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Opioid-induced Rewards, Locomotion, and Dopamine Activation: A Proposed Model for Control by Mesopontine and Rostromedial Tegmental Neurons

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Abstract

Opioids, such as morphine or heroin, increase forebrain dopamine (DA) release and locomotion, and support the acquisition of conditioned place preference (CPP) or self-administration. The most sensitive sites for these opioid effects in rodents are in the ventral tegmental area (VTA) and rostromedial tegmental nucleus (RMTg). Opioid inhibition of GABA neurons in these sites is hypothesized to lead to arousing and rewarding effects through disinhibition of VTA DA neurons. We review findings that the laterodorsal tegmental (LDTg) and pedunculopontine tegmental (PPTg) nuclei, which each contain cholinergic, GABAergic, and glutamatergic cells, are important for these effects. LDTg and/or PPTg cholinergic inputs to VTA mediate opioid-induced locomotion and DA activation via VTA M5 muscarinic receptors. LDTg and/or PPTg cholinergic inputs to RMTg also modulate opioid-induced locomotion. Lesions or inhibition of LDTg or PPTg neurons reduce morphine-induced increases in forebrain DA release, acquisition of morphine CPP or self-administration. We propose a circuit model that links VTA and RMTg GABA with LDTg and PPTg neurons critical for DA-dependent opioid effects in drug-naïve rodents.

Keywords

morphine; reward; pedunculopontine tegmental nucleus; laterodorsal tegmental nucleus; addiction; glutamate; GABA; acetylcholine

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1. Introduction

Opioids have analgesic, anxiolytic, and euphoric effects leading to widespread use, dependence and addiction in humans (Nutt and Lingford-Hughes, 2008). Development of long-lasting, potent synthetic opioids has heightened these risks, and increased use, rates of dependence, and death (Gruber et al., 2007; Kalant, 1997; Murthy, 2017).

In rodents, the critical midbrain sites of action for opioids, and especially the circuits connecting these sites, remain controversial (see reviews by Badiani et al., 2011; Barrot et al., 2012; Ikemoto, 2010; Le Merrer et al. 2009; Madhavan et al., 2010; Smith and Berridge, 2007). Opioid-mediated disinhibition of ventral tegmental area (VTA) dopamine (DA) neurons has been an influential theory to explain DA-dependent opioid reward. Initially, it was proposed that opioids act on μ -opioid receptors (μ -ORs) to inhibit VTA gamma-aminobutyric acid (GABA) neurons that provide tonic inhibition to neighboring VTA DA neurons, in turn disinhibiting mesolimbic DA neurons (Gysling and Wang, 1983; Johnson and North, 1992; Klitenick et al., 1992; Margolis et al., 2014; Matthews and German, 1984). More recently defined rostromedial tegmental nucleus (RMTg) GABA neurons project to and inhibit VTA DA neurons (Jhou et al., 2009a; 2009b; Lecca et al., 2012). RMTg GABA neurons are strongly inhibited by opioids and this is followed by disinhibition of VTA and substantia nigra pars compacta (SNc) DA neurons (Jalabert et al., 2011; Lecca et al., 2012; 2011; Matsui and Williams, 2011).

In our view, the most critical short-coming of previous circuit models is their inability to explain the critical role of mesopontine tegmental nuclei – the laterodorsal tegmental nucleus (LDTg) and the pedunculopontine tegmental nucleus (PPTg) – in the acquisition of opioid reward. Lesions of mesopontine tegmental nuclei or blockade of muscarinic acetylcholine receptors (mAChR) in the VTA inhibit the acquisition of opioid self-administration, conditioned place preference (CPP) or locomotion as effectively as pharmacological antagonists of DA receptors or mesolimbic DA lesions (e.g., Basile et al., 2002; Gerrits and Van Ree, 1996; Nader and van der Kooy, 1997; Pettit et al., 1984; Steidl and Yeomans, 2009; Vaccarino et al., 1986).

Here we review where opioids act in the brain to induce DA activation, locomotion, and reward in drug-naïve animals. We emphasize the involvement of PPTg and LDTg neurons and their reciprocal projections to the VTA and RMTg in the behavioral responses of drug-naïve animals to initial opioid exposure (Lavezzi et al., 2012; Lavezzi and Zahm, 2011; Omelchenko and Sesack, 2006, 2005; Wasserman et al., 2016; 2013; Watabe-Uchida et al., 2012). In this review, we primarily focus on acute opioid exposure and do not address the potential brain changes resulting from long-term exposure to opioids or neurobiological mechanisms of dependence and relapse.

2. Rewarding effects of opioids and dopamine

Intracranial morphine self-administration was first achieved with cannulae placed in the VTA near mesolimbic DA neurons (Bozarth and Wise, 1981a; David and Cazala, 1994; David et al., 2002; Devine and Wise, 1994; Welzl et al., 1989) where μ -ORs are found (Erbs

et al., 2015; Kitchen et al., 1997; Mansour et al., 1987). VTA infusions of morphine, or DAMGO, a μ -OR agonist, or endomorphin-1, an endogenous μ -OR ligand, also lead to the acquisition of CPP (Bals-Kubik et al., 1993; Phillips and LePiane, 1980) or support self-administration (Zangen et al., 2002). μ -OR blockers, such as naloxone, infused near the VTA block morphine CPP of systemic opioids (Olmstead and Franklin, 1997). Early studies also showed that DA receptor blockers or 6-OHDA lesions attenuated heroin CPP or self-administration (Bozarth and Wise, 1981b; Spyraiki et al., 1983). However, further studies showed that mesolimbic DA terminals or DA receptors in nucleus accumbens (NAcc) are not necessary for either heroin self-administration (Ettenberg et al., 1982; Pettit et al., 1984) or locomotion induced by intra-NAcc infusions of an enkephalin analog (Kalivas et al., 1983; Vaccarino et al., 1986).

In addition, morphine infusions into the NAcc, where high densities of opioid receptor subtypes are expressed (Erbs et al., 2015; Kitchen et al., 1997; Mansour et al., 1987) – downstream from dopaminergic synapses – support self-administration in rats and mice (Olds, 1982; David and Cazala 2000). Injections of the opioid antagonist methylnaloxonium into the NAcc, for example, have been shown to block locomotion induced by systemic heroin in rats (Amalric and Koob, 1985). However, while DA is not necessary for opioid-induced locomotion in rats, chronic blockade of dopaminergic transmission by either mesolimbic 6-OHDA lesions (Stinus et al., 1985) or prolonged systemic neuroleptic drugs (Stinus et al., 1986) results in supersensitivity to locomotion induced by NAcc injections of morphine, suggesting that a functional DA system in fact reduces sensitivity to NAcc opioid-induced locomotion.

3. Mesopontine cholinergic and glutamate neurons activate DA neurons and rewards

3.1. LDTg or PPTg activation excites mesolimbic or nigrostriatal dopamine pathways

The LDTg and PPTg provide sources of input to the VTA and SNc (Geisler and Zahm, 2005; Oakman et al., 1999; 1995; Watabe-Uchida et al., 2012; Woolf, 1991). Within each of the LDTg and PPTg, three largely separate populations of neurons are found: cholinergic neurons expressing choline acetyltransferase (ChAT), glutamatergic neurons expressing the vesicular glutamate transporter-2 (VGluT2), and GABAergic neurons expressing glutamic acid decarboxylase (GAD) (Wang and Morales, 2009). Electrical stimulation of the LDTg or PPTg in urethane-anesthetized rats increases DA efflux in the NAcc or striatum, respectively. These DA outputs depend on both cholinergic and glutamatergic receptors in the VTA and the neighboring SNc (Forster and Blaha, 2000; 2003). Increases in NAcc DA, immediately following LDTg electrical stimulation (Figure 1A), and increases in dorsal striatal DA, immediately following PPTg electrical stimulation, depend on nicotinic and ionotropic glutamate receptors in the VTA or SNc, respectively. After a decrease in basal DA levels (due to inhibitory M2 and M4 muscarinic autoreceptors in LDTg or PPTg), DA levels increase again and remain elevated for approximately 60 min. The late and prolonged phase in increased accumbal or striatal DA efflux is completely blocked in rats by the mAChR antagonist, scopolamine, in the VTA or SNc, respectively (Forster and Blaha, 2003; 2000), or by systemic knockout of M5 mAChRs in mice (Figure 1B; Forster et al., 2002b; Steidl et

al., 2011). Therefore, M5 mAChR – the only subtype of mAChR expressed by midbrain DA neurons (Vilaró et al., 1990) – are responsible for most of the excitatory effects of LDTg or PPTg electrical stimulation on forebrain DA efflux (Yeomans et al., 2001).

Electrophysiological recordings from VTA DA neurons support the critical role of the LDTg in regulating mesolimbic DA activity. DA neurons burst firing induces large transient increases in forebrain DA that are thought to be functionally relevant for encoding reward prediction and incentive salience (Berridge and Robinson, 1998; Cooper, 2002; Grace, 1991; Schultz, 1998). Following LDTg chemical inactivation, VTA DA neurons fail to burst fire, either in response to activation of PPTg inputs, which usually reliably elicit burst firing, or in response to direct VTA application of glutamate (Floresco et al., 2003; Lodge and Grace, 2006).

3.2. Rewarding effects of acetylcholine and glutamate in the ventral tegmental area

A role for cholinergic input to the VTA in reward function has been clearly established over the last 3 decades. Feeding, drinking, rewarding electrical brain stimulation of the lateral hypothalamus, or intravenous cocaine self-administration each increase ACh levels in VTA (Rada et al., 2000; You et al., 2008). In rats, brain-stimulation reward thresholds are dose-dependently increased by up to 100% by the muscarinic blockers atropine or scopolamine infused into the VTA, or by 20% by nicotinic blockers infused into the VTA (Kofman and Yeomans, 1988; Yeomans and Baptista, 1997; Yeomans et al., 1985). Inhibition of M5 gene expression in VTA by M5 antisense oligonucleotides similarly increases reward thresholds by 30–100% (Yeomans et al., 2000). Muscarinic blockers in the VTA also block the acquisition of food self-administration in rats (Sharf et al., 2006; Sharf and Ranaldi, 2006) and increase intravenous cocaine self-administration in rats (You et al., 2008). VTA infusions of the cholinergic receptor agonist carbachol induce CPP and support operant self-administration (Ikemoto and Wise, 2002; Yeomans et al., 1985). VTA infusions of the cholinesterase inhibitor neostigmine also support operant self-administration in rats (Ikemoto and Wise, 2002). VTA self-administration of carbachol is reduced by VTA co-administration of either a muscarinic or a nicotinic receptor antagonist.

VTA cholinergic signaling is also associated with conditioned rewarding effects. Muscarinic AChR antagonists in the VTA reduce conditioned responding for food-associated cues (Addy et al., 2015; Wickham et al., 2015) and cue-driven cocaine seeking (Solecki et al., 2013). Conversely, VTA microinjections of physostigmine, an inhibitor of acetylcholinesterase, increase cue-induced reinstatement responding of extinguished heroin seeking (Zhou et al., 2007). In addition, VTA ACh levels, measured by microdialysis in rats trained to self-administer intravenous cocaine, increase following cocaine or following conditioned cues associated with cocaine (You et al., 2008).

Unlike the cholinergic agonist carbachol, rats do not self-administer the glutamate receptor agonist AMPA into the VTA (Ikemoto et al., 2004), and VTA infusions of the glutamate receptor agonist NMDA are also only weakly reinforcing (Ikemoto, 2004). VTA glutamatergic signaling is also associated with conditioned rewarding effects. VTA glutamate levels, measured by microdialysis in rats self-administering intravenous cocaine

(You et al., 2007) or heroin (Wang et al., 2012), increase following conditioned cues for the drug, but not following unconditioned drug exposure.

3.3. Optogenetic activation of LDTg and PPTg cholinergic and glutamatergic inputs to the ventral tegmental area

Optogenetic techniques have allowed for excitation of LDTg inputs to the VTA in freely-moving rats or mice (Bernstein and Boyden, 2011; Britt and Bonci, 2013; Deisseroth, 2015; Fenno et al., 2011). In mice, optogenetic excitation of LDTg cell bodies that send projections to the VTA results in the acquisition of Pavlovian CPP (Lammel et al., 2012). In these experiments, mice are confined to a conditioning chamber where they receive experimenter-delivered optogenetic excitation. On a subsequent light-free choice test, mice show a preference for the conditioning chamber in which optogenetic excitation was previously received. In rats, optogenetic excitation of LDTg axons in the VTA reinforces lever pressing and these reinforcing effects depend on NAcc DA (Steidl and Veverka, 2015; Steidl et al., 2017b).

Selective excitation of LDTg cholinergic inputs to the VTA in mice results in rewarding effects. When given the opportunity in a place preference apparatus to enter a conditioning chamber to obtain optogenetic excitation of LDTg cholinergic inputs to the VTA, mice spend more time in that chamber and subsequently show a CPP for that chamber in the absence of optogenetic excitation (Steidl et al., 2017c). In rats, optogenetic excitation of LDTg or PPTg cholinergic inputs to the VTA, but not the SNc, results in the acquisition of Pavlovian CPP (Xiao et al., 2016).

The interpretation of studies showing that rats do not self-administer glutamatergic agonists into the VTA is complicated by the fact that, unlike ACh, where LDTg and PPTg are the only sources of VTA input, the LDTg and PPTg are only two of many sources of glutamatergic input to the VTA (Geisler et al., 2007). While the net effect of glutamatergic agonists in the VTA may not be reinforcing, this does not rule out the possibility that individual VTA glutamate afferents contribute to reward function. Selective optogenetic excitation of PPTg glutamatergic inputs to the VTA reinforces operant self-administration in mice (Yoo et al., 2017). When given the opportunity in a place preference apparatus to enter a conditioning chamber to obtain selective optogenetic excitation of LDTg glutamatergic inputs to the VTA, mice enter that chamber more than an alternate chamber. Mice remain in the light-paired chamber for very short periods of time (between ~0.3–8 sec per visit) but persist in returning to the stimulation-paired chamber throughout the testing session and in subsequent testing sessions. By contrast, with excitation of LDTg cholinergic inputs to the VTA mice remain in the light-paired chamber for much longer periods of time (between ~10–447 sec per visit). Under conditions in which chamber entry results in only 1.5 sec of VTA light stimulation – parameters that are similar to those used in operant intracranial self-stimulation paradigms and – mice behave similarly whether given excitation of LDTg cholinergic or glutamatergic inputs (Steidl and Veverka, 2015; Steidl et al., 2017c).

The behavioral differences resulting from optogenetic excitation of LDTg glutamatergic or cholinergic inputs to the VTA may be related to the different effects on NAcc DA efflux following LDTg electrical stimulation (Forster and Blaha, 2000; Forster et al., 2002b). That

is, VTA glutamate receptors induce only brief increases in NAcc DA, while VTA M5 mAChRs induce delayed NAcc DA increases that last for over 30 min. Therefore, optogenetic excitation of LDTg glutamatergic inputs to the VTA may induce rapid but short-lasting increases in NAcc DA levels. This induces transient rewarding effects, resulting in repeated entries into the stimulation-paired chamber but short stay durations. Conversely, optogenetic excitation of LDTg cholinergic inputs to the VTA may induce slower onset, but long-lasting increases in NAcc DA. This induces sustained rewarding effects, resulting in long stay durations.

4. Acetylcholine, glutamate, and opioids

4.1. VTA GABA disinhibition

Mu- and delta- opioid receptors in the VTA have been localized using receptor ligand autoradiography (Kitchen et al., 1997; Mansour et al., 1987) and genetic knock-in tagging of opioid receptors with fluorescent markers (Erbs et al., 2015). Mu-opioid receptors are predominantly found on VTA GABA neurons and not on DA neurons (Dilts and Kalivas, 1989; Garzon and Pickel, 2001). Opioids directly inhibit many VTA GABA neurons that provide tonic inhibition to neighboring VTA DA neurons (Gysling and Wang, 1983; Johnson and North, 1992; Klitenick et al., 1992; Margolis et al., 2014; Matthews and German, 1984; Steffensen et al., 2006). Thus, the “VTA GABA-DA Disinhibition Hypothesis” proposed that opioids induce reward by inhibiting VTA GABA neurons, thereby disinhibiting adjacent mesolimbic DA neurons (Johnson and North, 1992). Opioid-mediated disinhibition of VTA DA neurons continues to be an influential theory as it provides a neurophysiologically tested model linking VTA μ -ORs with the well-defined mesolimbic DA reward systems. Several experimental findings, however, suggest that opioid-mediated disinhibition of VTA DA neurons is, by itself, not sufficient as a sole mechanistic explanation for the DA-dependent and possibly DA-independent rewarding and locomotor-inducing effects of opioids. We discuss each of these below.

4.2. The rostromedial tegmental nucleus

GABAergic neurons that critically control the DA system are localized just caudal to the classically defined VTA. The GABAergic cells in this region – termed either the rostromedial tegmental nucleus (RMTg; Jhou et al., 2009a; 2009b) or, alternatively, the tail of the VTA (Barrot et al., 2012; Perotti et al., 2005) – are continuous with those of the VTA, and they also project to (Jhou et al., 2009a; 2009b) and inhibit VTA DA neurons (Lecca et al., 2012). RMTg GABA neurons express high level of μ -ORs (Jhou et al., 2009b; Kaufling and Aston-Jones, 2015; Wasserman et al., 2016). Despite similarities with the VTA, the RMTg is both anatomically and functionally distinct from the VTA. The RMTg represents a relatively pure GABAergic cell population with high-level expression of GAD67, and is distinguishable from the VTA and SNc by a lack of DA neurons (Jhou et al., 2009a, 2009b; Kaufling et al., 2010, 2009; Olson and Nestler, 2007; Perotti et al., 2005). Other markers that differentiate the RMTg from its surrounding areas include high levels of the neuropeptide nociceptin (Jhou et al., 2009b) and high expression of Fos and related immediate early genes following the administration of psychostimulants and certain aversive stimuli (Jhou et al., 2009a, 2009b; Lavezzi et al., 2012).

Electrophysiological recordings from RMTg GABA neurons show that they are strongly inhibited by systemic or locally administered opioids, followed by disinhibition of VTA and SNc DA neurons (Jalabert et al., 2011; Lecca et al., 2012; Matsui and Williams, 2011). Rats learn to self-administer opioids into the RMTg more vigorously than into the posterior portions of the VTA and opioids infused into the RMTg lead to the acquisition of CPP (Jhou et al., 2012). RMTg infusions of morphine (Steidl et al., 2017a) or the selective μ -OR agonist DAMGO (Kotecki et al., 2015) increase open-field locomotion. Morphine in RMTg, or in the posterior portions of the VTA, also reduces intravenous heroin self-administration (Steidl et al., 2015). Chemogenetic excitation of RMTg GABA neurons with hM3D designed muscarinic receptors (DREADDs, designer receptors exclusively activated by designer drugs; for a review see Sternson and Roth, 2014) inhibits systemic morphine-induced locomotion, while chemogenetic inhibition of RMTg GABA neurons via hM4D designed muscarinic receptors facilitates systemic morphine-induced locomotion (Wasserman et al., 2016). Similar experiments that test the effects of chemogenetic inhibition or excitation of VTA GABA neurons on systemic morphine-induced locomotion have not been conducted. These experiments would allow for a direct comparisons of the relative importance of VTA and RMTg GABA neurons to morphine-induced locomotion.

4.3. Contributions of VTA and RMTg acetylcholine and glutamate signaling on opioid reward, locomotion, and dopamine in drug-naïve rodents

PPTg or LDTg excitotoxic lesions block increases in striatal (Miller et al., 2002) or NAcc (Forster et al., 2002a) DA, respectively, usually observed following systemic morphine administration in rats (Figure 2A). PPTg or LDTg excitotoxic lesions also block morphine-induced locomotion or stereotypy (Bechara and van der Kooy, 1992; Forster et al., 2002a; Miller et al., 2002; Olmstead and Franklin, 1994a). PPTg excitotoxic lesions block the acquisition of morphine or heroin CPP and the acquisition of heroin self-administration in drug-naïve rats (Bechara et al., 1992; Nader and van der Kooy, 1997; Nader et al., 1994; Olmstead and Franklin, 1993; 1994b; Olmstead et al., 1998; Vargas-Perez et al., 2007).

Cholinergic and glutamatergic signaling in the VTA contributes to opioid reward and to opioid-induced locomotion. Cholinergic signaling in the RMTg also contributes to opioid-induced locomotion. The specific contributions of glutamatergic signaling in the RMTg to opioid reward and/or opioid-induced locomotion have not been tested to our knowledge. In the case of ACh, the only known sources of input to VTA are the PPTg and LDTg. Many PPTg and LDTg cholinergic neurons also project to RMTg (Wasserman et al., 2016). Some of the structures that are part of the basal forebrain cholinergic system – another major source of brain ACh – also project to the RMTg (e.g. medial septum, horizontal diagonal band, magnocellular preoptic area; Yetnikoff et al., 2015). However, these represent relatively minor sources of input to RMTg and it remains to be determined whether these RMTg projections arise specifically from cholinergic neurons. In the case of glutamate, the LDTg and PPTg are only two of several sources of glutamatergic input to the VTA, but, as described earlier, LDTg and/or glutamatergic afferents to VTA importantly contribute to mesolimbic DA activation and reward (Forster and Blaha, 2003; Geisler et al., 2007; Steidl et al., 2017c; Yoo et al., 2017). We are not aware of tract-tracing studies specifically outlining RMTg glutamatergic afferents.

M5 mAChRs on VTA DA neurons are necessary for systemic morphine-induced locomotion. For example, systemic morphine-induced locomotion is reduced by approximately half across a range of morphine doses in M5 receptor knockout (KO) mice. VTA injections of the mAChR antagonist atropine in wild-type (WT) mice block morphine-induced locomotion to a similar extent as systemic M5 gene knockout (Steidl and Yeomans, 2009) suggesting that VTA M5 mAChRs are critical for the locomotor effects of systemic morphine. Similarly, locomotion induced by intra-VTA morphine is blocked by VTA co-infusion of atropine (Steidl et al., 2017a).

The ability of intra-VTA morphine to increase NAcc DA efflux is completely blocked by VTA pretreatment with the mAChR antagonist scopolamine or by systemic M5 gene knockout (Steidl et al., 2011). Increases in NAcc DA and CPP induced by systemic morphine are reduced in M5 KO mice (Basile et al., 2002). Similarly, in rats, VTA atropine blocks the acquisition of morphine CPP (Rezayof et al., 2007) and the ability of systemic morphine to increase NAcc DA efflux is also completely blocked by VTA infusions of scopolamine (Miller et al., 2005; Figure 2B).

Similarly, site-specific rescue of M5 receptors in the VTA of M5 KO mice (by injecting herpes simplex virus [HSV] with added M5 receptors genes into VTA) restored morphine-induced locomotion. Further, HSV-M5 overexpression of M5 receptors in WT mice increased morphine-induced locomotion between 3–8 times above baseline (Wasserman et al., 2013; Figure 3). In RMTg, HSV expression of M5 receptors in WT mice, which are not endogenously expressed in this brain area, reduced morphine-induced locomotion between 64% and 83% at two doses of systemic morphine, with little effect on saline-induced locomotion (Wasserman et al., 2013). Therefore, cholinergic inputs to the VTA and the RMTg each contribute to the ability of opioids to induce reward, locomotion, and DA activation.

Glutamate blockers infused into anterior VTA sites increased heroin self-administration and blocked the acquisition of heroin CPP (Shabat-Simon et al., 2008). However, whether this is due to the blockade of mesopontine glutamatergic inputs to the VTA is difficult to determine as many brain regions provide glutamatergic input to the VTA (Geisler et al., 2007). Glutamatergic inputs to VTA DA neurons are thought to be especially important for the development of addiction. Sensitization – an enhancement in the ability of drugs of abuse to activate DA neurotransmission, increase locomotor activation, and support self-administration following previous drug exposure – is thought to play a major role in the maintenance of drug use and in the reinstatement of drug-seeking behaviors (Robinson and Berridge, 1993; Vanderschuren and Kalivas, 2000; Vezina 2004; Wolf, 1998). Changes in VTA glutamatergic signaling, have been implicated in behavioral changes that result from repeated exposure to opioids (Carlezon et al., 1999, 1997; Fitzgerald et al., 1996; Saal et al., 2003) and to stimulant drugs (Argilli et al., 2008; Borgland et al., 2004; Carlezon and Nestler, 2002; Churchill et al., 1999; Fitzgerald et al., 1996; Saal et al., 2003; Ungless et al., 2001; White et al., 1995; Zhang et al., 1997) and are thus thought to be critical in the development of behavioral sensitization. The origin of the critical glutamatergic projections giving rise to the VTA glutamatergic synapses at which changes important for the development of sensitization have not been determined. Interestingly, recent data also

suggest a potential role for changes in LDTg cholinergic signaling in morphine sensitization (Bajic et al., 2015).

4.4. Contributions of PPTg, LDTg, and RMTg to opioid reward, locomotion, and dopamine in drug-experienced rodents

In contrast to the effects described above in drug-naïve animals, PPTg lesions do not block the acquisition of morphine CPP in opioid dependent/withdrawn rats and do not affect established heroin self-administration (Bechara and van der Kooy, 1989; Bechara et al., 1992; Nader & van der Kooy, 1997; Nader et al., 1994; Olmstead et al., 1998). PPTg lesions using a toxin designed to target PPTg cholinergic neurons (Clark et al., 2007), also do not affect established heroin self-administration (Steidl et al., 2014). These lesions do, however, result in at least some loss of PPTg glutamatergic and GABAergic neurons in addition to almost complete loss of cholinergic neurons. Accordingly, the PPTg has been suggested to be part of a DA-independent mechanism, likely involving its descending projections (Heinmiller et al., 2009), important for opioid reward in drug-naïve animals (Bechara et al., 1998; Nader et al., 1997; Ting-A-Kee and van der Kooy, 2012). Dorsal tegmental outputs become less important for opioid CPP and self-administration after opioid dependence and withdrawal while DA output becomes more important (Bechara and van der Kooy, 1989; Bechara et al., 1998; 1992; Laviolette and van der Kooy, 2001; Laviolette et al., 2004; Nader and van der Kooy, 1997; Nader et al., 1997; 1994; Vargas-Perez et al., 2009; 2007; Ting-A-Kee and van der Kooy, 2012). Importantly, the effects of LDTg lesions on opioid reward in drug-experienced animals have not been tested. Similarly, the effects of AChR antagonism, or removal in the VTA, in drug-experienced animals, which blocks the acute effects of opioids on locomotion, CPP and DA release in drug-naïve animals, have not been extensively examined. One report (Steidl et al., 2013) shows that acute food deprivation, which reduces PPTg contributions to morphine CPP in rats (Nader and van der Kooy, 1994), reduces the contribution of M5 receptors to systemic morphine-induced locomotion in mice. This suggests that cholinergic signaling in the VTA, similar to PPTg lesions, plays less of a role in drug-experienced animals.

Several studies have investigated RMTg contributions to opioid effects in drug-withdrawn animals. Chronic morphine treatment (1 week) has been shown to result in partial tolerance to the ability of morphine to inhibit RMTg inputs to VTA following acute withdrawal in ex vivo slice preparations (Matsui et al., 2014). On the other hand, in experiments in which rats undergo long-term in vivo withdrawal from morphine (2 weeks) it has been shown that RMTg GABA neurons, unlike VTA DA neurons (Georges et al., 2006), do not show tolerance to the inhibitory effects of morphine. Interestingly, optogenetic excitation of RMTg GABA neurons inhibits VTA DA neurons similarly in drug-naïve and withdrawn rats, suggesting that the inhibitory influence of RMTg neurons on VTA DA neurons is preserved in morphine-withdrawn rats. By contrast, RMTg inhibition with a GABA-A receptor agonist, which increases DA activity in drug-naïve rats, loses its ability to do so in withdrawn rats. Changes in RMTg to VTA signaling may thus differ at different time points following the onset of withdrawal (Kaufling and Aston-Jones, 2015). The contributions of the RMTg to the motivational effects of opioid-withdrawal have not been investigated to our knowledge. While naloxone-precipitated withdrawal induces Fos activation in RMTg

neurons, RMTg lesions do not alter the physical signs of opioid withdrawal (Sánchez-Catalán et al., 2017). Future experiments may test the effect of RMTg lesions on the acquisition of conditioned place aversion to an environment paired with opioid withdrawal.

5. Towards a new model

Taken together, the data presented above make it clear that VTA or RMTg GABA-DA disinhibition alone does not account for all that is known about the rewarding effects of opioids. Below, we present a new model in which opioids inhibit RMTg and VTA GABA neurons each of which, in turn, reciprocally inhibit PPTg and LDTg cholinergic neurons. Now disinhibited, PPTg and LDTg cholinergic neurons can then activate VTA DA neurons via well-established mechanisms, with increased cholinergic signaling further modulating RMTg GABA neurons (Figure 4).

5.1. VTA opioids recruit ascending mesopontine inputs to the VTA

The PPTg and LDTg each receive afferent input from the VTA (Semba and Fibiger, 1992; Steiniger et al., 1992). Detailed anatomical studies on which VTA cell populations provide these afferents and which PPTg/LDTg cell populations are their targets are still lacking, but descending projections of VTA GABA neurons, which are inhibited by opioids (Johnson and North, 1992), to the PPTg have been suggested to be important for opioid reward in drug-naïve animals (Laviolette and van der Kooy, 2004; Laviolette et al., 2004). Whole-cell patch clamp recordings have shown that cholinergic neurons in the LDTg are inhibited by GABA application (Kohlmeier and Kristiansen, 2010). Substantia nigra pars reticulata GABA neurons, another source of GABAergic input to the PPTg, have been shown to directly inhibit PPTg cholinergic neurons (Takakusaki et al., 1997). Based on these findings we have previously suggested that one consequence of the inhibition of VTA GABA neurons by opioids is a decrease in their tonic inhibition of mesopontine cholinergic neurons, in turn increasing the well-established ascending excitatory input to mesolimbic and nigrostriatal DA pathways mainly via M5 mAChRs on VTA and SNc DA neurons (Steidl et al., 2011).

It is important to note that genetic knock-in tagging of opioid receptors with fluorescent markers (Erbs et al., 2015) shows the presence of μ -ORs (and delta opioid receptors) in LDTg and PPTg. Systemic opioids will, of course, act on these receptors as well. However, which LDTg and PPTg cell populations express μ -ORs is not known. Reverse dialysis of morphine into the LDTg reduces LDTg ACh levels (Mortazavi et al., 1999) suggesting that the direct effect of morphine in the LDTg inhibits cholinergic output (Lydic et al., 1993). However, the contributions of direct opioid effects in LDTg (and possibly PPTg) to the rewarding effects of opioids are expected to be relatively minor as they cannot explain the critical role of enhanced mesopontine cholinergic output to the VTA in opioid-induced DA release, locomotion, and reward. The indirect activation of VTA DA neurons through a mesopontine cholinergic relay provides an explanation for the following previously described experimental findings: (1) intra-VTA morphine does not enhance NAcc DA efflux either in M5 KO mice or in WT mice pretreated with VTA scopolamine (Steidl et al., 2011); (2) increases in NAcc or dorsal striatal DA efflux induced by systemic morphine are blocked by excitotoxic lesions of LDTg or PPTg, respectively, or by VTA or SNc scopolamine

pretreatment, respectively (Forster et al., 2002a; Miller et al., 2005; 2002); (3) morphine CPP is reduced in M5 KO mice or by cholinergic blockers in the VTA of rats (Basile et al., 2002; Rezayof et al., 2007); (4) intra-VTA atropine co-infusion completely blocks locomotion induced by intra-VTA morphine (Steidl et al., 2017a) and systemic morphine-induced locomotion is reduced by approximately half either in M5 KO mice or by VTA pretreatment with atropine in WT mice (Steidl and Yeomans, 2009); and (5) decreases in systemic morphine-induced locomotion in M5 KO mice are rescued by VTA viral transfection of missing M5 receptors, while overexpression of VTA M5 receptors increases systemic morphine-induced locomotion in WT mice (Wasserman et al., 2013).

5.2. RMTg opioids recruit ascending mesopontine inputs to the RMTg

RMTg neurons project to both the PPTg and LDTg (Jhou et al. 2009b). Which PPTg or LDTg cell populations (i.e., ACh, glutamate, or GABA) are the targets of RMTg outputs has not been determined. Injections of retrograde tracers into both VTA and the caudal PPTg in rats, result in double-labeling of many RMTg GABA neurons (Lavezzi et al., 2011). This suggests that RMTg GABA neurons may also tonically inhibit the caudal PPTg, where the highest density of cholinergic neurons are found, in addition to their established tonic inhibition of VTA DA neurons. The RMTg also receives projections from the PPTg and the LDTg (Yetnikoff et al., 2015) and, as recently shown, individual LDTg cholinergic neurons project to both the RMTg and the VTA. In addition, PPTg and LDTg cholinergic and GABAergic neurons each project to the RMTg (Wasserman et al., 2016). The bi-directional connectivity between the RMTg and the PPTg and LDTg suggests that, similar to the VTA, opioid inhibition of RMTg GABA neurons can result in the recruitment of the LDTg and PPTg which, in turn, results in increased cholinergic drive back to the RMTg and to the VTA. In the VTA this would lead to excitation of mesolimbic DA signaling as described above.

The effects of increased cholinergic feedback to the RMTg are less clear than those in the VTA and further experiments are needed to clarify our understanding. Broad-spectrum pharmacological blockade of RMTg mAChRs with atropine strongly enhances locomotion induced by intra-RMTg morphine suggesting that cholinergic feedback to RMTg that is recruited by RMTg morphine is inhibitory to morphine locomotion (Steidl et al., 2017a). Consistent with this, HSV expression of non-endogenous M5 mAChRs in RMTg GABA neurons, which effectively should enhance the effects of cholinergic feedback to the RMTg recruited by systemic morphine, decreases morphine-induced locomotion in WT mice (Wasserman et al., 2013). The results of these studies (systemic vs. intra-RMTg morphine) are not directly comparable of course and, importantly, neither the effects of RMTg atropine on systemic morphine-induced locomotion nor the effects of RMTg M5 overexpression on RMTg morphine-induced locomotion have been explicitly tested.

Within the RMTg, both M3 and M4 have been found within the vicinity of μ -OR labeled neurons and the proportion of M4 receptors is higher than that of M3 receptors (Wasserman et al., 2016). Whether M3 or M4 receptors in RMTg are associated with GABA neurons or with the axon terminals of RMTg afferents is less clear however. Analysis at the level of electron microscopy will be required for clarification. Furthermore, while M5 mAChRs are

not endogenously expressed in RMTg (Wasserman et al., 2013) whether other mAChR subtypes beyond M3 and M4 (e.g., M1 or M2) are found in RMTg is unknown. The presence of nicotinic acetylcholine receptors has not been demonstrated in RMTg either, but intra-RMTg nicotine excites RMTg GABA neurons and excitation is blocked by intra-RMTg mecamylamine treatment (Lecca et al., 2011).

Experiments testing the roles of RMTg M3 or M4 receptors in locomotion, or in any behavior for that matter, are limited. While RMTg atropine infusions, in contrast to VTA atropine infusions, elicit strong but short-lasting (~10 min) increases in locomotion in mice these data do not address the relative contributions of RMTg M3 and M4 receptors. However, RMTg infusions of the M3-selective antagonist 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP) induce strong locomotion lasting for approximately 2 hrs. Infusions of the M4-selective antagonist Tropicamide (1.3 µg) do not elicit locomotion at doses that are approximately equimolar to the 4-DAMP (~2 µg) and to atropine (3 µg) doses, each of which do. Furthermore, RMTg infusions of 10.3 µg Tropicamide also do not elicit significant increases in locomotion (Steidl et al., 2017a). In addition, while 4-DAMP and Tropicamide have higher affinities for the M3 and the M4 mAChRs over other mAChR subtypes, respectively, their selectivity is limited. Nonetheless, taken together, these data support a role for endogenous cholinergic input to the RMTg in the control of drug-free open-field exploratory behavior. These data also suggest an important role of M3 receptors in mediating these effects.

In the VTA, M3, but not M4, mAChRs are associated with GABAergic neurons (Michel et al., 2005; Tzavara et al., 2004). Conceivably, the effects of endogenous ACh release in the RMTg could, similar to VTA GABA neurons, excite RMTg GABA neurons leading to increased GABAergic inhibition of VTA DA neurons. RMTg infusions of 4-DAMP would then block the effect of endogenous cholinergic input to RMTg, resulting in increased inhibition of VTA DA neurons and thus increased DA-dependent locomotion. It is worth noting, however, that the lack of an M4 mAChR antagonist effect does not necessarily discount a role for the receptor. For example, while intra-RMTg infusions of the nAChR antagonist mecamylamine have no effect on RMTg GABA neuron activity, infusions of the nAChR agonist nicotine excite RMTg GABA neurons (Lecca et al., 2011). Similarly, while intra-RMTg infusions of the µ-OR antagonist naloxone have no effect on RMTg GABA neuron activity, infusions of the µ-OR agonist morphine inhibit RMTg GABA neurons (Jalabert et al., 2011; Lecca et al., 2011). Thus, the lack of an effect of RMTg Tropicamide on drug-free or on intra-RMTg morphine-induced locomotion does not rule out a potential role for RMTg M4 receptors in mediating the effects of opioid-driven enhanced cholinergic feedback to the RMTg. Future experiments will need to test whether locomotion induced by RMTg 4DAMP infusions (or by RMTg infusions of higher Tropicamide doses [>10.3 µg]) is in fact DA-dependent. It is also important to compare the described effects of M3- and M4-selective antagonist to those of agonists for the same receptors. Unfortunately, selective M3 or M4 agonists are currently not available. Available allosteric modulators of M3 and M4 receptors may provide a tool to further test their contributions in mediating the effects of endogenous cholinergic input to the RMTg on locomotion.

The relative contributions of RMTg M3 and M4 receptors to opioid-induced locomotion also require further clarification. If, as previously suggested, the higher numbers of M4 receptors in the vicinity of RMTg μ -OR labeled neurons are in fact associated with GABAergic cell bodies, then the consequence of increased cholinergic feedback to the RMTg would be additional inhibition of RMTg GABA neurons and thus a potentiation of locomotion – effectively a positive feedback loop. Furthermore, the concurrent effects of opioids in the VTA resulting from a systemic morphine injection would also result in increased cholinergic input to VTA and RMTg, further potentiating locomotion. However, co-infusions of the M4-selective antagonist Tropicamide (at doses of 1.3 μ g and 10.3 μ g) have no effect on RMTg morphine-induced locomotion while co-infusions of the M3-selective antagonist 4-DAMP approximately double RMTg morphine-induced locomotion. While interpretation of these data is subject to the same limitations as noted above (i.e., that the M4 receptor may be sufficient but not necessary to inhibit RMTg GABA neurons), they are consistent with the differential effects of RMTg 4-DAMP and Tropicamide on morphine-free locomotion and argue that the recruitment of PPTg/LDTg cholinergic feedback to the RMTg excites RMTg GABA neurons via M3 receptors to inhibit VTA DA neurons. This would effectively argue for a negative feedback loop. Importantly, the experiments conducted thus far attempting to distinguish between the contributions of RMTg M3 and M4 receptors using RMTg morphine infusions have not been extended to systemic morphine injections.

6. Future directions, limitations of our model, and conclusions

Opioid drugs have powerful euphoric effects. During the initial acquisition opioid drugs appear to recruit multiple brain pathways in parallel and this will contribute to the high risk for dependence and addiction resulting from continued use. Brain pathways recruited include the mesopontine LDTg and PPTg nuclei which lead to rapid DA activation via glutamatergic input to the VTA, and to sustained DA activation via cholinergic input to the VTA. Cholinergic input the RMTg will modulate RMTg GABAergic inputs to the VTA.

In the present model, opioid-mediated disinhibition of VTA DA neurons continues to influence rewarding and locomotor effects, but indirectly through LDTg and PPTg neurons. Notably, multiple experiments in rats and/or mice show that cholinergic signaling in the VTA, mediated mainly by M5 mAChR, is critical for the ability of systemic or intra-VTA opioids to increase forebrain DA extracellular levels or locomotion, and for the ability of systemic opioids to support the acquisition of CPP. The importance of opioid effects on GABA neurons in the caudally located RMTg, an area with particularly high levels of μ -ORs, is also not yet accounted for. Here, we present a model that attempts to reconcile these discrepancies by suggesting that another consequence of opioid inhibition of VTA or of RMTg GABA neurons is a reduction in GABAergic inhibition of LDTg and/or PPTg cell populations. In turn, this (1) enhances excitatory LDTg and/or PPTg inputs to the VTA, which excite mesolimbic DA signaling via well-established cholinergic and glutamatergic receptor mechanisms, and (2) enhances LDTg and/or PPTg inputs to the RMTg which further modulates opioid effects.

The present model makes several testable predictions that need to be addressed in future experiments. First and foremost, detailed anatomical studies are needed to outline the

connectivity between (1) VTA GABA neurons and LDTg/PPTg cell populations, and (2) RMTg GABA neurons and LDTg/PPTg cell populations. This may be accomplished using Cre-dependent transynaptic tracers (see for example Watabe-Uchida et al., 2012) in combination with immunofluorescence to, for example, investigate whether LDTg or PPTg cholinergic or glutamatergic neurons receive monosynaptic inputs from VTA or RMTg GABA neurons. Electrophysiological studies could further investigate the functional connectivity between identified cell populations in these areas. The present model predicts that opioid action in each of the VTA and the RMTg should facilitate mesopontine cholinergic inputs to each of the VTA and the RMTg. Accordingly, systemic opioid administration should result in measurable increases of VTA and/or RMTg ACh extracellular levels. Previously, cocaine-induced changes in VTA ACh levels (You et al., 2008) or morphine-induced changes in interpeduncular nucleus ACh levels (Taraschenko et al., 2007) have been characterized using microdialysis in rats. Comparisons between opioid-induced increases in the VTA and the RMTg using microdialysis would be very difficult in the mouse brain, but may be possible in the rat brain.

An important remaining question is the relative contribution of the two proposed cholinergic feedback loops (i.e. VTA – PPT/LDTg – VTA/RMTg and RMTg – PPTg/LDTg – RMTg/VTA) to overall locomotion when systemic morphine infusions are given. Conceivably, optogenetic inhibition of PPTg or LDTg cholinergic terminals in the VTA or in the RMTg could allow for a direct comparison of their contributions.

Finally, the present model is based primarily on locomotor and DA output measures. Whether the hypothesized contributions of mesopontine feedback to VTA and/or RMTg are equally important for opioid CPP and self-administration remains to be determined.

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Abbreviations

μ-OR	mu opioid receptor
ACh	acetylcholine
AChR	acetylcholine receptor
ChAT	choline acetyltransferase
CPP	conditioned place preference
DA	dopamine
DREADD	designer receptors exclusively activated by designer drugs
GABA	gamma-aminobutyric acid

GAD	glutamic acid decarboxylase
hM3D	muscarinic 3 designed receptor (DREADD)
hM4D	muscarinic 4 designed receptor (DREADD)
HSV	herpes simplex virus
KO	knockout
LDTg	laterodorsal tegmental nucleus
mAChR	muscarinic acetylcholine receptor
NAcc	nucleus accumbens
PPTg	pedunculo pontine tegmental nucleus
RMTg	rostromedial tegmental nucleus
SNc	substantia nigra pars compacta
VGluT2	vesicular glutamate transporter-2
VTa	ventral tegmental area
WT	wild-type

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Highlights

- Midbrain and pontine circuits are needed for opioid arousal and preference in rodents.
- Opioid use acquisition depends on midbrain mu opioid, and M5 cholinergic, receptors.
- Midbrain and pontine neurons and their links with dopamine neurons are reviewed.
- Our model links pontine ACh nuclei with critical RMTg and VTA GABA and DA neurons.
- Their possible roles in opioid use and addiction are discussed.

Figure 1. Increases in accumbal DA induced by LDTg electrical stimulation depend mainly on VTA muscarinic AChRs

(A) Chronoamperometric recordings depicting the time course of increases on NAcc DA efflux following LDTg electrical stimulation (arrow) in rats. Accumbal DA increases immediately following LDTg electrical stimulation (Phase 1) depend on nicotinic AChRs and ionotropic GLU receptors in the VTA. This is followed by decreases in DA (Phase 2) that depend on LDTg M2 and M4 muscarinic autoreceptors. DA levels then increase again and remain elevated for approximately 60 min (Phase 3). This late and prolonged phase in increased accumbal DA efflux is completely blocked by VTA infusions of the mAChR antagonist scopolamine (Figure adapted from Yeomans et al., 2001). (B) Chronoamperometric recordings depicting the time course of increases on NAcc DA efflux following LDTg electrical stimulation (arrow) in wild-type (top panel) and M5 knockout (bottom panel) mice. LDTg electrical stimulation in mice induces the same tri-phasic increases in accumbal DA efflux as in rats and systemic pretreatment with the muscarinic AChR antagonist scopolamine selectively blocks the late prolonged phase in increased accumbal DA efflux (top panel, red squares and blue triangles, respectively). Knockout mice lacking M5 receptors – the only mAChR subtype expressed by VTA DA neurons (Vilaró et al., 1990) – selectively abolishes the late prolonged phase in increased accumbal DA efflux (Figure adapted from Forster et al., 2002b).

Figure 2. Increases in accumbal DA induced by intravenous morphine are attenuated by excitotoxic lesions of the LDTg (A) or by VTA pretreatment with the muscarinic AChR antagonist scopolamine (B)

(A) Chronoamperometric recordings depicting the time course of increases in NAcc DA efflux induced by intravenous morphine (2 mg/kg) administration (arrow) in LDT-lesioned (LDT-X; red triangles) and sham control (blue squares) rats. In sham control rats, systemic injection of morphine induces long-lasting increases in accumbal DA. In LDTg-lesioned rats these increases are markedly attenuated. Points represent the mean changes in the chronoamperometric responses and solid lines the SEM. Red and blue dashed lines correspond, respectively, to the effects of pedunculopontine tegmental nucleus and sham lesions on morphine-evoked increases in striatal DA efflux (Figure adapted from Forster et al., 2002a). (B) Chronoamperometric recordings depicting time course of increases in NAcc DA efflux induced by intravenous morphine (2 mg/kg) administration following VTA pretreatment with scopolamine (SCO; 200 μ g) or vehicle (PBS). Systemic injection of morphine induces long-lasting increases in accumbal DA release (blue dashed line). VTA muscarinic AChR blockade strongly reduces these increases (red circles). Arrows indicate termination of intra-VTA infusion and commencement of i.v. injection, red circles represent mean changes in the chronoamperometric responses with respect to baseline (0%), and solid black lines the SEM. (Figure adapted from Miller et al., 2005).

Figure 3. Opposite effects of virally-mediated upregulation of M5 AChRs in VTA and RMTg (A) VTA sites where virally-mediated upregulation of M5 receptors increases morphine-induced locomotion in wild-type mice are shown with green spots. RMTg sites where virally-mediated upregulation of M5 receptors decreased morphine-induced locomotion are shown with red spots. Sites at which control viral vectors were infused are shown by blue spots (VTA) and orange spots (RMTg) (Franklin and Paxinos, 2007). (B) Virally-mediated upregulation of M5 receptors in VTA (left; green bars) significantly increased total locomotion at two doses of morphine relative to VTA control viral vectors (blue bars). No differences were observed for saline locomotion. By contrast, virally-mediated upregulation of M5 receptors in RMTg (right; red bars) significantly reduced total locomotion at the 30 mg/kg morphine dose relative to RMTg control vectors (orange bars). No differences were observed for saline locomotion (Figures adapted from Wasserman et al., 2013).

Figure 4. Model of midbrain and pontine control of morphine-induced locomotion

RMTg GABA neurons tonically inhibit VTA DA neurons and LDTg/PPTg ACh neurons. VTA GABA neurons also tonically inhibit VTA DA neurons and LDTg/PPTg ACh neurons. Opioids inhibit RMTg and VTA GABA neurons through agonism of the μ -opioid receptor, leading to disinhibition of VTA DA and LDTg/PPTg ACh neurons. The now disinhibited LDTg and PPTg ACh neurons increase ACh efflux to the VTA and the RMTg. ACh efflux in the VTA leads to activation of M5 muscarinic receptors on DA neurons, resulting in increased NAcc DA efflux and DA-mediated locomotion. ACh efflux in the RMTg further modulates opioid locomotion via M3 and M4 muscarinic ACh receptors. Plus signs (+) indicate excitatory signals, minus signs (–) indicate inhibitory signals. μ , μ -opioid receptor; GLU, glutamate; NAcc, nucleus accumbens.