

The Hippocampus Modulates Dopamine Neuron Responsivity by Regulating the Intensity of Phasic Neuron Activation

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Aberrant dopamine (DA) signaling has been advanced as a contributing factor to the pathophysiology of a number of psychiatric conditions including schizophrenia; however, the many factors involved in regulating DA system responsivity have not been completely delineated to date. We have shown previously that DA neuron activity states are independently regulated by distinct afferent pathways. We now provide evidence that these pathways interact to control the population of neurons that are phasically activated. As shown previously, infusions of NMDA into the ventral subiculum (vSub) increases the number of spontaneously active DA neurons (population activity), while having no effect on firing rate or average bursting activity. In contrast, NMDA activation of the pedunculopontine tegmental nucleus (PPTg) results in a significant increase in DA neuron burst firing without significantly affecting population activity. However, simultaneous excitation of the vSub and PPTg induces a significant increase in both DA neuron population activity and burst firing resulting in a ~4-fold increase in the number of high-bursting neurons observed per electrode track. These data suggest that DA neuron population activity is not simply associated with the tonic release of DA in forebrain regions, but rather represents a recruitable pool of DA neurons that can be further modulated by excitatory inputs to induce a graded phasic response. Taken as a whole, we propose that the synchronous activity of distinct afferent inputs to the VTA phasically activates selective populations of DA neurons, and hence may be a site of pathological regulation underlying aberrant DA signaling.

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INTRODUCTION

The tonic/phasic model of dopamine (DA) release was first proposed as a model for defining the operating modes of the DA system and regulation of its responsivity (Grace, 1991). This model proposed that mesolimbic DA release is regulated via two independent mechanisms: (1) transient or 'phasic' DA release that is mediated mainly through burst firing of the neuron and (2) extra synaptic or 'tonic' levels of DA which are mediated by basal DA neuron activity and regulated via presynaptic inputs (Floresco *et al*, 2003; Grace, 1991). Thus, DA neuron burst firing induces a large transient increase in synaptic DA (Chergui *et al*, 1994) and is considered to be the functionally relevant signal sent to postsynaptic sites to encode reward prediction or indicate incentive salience (Berridge and Robinson, 1998; Schultz, 1998). In contrast, tonic DA transmission occurs on

a much slower scale and has been suggested to set the background level of DA receptor activation; as such, it has been proposed to regulate the responsivity of the DA system through pre- and postsynaptic mechanisms (Grace, 1991).

Since this time, there has been increasing evidence for functionally related but independent afferent systems regulating either tonic or phasic DA release (Floresco *et al*, 2003). Thus, we have shown that a decrease in tonic GABAergic transmission to the VTA results in a significant and selective increase in DA neuron population activity that corresponds to increases in tonic DA efflux in the nucleus accumbens (Acb) (Floresco *et al*, 2003). In contrast, activation of excitatory afferents to the VTA results in a selective increase in DA neuron burst firing that is associated with a significant increase in phasic DA transmission (Floresco *et al*, 2003). Moreover, each of these transmission states can differentially affect afferent input to the Acb (Goto and Grace, 2005).

One important feature of this regulation is that activation of excitatory inputs to the VTA results in burst firing only in DA neurons that are already spontaneously active (Floresco *et al*, 2003). Thus, in those neurons that are inactive, presumably due to GABA-mediated hyperpolarization, activation of glutamatergic afferents has little or no effect, likely attributable to Mg^{2+} blockade of the NMDA

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receptor-channel complex. These findings suggest the presence of a unique interdependence of the GABAergic and glutamatergic VTA inputs, that is, only neurons that are not under GABA-mediated hyperpolarization are capable of entering a burst firing mode in response to a glutamatergic input. We now propose that changes in DA neuron population activity may not simply regulate extrasynaptic DA levels in forebrain regions but may also reflect which DA neurons are capable of exhibiting phasic activation in response to glutamatergic input.

The ventral subiculum (vSub) is the major output relay of the hippocampus, a structure vital for the encoding and recall of episodic memory (Squire, 1992). Moreover, it has been demonstrated that stimulation of the vSub results in a significant (~ 2 -fold) increase in DA neuron population activity, which is correlated with an increase in extracellular DA levels in the Acb (Blaha *et al*, 1997; Brudzynski and Gibson, 1997; Floresco *et al*, 2001, 2003; Legault and Wise, 1999). This ability of subiculum stimulation to selectively increase the number of spontaneously active DA neurons has been shown to be due to a multisynaptic pathway (Floresco *et al*, 2001). Thus, vSub activation excites Acb projection neurons that inhibit ventral pallidal GABAergic afferents to the VTA, thereby releasing DA neurons from a tonic inhibitory influence (Floresco *et al*, 2001). In contrast, the pedunculopontine tegmental nucleus (PPTg) is a glutamatergic/cholinergic region driven by a number of limbic afferents, including the prefrontal cortex, bed nucleus of the stria terminalis and central nucleus of the amygdala (Semba and Fibiger, 1992), and has been shown to be activated by auditory (Pan and Hyland, 2005; Reese *et al*, 1995), visual (Pan and Hyland, 2005) and somatosensory stimuli (Grunewerg *et al*, 1992). The PPTg was found to also directly regulate burst firing of DAergic neurons (Floresco *et al*, 2001; Lokwan *et al*, 1999). These previous studies focused on the independent actions of afferent systems regulating tonic and phasic DA neuron activity. In this study, we explore the possibility that these systems may actually act in concert with respect to regulating DA neuron activity states, with the vSub determining which subsets of DA neurons may be phasically activated via PPTg inputs.

METHODS

All experiments were performed in accordance with the guidelines outlined in the National Institute 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.

Surgery and Pharmacological Manipulations

Male Sprague–Dawley rats (31 rats in total: 250–400 g) were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally) and placed in a stereotaxic apparatus. Chloral hydrate was utilized since, under this anesthetic, DA neuron activity states more closely resemble that observed in freely moving rats (Hyland *et al*, 2002). Anesthesia was maintained by supplemental administration of chloral hydrate as required to maintain suppression of limb compression withdrawal reflex and a core body temperature of 37°C was

sustained by a thermostatically controlled heating pad. For acute administration of NMDA/vehicle, rats were implanted with 23 G injection cannulae 2.0 mm dorsal to the vSub (A/P -6.0 , M/L $+5.3$, D/V -4.5 mm from bregma) and/or PPTg (A/P -8.0 , M/L $+1.6$, D/V -5.0 mm from bregma) that were fixed in place with dental cement and two anchor screws. *N*-methyl-D-aspartate (Sigma, St Louis, MO) was dissolved in Dulbecco's phosphate-buffered saline (PBS) and infused ($0.75 \mu\text{g}/0.5 \mu\text{l}$; duration ~ 30 s) through a 30-G injection cannula protruding 2.0 mm past the end of the guide. The efficacy of NMDA infusions was demonstrated by a potent increase in firing rate of vSub neurons after intra-vSub NMDA compared to Dulbecco's PBS infusions (vehicle 1.0 ± 0.4 Hz: NMDA 5.3 ± 0.9 Hz, $p < 0.05$, Mann–Whitney rank-sum test, $n = 5$ rats). The control group for DA neuron population studies consisted of seven rats that received vehicle (Dulbecco's PBS) infusions into either the vSub, PPTg, or both regions. These groups all showed similar DA neuron population activity parameters so that their data were combined. All rats received only one injection per region and DA cell recordings were typically recorded from 10 min to 2 h following infusions.

Extracellular Recordings

Glass extracellular microelectrodes (impedance 6–14 M Ω) were lowered into the VTA (A/P -5.3 , M/L $+0.8$ mm from bregma and -6.5 to -9.0 mm ventral of brain surface) using a hydraulic microdrive, and the activity of the population of DA neurons was determined by counting the number of spontaneously active DA neurons encountered while making five to nine vertical passes, separated by 200 μm , throughout the VTA. Spontaneously active DA neurons were identified using previously established electrophysiological criteria (Grace and Bunney, 1983), and once isolated, their activity was recorded for 2–3 min. Three parameters of activity were measured: (i) population activity (defined as the number of spontaneously active DA neurons recorded per electrode track), (ii) basal firing rate, and (iii) the proportion of action potentials occurring in bursts (defined as the occurrence of two spikes with an interspike interval of < 80 ms, and the termination of the burst defined as the occurrence of an interspike interval of > 160 ms; Grace and Bunney, 1983).

Histology

At the cessation of the experiment, the recording site was marked via iontophoretic ejection of Pontamine sky blue from the tip of the recording electrode (30 μA constant current: 20–30 min). After dye injection, rats were killed by an overdose of anesthetic, decapitated and their brains removed, fixed for at least 48 h (8% (w/v) paraformaldehyde in PBS), and cryoprotected (25% (w/v) sucrose in PBS) until saturated. Brains were sectioned (60 μm coronal sections), mounted onto gelatin–chrom alum-coated slides and stained with cresyl violet for histochemical verification of electrode sites and thionin for verification of cannula sites. All histology was performed with reference to a stereotaxic atlas (Paxinos and Watson, 1986) and (for PPTg placements) alternating brain slices stained for NADPH-diaphorase (a marker for cholinergic neurons; Vincent *et al*, 1983)

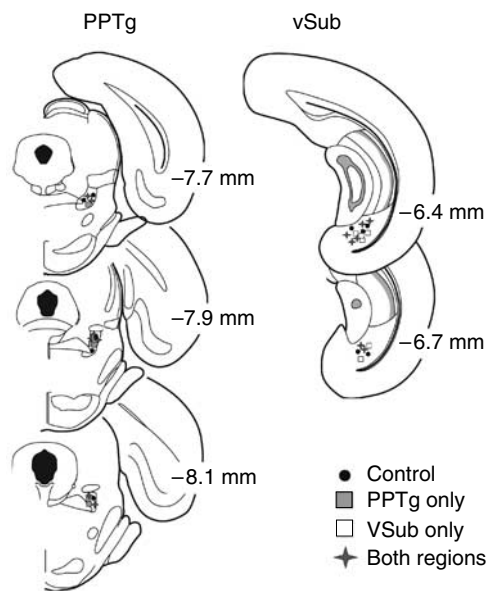


Figure 1 Histological localization of injection sites in the vSub and/or PPTg. Numbers beside each plate represent approximate A/P distance from bregma.

and thionin. The locations of injection sites are presented in Figure 1.

Analysis

Electrophysiological analysis of DA neuron activity was performed using custom-designed computer software (Neuroscope). All data are represented as the mean \pm standard error of the mean (SEM). Differences in standard electrophysiological recording parameters were examined using a one-way analysis of variance (ANOVA) followed by a Dunnett's *post hoc* test, whereas differences in the proportion of high- and low-bursting neurons were examined using a two-way ANOVA followed by a Student–Newman–Keuls *post hoc* test. The criteria for defining high and low burst firing neurons was determined from a bimodal Gaussian fit of the burst firing frequency distribution curve obtained with simultaneous PPTg and vSub activation. The cutoff values are defined as 2 SD from the means of each peak. As such, low-bursting neurons are defined as having $<33\%$ action potentials fired in burst (mean $13.6 \pm 9.9\%$), while high-bursting defined by $>56\%$ (mean $69.1 \pm 6.8\%$) spikes fired in burst.

All statistics were calculated using the SigmaStat software program (Jandel), while the bimodal Gaussian fit was determined using Origin Pro (OriginLab).

RESULTS

Rats that received control vehicle infusions ($n=7$ rats, 65 neurons) exhibited an average of 1.18 ± 0.1 spontaneously active DA neurons per electrode track that fired at an average rate of 3.9 ± 0.2 Hz with $29 \pm 3\%$ of action potentials fired in bursts (Figure 2a–c), consistent with previous findings (Floresco *et al.*, 2001, 2003). Intrastubulum infusion of NMDA ($n=6$ rats, 86 neurons) resulted in a

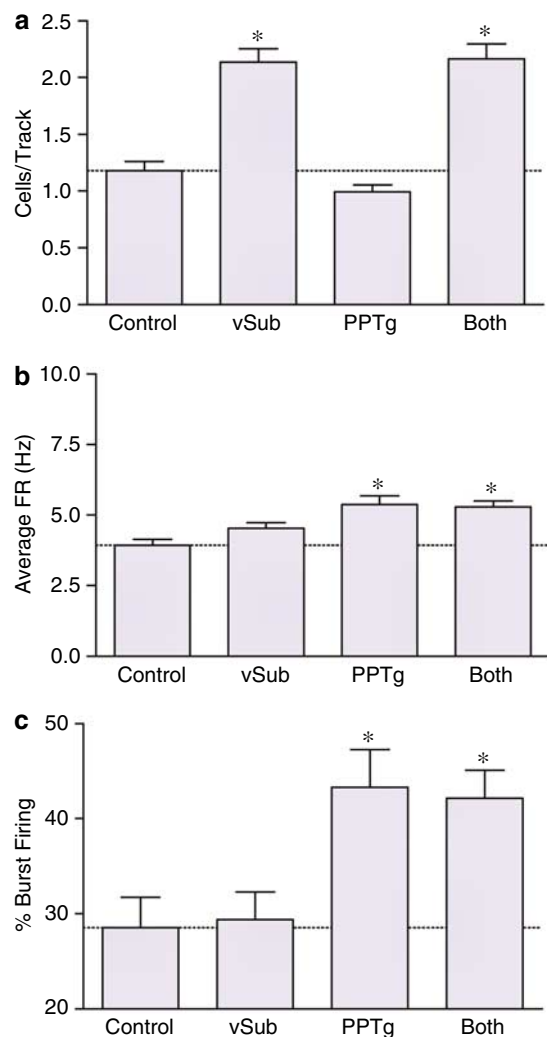


Figure 2 NMDA infusions alter population activity and firing pattern of VTA DA neurons. NMDA ($0.75 \mu\text{g}/0.5 \mu\text{l}$) was infused into the vSub, PPTg, or both regions, and the activity of spontaneously active VTA dopamine neurons examined. Three parameters of activity were recorded: population activity (a), average firing rate (b), and average percent burst firing (c). *Statistically significant difference from control (vehicle infusions) ($p < 0.05$ one-way ANOVA; Dunnett's *post hoc*; $n=6$ –7 rats/group).

significant (~ 2 -fold, $P < 0.05$) increase in DA neuron population activity (defined as number of neurons active; Figure 2a) without significantly affecting average burst firing or firing rate relative to control (Figure 2b and c). Analysis of the burst firing frequency distribution curves demonstrated no significant differences between control and vSub activation groups, further demonstrating that vSub stimulation does not modulate average DA neuron burst firing. Furthermore, vSub stimulation induced a concomitant (~ 2 -fold) increase in both high- and low-bursting neurons, operationally defined on the basis of distribution of burst events across the population of neurons recorded, as neurons with $>56\%$ or $<33\%$ action potentials in bursts, respectively (see Methods; Figure 3).

Activation of a glutamatergic/cholinergic pathway to the VTA by an intra-PPTg infusion of NMDA ($n=6$ rats, 45 neurons) resulted in significantly more burst firing of these cells (Figure 2c) without significantly affecting population

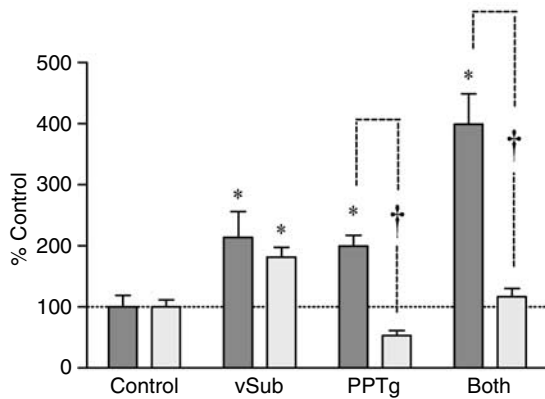


Figure 3 Activation of the PPTg and the vSub by NMDA has additive effects on the number of high-bursting DA neurons recorded in the VTA. NMDA (0.75 μ g/0.5 μ l) was infused into the vSub, PPTg, or both regions, and the activity of spontaneously active VTA dopamine neurons examined. Dark bars represent high-bursting neurons defined as cells firing >56% action potentials in burst, while light bars represent low-bursting neurons (<33% action potentials in burst). *Significant difference compared to control (vehicle infusions); †significant difference between high- and low-bursting neurons ($p < 0.05$ two-way ANOVA; Student–Newman–Keuls *post hoc*; $n = 6$ –7 rats/group).

activity (Figure 2a). This transition from low to high burst firing is reflected in a ~ 2 -fold increase in high-bursting neurons (Figure 3).

Simultaneous activation of the PPTg and vSub ($n = 7$ rats, 101 neurons) resulted in a significant increase in both DA neuron population activity and average burst firing (Figure 2a and c). Interestingly, the net effect of this activation was a four-fold increase in the number of high-bursting neurons per electrode track (Figure 3).

It is important to note that both PPTg and simultaneous vSub/PPTg infusions of NMDA resulted in a significant increase in average firing rate (Figure 2b) and is likely reflective of the increased burst firing induced by these treatments (Grace and Bunney, 1984).

DISCUSSION

Using *in vivo* extracellular recordings in chloral hydrate anesthetized rats, we report that although individual manipulations of afferent pathways exert independent control over DA neuron firing characteristics, simultaneous activation of multiple regions can result in a supraphasic increase in DAergic transmission. Specifically, the present study confirms that infusions of NMDA into the vSub induce an increase in the number of spontaneously active DA neurons (population activity), while having no effect on firing rate or average bursting activity. In contrast, NMDA activation of the PPTg resulted in a significant increase in DA neuron burst firing without affecting population activity. Interestingly, the simultaneous excitation of the vSub and PPTg induced a significant increase in both DA neuron population activity and average burst firing resulting in a ~ 4 -fold increase in the number of high-bursting neurons observed per electrode track. These data suggest that DA neuron population activity is not only associated with the tonic release of DA in forebrain regions but also

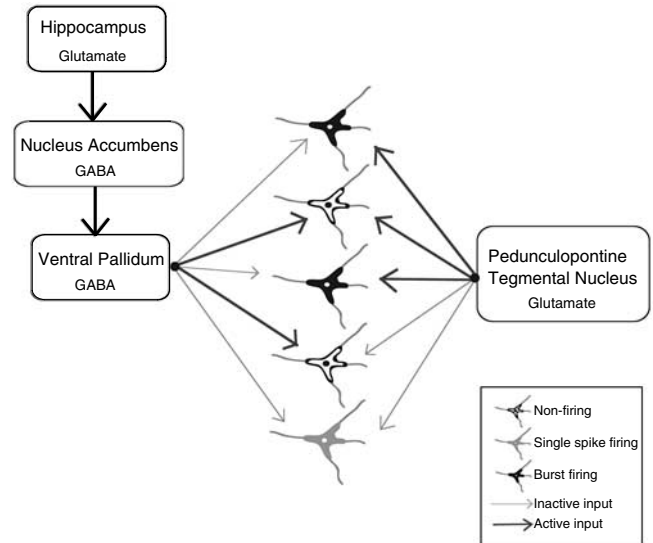


Figure 4 A model to explain how selective neural circuits enable the hippocampus to selectively modulate the population of DA neurons capable of exhibiting phasic activation. Stimulation of hippocampal afferents excites Acb projection neurons that inhibit VP GABAergic afferents to the VTA, thereby releasing DA neurons from a tonic inhibitory influence. Concurrent PPTg activation directly influences burst firing only in DA neurons that have been removed from the tonic GABA-mediated hyperpolarization.

represents a recruitable pool of DA neurons that can be further modulated by excitatory inputs to induce a graded phasic response. Taken as a whole, we hypothesize that the synchronous activity of select afferent inputs to the VTA are required to induce the most robust DA signal and hence pathologies of afferent systems may result in aberrant DA signaling. Moreover, the hippocampus subiculum would be positioned to select which subpopulations of DA neurons are capable of exhibiting phasic activation by the PPTg, thereby providing independent means for regulating DA neuron output (Figure 4).

As previously demonstrated, vSub activation by NMDA resulted in a ~ 2 -fold increase in the number of spontaneously active DA neurons observed per electrode track without affecting percent bursting or average firing rate. The finding that a concomitant increase in both the number of high- and low-bursting neurons was observed suggests that quiescent DA neurons are subject to the same tonic regulation as spontaneously active neurons once they are released from GABA-mediated inhibition. Interestingly, PPTg activation induced a similar ~ 2 -fold increase in the number of high-bursting DA neurons albeit through a different mechanism, that is, a transition from low to high burst firing resultant from the increased glutamatergic (and possibly cholinergic) tone to the VTA as suggested by previous studies of glutamate afferents and DA neuron burst firing (Lokwan *et al*, 1999; Smith and Grace, 1992). The present study demonstrates that simultaneous activation of the vSub and PPTg results in a summation of these effects resulting in a ~ 4 -fold increase in the number of high-bursting neurons, confirming the interdependence of the GABAergic and glutamatergic inputs to the ventral mesencephalon.

Schultz and others have shown that presentation of reward-related signals induces transient burst firing in DA neurons only when the reward-related signal is unpredicted (ie novel) (Schultz, 1998). Furthermore, the PPTg has been shown to respond to sensory stimuli (Grunberg *et al*, 1992; Reese *et al*, 1995) and it has been suggested that this region may be involved in signal detection independent of its behavioral salience (Pan and Hyland, 2005); therefore, the PPTg may be involved in detecting a signal with the hippocampus evaluating its novelty (Lisman and Grace, 2005). Since the computation of novelty requires the comparison of incoming information with stored memories, this likely occurs in the hippocampus (Knight, 1996). As such, it is possible that novelty detection by the hippocampus gates information processing in the VTA by amplifying the DA neuron response to excitatory inputs. This ability of the hippocampus to gate DAergic responses may have important implications for understanding the etiology of schizophrenia, which has been proposed to be associated with both hippocampal dysfunction and DA dysregulation (Grace, 2000; Harrison, 2004). Evidence for a DA involvement in schizophrenia was initially based on the efficacy of DA antagonists in treating the disease and more recently on the increased amphetamine-induced DA release observed in schizophrenia patients (Laruelle and Abi-Dargham, 1999). The hippocampus has been implicated in this disease due to a reduction in volume, enhancement of basal metabolism, and deficits in hippocampal memory processes observed in schizophrenia patients (Harrison, 2004). One possible consequence of this pathologically enhanced hippocampal activity (given the results of the current study) may be an increased salience of previously insignificant stimuli, that is, it is plausible that hippocampal damage such as that observed in schizophrenia may result in an increased DA neuron population activity that may lead to a considerably amplified phasic DA signal in response to nonsalient events or objects (Kapur, 2003).

Conclusions

Taken as a whole, the present study demonstrates that although individual manipulations of afferent pathways exert independent control over DA neuron firing characteristics, the synchronous activity of multiple regions is required to induce the most robust DA signal. Moreover, that modulation of DA neuron population activity is not only associated with altered tonic DA levels in forebrain regions but also appears to determine which subsets of the DA neuron population may be further modulated by excitatory inputs to induce a graded phasic response. These data further suggest that pathologies of afferent systems, such as the hippocampus, may result in aberrant DA signaling and could explain why schizophrenia patients fail to differentiate relatively nonsalient stimuli.

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