Opiate-Induced Molecular and Cellular Plasticity of Ventral Tegmental Area and Locus Coeruleus Catecholamine Neurons

Michelle S. Mazei-Robison and Eric J. Nestler

Fishberg Department of Neuroscience and Friedman Brain Institute, Mount Sinai School of Medicine, New York, New York 10029

Correspondence: eric.nestler@mssm.edu

The study of neuronal adaptations induced by opiate drugs is particularly relevant today given their widespread prescription and nonprescription use. Although much is known about the acute actions of such drugs on the nervous system, a great deal of work remains to fully understand their chronic effects. Here, we focus on longer-lasting adaptations that occur in two catecholaminergic brain regions that mediate distinct behavioral actions of opiates: ventral tegmental area (VTA) dopaminergic neurons, important for drug reward, and locus coeruleus (LC) noradrenergic neurons, important for physical dependence and withdrawal. We focus on changes in cellular, synaptic, and structural plasticity in these brain regions that contribute to opiate dependence and addiction. Understanding the molecular determinants of this opiate-induced plasticity will be critical for the development of better treatments for opiate addiction and perhaps safer opiate drugs for medicinal use.

Because of their potent analgesic properties, opiate drugs have been used for centuries. Opiates include compounds derived from the opium poppy such as morphine and codeine, as well as many synthetic derivatives such as heroin, oxycodone, and hydrocodone. For the purposes of this review, we focus on the actions of morphine and heroin, as these have been the most studied in model systems. Despite effectiveness in treating acute pain, there are serious complications with long-term opiate use, including tolerance, physical dependence, and addiction (Ballantyne and LaForge 2007). Abuse of prescription drugs, and specifically pain-relieving opiates, has increased greatly in recent years

in both the adult and adolescent U.S. populations (Compton and Volkow 2006; Manchikanti et al. 2010). The medical use of opiates has also risen steadily as treatment for chronic pain disorders has become more aggressive (Kuehn 2007). Although the ethics of chronic pain treatment and the potential over or under use of opiate drugs can be debated (Fields 2011), there is no question that chronic opiate use causes neuroadaptations that lead to undesirable effects.

Physical dependence and addiction to opiates were once considered closely linked; however, these processes are now believed to be mediated by distinct mechanisms and circuits within the brain (Koob and Le Moal 2001). Physical

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dependence is manifested as negative physical symptoms (e.g., sweating, abdominal pain, diarrhea) when the drug is withdrawn. Addiction, or “substance dependence” as defined by the Diagnostic and Statistical Manual of Mental Disorders, has a profound long-term impact on health and productivity and is characterized by the compulsion to seek and take drug despite negative consequences. Part, but not all, of this addiction phenotype likely reflects “psychological dependence,” that is, negative emotional symptoms that occur during drug withdrawal.

In this review, we discuss what is known about the neuroadaptations, or opiate-induced plasticity, that occur in two brain regions rich in catecholamine neurons, that play critical roles in opiate addiction and physical dependence, respectively: dopaminergic neurons within the midbrain ventral tegmental area (VTA) and noradrenergic neurons within the pontine locus coeruleus (LC). This discussion focuses on three types of opiate-induced plasticity in these regions: synaptic plasticity—persistent changes in glutamatergic and GABAergic synaptic transmission (Dacher and Nugent 2011b; Luscher and Malenka 2011); cellular plasticity—homeostatic changes in intracellular signaling cascades (Williams et al. 2001; Nestler 1992, 2004); and structural plasticity—long-lasting changes in neuronal morphology (Russo et al. 2010). Identifying the molecular determinants of these three types of plasticity in the brain’s catecholaminergic neurons serves as a model of the plasticity induced in other important neural substrates of addiction and will be key to developing better therapies for opiate addiction and possibly safer opiate drugs for analgesia.

VENTRAL TEGMENTAL AREA

Background

The VTA has been widely studied in drug abuse given its fundamental role in reward. Dopamine (DA) neurons in VTA project to multiple brain regions including the nucleus accumbens (NAc), where increased DA release has been noted in response to every class of abused drug (Di Chiara and Imperato 1988). However, while DA neu-

rons are a prominent portion (~60%–65%) of this midbrain nucleus, there is considerable cellular diversity, with a significant portion of GABA neurons (30%–35%) as well as descriptions of glutamatergic neurons (2%–3%) (Swanson 1982; Nair-Roberts et al. 2008; Sesack and Grace 2010). The DA and GABA neurons within the ventral midbrain, in general, project topographically (medial to lateral) with the main output structures consisting of NAc, prefrontal cortex (PFC), and amygdala (AMY) (extensively reviewed in Sesack and Grace 2010) (Fig. 1). The primary afferents to VTA include excitatory inputs from PFC, pedunculopontine and laterodorsal tegmentum (PPTg and LDT), as well as many other recently defined structures (Geisler et al. 2007). The inhibitory input to the VTA is less well defined, but inputs from NAc, ventral pallidum, and mesopontine rostromedial tegmental nucleus (RMTg) have been reported (Sesack and Grace 2010). Research to date has focused disproportionately on DA neurons in VTA, and specifically those that project to NAc, because of the critical role of this projection in reward (Nestler 2004).

Acute Opiate-Induced Changes in Neuronal Activity

Given the ability of acute morphine into the VTA to elicit increased DA release in the NAc (Leone et al. 1991), a substantial amount of work has examined the acute effects of opiates in the VTA. Acute morphine increases the firing rate of DA neurons in VTA (Gysling and Wang 1983). This effect is mediated at least in part by the binding of morphine to the $G_{i/o}$ -coupled μ -opioid receptor (MOR) on local GABA neurons, thereby decreasing their activity and subsequent GABA release on DA neurons and resulting in disinhibition of DA neurons (Johnson and North 1992). However, interpretation of much of the early electrophysiology work is complicated by evidence highlighting the near indistinguishable nature of VTA DA and GABA neurons (by size, morphology, and electrophysiological properties) (Margolis et al. 2006), clarifying the need to identify VTA neurons studied more definitively (e.g., by immunohistochemistry, use of

Figure 1. Cartoon of a sagittal section of rodent brain illustrating the VTA and LC and their major afferent and efferent projections. DAergic (red) and GABAergic (blue) neurons in VTA project to limbic and cortical structures and receive glutamatergic (black-dash, PFC) and GABAergic input (blue-dash, NAc, VP). Noradrenergic neurons (green) in LC innervate multiple regions including HIPP and PFC and receive glutamatergic input from PGI. Abbreviations: AMY, amygdala; HIPP, hippocampus; LC, locus coeruleus; NAc, nucleus accumbens; PFC, prefrontal cortex; PGI, nucleus paragigantocellularis; VP, ventral pallidum; VTA, ventral tegmental area.

GFP reporter mice, etc.), a point that will be discussed in detail later in this review. Here, we mainly focus on opiates that act as agonists at the MOR in VTA, such as morphine, as these drugs produce the rewarding effects most often studied in the drug abuse field. However, it is known that κ -opioid receptors (KOR) are also expressed on VTA DA neurons, and that activation of these receptors can directly inhibit the firing rate of DA neurons (Margolis et al. 2003), likely contributing to the aversive effects of kappa agonists. The ability of opiates to produce both VTA DA neuron activation and inhibition, and rewarding and aversive effects, is intriguing, and this “yin-yang” modulation and the role of endogenous opioid peptides in reward deserves to be a focus of future study.

Acute Opiate-Induced Synaptic Plasticity

In addition to changes in neuronal activity, there are many reports of synaptic plasticity induced by acute opiates. As with cocaine and other abused drugs, a single injection of morphine was found to increase the ratio of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) to *N*-methyl-D-aspartic acid (NMDA) excitatory postsynaptic currents (EPSCs) 24

hours after administration, consistent with long-term potentiation (LTP) of glutamatergic synapses onto DA neurons (Saal et al. 2003). Recently, it has also been reported that acute morphine induces AMPAR receptor (AMPA) redistribution in VTA in a manner similar to cocaine, specifically an insertion of GluA2-lacking AMPARs (Brown et al. 2010). Brown et al. observed an increased rectification index and increased cytoplasmic GluA2 AMPAR in response to acute morphine, an effect that is recapitulated by direct stimulation of DA neurons in VTA using selective channelrhodopsin 2 expression (Brown et al. 2010), directly implicating DA activity/signaling within VTA to glutamatergic regulation. These data are consistent with earlier work that GluA1, but not GluA2, overexpression in VTA sensitizes animals to morphine’s locomotor-activating and rewarding behaviors (Carlezon et al. 1997).

Acute opiates also influence plasticity at GABAergic synapses in VTA. High-frequency stimulation has been found to elicit LTP at GABA terminals (LTP_{GABA}) on VTA DA neurons, an effect that is dependent on activation of postsynaptic NMDA receptors (NMDAR) and release of nitric oxide (NO) as a retrograde messenger from DA neurons (Nugent et al. 2007).

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NO then increases guanylyl cyclase (GC) activity in the GABA neuron, leading to increased GABA release and LTP_{GABA}. A single dose of morphine inhibits LTP_{GABA} by interrupting the NO–GC–protein kinase G (PKG) signal cascade, causing a loss of normal inhibitory control (observed 2 and 24 hours following injection, but not 5 days) (Nugent et al. 2007, 2009; Niehaus et al. 2010). Thus, disruption of LTP_{GABA} provides another mechanism for the ability of acute opiates to increase VTA DA neuronal activity.

More recently, another form of VTA GABAergic plasticity has been described: long-term depression of GABAergic synapses onto DA neurons (LTD_{GABA}) (Dacher and Nugent 2011a). Using low-frequency stimulation (LFS), a stable LTD_{GABA} in DA cells was induced that, in contrast to LTP_{GABA}, was expressed postsynaptically and did not depend on NMDAR. This effect was also not dependent on endocannabinoid signaling, but was blocked by the dopamine D2 receptor (D2R) antagonist sulpiride. Interestingly, a single morphine injection was sufficient to prevent LFS-induced LTD_{GABA} 24 hours after administration, suggesting that morphine can bidirectionally regulate GABA plasticity in VTA (Dacher and Nugent 2011a).

Chronic Opiate-Induced Synaptic Plasticity

Although the synaptic changes that occur with acute opiates have been relatively well characterized, the chronic changes have not. To date, few if any studies have examined changes in either glutamatergic or GABAergic plasticity in response to chronic opiate administration. This includes lack of knowledge as to whether there are differences in passive vs. active drug administration, an important consideration given the recent work showing that the persistence of LTP in the VTA of animals abstinent from cocaine self-administration (up to 3 months) occurs only with contingent cocaine exposure (Chen et al. 2008).

However, it is known that chronic morphine, like acute morphine, increases DA neuronal activity. In vivo recordings following chronic morphine show increases in both basal firing

rate and burst activity that return to baseline during withdrawal (Georges et al. 2006). This is in contrast to previous work that observed a persistent decrease in DA activity in morphine-withdrawn rats (Diana et al. 1995, 1999). One potential reason for these differences is the administration method used. For example, the Georges et al. study used a subcutaneous (s.c.) sustained release pellet paradigm, which has been shown to have a much different pharmacodynamic profile than the chronic escalating dose paradigm used in the earlier Diana et al. studies. As previously reported (Fischer et al. 2008), 24 hr after the last morphine pellet, blood morphine levels are not decreased, remaining relatively stable with the peak (~3000 ng/ml), while the chronic injection model produces a much higher peak (~10,000 ng/ml) at 1 hr, with blood levels below 100 ng/ml after 4 hr and negligible by 12 hr. The change in DA firing rate induced by withdrawal from chronic morphine, whether a return to baseline or decrease below baseline, appears to be dependent on changes in GABA release. Withdrawal from chronic morphine increases GABA inhibitory postsynaptic currents (IPSCs) and GABA release onto VTA DA neurons (Bonci and Williams 1997), an effect that has recently been found to be dependent on recycling of the MOR and on cyclic adenosine-5'-monophosphate (cAMP) signaling (Madhavan et al. 2010).

Another potential contributor to differences between studies is the heterogeneity of VTA compared to LC (as described below). Not only is there the complexity of multiple cell types (primarily GABA vs. DA), but the distribution of cell types also varies along the rostral-caudal VTA axis (Fig. 2). Specifically, the proportion of DA to GABA neurons is much higher in rostral VTA subregions (IFN, RL) compared to caudal subregions (PN, PIF) (Nair-Roberts et al. 2008). This difference has functional relevance to morphine-induced behavioral changes. HSV-GluA1 overexpression increased morphine reward behavior with injection into rostral VTA, whereas it induced aversive behavior in caudal VTA, an effect also observed on viral overexpression of cAMP-response-element binding protein (CREB) or phospholipase

Figure 2. Cellular and projection complexity within VTA. The proportion of DA (red) to GABA (blue) neurons varies among VTA subnuclei with higher DA:GABA ratios observed in more rostral subregions such as rostral linear nucleus (RL) compared to more caudal subnuclei such as paranigral (PN) and parainterfascicular (PIF) regions. Additionally, DA neuronal projections differ throughout with VTA with more lateral regions such as parabrachial nucleus (PBP) projecting to NAc lateral shell (Lat Sh), whereas medial regions such as PN have diverse projections including amygdala (AMY), prefrontal cortex (PFC), NAc core, and NAc medial shell (Med Sh). Limited work has examined GABA neuronal projections; there is some evidence that GABA neurons in rostral PBP have a strong projection to PFC, whereas there are few rostral PBP DA neurons that project to PFC, but a large caudal DA PBP projection; this suggests that the PBP-PFC projection is not only defined regionally, but is also neuronal-subtype specific (Lammel et al. 2008). (Cell counts used are from Nair-Roberts et al. 2008 and projections are from retrograde labeling studies by Lammel et al. 2008.)

C gamma (PLC γ) (Carlezon et al. 2000; Bolanos et al. 2003; Olson et al. 2005). This difference can be seen at the molecular level as well, as chronic morphine induced cAMP response element (CRE)-mediated transcription in DA neurons in rostral and caudal VTA, but was only observed in non-DA neurons in rostral VTA (Olson et al. 2005). Ultrastructural studies confirm such rostral-caudal differences, and suggest the added complexity of treatment regimen and projection output. GluA1 was increased in both tyrosine hydroxylase (TH)-positive (DAergic) and TH-negative (likely GABAergic) den-

drites in the parabrachial (PBP) VTA with a single morphine injection. In contrast, with chronic morphine, there was an increase in GluA1 in the paranigral (PN) VTA in addition to the PBP region (Lane et al. 2008).

Differences among VTA DA neurons, based on their output region, have been of great interest recently, as it is now well established that the electrophysiological properties of DA neurons vary by projection. VTA DA neurons projecting to NAc have a much smaller I_h current than neurons projecting to basolateral amygdala (BLA) (Ford et al. 2006), and there are differences in

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projections within NAc itself, with DA neurons projecting to NAc lateral shell displaying much higher I_h current than DA neurons that project to NAc medial shell (Lammel et al. 2011). Action potential (AP) duration of DA neurons also varies by projection, as NAc-projecting DA neurons have the longest AP duration, while PFC-projecting neuron AP duration is shorter, and AMY-projecting DA neurons have the shortest duration (Margolis et al. 2008). Importantly, responsiveness to opiates also appears to differ within the VTA depending on projection type: DA neurons projecting to NAc responded more to KOR agonists than BLA-projecting neurons, whereas the opposite effect was noted for responsiveness to a MOR/delta (DOR) agonist, which had a greater effect on BLA-projecting neurons (Ford et al. 2006). This translated to presynaptically-mediated opiate effects as well, as a KOR agonist caused a greater inhibition of GABA_A IPSCs of DA neurons projecting to BLA, while there was a greater KOR agonist-mediated inhibition of GABA_B IPSCs in neurons projecting to NAc (Ford et al. 2006). Additionally, it has recently been observed that modulation of excitatory synapses on DA neurons differs depending on projection (Lammel et al. 2011). Lammel and colleagues (2011) found that AMPA/NMDA ratio was increased by cocaine in DA neurons that projected to NAc, but not in DA neurons that projected to PFC. However, AMPA/NMDA ratio was increased in DA cells projecting to PFC in response to an aversive stimulus (hind-paw formalin injection), an effect that was also observed in DA neurons that projected to NAc lateral shell, but absent in DA neurons projecting to NAc medial shell—showing heterogeneity in response within subregions of this projection target (Lammel et al. 2011). Clearly these studies indicate that a more thorough understanding of the synaptic adaptations that occur with both acute and chronic opiates will need to integrate information on the output of the DA neurons studied. The development of neuron- and projection- specific techniques will serve to clarify these issues, by allowing specific modulation in this heterogeneous region.

Opiate-Induced Structural and Cellular Plasticity

The relevance of drug-induced structural plasticity to synaptic and behavioral changes has been reviewed recently (Russo et al. 2010). Most studies of structural plasticity to date have examined changes in spine morphology or dendritic branching of neurons in VTA target regions, but our laboratory has investigated another structural adaptation in response to chronic opiate administration, an alteration of VTA DA neuron soma size. We first observed that rat VTA DA neuron surface area decreases ~25% in response to chronic, but not acute, morphine administration (Sklair-Tavron et al. 1996). This effect was specific for DA neurons in VTA, as TH-negative cells (likely GABAergic) were not altered. Additionally, this change could be blocked by systemic naltrexone, suggesting that MOR signaling was required, and local brain-derived neurotrophic factor (BDNF) infusion in VTA also prevented the decrease, suggesting that decreased neurotrophic signaling may underlie the morphological change. Importantly, this reduction in VTA DA neuron soma size is observed with chronic administration of heroin as well as morphine (Russo et al. 2007), in passive and self-administration protocols (Spiga et al. 2003; Chu et al. 2007; Russo et al. 2007), and across species, as we have recently characterized this effect in mouse and in postmortem tissue from human heroin abusers (Mazei-Robison et al. 2011). Follow-up studies found no evidence of VTA DA neuronal death or injury (Sklair-Tavron et al. 1996; Russo et al. 2007) and that the decrease in cell size persists for 14 days after chronic morphine administration, but returns to baseline by 30 days. This time-line mirrors reward tolerance (Russo et al. 2007), in which repeated drug use decreases the rewarding effect of the drug and leads to an escalation of drug intake, as seen in humans (O'Brien 2001).

Given that BDNF could rescue the chronic morphine-induced structural change, we wanted to examine whether downstream neurotrophic signaling pathways mediated this structural plasticity. Although there is some controversy as to whether BDNF levels themselves are altered

in VTA in response to chronic opiate administration (Numan et al. 1998; Chu et al. 2007; Koo et al. 2010), regulation has been reported in the three main signaling pathways downstream from BDNF: PLC γ , phosphatidylinositol 3'-kinase (PI3K), and mitogen-activated protein kinase (MAPK) (Russo et al. 2009). Chronic morphine increases activity of the PLC γ pathway (Wolf et al. 1999, 2007), decreases activity of the PI3K pathway, as measured by decreased insulin receptor substrate-2 (IRS2) and phospho-AKT levels (Wolf et al. 1999; Russo et al. 2007; Mazei-Robison et al. 2011), and increases MAPK signaling, as measured by increased phosphorylation and catalytic activity of extracellular-related protein kinase (ERK) (Ortiz et al. 1995; Berhow et al. 1996; Liu et al. 2007). Using viral-mediated overexpression, we found that it was the chronic morphine-induced change in PI3K signaling that contributes to the morphological change: overexpression of a dominant-negative IRS2 (IRS2dn) or AKTdn was sufficient to decrease VTA DA soma size, while overexpression of wild-type IRS2 prevented the morphine-induced decrease and overexpression of a constitutively active AKT (AKTca) increased soma size (Russo et al. 2007; Mazei-Robison et al. 2011). In contrast, overexpression of either PLC γ or ERK was not sufficient to alter VTA DA soma size (Russo et al. 2007). Importantly, overexpression of IRS2 was also able to prevent morphine reward tolerance, implicating a role for structural plasticity in behavioral response.

Our recent work suggests that this structural change may be intimately linked to the activity changes induced by chronic opiates. Similar to the *in vivo* study by Georges et al. discussed above, we found that VTA DA firing rate was increased at the same time-point at which soma size is decreased in mice exposed to chronic morphine (Mazei-Robison et al. 2011). However, we found that DA output to the NAc, as measured by *in vivo* cyclic voltammetry, is actually decreased, suggesting a break in the normal activation and output in the mesolimbic reward circuit. We further characterized this result and found that IRS2dn overexpression in VTA, which is sufficient to decrease DA soma size, decreased DA output to NAc and also

decreased the expression of several K⁺ channel subunits, in a manner similar to chronic morphine. In our efforts to identify the signaling pathways downstream from IRS2/AKT that mediate the chronic morphine-induced neuroadaptations, we made the surprising observation that mammalian target of rapamycin (mTOR) complex 1 (mTORC1) signaling, a well-established pathway in cellular growth, was actually increased by chronic morphine. In contrast, we observed a decrease in mTOR complex 2 (mTORC2) signaling, which we went on to show is both necessary and sufficient for morphine-induced changes in soma size and neuronal activity. Specifically, we found that overexpression of rapamycin-insensitive companion of mTOR (Rictor), an essential component protein of mTORC2, was sufficient to prevent the decrease in soma size and also prevented the increase in DA neuron firing rate in a cell-autonomous way: only DA cells in VTA that overexpressed Rictor had an attenuated firing rate, whereas nearby DA cells still showed the increase. This suggests that signaling changes intrinsic to DA neurons can mediate excitability changes induced by chronic opiates, possibly by altering AKT modulation of GABA_A currents (Krishnan et al. 2008) or the expression of K⁺ channels (Mazei-Robison et al. 2011) (Fig. 3). As with IRS2 overexpression, we found that alteration of mTORC2 activity correlated with morphine reward behavior, as decreasing mTORC2 activity decreased morphine conditioned place preference (CPP), while increasing mTORC2 activity was sufficient to induce CPP to a low dose of morphine that does not induce place conditioning in control animals.

It is unlikely that soma size change is the only structural adaptation induced by chronic opiates in the VTA. Given the decreased dendritic spine number and dendritic complexity of branching of NAc medium spiny neurons of rats previously exposed to chronic morphine (Robinson and Kolb 1999; Robinson et al. 2002), we expect that dendritic changes are also occurring in VTA DA neurons. Current studies are underway to characterize spine morphology changes, a huge gap in the field, as only one study to date has examined drug-induced changes in VTA dendritic

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Figure 3. Chronic morphine decreases VTA DA soma size yet increases neuronal excitability, while DA transmission to NAc is decreased. The net effect of morphine is a less responsive reward pathway, i.e., reward tolerance. Down-regulation of IRS2-AKT signaling (blue) in VTA mediates the effects of chronic morphine on soma size and electrical excitability; the effect on excitability is mediated via decreased GABA_A currents and suppression of K⁺ channel expression. Morphine-induced down-regulation of mTORC2 activity in VTA is crucial for these morphine-induced morphological and physiological adaptations as well as for reward tolerance. In contrast to mTORC2, chronic morphine increases mTORC1 activity (red), which does not appear to directly influence these morphine-induced adaptations. Chronic morphine also decreases DA output to NAc, as well as decreasing dendritic branching and the number of dendritic spines on medium spiny GABA neurons in NAc, further suppressing normal DA signaling in the mesolimbic circuit.

architecture. This study found an increase in dendritic spine density in one subtype of VTA neuron in response to an acute cocaine injection, the same subtype shown to show increased NMDA/AMPA ratio (Sarti et al. 2007). Data from our previous work, that length of VTA DA processes is decreased (~30%) in rats treated with chronic morphine (Sklair-Tavron et al. 1996), is consistent with global changes in VTA DA architecture. This change could also help to explain the decrease in DA output to the NAc after chronic morphine, as we have previously reported decreased axonal transport and levels of neurofilament proteins in VTA (Beitner-Johnson et al. 1992, 1993), suggesting that

chronic morphine also affects axonal structure and function. Given the regional and projection complexity in VTA DA neurons noted above, we are currently examining whether these structural changes are induced in a particular subset of VTA DA neurons using fluorescent retrograde tracers. These data will be critical to understanding the structural and electrophysiological changes induced by chronic opiates and the relevant output circuits involved.

As alluded to earlier, several studies, both molecular and electrophysiological, have provided evidence that chronic opiate administration activates the cAMP-CREB pathway in the VTA

(Bonci and Williams 1997; Olson et al. 2005; Madhavan et al. 2010). Also, a microarray study defined the global changes in gene expression that occur in VTA in response to chronic morphine (McClung et al. 2005). Work is now needed to better define the cellular specificity of these neuroadaptations as well as delineate their functional consequences. Moreover, while most work on VTA has focused on opiate-induced neuroadaptations presumed to occur in DA neurons, it is essential to explore drug-induced plasticity that occurs in the VTA's GABAergic neurons, which are one of the key initial targets of opiate action in this brain region.

LOCUS COERULEUS

Background

The LC is the main site of norepinephrine (NE)-containing neurons in the brain (Dahlstrom and Fuxe 1965). As reviewed previously (Aston-Jones and Bloom 1981a; Aston-Jones et al. 1991b; Berridge and Waterhouse 2003; Van Bockstaele et al. 2010), LC is a discrete, compact, homogeneous nucleus, consisting of almost exclusively NE neurons. The major inputs to LC are from the medullary nucleus paragigantocellularis (PGi) and nucleus prepositus hypoglossus, and LC outputs are widespread including forebrain, cerebellum, brainstem, and spinal cord (Fig. 1) (Berridge and Waterhouse 2003). LC neuronal activity is highly synchronous both basally and in response to stimuli (Foote et al. 1980; Aston-Jones and Bloom 1981b; Aston-Jones et al. 1991a; Ishimatsu and Williams 1996). LC neurons are spontaneously active (Williams et al. 1991) and their activation elicits NE release in several forebrain regions including cortex and hippocampus. The LC largely serves as a relay nucleus, with limited synaptic plasticity noted to date, although glutamate afferents control LC activity, notably from PGi (Ennis et al. 1992). LC neurons express the three main classes of opioid receptors: MOR, DOR, and KOR with distinct distribution, although, as with the VTA, our discussion is limited to the MOR, which is most directly implicated in opiate dependence and addiction.

Opiate-Induced Cellular Plasticity

Although there is no evidence of traditional synaptic plasticity (i.e., LTP and LTD) in LC, there is well-described cellular plasticity. A unique feature of LC is that many of its *in vivo* responses to chronic opiates can be recapitulated and studied at the single-cell level (Nestler et al. 1994; Nestler and Aghajanian 1997; Nestler 2004). Binding of opiates (e.g., morphine) to the MOR leads to decreased adenylyl cyclase (AC) activity and cAMP signaling (Duman et al. 1988). Acute binding of opiates to the MOR also decreases the pacemaker activity of LC neurons, largely by activating G protein-gated inwardly-rectifying K^+ (GIRK) channels (Williams et al. 1982; Torrecilla et al. 2002). However, with chronic opiate administration, both the firing rate and cAMP signaling return to baseline because of an up-regulation of the cAMP pathway, illustrating tolerance (Aghajanian 1978; Duman et al. 1988; Nestler and Tallman 1988; Guitart and Nestler 1989; Kogan et al. 1992; Ivanov and Aston-Jones 2001). This plasticity induced by chronic opiate administration (i.e., cAMP pathway up-regulation) becomes functionally evident on withdrawal of the opiate, when the firing rate of LC neurons is significantly increased along with a large increase in cAMP activity, illustrating dependence and withdrawal (Fig. 4) (Aghajanian 1978; Rasmussen et al. 1990).

These adaptations are mediated via the up-regulation of several signaling proteins in the cAMP pathway including AC1/8 (Matsuoka et al. 1994; Lane-Ladd et al. 1997; Zachariou et al. 2008), cAMP-dependent protein kinase (PKA) (Nestler and Tallman 1988), CREB (Guitart et al. 1992; Shaw-Lutchman et al. 2002; Han et al. 2006), and TH and BDNF—both downstream CREB targets (Guitart et al. 1989; Akbarian et al. 2002). Chronic opiates also induce GIRK2/3 expression in LC (Cruz et al. 2008) as well as numerous other genes as revealed by microarray analysis (McClung et al. 2005). Furthermore, it has recently been shown, using an LC slice culture model, that the increased intrinsic electrical activity of LC neurons induced by chronic opiates is caused by the direct activation of MOR on LC NE neurons, implicating an

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Figure 4. Up-regulation of the cAMP pathway in LC as a mechanism of opiate tolerance and dependence. *Top* panel, Opiates acutely inhibit the functional activity of the cAMP pathway (indicated by cellular levels of cAMP and cAMP-dependent protein phosphorylation). With continued opiate exposure, functional activity of the cAMP pathway gradually recovers, and increases far above control levels following removal of the opiate (e.g., by administration of the opioid receptor antagonist naloxone). These changes in the functional state of the cAMP pathway are mediated via induction of adenylyl cyclases (AC) and protein kinase A (PKA) in response to chronic administration of opiates. Induction of these enzymes accounts for the gradual recovery in the functional activity of the cAMP pathway that occurs during chronic opiate exposure (tolerance and dependence) and activation of the cAMP pathway observed on removal of opiate (withdrawal). *Bottom* panel, Opiates acutely inhibit LC neurons by increasing the conductance of an inwardly rectifying K^+ channel via coupling with subtypes of $G_{i/o}$ and, possibly, by decreasing a Na^+ -dependent inward current via coupling with $G_{i/o}$ and the consequent inhibition of AC, reduced levels of PKA activity, and reduced phosphorylation of the channel or pump responsible. Inhibition of the cAMP pathway also decreases the phosphorylation of many other proteins and, thereby, affects numerous other neuronal processes. For example, it reduces the phosphorylation state of cAMP response element-binding protein (CREB), which initiates some of the longer-term changes in LC function. Chronic administration of morphine increases the levels of ACI, ACVIII, PKA catalytic (cat.) and regulatory subunits, and several phosphoproteins, including CREB and tyrosine hydroxylase (TH) (indicated by red arrows). (Legend continues on facing page.)

intrinsic homeostatic adaptation (Cao et al. 2010). This approach identified a crucial role for CREB in both the pacemaker activity and morphine-induced increase in LC firing rate (Han et al. 2006; Cao et al. 2010), an effect that was also observed in mice with an early developmental knockout of CREB specific to NE neurons (Parlato et al. 2010). Finally, this activation of LC neuronal firing, and the up-regulated cAMP-CREB pathway, which mediates the increased firing, have been shown in numerous studies to be both necessary and sufficient to mediate several symptoms of physical opiate withdrawal (Lane-Ladd et al. 1997; Punch et al. 1997; Han et al. 2006).

Although most of the opiate-induced plasticity described here is postulated to be intrinsic to LC NE neurons, there is some evidence that chronic morphine can also influence excitatory input to LC as there is an increase in spontaneous EPSC frequency in slices from morphine-treated mice (Torrecilla et al. 2008). Additionally, there is an increase in glutamate and aspartate release in LC in vivo in morphine-withdrawn rats and local application of excitatory amino acid antagonists in LC partially blocks the withdrawal-induced increase in LC activity (Akaoka and Aston-Jones 1991; Aghajanian et al. 1994).

Some controversy remains as to whether the changes in cAMP-CREB signaling in LC neurons and in LC neuronal activity mediate opiate withdrawal behaviors. For example, lesions of LC, or developmental knockout of CREB activity in LC NE neurons, fail to detectably alter withdrawal symptoms (Christie et al. 1997; Parlato et al. 2010). In contrast, we have shown that modulation of the activity of the cAMP pathway or of CREB in LC of adult animals consistently blocks several withdrawal behaviors (Lane-Ladd et al. 1997; Punch et al. 1997; Han et al. 2006).

We believe that several key considerations explain these differing findings. First, LC is just one of several brain areas important for physical opiate dependence and withdrawal (Koob and Le Moal 2001). It is not surprising that animals with lesioned LCs still develop profound physical dependence mediated by increased reliance on these other neural substrates. Second, it is very plausible that some of the tools used to manipulate cAMP pathway activity in LC (e.g., local infusion of PKA activators or inhibitors) influence glutamatergic afferents in this region, which also appear to show plastic changes (including cAMP pathway up-regulation) after chronic morphine (Nestler 1992; Christie et al. 1997). Third, despite a likely role for these glutamatergic afferents, there is no question that plasticity intrinsic to LC NE neurons is also involved, because local knockout of CREB from the adult LC (which cannot affect afferent nerve terminals) blocks the morphine-induced increased excitability of LC NE neurons and attenuates withdrawal (Cao et al. 2010; V Zachariou and EJ Nestler, unpubl.). The lack of effect of CREB knockout from these neurons in conditional knockout mice (Parlato et al. 2010) highlights the developmental compensations that complicate the use of early knockout models and emphasizes the importance of using gene manipulations in the fully differentiated adult brain when studying adult plasticity.

Thus, a wealth of experimental evidence establishes up-regulation of the cAMP-CREB pathway as a mechanism of intrinsic homeostatic plasticity in LC NE neurons in the development of opiate physical dependence. It is also important to emphasize the historical importance of this work on LC, as it served as a model system for the long-term actions of opiates on the brain: based on these earlier investigations of LC, up-regulation of the cAMP-CREB

Figure 4. (*Continued*) These changes contribute to the altered phenotype of the drug-addicted state. For example, the intrinsic excitability of LC neurons is increased by enhanced activity of the cAMP pathway and Na^+ -dependent inward current, which contributes to the tolerance, dependence, and withdrawal showed by these neurons. Up-regulation of ACVIII and TH is mediated via CREB, whereas up-regulation of ACI and of the PKA subunits appears to occur via an unidentified, CREB-independent mechanism.

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pathway has since been shown to be a common mechanism of opiate tolerance, dependence, and withdrawal in numerous regions of the central and peripheral nervous systems and indeed represents one of the best established models of the molecular basis of drug addiction (Nestler 2001, 2004).

OPIATE-INDUCED STRUCTURAL PLASTICITY

To date, there has not been a description of structural plasticity in LC neurons in response to chronic opiate administration. We are currently assessing whether any soma size changes occur in these neurons analogous to the changes observed in DA neurons in VTA. However, two lines of evidence suggest that this type of change may not be relevant in LC. First, normal axonal transport and levels of neurofilament proteins were observed in LC after chronic morphine in contrast to VTA (Beitner-Johnson et al. 1992; Beitner-Johnson and Nestler 1993), suggesting that trophic support of neuronal structure may not be affected. Second, given our finding that increased firing rate is a key contributor to changes in soma size, the differences between opiate regulation of firing rates in LC and VTA may be important. Namely, in VTA, opiates acutely and chronically increase firing rate in slices and in vivo, and we observe a decreased cell size coincident with and as a consequence of this increase in firing rate. This increased rate then normalizes, or even decreases below baseline, in animals withdrawn from the opiate. Because there is evidence from our own work (Russo et al. 2007), and others (Spiga et al. 2003), that the soma size is also decreased at these later time-points, when firing rate has decreased, it may be the initial sustained increase in firing rate that is vital for induction or maintenance of the morphological change. In contrast, LC neuronal activity is acutely decreased by morphine administration, returns toward baseline levels in vivo with chronic administration, and only increases above normal levels on opiate withdrawal. (These in vivo observations differ from what occurs in brain slice cultures, in which the increased firing rate and cAMP-CREB

pathway up-regulation occur in the chronic morphine-treated [dependent] state, without withdrawal [Cao et al. 2010].) These considerations suggest that, whereas chronic morphine may not elicit a change in structural plasticity in LC neurons in vivo, withdrawal from morphine might. In support of this idea, results from our microarray study of LC found that several genes involved in cell growth and structure are decreased or unchanged with chronic morphine, but are increased with withdrawal (McClung et al. 2005). It is known that prolonged decreases in the basal firing rate of LC neurons is not sufficient to alter soma size, as early CREB knockout from LC NE neurons did not alter neuronal size but decreased basal activity (Parlato et al. 2010). However, we also did not detect a difference in VTA DA soma size when we over-expressed a K^+ channel to decrease firing rate (Mazei-Robison et al. 2011), so the Parlato et al. observations do not preclude the possibility of a morphine withdrawal-induced change. Still, it should be noted that the mechanism mediating the changes in firing rate between the two brain regions is very different, with changes in AKT signaling, GABA_A currents, and K^+ channel expression implicated in VTA and cAMP-CREB signaling implicated in LC.

CONCLUDING REMARKS

Together, data from VTA and LC illustrate the complex and important changes in synaptic, cellular, and structural plasticity that mediate the lasting effects of opiate drugs on the brain's catecholamine neurons and other neuronal types in these regions, which in turn influence drug reward and dependence. Although the plasticity that underlies acute opiate action in both regions, and chronic opiate action in LC, is fairly well characterized, future studies are needed to delineate the plasticity that occurs with chronic opiate administration in VTA with respect to differences seen across multiple cell types and across multiple input-output patterns even for a single cell type. Such advances will contribute to a better understanding of how opiates influence this brain region to control reward and ultimately addiction. Such an understanding of

the long-lasting adaptations induced by opiates in VTA and LC will improve not only our knowledge of the etiology of opiate dependence and addiction, but will also help us elucidate novel therapeutic interventions.

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Opiate-Induced Molecular and Cellular Plasticity of Ventral Tegmental Area and Locus Coeruleus Catecholamine Neurons

Michelle S. Mazei-Robison and Eric J. Nestler

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