of individual dopamine neurons in response to reward-related stimuli would not produce a discernable impact on dopamine release measured with conventional neurochemical assays. In contrast, we propose that increases in tonic levels of dopamine, mediated by dopamine neuron population activity and presynaptic glutamatergic regulation[8], and acting via volume transmission to affect a large population of striatal neurons, would subserve functions distinctly different from those mediated by phasic dopamine release.

Perturbations in the dissociable mechanisms that regulate tonic and phasic levels of dopamine may underlie the pathophysiology of certain psychiatric and neurological disorders. For example, hippocampal and cortical pathology is observed in schizophrenia[29]. Based on the present data, a reduction of excitatory afferent input from these regions to the ventral striatum would be expected to cause an overall decrease in tonic levels of dopamine via a reduction in dopamine neuron population activity[30] and decreased glutamatergic regulation of presynaptic dopamine terminals. This diminished tonic dopamine tone would lead to a reduction in the negative feedback on dopamine autoreceptors at the terminal region. Thus, environmental stimuli that promote an increase in burst firing of dopamine neurons would result in overstimulation via phasic dopamine release, which may underlie the increased dopamine system responses observed in schizophrenia[31].

METHODS

Surgery and pharmacological manipulations. The surgical preparation has been described in detail elsewhere[9]; procedures were in accordance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Male Sprague-Dawley rats weighing 290–450 g were anesthetized with chloral hydrate (400 mg/kg, i.p.) with supplementation and placed in a stereotaxic apparatus (flat skull). Subsets of rats were implanted with a 23-gauge guide cannula 2.0 mm dorsal to the VP (A/P, 0.0 mm from bregma; M/L, +2.3 mm from midline; D/V, –6.0 mm from dura), the PPTg (A/P, –8.0 mm; M/L, +1.8 mm; D/V, 4.5 mm) or the ventral subiculum (A/P, –6.0 mm; M/L, +5.3 mm; D/V, 4.0 mm). For the microdialysis studies, rats were implanted with guide cannulae in the VP or the PPTg, and a microdialysis probe in the medial NAc (A/P, +1.5 mm; M/L, +1.1 mm; D/V, 7.5 mm). The locations of the surgical placements for all brain regions are presented in **Fig. 4**. Baclofen HCl (0.2 μg), muscimol HBr (0.2 μg), bicuculline methbromide (0.1 μg) and *N*-methyl-D-aspartate (NMDA, 0.75 μg) were mixed fresh in Dulbecco's buffer and infused through a 30-gauge injection cannula protruding 2.0 mm past the end of the guide, at an injection volume of 0.5 μl every 2 min. Brain nuclei were inactivated by infusion of a $GABA_A/GABA_B$ agonist cocktail (muscimol/baclofen, 200 ng each), and were activated by administration of either the $GABA_A$ antagonist bicuculline (100 ng in the VP or PPTg) or NMDA (ventral subiculum, 750 ng). The dosages were selected from previous studies in which these compounds were shown to be behaviorally active when infused into the PPTg, VP or ventral subiculum[32–35]. The control group consisted of eight rats that received vehicle infusions into the VP ($n = 3$), the PPTg ($n = 2$) or the ventral subiculum ($n = 3$). These groups all showed similar dopamine neuron population activity parameters ($F_{2,5} = 1.9$, n.s.), so their data were combined.

Extracellular recordings and experimental protocol. Extracellular recording microelectrodes (2.0 mm OD borosilicate glass capillary tubing, ~1 μm tip diameter, impedance 5–10 MΩ) were filled with 2 M NaCl containing 2% pontamine sky blue dye. The electrode signal was amplified, filtered and discriminated from noise using a combination amplification and window discrimination unit, and data were acquired, stored and analyzed using custom-designed computer software. Following intracranial infusions, a recording electrode was lowered into the VTA (+3.3 mm anterior from lambda, 1.0 mm lateral, 6.5–8.5 mm ventral from brain surface) with a hydraulic microdrive. The electrophysiological properties of spontaneously active VTA dopamine neurons were sampled by making 6–9 vertical passes (separated by 200 μm) of the electrode through the dopamine cell body region. Dopamine neurons were identified using established electrophysiological criteria[36], and once isolated, spontaneous activity was recorded for 2–3 min. Three parameters of activity were sampled, the first being the number of spontaneously active dopamine neurons recorded per electrode track (*i.e.*, population activity). The validity of this index as a reliable measure of dopamine neuron activity change has been discussed previously[37]. This index has been shown to be reliable and consistent across animals and across laboratories. Furthermore, treatments that alter the number of spontaneously active dopamine neurons per electrode track have been shown to provoke corresponding changes in dopamine efflux[38]. The other two measures were (i) basal firing rate and (ii) the proportion of spikes fired that occurred in bursts (defined as the occurrence of two spikes with an interspike interval <80 ms,

and the termination as the occurrence of an interspike interval >160 ms)[36]. These measures were calculated as an average value for each rat and analyzed using three separate one-way ANOVAs and Dunnett's tests where appropriate.

Microdialysis and HPLC measures of dopamine levels in NAc. Concentric microdialysis probes with ~2 mm of exposed membrane (240–320 μM diameter, ~6,000 Da permeability) were implanted into the Nac and were perfused with artificial cerebral spinal fluid containing 145 mM Na^+, 2.7 mM K^+, 1.0 mM Mg^{2+} and 1.2 mM Ca^{2+}, maintained at pH 7.4 with 2 mM sodium-phosphate buffer at a rate of 2 μl/min as described previously[39]. Samples were collected every 10 min into 20 μL of HPLC mobile phase to minimize degradation of neurotransmitters and injected immediately into an HPLC system via an ESA model 540 autosampler (ESA, Inc.) and separated with an ESA analytical column; (150 × 3.2 mm, MD-150/RP-C18) and MD-TM 70-1332 mobile phase adjusted to pH 4.9 using 1*N* NaOH (flow rate, 0.7 ml/min). Dopamine was detected by oxidation using an ESA Coulochem II detector equipped with a guard cell (+300 mV) and a dual-electrode analytical cell (ESA 5014; E1 = –150 mV, E2 = +150 mV). Chromatographic data were acquired and processed using an ESA 501 data system. The HPLC system was calibrated using external standards (detection limit of dialysate dopamine, ~2 fmol). In these experiments, we obtained dialysate samples until stable baselines were achieved (>20% variation for 40 min) after which drugs were infused in the VP or PPTg 4 min before the next sample. For experiments in which dopamine reuptake was blocked, initial baselines were established, after which nomifensine (10 μM) was continuously perfused through the dialysis probe for 70 min to achieve a second, elevated baseline. We then infused drugs into the VP or PPTg. Neurochemical data were transformed into percent change from baseline, where 100% represented the average concentration of the three samples preceding the final baseline sample, and analysed with one-way repeated measures ANOVA and Dunnett's test where appropriate.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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