The lateral mesopontine tegmentum regulates both tonic and phasic activity of VTA dopamine neurons.

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Running Head: Afferent regulation of VTA dopamine neurons
Abstract

Anatomical studies have demonstrated that the mesolimbic dopamine system receives a substantial afferent input from a variety of regions ranging from the prefrontal cortex through to the brainstem. However, how these afferents regulate dopamine neuron activity is still largely unknown. The mesopontine tegmentum provides a significant input to ventral tegmental area (VTA) dopamine neurons and it has been demonstrated that discrete subdivisions within this region differentially alter dopamine neuron activity. Thus, the laterodorsal tegmental nucleus provides a tonic input essential for maintaining burst firing of dopamine neurons, while the pedunculopontine tegmental (PPTg) nucleus regulates a transition from single-spike firing to burst firing. In contrast, the recently identified rostromedial tegmental nucleus provides an inhibitory input to the VTA and decreases spontaneous dopamine neuron activity. Here we demonstrate that an area adjacent to the PPTg regulates both population activity as well as burst firing of VTA dopamine neurons. Specifically, NMDA activation of the lateral mesopontine tegmentum produces an increase in the number of spontaneously active dopamine neurons and an increase in the average percent burst firing of dopamine neurons. This increase in neuronal activity was correlated with extracellular dopamine efflux in the nucleus accumbens, as measured by in vivo microdialysis. Taken together we provide further evidence that the mesopontine tegmentum regulates discrete dopamine neuron activity states that are relevant for the understanding of dopamine system function in both normal and disease states.

Keywords

Dopamine, mesopontine tegmentum, extracellular recording, afferent input, burst firing.
Introduction

Aberrant dopamine signaling has been advanced as a contributing factor for a variety of psychiatric diseases, including schizophrenia (Abi-Dargham 2004; Laruelle and Abi-Dargham 1999; Lodge and Grace 2011) and drug abuse (Koob 1992; Schultz 2002; 1998; Wise 2004). Interestingly, in most of these conditions there are no observable pathologies within the midbrain dopamine neurons themselves, suggesting that it may be the regulation of these neurons that is altered in psychiatric disease. Indeed, there is significant evidence that schizophrenia, for example, is associated with aberrant hippocampal drive of mesolimbic dopamine neurons in the ventral tegmental area (VTA) (Lodge and Grace 2011). Such information is clearly relevant to a better understanding of disease pathophysiology and, subsequently, the generation of more appropriate therapies, i.e. those that act upstream of the dopamine system.

To better understand how pathology within afferent structures relates to aberrant dopamine signaling, it is essential to understand how the dopamine system is regulated under ‘normal’ conditions. At present, there is significant anatomical information describing the afferent and efferent connections of the VTA and substantia nigra (SN) (Carr and Sesack 2000; Geisler et al. 2007; Oades and Halliday 1987; Omelchenico and Sesack 2005; Phillipson 1979). In addition, recent studies combining viral tracing in transgenic mice have provided information detailing the afferent input to dopamine neurons specifically, and have confirmed a widespread innervation from cortical and subcortical regions (Watabe-Uchida et al. 2012).

While there is substantial information regarding the afferent input to the VTA dopamine neurons, the way that these afferents effect dopamine transmission is less well studied. Electrophysiological studies in rodents have demonstrated that different afferent regions can modulate discrete activity states of the dopamine neuron (Floresco et al. 2003; Jhou et al. 2009a; Jhou et al. 2009b; Lodge 2011; Lodge and Grace 2006a; b). For example, the ventral pallidal input to the VTA directly regulates the number of dopamine neurons firing spontaneously (Floresco et al. 2003), a measure of the activity of the population of neurons in the VTA that is thought to provide a gain function to the system (Grace et al. 2007). In contrast, afferents from the medial prefrontal and orbitofrontal cortices have been demonstrated to bi-directionally regulate the firing rate of individual dopamine neurons (Aston-Jones et al. 2009; Lodge 2011; Overton et al. 1996; Takahashi et al. 2011; Tong et al. 1996; Tong et al. 1998).
A significant innervation of the VTA arises from the mesopontine tegmentum, specifically the pedunculopontine (PPTg), laterodorsal (LDTg), and rostromedial (RMTg) tegmental nuclei (Hallanger and Wainer 1988; Jhou et al. 2009a; Jhou et al. 2009b; Oakman et al. 1995; Omelchenko and Sesack 2006; Woolf and Butcher 1986). Thus, activation of the PPTg has been demonstrated to induce burst firing of dopamine neurons (Floresco et al. 2003; Lodge and Grace 2006a), thought to provide a functionally relevant signal indicating reward or encoding an error prediction signal. It should be noted that spontaneous burst firing is not typically observed in in vitro preparations where mesopontine tegmental inputs are severed during slice preparation (Grace and Onn 1989). We have previously demonstrated that this loss of burst firing is due to inputs arising from the LDTg since inactivation of this region results in a cessation of spontaneous, afferent- and glutamate- induced burst firing (Lodge and Grace 2006b). This is likely attributable to cholinergic afferents as transgenic mice lacking the β2 subunit of the nicotinic receptor display a similar reduction in burst firing of VTA dopamine neurons (Mameli-Engvall et al. 2006). Thus, the mesopontine tegmentum appears to provide a critical regulation of phasic activity within the VTA. Indeed, this is further evident based on recent examinations of the GABAergic input from the RMTg, which provides an inhibitory signal to ‘pause’ dopamine neuron activity in response to aversive inputs (Jhou et al. 2009a; Jhou et al. 2009b).

Taken together, therefore, the mesopontine tegmentum is well situated to bidirectionally modulate phasic dopamine neuron activity in the VTA. Here we report on an additional afferent input to the VTA dopamine neurons arising from a region adjacent to the PPTg (including the medial paralemniscal nucleus (MPL) and anterior A7 nuclei) that not only augments burst firing of dopamine neurons, but also influences the number of spontaneously active dopamine neurons, a standard measure of population activity thought to reflect the gain of the dopamine system (Grace et al. 2007). While there is relatively little literature detailing the ascending pathways of the MPL and A7 cell groups, it has been demonstrated that the MPL plays a role in stress reactivity via it projections to the hypothalamus (Fegley et al. 2008; Palkovits et al. 2004), whereas spinal projections from the A7 cell group are thought to participate in nociceptive signaling (Pertovaara 2006; Westlund et al. 1983).
Materials and Methods

All experiments were performed in accordance with the guidelines outlined in the USPHS Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center, San Antonio.

Extracellular Recordings and Drug Administration

Male Sprague-Dawley rats (250-350 g) were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic apparatus. Anesthesia was maintained by supplemental administration of chloral hydrate as required to maintain suppression of limb compression withdrawal reflex. A core body temperature of 37°C was sustained by a thermostatically controlled heating pad (KOPF, TCAT-2LV, USA). For acute administration of N-methyl-D-aspartic acid [NMDA, 1.5 mg/ml], baclofen/muscimol (0.4 mg/ml each), yohimbine (2mg/ml) or vehicle (Dulbecco’s phosphate-buffered saline), rats were implanted with 23 G injection cannulae 2.0 mm dorsal to the lateral mesopontine tegmentum (A/P -8.4, M/L +2.4, D/V -5.5 mm from bregma). Yohimbine was used to selectively augment activity from the adrenergic A7 cell group as these neurons are tonically active (Min et al. 2008) and blocking α2 autoreceptors has been shown to increase noradrenergic neuron activity in other catecholamine cell groups (Cedarbaum and Aghajanian 1976).

Glass extracellular microelectrodes (impedance 6-14MΩ) were lowered into the VTA (A/P -5.3, M/L +0.6 mm from bregma and -6.5 to -9.0 mm ventral of brain surface) using a hydraulic microdrive (KOPF-model 640) and the activity of the population of DA neurons was determined by counting the spontaneously active dopamine neurons encountered while making 6-9 vertical passes, separated by 200 µm, throughout the VTA. Spontaneously active dopamine neurons were identified using previously established electrophysiological criteria (Grace and Bunney 1983). NMDA (0.75 µg/0.5 µl), baclofen/muscimol (0.2 µg/0.5 µl), yohimbine (1 µg/0.5 µl) or Dulbecco’s PBS (0.5 µl) were infused through a 30 G injection cannula protruding 2.0 mm past the end of the implanted guide cannula. The injection cannula was left in situ for the duration of the experiments to ensure adequate diffusion of drug into the surrounding tissue. Rats received only one injection per region and each dopamine neuron encountered was typically recorded for 2-3 mins. Multiple dopamine neurons were recorded per animal and a typical experiment lasted up to 2 hours following drug infusion.
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 1984)). A subset of rats received an injection of blue dye (0.5 µl) into the lateral mesopontine tegmentum to approximate the spread of the intracranial drug injections. At the cessation of the experiments, rats were decapitated and their brains removed, fixed for 24 hours (3.7% formaldehyde), cryoprotected (10% w/v sucrose in PBS) and sectioned (25 µm coronal sections) on a cryostat. Sections containing electrode and cannula tracks were mounted onto gelatin-chrom alum coated slides and processed with a Nissl stain for histological verification (Paxinos and Watson 1998) – Figure 1A.

Microdialysis & HPLC
Extrasynaptic levels of dopamine in the nucleus accumbens (NAc) were determined by High Performance Liquid Chromatography (HPLC) with electrochemical detection. Male Sprague-Dawley rats (250-350 g) were anaesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic apparatus. Anesthesia was maintained by supplemental administration of chloral hydrate as required to maintain suppression of limb compression withdrawal reflex. A core body temperature of 37°C was sustained by a thermostatically controlled heating pad (KOPF, TCAT-2LV, USA) and a hole was drilled overlying the NAc (AP, +1.5; ML, +/-2.0; DV, -5.5 – 7.5). A concentric microdialysis probe (BAS inc – BR-2; 2 mm exposed membrane, 30KDa MW cut-off and 320µm OD) was lowered into the NAc and the probe perfused (2µl/min) with Dulbecco’s phosphate buffered saline, and allowed to equilibrate for 60 minutes. Dialysate was continuously collected and dopamine levels quantified by HPLC every 20 min. Once a stable baseline was achieved, the lateral mesopontine tegmentum was activated with NMDA as detailed above. Samples were collected at 20-min intervals into 10 µl of HPLC mobile phase (ESA: MD-TM 70-1332) to minimize neurotransmitter degradation. Samples were immediately injected into an HPLC system (Dionex) and separated on an MD-150 analytical column (150 × 3.2 mm) perfused with HPLC mobile phase (0.6 ml/min). Dopamine was detected by oxidation using a Dionex Coulcoum III detector equipped with an ESA Model 5020 guard cell (+350 mV) and an Model 5014B microdialysis cell (E1, -150 mV; E2, +220 mV). The HPLC system was calibrated at the
start of each experiment using external dopamine standards. At the cessation of the experiments, rats were decapitated and their brains removed, fixed for 24 hours (3.7% formaldehyde), cryoprotected (10% w/v sucrose in PBS) and sectioned (25µm coronal sections) on a cryostat (Shandon). Sections containing dialysis probe placements were mounted onto gelatin-chrom alum coated slides and processed with a Nissl stain for histological confirmation of probes & cannula tracks (Paxinos and Watson 1998) – Figure 1B.

Retrograde tracing and Immunohistochemistry

Male Sprague-Dawley rats (250-350 g) were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.) and placed in a stereotaxic apparatus. Anesthesia was maintained by supplemental administration of sodium pentobarbital as required to maintain suppression of limb compression withdrawal reflex. A core body temperature of 37°C was sustained by a thermostatically controlled heating pad and a hole was drilled in the skull overlaying the VTA. Glass micropipettes were backfilled with cholera toxin subunit B conjugated to Alexa Fluor 488 (CtB-488: Life technologies) and lowered stereotaxically into the VTA (A/P -5.3, M/L +0.6 mm, V/D -7.5 mm from bregma). CTb-488 was injected (0.75 µl) by air pressure and the pipette left in situ for 15 min. The skin was sutured and rats were allowed to recover for one week before being anaesthetized with sodium pentobarbital (120 mg/kg, i.p.) and transcardially perfused with 3.7% formaldehyde. Brains were removed, stored in 3.7% formaldehyde, cryoprotected (10% w/v sucrose in PBS) and sectioned (50µm coronal sections) on a cryostat (Shandon/Leica). Sections were blocked in PBS containing 2% rabbit serum albumin and 0.3% Triton X-100 for 30 min and then incubated at 4°C for 48 h with a goat anti-Choline Acetyltransferase (ChAT) antibody (AB144P-Millipore, 1:500 in PBS containing 1% rabbit serum albumin and 0.3% Triton X-100). Next, sections were incubated for 2 h at room temperature with rabbit anti-goat IgG conjugated to Alexa Fluor 594 (1:1000: Life technologies) and were mounted onto slides and cover slipped with Prolong Gold antifade mountant (Life technologies).

Analysis

Electrophysiological analysis of dopamine neuron activity was performed using commercially available computer software (LabChart v7.1). Chromatographic data were acquired and analyzed using Chromeleon software (Dionex). Combined retrograde tracing and immunohistochemistry was
examined on an Axio Lab.A1 Fluorescence Microscope and images taken with an attached AxioCam
ICc1 digital camera. All data are represented as the mean ± the standard error of the mean (S.E.M.)
unless otherwise specified. All statistics were calculated using SigmaPlot (Systat Software Inc) and
posthoc tests examined multiple comparisons versus the control group only.

**Results**

**VTA Dopamine Neuron Activities**

Rats that received control vehicle infusions (n = 8 rats, 64 neurons) exhibited an average of 0.96 ±
0.07 spontaneously active dopamine neurons per electrode track that fired at an average rate of 3.86
± 0.09 Hz with 18.1 ± 2.2% of action potentials fired in bursts (Figure 2A-C). Intracranial infusion of
NMDA into the lateral mesopontine tegmentum (n = 8 rats, 110 neurons) resulted in a significant
increase in dopamine neuron population activity (1.74 ± 0.07: 1-way ANOVA, F=29.124, Holm Sidak,
t=7.527, p<0.05: Figure 2A), average firing rate (4.64 ± 0.17 Hz: 1-way ANOVA on Ranks, H=8.272,
Dunn’s Method, Q=2.805, p<0.05: Figure 2B) and burst firing (37.1 ± 2.4%: 1-way ANOVA, F=7.700,
Holm Sidak, t=4.705, p<0.05: Figure 2C) relative to control. Injection of either baclofen/muscimol (n =
7, 52 neurons) or yohimbine (n = 8, 75 neurons) into the lateral mesopontine tegmentum failed to
significantly alter any parameter of dopamine neuron activity (cells/track: B/M 0.86 ± 0.09, YHB 1.04
± 0.07; Holm Sidak, t=0.935 & 0.797 respectively, p>0.05; firing rate: B/M 4.15 ± 0.19 Hz, YHB 4.12 ±
0.27 Hz, Dunn’s Method, Q=0.930 & 0.935 respectively, p>0.05; burst firing: B/M 24.2 ± 2.4%, YHB
27.6 ± 3.9%, Holm Sidak, t=1.460 & 2.353 respectively, p>0.05, Figure 2A-C).

**HPLC**

Baseline dopamine levels were (PBS: 0.021 ± 0.005 ng/ml; NMDA: 0.038 ± 0.03 ng/ml, n=6/group).
NMDA activation of the lateral mesopontine tegmentum significantly increased extrasynaptic
levels of dopamine in the NAc which peaked (NMDA 466.8 ± 90.8% of baseline, c.f. Vehicle 100.3 ±
14.8% of baseline: 2-way ANOVA \( F_{(Treatment)}=3.987 \), Holm-Sidak \( t=2.305 \), \( p<0.05 \)) soon after
administration and returned to baseline level after ~100 mins (Figure 3).

**Retrograde tracing/Immunohistochemistry**

Retrograde tracing with cholera toxin B demonstrated a strong innervation of the VTA from the
mesopontine tegmentum including both the PPTg as well as ventro-lateral regions (Figure 4). In contrast, immunohistochemistry for choline acetyltransferase was largely restricted to the PPTg with the lateral mesopontine tegmentum being largely devoid of cholinergic neurons (Figure 4).

Discussion

Afferents from the mesopontine tegmentum are known to modulate dopamine neuron activity (Floresco et al. 2003; Jhou et al. 2009a; Jhou et al. 2009b; Lodge and Grace 2006b) – Figure 5. Here we report on a subregion of the pontine tegmentum that is lateral of the PPTg, comprising the MPL and A7 nuclei. Thus, similar to the PPTg, activation of this region results in significant increases in burst firing of VTA dopamine neurons (Floresco et al. 2003; Lodge and Grace 2006a). However, an increase in the number of spontaneously active dopamine neurons observed per electrode track was also observed following NMDA administration to the lateral mesopontine tegmentum. Given that increases in dopamine neuron population activity are thought to increase the gain of the system to phasic activation, such an afferent drive is likely to induce robust alterations in dopamine system function. Indeed, our microdialysis data demonstrate that activation of this region significantly increases dopamine overflow in target regions of the forebrain. Interestingly, this region does not appear to provide a tonic input to the dopamine neurons, at least in the chloral hydrate anesthetized rat. Thus, administration of the GABA$_A$ and $B$ agonists, muscimol and baclofen, respectively, did not significantly alter spontaneous dopamine neuron activity. This is in contrast to nearby regions including the PPTg and LDTg that provide a tonic regulation of dopamine system function (Floresco et al. 2003; Lodge and Grace 2006b). Specifically, the tonic input from the LDTg appears necessary to support both glutamate and afferent-induced burst firing of VTA dopamine neurons (Lodge and Grace 2006b). In addition, muscimol/baclofen inactivation of the LDTg has been demonstrated to normalize the firing pattern of dopamine neurons, resulting in a pacemaker rhythm not typically observed in vivo, but widely reported in slice electrophysiological studies (Lodge and Grace 2006b). This is likely attributable to alterations in cholinergic transmission as transgenic mice lacking the $\beta_2$ subunit of the nicotinic receptor display a similar reduction in burst firing of VTA dopamine neurons (Mameli-Engvall et al. 2006). Thus, it is perhaps not surprising that inactivation of regions adjacent to the PPTg did not alter baseline activity as this area is largely devoid of cholinergic neurons (see Figure 4).
The mechanisms by which the PPTg induces burst firing are not entirely known as the PPTg contains both glutamatergic and cholinergic projections to the VTA (Geisler et al. 2007; Wang and Morales 2009). The observation that lateral regions of the mesopontine tegmentum also produce robust increases in dopamine neuron burst firing are more likely attributable to a direct glutamatergic projection, as this region appears largely devoid of cholinergic neurons (Wang and Morales 2009). Indeed, the afferents to the VTA are not from distinct sub-regions of the brain, but rather, form a continuum extending from the prefrontal cortex through to the brainstem (Geisler et al. 2007; Watabe-Uchida et al. 2012). Similarly, the distribution of glutamatergic neurons in the mesopontine tegmentum, as determined by vGluT2 mRNA expression, are not discretely localized and those projecting to the VTA are from a diffuse area including, but not limited to the PPTg and LDTg (Geisler et al. 2007).

In addition to inducing burst firing of VTA dopamine neurons, activation of the lateral regions of the mesopontine tegmentum also produced robust increases in the number of spontaneously active VTA dopamine neurons. This increase in dopamine neuron population activity is not reported following activation of the adjacent PPTg (Floresco et al. 2003; Lodge and Grace 2006a), but has been observed with activation of the LDTg (Lodge and Grace 2006b). The ability to alter the number of dopamine neurons firing spontaneously has been suggested to provide a gain of function to the dopamine system and is made possible due to a tonic GABAergic input arising from the ventral pallidum (Grace et al. 2007). Thus, dopamine neurons of the VTA are bombarded by inhibitory postsynaptic potentials (IPSPs) (Grace and Bunney 1985) that are thought to tonically inhibit a proportion of VTA dopamine neurons (estimated at greater than 50%). By directly or indirectly altering this pallidal input to dopamine neurons, afferent regions can regulate the number of spontaneously active neurons in the VTA. This increase in population activity will result in two functional outcomes 1) a tonic increase in dopamine efflux in the nucleus accumbens and 2) an enhanced phasic dopamine signal in response to afferent drive (Floresco et al. 2003; Lodge and Grace 2006a). Indeed, increases in dopamine neuron population activity have been reported to increase dopamine efflux in the NAc as measured by microdialysis (Floresco et al. 2003). This is consistent with the data presented here where NMDA activation of the lateral mesopontine tegmentum produced a significant increase in NAc dopamine efflux. In addition to increasing tonic dopamine transmission, increases in dopamine neuron population activity also alter the phasic dopamine signal by determining the proportion of neurons...
that can burst fire (Lodge and Grace 2006a). Taken together, by altering both dopamine neuron population activity and burst firing, the lateral mesopontine tegmentum is situated to dramatically influence dopamine signaling.

Here we expand our previous observations regarding the regulation of dopamine neuron activity by the mesopontine tegmentum and demonstrate that this region provides substantial control over VTA dopamine neuron activity. Moreover, this appears to be independent of the A7 cell group as infusions of the α2 autoreceptor antagonist, yohimbine, failed to alter VTA activity. Thus, ascending inputs from the MPL likely contribute to the activating effects of lateral mesopontine tegmentum activation. While little is known about the function of the MPL, it has been demonstrated to contain tuberoinfundibular peptide of 39 which regulates some aspects of stress reactivity via projections to the hypothalamus (Fegley et al. 2008; Palkovits et al. 2004). Thus, the mesopontine tegmentum, as a whole, is well situated to integrate a variety of inputs from both forebrain and brainstem to regulate dopamine system function. Although we have demonstrated a direct pathway from the lateral mesopontine tegmentum to the VTA, it is still unknown whether the electrophysiological effects observed with NMDA activation of this area are attributable to this direct projection. Indeed, regulation of dopamine neuron activity may occur secondary to activation of either direct or indirect projections. Moreover, it is important to note that the VTA is not homogenous and that there are discrete projections to forebrain targets that may be differentially modified by motivationally relevant stimuli or afferent inputs (Lammel et al. 2011). While we are unable to determine which ascending dopamine pathways were activated by the lateral mesopontine tegmentum, or whether this was attributable to direct or indirect projections, we clearly demonstrate that the net effect of lateral mesopontine tegmentum activation is to augment both dopamine neuron population activity and burst firing.

By better understanding the physiology of the afferent inputs to the dopamine neurons under ‘normal’ conditions, we will be in a better position to determine how pathology results in aberrant dopamine signaling related to disease. Indeed, the mesopontine tegmentum has been advanced as a therapeutic target for the treatment of Parkinson's Disease, specifically the PPTg is a target for deep brain stimulation and is effective at ameliorating gait and postural symptoms (Stefani et al. 2007).
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Disclosure

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Figure Captions

Figure 1: Histological localization of injection sites in the lateral mesopontine tegmentum for electrophysiological (A) and neurochemical (B) studies. The arrow in (A) points to a representative dye injection (blue) depicting the approximate spread of intracranial drug injections. Numbers beside each plate represent approximate A/P distance from bregma. Abbreviations: A7 noradrenaline cells (A7), Killiker-Fuse nucleus (KF), medial paralemniscal nucleus (MPL), pedunculopontine tegmental nucleus (PPTg), rubrospinal tract (rs), superior cerebellar peduncle (scp). Adapted from (Paxinos and Watson 1998).

Figure 2: Activation of the lateral mesopontine tegmentum alters population activity and burst firing of VTA dopamine neurons. NMDA (0.75 µg/0.5 µl), baclofen/muscimol (0.2 µg/0.5 µl), or yohimbine (1 µg/0.5 µl) were infused into the lateral mesopontine tegmentum and the activity of spontaneously active VTA dopamine neurons examined. Representative neuronal activity following administration of vehicle or NMDA is depicted in (A & B) respectively. Scale bars represent 5 seconds for electrophysiological traces and 1ms for average waveforms (inset). Three parameters of activity were recorded (C): population activity, average firing rate and average percent burst firing. * Statistically significant difference from control (vehicle infusions) (p < 0.05, one-way ANOVA or ANOVA on ranks: n=7-8 rats/group).

Figure 3: Activation of the lateral mesopontine tegmentum increases dopamine efflux in the nucleus accumbens. NMDA (0.75 µg/0.5 µl) or vehicle (0.5 µl) were infused into the lateral mesopontine tegmentum and dopamine efflux collected by microdialysis and measured by HPLC. * Statistically significant difference from control (vehicle infusions) (p < 0.05, two-way ANOVA; Holm-Sidak post hoc: n=6 rats/group).
Figure 4: Histological confirmation of a projection from the lateral mesopontine tegmentum to the VTA. Alexa fluor 488 conjugated to cholera toxin B was injected into the VTA (A & B) and retrogradely labelled neurons (green) were observed throughout the mesopontine tegmentum (C). Cholinergic neurons (red) were identified by choline acetyltransferase immunohistochemistry. (a1-3) depict the mesopontine tegmentum at 10x whereas (b1-3 and c1-3) are higher magnifications (20x) of the PPTg and lateral mesopontine tegmentum, respectively.

Figure 5: Schematic depicting the mesopontine tegmental regulation of dopamine neuron activity states. Activation of the PPTg induces a transition from single spike firing to burst firing. The RMTg provides an inhibitory input to the VTA and attenuates spontaneous activity of dopamine neurons. The LDTg is known to provide a permissive factor that is essential for glutamate and afferent induced burst firing. Finally, the lateral mesopontine tegmentum, described here, produces a robust increase in both dopamine neuron population activity (i.e. the number of spontaneously active dopamine neurons) and burst firing.
Lateral Mesopontine Tg
(Increases Burst Firing and proportion of active neurons)

PPTg
(Increases Burst Firing)

RMTg
(Inhibits Activity)

LDTg
(Permits Burst Firing)

GABA

Glu/Ach

Glu