Glutamatergic Systems and Memory Mechanisms Underlying Opioid Addiction

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Glutamate is the main excitatory neurotransmitter in the brain and is of critical importance for the synaptic and circuit mechanisms that underlie opioid addiction. Opioid memories formed over the course of repeated drug use and withdrawal can become powerful stimuli that trigger craving and relapse, and glutamatergic neurotransmission is essential for the formation and maintenance of these memories. In this review, we discuss the mechanisms by which glutamate, dopamine, and opioid signaling interact to mediate the primary rewarding effects of opioids, and cover the glutamatergic systems and circuits that mediate the expression, extinction, and reinstatement of opioid seeking over the course of opioid addiction.

Addiction to opioid drugs, including opiates like heroin and morphine and synthetic opioids like fentanyl, starts with drug exposure and ultimately ends in chronic relapse. The final stage of this complex disorder is characterized by compulsive drug seeking despite negative consequences. Drug craving and the resultant drug seeking are often triggered by stimuli that become associated with aspects of the drug experience through a process of conditioning. Such aspects of the drug experience can include the rewarding sensation of opioids, or the aversive experience of opioid withdrawal, as well as behaviors linked with drug attainment and drug use. In addition, craving and drug memories can be triggered by stressful life events.

Although the primary rewarding effects of opioids are mediated by the μ opioid receptor (MOR), opioid reward also requires glutamatergic neurotransmission, and glutamate is critical for the formation and maintenance of opioid memories. These indirect glutamatergic effects of opioid drugs involve the prefrontal cortex, amygdala, hippocampus, paraventricular nucleus of the thalamus (PVT), and pedunculo-pontine tegmentum (PPTg), all of which converge onto nucleus accumbens (NAc) neurons (Brog et al. 1993; Finch 1996). These systems also directly and indirectly innervate neurons in the ventral pallidum and ventral tegmental area (VTA) downstream of the accumbens, and the combined output of this ventral basal ganglia

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system determines whether drug seeking occurs (Fuller et al. 1987; Geisler et al. 2007; Kalivas 2009).

Relapse may occur when memories associated with opioid use trigger craving for the drug. Over the course of opioid use, environmental stimuli such as drug paraphernalia (e.g., heroin syringes) or the context in which opioids were used become associated with the interoceptive experiences of opioid reward and withdrawal and drug-related behaviors that together comprise the opioid experience. Through this conditioning process, powerful memories are created that can drive opioid craving when abstinent users are confronted with reminder cues, thus triggering relapse, particularly when inhibitory cognitive control over behavior is compromised by opioid use (Schippers et al. 2012).

Like other memories, the associations formed during opioid use undergo three different phases of learning. Acquisition describes the process that underlies the initial formation of memory, and consolidation describes the process whereby memories are stored. These processes can be manipulated by administering treatments prior to learning or immediately after learning, respectively. Following consolidation, memories reemerge to drive behavior in a process called expression, and this process can be manipulated by treatment given prior to memory tests. Importantly, the acquisition of opioid memories primarily reflects primary opioid reward, whereas the expression of opioid memories reflects conditioned opioid reward and opioid reinforcement as described below.

PRIMARY AND CONDITIONED OPIOID REWARD

The rewarding effects of opioids have been extensively studied using the conditioned place preference (CPP) model. In this model, animals are confined to two distinct environmental contexts, one of which is paired with the rewarding state produced by opioid drugs. Subsequently, opioid reward memory is measured as the time an animal chooses to spend in the opioid-conditioned context over time spent in the unpaired context. Primary and conditioned opioid reward

can be distinguished using the CPP model. Primary opioid reward describes the acutely rewarding effects of opioids, and is assessed by whether manipulations prevent the acquisition of opioid CPP. Once the memory is acquired and consolidated, conditioned opioid reward can be distinguished by manipulations that prevent the expression of opioid CPP. However, it should be noted that the CPP model cannot distinguish interventions that block primary opioid reward from interventions that block general reward learning.

The primary rewarding effects of opioids are mediated by MOR expressed on long-range inhibitory inputs onto VTA dopamine neurons, and to a much lesser extent, MOR expressed on local GABAergic VTA interneurons (Wise 1989; Jalabert et al. 2011; Hjelmstad et al. 2013; Matsui et al. 2014). Of the GABAergic inputs to the VTA, MOR expressed on striatal dopamine D1 receptor-expressing medium spiny neurons (MSNs), which innervate the VTA and neighboring substantia nigra dopamine neurons, is sufficient for primary opioid reward and at least partially sufficient for opioid reinforcement, the ability of opioids to drive behavioral responding (Cui et al. 2014). However, in addition to MOR activation, glutamatergic modulation is also required for the activating effects of opioids on dopamine neurons and for primary opioid reward (Jalabert et al. 2011). Glutamatergic neurotransmission outside the VTA also regulates opioid reward and the contributions of distinct ionotropic and metabotropic glutamate receptors in this process are discussed below.

OPIOID REWARD—IONOTROPIC GLUTAMATE RECEPTORS

The *N*-methyl-D-aspartate receptor (NMDAR) is strongly linked to opioid reward. NMDARs are heterotetramers composed of different combinations of GluN1, GluN2A-D, and GluN3 subunits, which require the binding of glutamate and a coagonist (glycine or D-serine), as well as membrane depolarization for activation. These receptors are critical regulators of the synaptic plasticity that underlies learning and memory processes (Lüscher and Malenka

2012). NMDAR activation is necessary for the acquisition and expression of morphine CPP (Tzschentke and Schmidt 1995, 1997; Popik et al. 1998, 2003a,b; Suzuki et al. 2000; Papp et al. 2002; Ribeiro Do Couto et al. 2004; Yonghui et al. 2006) as shown by NMDAR antagonists injected prior to conditioning sessions or CPP expression tests. These effects have been localized to a number of brain regions, including the NAc, the ventral pallidum, and the VTA (Popik and Kolasiewicz 1999; Harris et al. 2004; Dallimore et al. 2006).

GluN2B-selective antagonists also block the acquisition of opioid CPP (Suzuki et al. 1999; Narita et al. 2000; Ma et al. 2006, 2011b), and these systemic effects are likely mediated by the NAc, where morphine increases GluN2B expression (Narita et al. 2000; Ma et al. 2006), and where knockdown of GluN2B or local infusion of a GluN2B antagonist blocks the acquisition of morphine CPP (Kao et al. 2011; Xu et al. 2012). Importantly, comparable doses of GluN2B antagonists do not alter spatial learning and memory (Ma et al. 2011b), and doses of NMDAR antagonists that block morphine CPP do not block food CPP (Papp et al. 2002; Popik et al. 2003b; Ma et al. 2006; Yonghui et al. 2006). These data suggest that opioid-specific neuroadaptations involving the NMDAR, and in particular GluN2B-containing receptors, underlies opioid reward.

The α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) also mediates opioid reward and AMPAR antagonists block both the acquisition and expression of opioid CPP (Tzschentke and Schmidt 1997). These effects depend on AMPAR in the NAc (Lay et al. 1993), the ventral pallidum (Dallimore et al. 2006), and the VTA (Harris et al. 2004; Shabat-Simon et al. 2008). It should be noted that AMPAR antagonists used in these studies also block ionotropic kainate glutamate receptors, and the different contribution of these two types of ionotropic glutamate receptors on opioid reward needs to be clarified. However, selective AMPAR interventions also perturb opioid reward. AMPARs are heterotetramers that consist of combinations of four subunits (GluA1–4; also referred to as GluR1–4) and the absence of

GluA2 controls the calcium permeability of these channels. AMPARs are expressed at the cell surface in a dynamic equilibrium between receptor insertion into the membrane versus receptor endocytosis, both of which are critical for drug memory formation and recall (Lüscher and Malenka 2011). In line with this, interfering with the endocytosis of GluA2-containing AMPAR using a blocking peptide prevents the acquisition, but not the expression of opioid CPP (Lin et al. 2016; but see Dias et al. 2012), and this effect is localized to the nucleus accumbens shell (NAshell) (Graziane et al. 2016).

OPIOID REWARD—METABOTROPIC GLUTAMATE RECEPTORS

Aside from ionotropic glutamate receptors, G-protein-coupled metabotropic glutamate receptors (mGluRs) are also implicated in opioid reward. These receptors are classified into three distinct subtypes. Type I mGluRs (mGluR1 and 5) activate neurons through phospholipase C (PLC) signaling (Kenny and Markou 2004), and mGluR5 antagonists block both the acquisition and expression of morphine CPP (Popik and Wrobel 2002; Aoki et al. 2004; Herzig and Schmidt 2004; Veeneman et al. 2011). At equivalent doses, mGluR5 antagonists do not alter spatial learning and memory (Popik and Wrobel 2002). The effects of mGluR5 on opioid memory are localized to the NAc (and olfactory tubercle) where morphine increases the expression of mGluR5 and protein kinase C, its primary intracellular mediator downstream of PLC (Aoki et al. 2004). Although these effects are comparable to the above-mentioned effects of NMDAR antagonists, the effects of mGluR5 antagonists (Herzig and Schmidt 2004), but not NMDAR antagonists (Tzschentke and Schmidt 1997; Papp et al. 2002), may be explained by a state dependence of the opioid memory. Other studies have reported no effects of mGluR5 antagonists on morphine CPP, at moderate or high doses (McGeehan and Olive 2003; Veeneman et al. 2011), or even a potentiation of morphine CPP (van der Kam et al. 2009a,b; Rutten et al. 2011). Although the reason for these discrepancies is unknown, mGluR5 blockade or substi-

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tution of primary opioid reward, and the state dependence induced by mGluR5 antagonism, may be mediated by distinct brain regions. For instance, the importance of mGluR5 signaling for opioid reward has been localized to the NAc (Aoki et al. 2004; Roohi et al. 2014; Qi et al. 2015). Morphine increases mGluR5 membrane expression in the NAc shell, but not the nucleus accumbens core (NAcore), and microinjecting a mGluR5 antagonist directly into the NAc shell blocks the expression of morphine CPP (Qi et al. 2015).

Type II and III mGluR have also been implicated in opioid reward. These receptors reduce neuronal activity by inhibiting adenylyl cyclase and calcium channels, by activating potassium channels, and by directly interfering with presynaptic vesicle release (Blackmer et al. 2001; Kenny and Markou 2004). Morphine exposure causes the up-regulation of type II metabotropic glutamate receptors (mGluR2 and 3) in the NAc and VTA (Manzoni and Williams 1999; Martin et al. 1999), and activating mGluR2/3 in the NAc prevents the acquisition and expression of morphine CPP (Baharlouei et al. 2015). Morphine CPP is also blocked by a systemic agonist for the type III metabotropic glutamate receptor 7 (mGluR7), which at the same dose does not affect sucrose consumption or spatial memory performance (Hajasova et al. 2018). Figure 1 summarizes the role of these different glutamate receptors and systems in primary and conditioned opioid reward.

OPIOID REWARD—GLUTAMATE TRANSPORTERS

Glutamate transporters are critical for the regulation of extracellular glutamate levels, and alter the level of glutamate available for binding to its receptors. In the forebrain, extracellular glutamate levels are controlled by sodium-dependent excitatory amino acid transporters 1–3 (EAAT) and the cystine glutamate exchanger. Of these, EAAT1 (also known as glutamate and aspartate transporter [GLAST]) and EAAT2 (also known as glutamate transporter 1 [GLT-1]) are expressed predominantly in astrocytes, while

EAAT3 is primarily expressed in neurons (Danbolt 2001). Glutamate transporters play an important role in opioid reward. Morphine increases the expression of EAAT3 in the medial prefrontal cortex (mPFC), NAc, and VTA (Wan et al. 2017). Glutamate transporter blockers, which increase synaptic glutamate levels, facilitate the acquisition of morphine CPP (Sekiya et al. 2004). By contrast, interventions that promote glutamate uptake interfere with the acquisition of morphine CPP (Tzschenke and Schmidt 1998; Fujio et al. 2005; Nakagawa et al. 2005a; Wan et al. 2017). These studies suggest that indirect glutamate receptor agonists and antagonists that act through glutamate transporters can enhance or occlude opioid memories, respectively.

OPIOID WITHDRAWAL AND AVERSION

Opioid withdrawal produces a negative affective state that can become a powerful driver of opioid seeking (Koob 2020), and this process is mediated by changes in glutamatergic neurotransmission. In particular, metabotropic glutamate receptors are linked to the manifestation of opioid withdrawal (Rasmussen et al. 2004, 2005). Withdrawal from morphine down-regulates mGluR2 expression in the NAc shell (Qian et al. 2019), and mGluR2 knockout exacerbates opioid withdrawal symptoms (Gao et al. 2018). Acute opioid withdrawal also increases mGluR5 binding in a wide range of brain regions and the negative affective state that is produced by opioid withdrawal can be reversed with a mGluR5 antagonist (Zanos et al. 2016).

The aversive state produced by opioid withdrawal can elicit a conditioned place aversion (CPA) that is comparable to opioid reward-induced CPP. In the CPA model, acute opioid withdrawal is often mimicked by injections of the MOR antagonist naloxone in opioid-dependent animals. The acquisition and expression of opioid CPA memories is mediated by glutamatergic neuroadaptations comparable to those that underlie opioid CPP. For instance, both the acquisition and expression of naloxone CPA are also blocked by NMDAR antagonists (Blokhina et al. 2000; Maldonado et al. 2003;

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Figure 1. Glutamate receptors and systems involved in opioid reward and aversion. Primary opioid reward is primarily mediated by μ opioid receptor inhibition of long-range GABAergic projections onto ventral tegmental area (VTA) dopamine neurons (originating from the nucleus accumbens shell [NAshell], ventral pallidum [VP] and the rostromedial tegmental [RMTg] nucleus). Primary opioid reward also requires glutamatergic neurotransmission onto dopamine neurons, originating mainly from the pedunclopontine tegmentum (PPTg), as well as orexinergic inputs from the lateral hypothalamus (LH). Conditioned opioid reward (and to a much lesser extent also primary opioid reward) depends on glutamatergic neurotransmission in allocortical (basolateral amygdala [BLA] and hippocampal formation [HPC]), prefrontal infralimbic (IL) and prelimbic (PL) cortices, NAshell, and central amygdala (CeA). Opioid withdrawal is predominantly mediated by the paraventricular nucleus of the thalamus (PVT), the periaqueductal gray (PAG), the CeA, and bed nucleus of the stria terminalis (BNST). In addition to these circuit mechanisms, the local and central/systemic pharmacological effects of glutamatergic drugs on opioid memories are represented by symbols as described in the figure legend (*left*). Green arrows = drives CPP/CPA; blue arrows = inhibits CPP/CPA; dashed arrows = indirect evidence for pathway involvement.

Kawasaki et al. 2005). Similar effects on the acquisition of naloxone CPA have been reported with AMPAR and broad-spectrum mGluR antagonists, and these effects were localized to the NAc (Kawasaki et al. 2011; Russell et al. 2016). Figure 1 summarizes the role of these different glutamate receptors and systems in opioid withdrawal. Similar to their role in regulating opioid reward memories, glutamate transporters also mediate opioid withdrawal, and increasing synaptic glutamate levels with glutamate transporter blockers facilitates the acquisition of naloxone CPA (Sekiya et al. 2004).

CONDITIONED OPIOID REINFORCEMENT

Besides conditioned reward, glutamatergic neurotransmission also regulates conditioned opioid reinforcement. Conditioned reinforcement

describes the mechanisms by which drug-associated stimuli can drive behavioral responding for opioids, and this is typically assessed using opioid self-administration models where animals can control their intake of a drug through operant responses such as nose pokes or lever presses. Because the response-contingent self-administration model takes into account cognitive aspects of drug seeking like expectation, reward valuation, and decision making, it has enhanced validity over noncontingent models (e.g., CPP) and is considered the gold standard for studying addiction. Notably, important distinctions have been reported in glutamate systems following contingent versus noncontingent models. For instance, opioid self-administration elicits substantially different patterns of gene expression, and produces different effects on dendritic spine density in limbic brain re-

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gions (Robinson et al. 2002; Jacobs et al. 2005; Kuntz et al. 2008).

However, manipulations that affect glutamatergic neurotransmission produce overall similar effects on conditioned reward and reinforcement. Consistent with their ability to decrease opioid reward in CPP models, systemic AMPAR and NMDAR antagonists increase heroin self-administration (Semenova et al. 1999; Xi and Stein 2002). These effects are mimicked by AMPAR blockade in the VTA, and by low doses of NMDAR antagonists infused into this structure. By contrast, high systemic or intra-VTA doses of NMDAR antagonists block heroin self-administration, perhaps due to the dissociative effects of these drugs (Semenova et al. 1999; Xi and Stein 2002). In addition to ionotropic glutamate receptors, metabotropic receptors are also involved in opioid reinforcement. Antagonists of mGluR5 reduce rates of opioid self-administration (van der Kam et al. 2007; Brown et al. 2012) and, in line with the substituting effects of mGluR5 antagonists for opioid reward in the CPP model, rats self-administer these drugs intravenously following heroin self-administration (van der Kam et al. 2009b). The mGluR2/3 is also involved in opioid reinforcement, and even though acute treatment with mGluR2/3 agonists does not affect heroin self-administration (Bossert et al. 2005a), constitutive knockout of mGluR2 predisposes rats toward escalation of heroin intake (Gao et al. 2018). However, some differences between the glutamatergic regulation of conditioned opioid reward and reinforcement have also been reported. For instance, while NMDAR blockade in the NAc blocks the expression of morphine CPP (Popik and Kolasiewicz 1999), it does not alter rates of heroin self-administration (Pulvirenti et al. 1992). In addition, AMPAR or NMDAR blockade in the prelimbic (PL) mPFC reduces heroin self-administration, but these manipulations facilitate the acquisition of morphine CPP (Bishop et al. 2011; De Jaeger et al. 2013; Abou Talebi et al. 2018). However, these apparent discordant findings may be explained by an increased sensitivity to the rewarding effects of opioids following ionotropic glutamate receptor blockade in the PL mPFC (Bishop et al. 2011),

which involves downstream activation of VTA dopamine neurons (Tan et al. 2014).

Combined, these findings suggest that most manipulations that affect glutamatergic neurotransmission and disrupt opioid CPP or self-administration likely act on primary opioid reward mechanisms. To isolate the opioid memories from the acute effects of opioids, extinction, reconsolidation, and reinstatement procedures are used.

OPIOID MEMORY PROCESSES

Following acquisition and consolidation, opioid memories can undergo updating in a process called reconsolidation (see below), or can be actively suppressed through a process termed extinction. In the case of extinction, opioid memories may return to drive opioid seeking when triggered by reminder cues in a process called reinstatement, or a reminder context during renewal. The memories may also spontaneously return to drive drug seeking after a bout of drug abstinence in a process called spontaneous recovery. Most studies into the glutamatergic mechanisms underlying opioid seeking use extinction procedures followed by reinstatement. During extinction, the opioid drug is unavailable, and over the course of repeated trials, animals learn to stop expressing place preference, or stop making operant responses. During the extinction phase, specific drug-associated cues become dissociated from the conditioned behavioral response. In the CPP model, these cues include the combined contextual stimuli of the drug-paired chamber, and in the self-administration model, these can either be the contextual or discrete cues paired with the self-administration chamber or drug infusion, respectively. Of note, the term extinction also describes the process whereby a new memory is formed, which actively suppresses the original conditioned memory. Following completion of the extinction process, opioid seeking can be reinstated by reexposure to stimuli that have not been extinguished, for instance, by reintroducing animals to a small priming dose of the unconditioned stimulus (i.e., the opioid drug). Reinstatement can also be elicited in extin-

guished animals by returning nonextinguished conditioned stimuli (e.g., lights or tones previously paired with the drug infusion) or by exposing the animals to stress. In addition, reinstatement can be triggered by returning animals to a drug-conditioned context after extinction in a distinct context, a process known as renewal of drug seeking (Bossert et al. 2005b; Scofield et al. 2016).

RECONSOLIDATION

Following retrieval, memories may enter a labile state during which memory content can be updated and memories become sensitive to disruption. This process, termed reconsolidation, requires protein synthesis (Nader et al. 2000). Retrieval of opioid CPP memory increases NAc shell surface expression of GluA1, and increases the expression of Arc and phosphorylation of extracellular signal-regulated kinases (ERK1/2) and cyclic AMP response element-binding protein (CREB). Blocking ERK signaling or Arc translation interferes with the reconsolidation of the opioid memory and reduces the expression of opioid CPP during subsequent tests (Lv et al. 2015). Reconsolidation of morphine CPP, but not naloxone CPA, also requires NMDAR activation in the NAc core (Wu et al. 2012b).

Briefly following retrieval of a memory, while the memory trace is in a labile state, the original memory may be updated with newly learned information. For instance, when extinction is conducted during this labile period, the memory is reconsolidated with an updated oppositional memory trace. This retrieval-extinction procedure can produce a lasting reduction in the behavioral expression of memories (Monfils et al. 2009). Retrieval-extinction is also capable of reducing heroin-primed relapse after heroin self-administration and the expression of heroin CPP (Xue et al. 2012). Evidence suggests that after protracted withdrawal from heroin self-administration when memories are remote as opposed to recent, retrieval-extinction procedures are less effective. If instead of drug-conditioned cues, the unconditioned stimulus, heroin, is used to trigger memory retrieval,

remote drug memories can again become labile and susceptible to interference by retrieval-extinction procedures (Yuan et al. 2019). This process may be mediated by the ability of drugs to temporarily reverse withdrawal-induced changes in glutamatergic synapses (Jedynak et al. 2016; Spencer et al. 2017; Madayag et al. 2019). Heroin-elicited destabilization of remote heroin memories requires neuronal activity and GluA1 and GluA2 endocytosis in the basolateral amygdala (BLA). Conversely, artificially activating glutamatergic BLA neurons during remote memory retrieval facilitates the destabilization of remote heroin memories, and restores the ability of weaker conditioned drug cues to initiate heroin memory reconsolidation. Collectively, these data suggest that remote memories for opioid reinforcement are at least in part stored in the BLA, and that modification of these opioid memories depends on synaptic neuroadaptations and neuronal activity in this structure (Yuan et al. 2019). In addition to AMPAR, NMDARs are also required for the reconsolidation of morphine CPP memories (Zhai et al. 2008).

EXTINCTION AND REINSTATEMENT—IONOTROPIC RECEPTORS

Ionotropic glutamate receptor signaling is necessary for extinction and reinstatement of opioid seeking in rodents, and is implicated in opioid addiction in humans, where polymorphisms in the AMPAR regulatory protein gene *CNIH3* are linked to opioid dependence (Nelson et al. 2016). Systemic AMPAR antagonist treatment, or blockade of GluA2-AMPA endocytosis, facilitates extinction and blocks the reinstatement of morphine CPP (Dias et al. 2012; Lin et al. 2016; Siahposht-Khachaki et al. 2017). However, AMPAR endocytosis may differentially affect the reinstatement of opioid CPP depending on the brain region targeted and the time point of intervention. Following protracted morphine withdrawal, local NAc shell blockade of GluA2-AMPA endocytosis potentiates the reinstatement of morphine CPP, which may suggest an inhibitory role for NAc shell neurons on opioid seeking at this time point (Madayag et al. 2019).

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By contrast, in the adjacent NAc core AMPAR or NMDAR antagonists prevent reinstatement elicited by discrete cues and heroin priming after extinction in a self-administration model (LaLumiere and Kalivas 2008; Shen et al. 2011).

Similar to the effects of AMPAR manipulations, NMDAR antagonists also facilitate extinction learning and reduce the reinstatement of morphine CPP (Siahposht-Khachaki et al. 2016). Interestingly, GluN2B-selective antagonists also reduce morphine CPP expression when they are administered without extinction training during abstinence (Ma et al. 2011b). In line with this observation, NMDAR- and GluN2B-selective antagonists prevent reinstatement when they are administered prior to extinction training in the days preceding a reinstatement test (Popik et al. 2006; Ma et al. 2011b). Combined, these findings indicate that NMDAR activation is necessary for the continuous maintenance of opioid memories. Indeed, when given during extinction, NMDAR antagonists facilitate extinction learning (Siahposht-Khachaki et al. 2016) without affecting the consolidation of extinction memory (Popik et al. 1998).

Morphine-primed and stress-induced reinstatement of morphine CPP increase GluN2B expression in the NAc (Ma et al. 2007), and when given prior to CPP reinstatement tests, GluN2B-selective and general NMDAR antagonists reduce drug-primed but not stress-primed reinstatement of morphine CPP (Ma et al. 2007; Siahposht-Khachaki et al. 2016). In the opioid self-administration model, withdrawal from self-administered heroin also increases GluN2B surface expression in the NAc core and GluN2B knockdown, or GluN2B antagonists infused directly into the NAc core reduce cue- or heroin-primed reinstatement of heroin seeking. These manipulations also prevent reinstatement-associated changes in dendritic spine morphology in this region (Shen et al. 2011). In humans, polymorphisms in the GluN2B-coding gene *GRIN2B* are also associated with opioid dependence (Xie et al. 2014).

D-cycloserine (DCS) is a partial NMDAR agonist that acts on the glycine-binding site, and shows promise as a facilitator of extinction

learning (Davis et al. 2006; Peters and De Vries, 2013). Indeed, systemic or infralimbic (IL) DCS administration facilitates the extinction of naloxone CPA (Myers and Carlezon 2010), which requires the phosphorylation of ERK and CREB, and the epigenetic enhancement of brain-derived neurotrophic factor (BDNF) transcription (Wang et al. 2012). However, DCS may not facilitate extinction, or reduce the reinstatement of morphine CPP (Lu et al. 2011). DCS also fails to alter the acquisition or expression of morphine CPP (Lu et al. 2011), although the related NMDAR coagonist D-serine prevents the acquisition of morphine CPP (Wu et al. 2017).

The NMDAR antagonist memantine has received considerable interest because of its approved status for clinical use in humans. Memantine blocks the acquisition and reinstatement of morphine CPP (Popik et al. 2003b, 2006; Ribeiro Do Couto et al. 2004, 2005; Mehri et al. 2018). However, although memantine reduces heroin reward in humans, this drug is not effective at reducing heroin reinforcement (Comer and Sullivan 2007). This finding illustrates the importance of distinguishing between drug use and drug seeking for clinical treatments of opioid addiction, which should target drug seeking rather than drug use to prevent relapse. Figure 2 summarizes the role of these different glutamate receptors and systems in opioid seeking.

EXTINCTION AND REINSTATEMENT— GLUTAMATE TRANSPORTERS AND METABOTROPIC RECEPTORS

Drugs of abuse disrupt homeostasis between the mechanisms that regulate synaptic glutamate, and this process has been extensively characterized in the NAc (Kalivas 2009). These synaptic changes include a reduced functioning of presynaptic glutamate receptors, a reduction in astroglial glutamate uptake, and the retraction of perisynaptic astroglial processes from the synapse. Combined, these neuroadaptations cause the extrasynaptic spillover of glutamate, which in turn activates glutamate receptors located in extrasynaptic compartments and on neighboring synapses (Schofield et al. 2016).

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Figure 2. Glutamate receptors and systems involved in opioid seeking. Opioid seeking requires glutamatergic projections from the medial prefrontal infralimbic (IL) and prelimbic (PL) cortices, and hippocampal formation (HPC) to the nucleus accumbens. While glutamate receptor activation in the ventral tegmental area (VTA) can modulate opioid seeking, the inputs responsible for this effect are unknown. The basolateral amygdala (BLA) is also critical to opioid seeking and provides glutamatergic projections to all of the other circuit nodes, but no direct projections have been implicated in opioid seeking thus far. In addition to these circuit mechanisms, the local and central/systemic pharmacological effects of glutamatergic drugs on opioid memories are represented by symbols as described in the figure legend (left). Green arrows = drives opioid seeking; blue arrows = inhibits opioid seeking; dashed arrows = indirect evidence for pathway involvement; gray arrows = hypothesized pathway involvement.

Withdrawal from heroin self-administration causes the down-regulation of GLT-1 on glial cells in the NAc (Shen et al. 2014). In addition, withdrawal leads to the retraction of perisynaptic astroglial processes from glutamatergic synapses (Kruyer et al. 2019). Combined, these processes contribute to the increased spillover of glutamate and activation of extrasynaptic glutamate receptors (Shen et al. 2014).

These disruptions in the functioning of glutamatergic synapses following heroin self-administration are reversed by *N*-acetylcysteine, an over-the-counter antioxidant and glutamatergic prodrug that restores glial GLT-1 expression. Indeed, pretreatment with *N*-acetylcysteine facilitates the extinction and reduces reinstatement of heroin seeking (Zhou and Kalivas 2008). *N*-acetylcysteine protects heroin self-administering rats from both cue- or heroin-primed reinstatement for up to 40 days following treatment (Zhou and Kalivas 2008), and is also capable of reducing heroin intake in self-administering rats but only after prolonged exposure to the drug (Hodebourg et al. 2019).

In addition to *N*-acetylcysteine, the β -lactam antibiotic ceftriaxone is also capable of up-reg-

ulating the expression of GLT-1 (Knackstedt et al. 2010), and ceftriaxone treatment restores the functioning of glutamatergic synapses in the NAc following opioid withdrawal (Shen et al. 2014; Hearing et al. 2016). Ceftriaxone also blocks morphine-primed reinstatement of CPP in mice after protracted withdrawal, and reduces the reinstatement of operant heroin seeking in a GLT-1-dependent manner (Shen et al. 2014; Hearing et al. 2016). The acquisition of morphine CPP is also prevented by ceftriaxone and the closely related β -lactam drug clavulanic acid (Schroeder et al. 2014), or by overexpressing GLT-1 in the NAc (Fujio et al. 2005).

Opioid withdrawal disrupts mGluR2/3 functioning in the NAc (Robbe et al. 2002; Wu et al. 2012a; Shen and Kalivas 2013; Qian et al. 2019). Restoring mGluR2/3 function with systemic or intra-accumbens infusions of mGluR2/3 agonists reduces reinstatement of heroin seeking (Bossert et al. 2005a, 2006; Zhu et al. 2017), likely by protecting against excessive presynaptic glutamate release (Bossert et al. 2006; LaLumiere and Kalivas 2008). However, the possibility remains that mGluR3 receptors located on glia may participate in these effects as

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well (Yao et al. 2005). Infusion of a mGluR2/3 agonist into the NAc also facilitates the extinction and prevents the reinstatement of morphine CPP (Baharlouei et al. 2018). In line with these observations, the cognitive enhancing drug, modafinil, is capable of preventing the reinstatement of morphine CPP, and this effect is mediated by mGluR2/3 receptors (Tahsili-Fahadan et al. 2010). Indirect evidence suggests that heroin addiction in humans may also be caused by dysregulated glutamatergic synapses, since polymorphisms in the mGluR3 gene *Grm3* are associated with heroin dependence (Jia et al. 2014). Group III metabotropic glutamate receptors may also regulate glutamate homeostasis, since both systemic and intra-accumbens mGluR7 agonists facilitate extinction and reduce reinstatement of morphine CPP (Hajasova et al. 2018; Vatankhah et al. 2018).

The above-mentioned reduced functioning of mGluR2/3, down-regulation of GLT-1, and retraction of astroglial processes from the synapse promotes excessive glutamate release during reinstatement, and spillover of glutamate into perisynaptic compartments (Scofield et al. 2016). Glutamate spillover activates extrasynaptic mGluR5 and GluN2B receptors, which initiate a signaling cascade that results in the production of nitric oxide, the activation of extracellular matrix metalloproteases (MMPs), and increased spine size, spine density, and glutamatergic synaptic function (Shen et al. 2011; Smith et al. 2017). Indeed, reinstatement of cocaine or heroin seeking increases MMP-9 activity in the NAc core, and local inhibition of MMP-9 reduces the reinstatement of cocaine seeking (Smith et al. 2014), while brain-wide pharmacological inhibition of MMP activity reduces heroin seeking (Van den Oever et al. 2010). Conversely, reinstatement of cocaine and heroin seeking and cocaine-seeking-induced MMP activation are also blocked by NAc core inhibition of mGluR5 signaling (Brown et al. 2012; Lou et al. 2014; Smith et al. 2017). Given that reinstatement of heroin seeking also activates MMP-9 in the NAc core (Smith et al. 2014) and elicits similar changes in structural and functional plasticity in glutamatergic synapses in the accumbens (Shen et al. 2011, 2014),

the above-mentioned signaling pathway may be a shared mechanism between reinstatement of cocaine and heroin seeking (Shen et al. 2011; Scofield et al. 2016). However, MMP activation in the prefrontal cortex is also implicated in the acquisition of conditioned drug reward and reinstatement of heroin seeking (Brown et al. 2008; Van den Oever et al. 2010). Brain-wide inhibition of MMP activity prevents heroin reinstatement-induced neuroadaptations in the mPFC, and interferes with the reconsolidation of cocaine CPP memory (Brown et al. 2007; Van den Oever et al. 2010).

In addition to the direct effect of glutamate receptors and transporters, indirect synaptic regulators of G-protein receptor function are also implicated in opioid memory formation and recall. Regulator of G-protein signaling (RGS) proteins antagonize G-protein-coupled receptor (GPCR) signaling by catalyzing GTPase activity and RGS4 knockdown in the NAc potentiates morphine CPP by increasing the phosphorylation of GluA1 and GluN2B, whereas overexpression of RGS4 blocks morphine CPP (Han et al. 2010; Kim et al. 2018). Similarly, conditional deletion of RGS7 in the NAc increases morphine CPP and self-administration, and potentiates morphine-induced synaptic strength (measured as an increase in AMPAR currents compared to NMDAR currents recorded from the same cell; henceforth this will be called A/N ratio) onto NAc neurons (Sutton et al. 2016).

REGULATION OF OPIOID MEMORIES BY GLUTAMATERGIC SYSTEMS AND SYNAPSES

Both ionotropic and metabotropic glutamate receptors are critically involved in the synaptic plasticity that underlies opioid memories. Common changes in glutamatergic synapses observed following exposure to drugs of abuse include altered synaptic neurotransmitter release, changes in the induction of synaptic long-term potentiation and depression (LTP/LTD), and changes in the number or composition of AMPAR (often a shift from GluA2-containing AMPAR toward Ca^{2+} permeable and high-conductance GluA2-lacking AMPAR) (Lüscher and Malenka 2011).

As mentioned above, glutamatergic neurotransmission onto NAc neurons, in particular glutamate originating from the prefrontal cortex, is critical for the learning and memory processes that underlie opioid memories. NAc projection neurons comprise two distinct populations of MSNs of equal proportion that can be distinguished based on their expression of dopamine D1 or D2 receptors (D1-/D2-MSNs) (Smith et al. 2013). Both D1- and D2-MSNs receive inputs from the prefrontal cortex, and are also innervated by glutamatergic inputs from the BLA, the PVT, the HPC, and the VTA (Brog et al. 1993; Stuber et al. 2010). These regions also innervate each other, and most of these regions innervate the ventral pallidum, one of the main output structures for D1- and D2-MSN projections, which is also implicated in opioid addiction (Rogers et al. 2008). Combined, these systems are critical for opioid reward and reinforcement, withdrawal-mediated aversion, and the reinstatement of heroin seeking following self-administration (Corrigall and Linseman 1988; Tzschentke and Schmidt 1999; Bossert et al. 2004, 2006, 2011, 2012, 2016; Harris et al. 2004; Dallimore et al. 2006; Rogers et al. 2008; Bishop et al. 2011; Lintas et al. 2011; Bossert and Stern 2014; O'Neal et al. 2019). We highlight below how changes in glutamatergic synapses and interactions between these brain regions regulate opioid memories.

PREFRONTAL PATHWAYS

The mPFC provides a major input to the NAc (Brog et al. 1993), which can be subdivided into a dorsomedial projection from the PL mPFC to the NAc core, and a ventromedial projection from the IL mPFC to the NAc shell (Sesack et al. 1989). Heroin seeking elicited by conditioned cues and priming doses of heroin requires glutamate release from PL-NAc core projections (LaLumiere and Kalivas 2008), and NMDAR or AMPAR antagonists applied locally to the NAc core block cue- or heroin-primed reinstatement of heroin seeking (LaLumiere and Kalivas 2008; Shen et al. 2011). Opioid withdrawal also produces synaptic changes in the PL-NAc core pathway that occlude LTP and LTD (Robbe et al. 2002; Shen

and Kalivas 2013; Qian et al. 2019), which are mediated in part by reduced mGluR2/3 functioning (Robbe et al. 2002; Qian et al. 2019) and an increase in the surface expression of NR2B-containing NMDAR on NAc neurons (Shen et al. 2011, 2014). These changes in synaptic functioning of the PL-NAc core pathway are likely induced by opioid withdrawal, as neurotransmission in the PL-NAc core is not affected by repeated morphine injections alone (Yuan et al. 2017). In addition to regulating the reinstatement of heroin seeking (LaLumiere and Kalivas 2008; Rogers et al. 2008), PL-NAc core projections are also necessary for reinstatement of other drugs, which has led to the notion that this may be a “final common pathway” for drug relapse (Kalivas 2009).

Reinstatement of heroin seeking may also depend on glutamatergic neurotransmission in the IL-NAc shell projection. Inactivation of either the PL or IL mPFC prevents cue- or heroin-primed reinstatement of heroin seeking, and these effects may depend on downstream activity in the NAc shell, as inactivation of this region also prevents heroin-primed reinstatement (Rogers et al. 2008). The IL contains at least a subset of neurons that encode drug-context associations, and the selective lesioning of this ensemble blocks the contextual renewal of heroin seeking (Bossert et al. 2011). Renewal of heroin seeking also activates IL-NAc shell projection neurons, and renewal is blocked by the unilateral inhibition of neuronal activity in the IL mPFC combined with the contralateral microinfusion of dopamine D1 receptor antagonists in the NAc shell (Bossert et al. 2012). By contrast, in cocaine self-administering animals the IL-NAc shell projection mediates the suppression of drug seeking after extinction. Inhibiting neuronal activity in this projection induces reinstatement in extinguished rats (Peters et al. 2008), and stimulating IL-NAc shell projection neurons reduces reinstatement (Augur et al. 2016). However, cocaine seeking and cocaine extinction are encoded by distinct ensembles of neurons in the IL mPFC (Warren et al. 2019). Thus, extinction versus expression of opioid memories may also be encoded by distinct neuronal ensembles in the IL mPFC. Indeed, a few studies have suggested

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that the IL mPFC may inhibit opioid memories after extinction training, as it does for cocaine. For example, inhibiting the IL mPFC causes the reinstatement of extinguished morphine CPP (Ovari and Leri 2008). In addition, extinction of morphine CPP induces differential expression of genes in the IL mPFC implicated in synaptic plasticity, including mGluR8 and BDNF (Martínez-Rivera et al. 2019). Furthermore, inhibiting the processes underlying memory maintenance within the IL mPFC blocks the retrieval of extinction memory for both naloxone CPA and morphine CPP (He et al. 2011). Lesions of the IL mPFC also interfere with naloxone CPA extinction, and CPA extinction requires NMDAR activation (and downstream ERK-CREB activation and BDNF signaling) in the IL mPFC (Wang et al. 2012). These studies indicate that opioid extinction memory may be stored in the IL mPFC and are supported by the following observations from heroin self-administration models. Blocking the endocytosis of GluA2-containing AMPAR locally within the IL, but not PL, mPFC reduces cue-induced reinstatement of heroin seeking and prevents a concordant synaptic depression in IL mPFC neurons (Van den Oever et al. 2008). In addition, potentiation of AMPAR function in the IL mPFC facilitates extinction learning after heroin self-administration, and reduces cue-induced reinstatement of heroin seeking (Chen et al. 2016). However, it remains unknown which IL mPFC projection target mediates facilitated extinction and suppressed reinstatement of opioid seeking, and what the precise conditions are under which IL mPFC promotes versus inhibits opioid seeking.

Distinct roles of IL and PL mPFC have also been reported for primary drug reward. Lesions of the IL mPFC prevent the acquisition of morphine CPP, whereas lesions of the PL mPFC prevent cocaine CPP (Tzschentke and Schmidt 1999). By contrast, blockade of AMPAR or NMDAR in PL, but not IL, mPFC potentiates morphine CPP (Bishop et al. 2011; De Jaeger et al. 2013). Following morphine CPP conditioning, firing of PL neurons is increased when animals enter the morphine-conditioned compartment, and this effect disappears after extinction training (Sun et al. 2011).

Opioids elicit their primary rewarding effects through MOR activation in the VTA as described above, but the formation of opioid memories also depends on opioid receptors expressed in the prefrontal cortex. MORs are predominantly expressed on cortical GABAergic interneurons (Taki et al. 2000; Ferezou et al. 2007), and morphine acting at MORs on parvalbumin interneurons reduces GABA release and disinhibits pyramidal neurons. Morphine in the mPFC also acts on δ opioid receptors (DORs), which are known regulators of MOR activity. DOR activation on somatostatin interneurons indirectly disinhibits pyramidal neurons through a mechanism that involves inhibition of adjacent parvalbumin neurons, and knockdown of DORs in somatostatin interneurons prevents the acquisition of morphine CPP (Jiang et al. 2019). Excessive MOR activation in the mPFC also causes motivational dysregulation due to the simultaneous disinhibition of prefrontal projections to the hypothalamus and NAc (Baldo 2016). This opioid-induced dysregulation of prefrontal circuits may underlie impulsive behavior elicited by opioid use, as heroin self-administration increases impulsive choice in rats during self-administration, and this behavior normalizes following abstinence from the drug (Schippers et al. 2012). Interestingly, MOR activation in the mPFC also activates downstream orexin neurons in the lateral hypothalamus (LH), which may contribute to the formation of opioid CPP memory and the regulation of opioid-induced neuroadaptations in the VTA by orexin (Richardson and Aston-Jones 2012; Baimel and Borgland 2015; Baldo 2016).

AMYGDALA PATHWAYS

BLA neurons and their projections are important regulators of opioid reward and reinforcement, and opioid memories may at least in part be stored in the BLA. Neuronal activity in the BLA is necessary for cue- and drug-primed reinstatement of heroin seeking (Fuchs and See 2002; Rogers et al. 2008), and BLA neurons also encode opioid withdrawal. Naloxone conditioned cues are capable of reducing heroin

self-administration in dependent animals, and the reconsolidation of these memories requires activation of the transcription factor Zif268 in the BLA (Hellemans et al. 2006). BLA neurons are also implicated in the extinction of morphine CPP. Extinction increases BDNF and GluA2 expression in the BLA (Xue et al. 2014), and inhibiting the BLA delays the extinction of morphine CPP (Sun and Laviolette 2012).

The maintenance of morphine CPP memories is also blocked by local infusions of PKM ζ inhibitory peptide (ZIP) in either the BLA or the NAc (He et al. 2011; Li et al. 2011b). Maintenance of long-term memories and associated LTP in glutamatergic synapses requires PKM ζ as well as PKC λ activity, and both kinases are blocked by ZIP, which explains effects of ZIP in PKM ζ -null mice (Sacktor and Hell 2017). PKM ζ maintains memories by inhibiting the endocytosis of GluA2-AMPA, and ZIP interference of opioid CPP memory maintenance is prevented by blocking GluA2 endocytosis (Li et al. 2011b). The BLA-NAc projection also mediates opioid reward. Tetanic stimulation of BLA projections to the accumbens induces an NMDAR- and dopamine D1 receptor-dependent LTP (Floresco et al. 1998, 2001), and BLA-NAc projections gate the responding of NAc neurons to morphine in an opioid experience- and NMDAR-dependent manner. In opioid-naïve rats, dopamine D1 receptor activation in the BLA is required for increased NAc neuronal firing in response to morphine, whereas dopamine D2 receptors are required for increased NAc neuronal activity in opioid-dependent rats (Lintas et al. 2011). NMDAR activation in BLA-NAc projections is also required for morphine CPP (Lintas et al. 2012). In line with these findings, repeated morphine exposure potentiates glutamate release and augments synaptic strength (increased A/N ratio) in BLA-accumbens projections onto D1-MSNs (Zhu et al. 2016; Cui et al. 2014). Thus, BLA-accumbens projections are likely important mediators of opioid reward.

The BLA also sends a dense projection to the mPFC (Sarter and Markowitsch 1983), and this projection may be important for opioid reward.

AMPA or NMDAR antagonists applied locally within the PL mPFC enhance CPP to subthreshold doses of morphine, and this effect depends on neuronal activity within the BLA (Bishop et al. 2011; De Jaeger et al. 2013). Protein synthesis in both the BLA and mPFC is also required for the consolidation of morphine CPP memory, and increased PL neuronal firing in a morphine-conditioned context is abolished following protein synthesis blockade in the BLA (Gholizadeh et al. 2013). In addition, the BLA-PL projection could provide an indirect mechanism whereby BLA neurons can activate the NAc to drive drug relapse (Stefanik and Kalivas 2013). However, the precise mechanisms by which BLA-PL projections drive drug seeking requires further investigation given that BLA projections tonically suppress neuronal population activity in the PL mPFC (Lintas et al. 2012), but are capable of increasing the firing of a subset of mPFC-accumbens-projecting neurons (McGinty and Grace 2008). The BLA and mPFC are reciprocally connected, but evidence on the role of PL-BLA projections in addiction is lacking. In addition, the BLA and HPC are heavily interconnected, and interactions between these brain regions regulate the contextual renewal of cocaine seeking (Fuchs et al. 2007). Both the HPC and BLA are also involved in the reinstatement of heroin seeking, possibly through similar direct interactions (Rogers et al. 2008; Bossert and Stern 2014).

The central amygdala (CeA) is located adjacent to the BLA and is heavily innervated by it (Royer et al. 1999). Ionotropic glutamatergic neurotransmission in the CeA is required for the acquisition of morphine CPP and naloxone CPA (Watanabe et al. 2002; Rezayof et al. 2007; Glass et al. 2008a). The expression of morphine CPP also requires NMDAR and downstream ERK activation in the CeA (Watanabe et al. 2002; Li et al. 2011a), and NMDAR and ERK signaling in the CeA are necessary for the increased expression, or incubation, of morphine CPP after protracted withdrawal (Li et al. 2008). MORs in the CeA colocalize with AMPARs and NMDARs in dendritic compartments, and morphine selectively down-regulates GluA2-, but not GluA1-containing AMPARs colocalized with MORs (Glass et al. 2009; Beckerman et al.

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2013). Morphine injections also transiently up-regulate CeA neuronal GluA1 surface expression, and this process is required for morphine reward, as viral overexpression of GluA1 potentiates and viral GluA1 knockdown delays the acquisition of morphine CPP. Interestingly, knockdown of GluA1 in the CeA also delays the extinction of morphine CPP, suggesting that this manipulation induces a general learning deficit (Cai et al. 2013). Protracted withdrawal from morphine self-administration also up-regulates GluA1 expression in the CeA (Hou et al. 2015). When chronic pain is experimentally induced in rats prior to morphine self-administration, extinction is impaired, and this can be reversed by viral knockdown of GluA1 in the CeA or by the overexpression of the epigenetic regulator MeCP2, which suppresses GluA1 transcription (Hou et al. 2015). Combined, these studies indicate that ionotropic glutamate receptor signaling and changes in CeA AMPAR subunit composition may be critical for the formation and expression of opioid memories. Indeed, GluA2-lacking AMPAR in the CeA are required for morphine CPP memory formation (Cai et al. 2013).

The CeA might also mediate the aversive state associated with opioid withdrawal by means of its projections to other regions, including the VTA, bed nucleus of the stria terminalis (BNST), the PVT (see below), and the periaqueductal gray (PAG) (Rizvi et al. 1991; Geisler and Zahm 2005; Zahm et al. 2011; Chang et al. 2019). Of these regions, the PAG is particularly well known for its role in opioid withdrawal symptoms, which are mediated by local inflammatory factors and prevented by increasing local endogenous opioid levels (Maldonado et al. 1992; Hao et al. 2011). Endogenous opioids released from CeA in the PAG produce analgesic effects, and this process may be disrupted during opioid withdrawal (Pavlovic et al. 1996). CeA interactions with the BNST are also implicated in opioid withdrawal. Naloxone-precipitated opioid withdrawal activates both regions (Gracy et al. 2001), and withdrawal-induced changed functioning of this extended amygdala circuit may drive a persistent aversive state (Harris and Aston-Jones 2007). Lesions of either the BNST or

CeA attenuate naloxone CPA and prevent the naloxone-induced expression of Fos across the extended amygdala (Nakagawa et al. 2005b). Both the CeA and the BNST are also required for heroin-, cue-, and stress-induced reinstatement of heroin seeking (Shalev et al. 2001; Rogers et al. 2008). In addition, the CeA is required for stress-induced reinstatement of morphine CPP (Ma et al. 2008), and BNST Fos expression correlates with the magnitude of morphine CPP only in morphine-dependent rats (Harris and Aston-Jones 2003). Combined, these data indicate that opioid withdrawal requires functional connections between the CeA and BNST (Nakagawa et al. 2005b). In turn, the BNST directly innervates the VTA and morphine withdrawal increases synaptic strength (increased A/N ratio), specifically in BNST neurons projecting to the VTA (Dumont et al. 2008).

PARAVENTRICULAR NUCLEUS OF THE THALAMUS PATHWAYS

The PVT has recently emerged as an important regulator of aversive motivated states and of opioid withdrawal symptoms (Zhu et al. 2016). During early withdrawal from repeated morphine injections, increased synaptic strength (increased A/N ratio) is observed onto PVT neurons as well as an increase in neuronal activity (McDevitt and Graziane 2019). Early morphine withdrawal also increases synaptic strength in the PVT-NAshell projection (increased A/N ratio and increased surface expression of GluA2-lacking AMPAR). These changes in synaptic functioning occur specifically in PVT projections to NAc D2-MSNs. Inhibition or optogenetic depotentiation of PVT-accumbens projections reduces naloxone-precipitated withdrawal symptoms and blocks the expression of naloxone CPA (Zhu et al. 2016). In addition to the NAc, the PVT also innervates a number of regions implicated in opioid reward and withdrawal, including the BLA, CeA, and BNST (Vertes and Hoover 2008; Do-Monte et al. 2015; Penzo et al. 2015) and PVT neurons projecting to these areas express MOR (Goeddecke et al. 2019). However, the precise role of the PVT in opioid addiction requires further investiga-

tion given that PVT neurons are not activated during the expression of morphine CPP (Harris and Aston-Jones 2003) and that stimulation of PVT neurons suppresses stress-potentiated heroin seeking following abstinence in a self-administration model (Chisholm et al. 2019). In light of these findings, it will be important to clarify the role of PVT neurons and their projections during opioid withdrawal and reinstatement of opioid seeking following opioid self-administration. Given that the PVT is also involved in pain processing (Chang et al. 2019), it may regulate both the negative affective state and the hyperalgesia that is associated with opioid withdrawal (Tilson et al. 1973).

HIPPOCAMPAL PROJECTIONS

The hippocampus and associated subiculum and dentate gyrus comprise the hippocampal formation (HPC), and these structures are heavily interconnected with the BLA, NAc, and prefrontal cortex (van Strien et al. 2009). HPC inputs to the NAc can drive MSNs toward a depolarized “up state,” which renders them more responsive to inputs from the BLA and mPFC, and these up states are prolonged in the presence of dopamine (O'Donnell et al. 1999). The ventral, but not dorsal, subiculum of the HPC and its projections to the NAc are necessary for context-induced reinstatement of heroin seeking (Bossert et al. 2012, 2016). Although projections from the ventral subiculum to the IL mPFC are not necessary for reinstatement of opioid seeking, they become activated during reinstatement (Bossert et al. 2016) and may instead mediate extinction of opioid memories as shown for fear memories (Soler-Cedeño et al. 2019). Although reductions in spine density within the HPC only occur following self-administered morphine (Robinson et al. 2002), the HPC does mediate the primary rewarding effects of opioids (Corrigall and Linseman 1988; Sell et al. 2000), and the acquisition and reinstatement of morphine CPP requires functional connectivity between the HPC and mPFC (Wang et al. 2019). Morphine acutely down-regulates GluA2 in the HPC, and the recall of morphine-context associations increases

the phosphorylation of GluA1 and surface expression of GluA2-lacking AMPAR in the HPC. Interfering with GluA1 phosphorylation blocks context-specific expression of morphine sensitization (Xia et al. 2011). Extinction of morphine CPP also increases the surface expression of phosphorylated GluA1 in the HPC (Billa et al. 2009). In addition to AMPAR regulation in this structure, morphine also increases local GluN2B expression in the HPC (Ma et al. 2006), and NMDAR antagonists applied locally to the HPC block the acquisition, but not the expression, of morphine CPP and naloxone CPA (Zar-rindast et al. 2007; Hou et al. 2009). NMDAR in the dorsal HPCs are also necessary for the extinction of opioid memories. NMDAR antagonists block naloxone CPA extinction learning by preventing the recruitment of ERK signaling downstream in the BLA (Wang et al. 2015).

Chronic opioid use may reduce HPC function, and human heroin addicts show abnormal functional HPC connectivity (Ma et al. 2011a). In addition, chronic morphine reduces HPC neurogenesis (Eisch et al. 2000). MOR activation in the HPC also impairs the acquisition of HPC-dependent spatial memory tasks and disrupts LTP in this structure (Spain and Newsom 1991; Pu et al. 2002). These effects are caused by the MOR-mediated dephosphorylation of GluA1 and down-regulation of hippocampal AMPAR, which is reversed with a MOR antagonist (Kibaly et al. 2016). By contrast, chronic MOR antagonism with naloxone potentiates spatial learning and memory in opioid-naïve animals (Spain and Newsom 1991; Kibaly et al. 2016).

NUCLEUS ACCUMBENS PROJECTIONS AND CELL-TYPE-SPECIFIC SYNAPTIC NEUROADAPTATIONS

Opioids induce neuroadaptations in glutamatergic synapses onto NAc neurons. Protracted opioid withdrawal increases the frequency and amplitude of glutamatergic neurotransmission onto D1-MSNs, while reducing transmission onto D2-MSNs (Hearing et al. 2016). Opioid withdrawal also increases the surface expression of GluA2-lacking AMPAR onto D1-MSNs. These processes and the reinstatement of morphine

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CPP following protracted withdrawal are reversed by the optogenetic depotentiation of glutamatergic inputs from the IL prefrontal cortex onto NAc shell neurons, indicating that synaptic adaptations in this pathway may underlie opioid memories (Hearing et al. 2016). Reexposure to morphine also depotentiates IL-NAc shell synapses, which is thought to promote the reinstatement of morphine CPP (Madayag et al. 2019). Thus, optogenetic activation of the IL-NAc shell pathway may occlude this endogenous depotentiation and thereby prevent reinstatement.

Following chronic treatment, morphine acutely reduces GluA1 surface expression in D1-MSNs (Glass et al. 2008b), and, compared to acute withdrawal from heroin self-administration, protracted withdrawal further down-regulates GluA1 expression in the NAc (Kong et al. 2014). Through a similar process, repeated morphine injections also cause the internalization of GluA2-containing AMPAR in D2-MSNs. This leads to the generation of silent synapses that only express NMDAR and do not respond to synaptically released glutamate. Following protracted opioid withdrawal, silent synapses are pruned on D2-MSNs, which produces a loss of spine density in the NAc shell. Silent synapse formation in D2-MSNs is prevented by blocking GluA2-AMPA endocytosis in the NAc shell prior to morphine injections, and this intervention also blocks the acquisition of morphine CPP (Graziane et al. 2016).

Acute withdrawal from self-administration of the short-acting opioid remifentanyl also induces neuroadaptations in NAc shell D1-MSNs. Remifentanyl withdrawal does not alter the intrinsic excitability of D1- or D2-MSNs or glutamatergic synaptic input to these cells, but it selectively blunts the functioning of release-inhibiting MORs expressed on glutamatergic inputs to D1-MSNs, thereby enhancing glutamate release onto these cells (James et al. 2013). Reduced mGluR2/3 function following opioid withdrawal also augments the synaptic release of glutamate in the NAc, causing increased synaptic strength and increased intrinsic excitability of MSNs (Robbe et al. 2002; Wu et al. 2012a; Shen and Kalivas 2013; Qian et al. 2019). These opioid withdrawal-induced changes in gluta-

matergic synaptic neurotransmission are prevented by coadministering a mGluR2/3 agonist with morphine treatment (Wu et al. 2012a).

Combined, these synaptic neuroadaptations onto NAc neurons produce a shift in accumbens activation of D1-MSNs relative to D2-MSNs, which likely promotes motivation for opioids, opioid reward, and reinforcement (Lobo and Nestler 2011).

Both D1- and D2-MSNs innervate neurons in the downstream ventral pallidum, whereas only D1-MSNs innervate the VTA and substantia nigra (Kupchik et al. 2015). NAc projections to the ventral pallidum and VTA differentially regulate heroin seeking in a subset of addiction-prone rats (O'Neal et al. 2019). The NAc to the ventral pallidum pathway has an overall inhibitory effect on heroin seeking during cue-induced reinstatement in extinguished rats. By contrast, D1-MSN projections from the NAc shell to the VTA drive heroin seeking during cue-induced reinstatement (O'Neal et al. 2019). Despite the involvement of MOR expressed on striatal D1-MSN terminals on the primary rewarding and reinforcing effects of opioids (Cui et al. 2014; Matsui et al. 2014), manipulating activity in accumbens-pallidal or accumbens-VTA projections does not alter the motivation to take heroin in a progressive ratio test (O'Neal et al. 2019).

VENTRAL TEGMENTAL DOPAMINE PROJECTIONS

The VTA sends dopamine projections to all limbic brain regions (Morgane et al. 2005). Dopaminergic neurons in the VTA and their projections are critically important for opioid reward and reinforcement (Wise 1989; Vargas-Perez et al. 2009; Lintas et al. 2011). Inhibition of dopamine neurons in the VTA reduces heroin self-administration (Corre et al. 2018), whereas stimulation of VTA-accumbens projections potentiates morphine CPP (Koo et al. 2012). Opioids directly increase dopamine neuron firing (Koo et al. 2012) and this effect occludes the behavioral reinforcing effects of optogenetic self-stimulation of dopamine neurons (Corre et al. 2018). In the VTA, BDNF signaling in

dopamine neurons partially reduces the effects of opioids on these neurons, and blocking the actions of endogenous BDNF in VTA dopamine neurons potentiates morphine CPP (Koo et al. 2012). However, the role of BDNF in the VTA is complex because, following opioid withdrawal, BDNF also induces a switch from inhibitory to excitatory GABA_A receptor-mediated responses in local GABAergic interneurons, which may potentiate the tonic suppression of dopamine neurons by these cells (Laviolette et al. 2004; Vargas-Perez et al. 2009; Madhavan et al. 2010). This switch is necessary for rendering opioid reward dependent on mesolimbic dopamine signaling and independent of nondopaminergic signaling in the PPTg (Vargas-Perez et al. 2009, 2014).

NMDAR activation and downstream protein kinase A (PKA) signaling in the VTA are necessary for the acquisition and expression of morphine CPP (Popik and Kolasiewicz 1999; Harris et al. 2004; Hu et al. 2014). However, unlike the accumbens, VTA GluN2B-NMDAR are not involved in morphine CPP (Kao et al. 2011). The acquisition of morphine CPP also requires the activation of AMPAR in the rostral VTA and downstream CREB-mediated transcription of GluA1 and tyrosine hydroxylase (Carlezon et al. 2000; Olson et al. 2005; Shabat-Simon et al. 2008). However, activation of the same signaling cascade in the caudal VTA results in conditioned aversion to morphine (Carlezon et al. 2000; Olson et al. 2005).

Glutamatergic neurotransmission in the VTA also mediates heroin seeking in a self-administration model, where intra-VTA mGluR2/3 agonists reduce renewal of drug seeking (Bossert et al. 2004). This effect is likely due to a mGluR2/3-mediated reduction in presynaptic glutamate release (Kenny and Markou 2004). VTA neurons receive glutamatergic inputs from a large number of brain regions (Geisler et al. 2007), and glutamatergic PPTg projections to the VTA are especially important for opioid reward and reinforcement (Vargas-Perez et al. 2007; Yoo et al. 2017). PPTg NMDAR activation is rewarding and NMDAR antagonists in the PPTg block the acquisition of morphine CPP (Heinmiller et al. 2009). Although PPTg projections to the VTA contain both glutamatergic

and cholinergic afferents, lesioning PPTg cholinergic neurons does not alter heroin self-administration or heroin CPP (Steidl et al. 2014). Indeed, glutamatergic PPTg inputs to the VTA preferentially target dopaminergic neurons, and optogenetic stimulation of this pathway drives operant reinforcement (Yoo et al. 2017).

Synaptic inputs onto VTA neurons undergo neuroadaptations following exposure to opioids. Increased activity of dopamine neurons following opioid exposure results from the combined effects of increased glutamatergic and decreased GABAergic neurotransmission onto these neurons (Baimel and Borgland 2015). Morphine increases the frequency and amplitude of glutamatergic neurotransmission and the strength of glutamatergic inputs (increased A/N ratio) onto VTA dopamine neurons (Saal et al. 2003; Baimel and Borgland 2015). Morphine exposure also disrupts an NMDAR- and nitric oxide-dependent potentiation of GABAergic neurotransmission onto dopamine neurons (Nugent et al. 2007). Both rostral and caudal VTA neurons receive inputs from orexin neuropeptide-expressing neurons in the LH, and increased Fos expression in these cells is associated with increased expression of morphine CPP (Richardson and Aston-Jones 2012). VTA orexin signaling is necessary for both the above-mentioned glutamatergic and GABAergic synaptic neuroadaptations produced by morphine onto VTA dopamine (Baimel and Borgland 2015).

Both naloxone-induced and protracted opioid withdrawal produce neuroadaptations onto VTA dopamine neurons as well, which render these neurons unresponsive to MOR agonists (Georges et al. 2006). Under these conditions, MOR agonists fail to inhibit GABA release onto dopamine neurons from rostromedial tegmental (RMTg) and NAc terminals, but not from local interneurons (Matsui et al. 2014). Protracted withdrawal-induced changes in MOR inhibition of GABA release is accompanied by a reduction in the activity of RMTg neurons, as well as a reduction in ionotropic glutamate neurotransmission onto dopamine neurons (Jalabert et al. 2011; Kaufling and Aston-Jones 2015). Together, these neuroadaptations likely

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underlie components of the negative affective state associated with opioid withdrawal.

The VTA is the main source of dopamine to the mPFC, amygdala, and HPC (Morgane et al. 2005), and systemic pharmacological manipulations of dopamine signaling impact all of these regions. The specific role of dopamine projections to these regions can also be ascertained using selective 6-hydroxy-dopamine (6-OH-DA) dopamine lesions. VTA-mPFC mesocortical dopamine projections are necessary for the rewarding effects of opioids, and 6-OH-DA lesions of this projection block CPP produced by opioids injected into the VTA (Narita et al. 2010). Dopamine signaling at D1 receptors in the mPFC is also required for the acquisition of morphine CPP and for stress- and cue-induced reinstatement of heroin seeking following extinction of heroin self-administration (See 2009; Tobin et al. 2013; Wang et al. 2019).

Dopamine projections to the BLA also mediate the acquisition of morphine CPP, and the specific type of dopamine receptor involved depends on the history of opioid use (Lintas et al. 2011). In opioid-naïve rats, blockade of BLA D1 receptors and downstream ERK signaling prevents the acquisition of morphine CPP, whereas morphine CPP is mediated by D2 receptors and calmodulin-dependent kinase 2 (CaMKII) signaling in opioid-dependent rats (Lintas et al. 2011; Lyons et al. 2013). Chronic opioid administration also produces a switch in the effects of dopamine on BLA neurons from inhibition to excitation, which is mediated by a dopamine D1 receptor- and PKA-dependent enhancement of presynaptic glutamate release. In opioid-dependent rats, blockade of D1 receptors in the BLA prevents the acquisition of naloxone CPA (Li et al. 2011c). However, the effects of BLA D1 signaling on the acquisition of opioid memories may be explained by a state dependence of these memories (Ting-A-Kee et al. 2013). D1 receptor antagonists in the BLA also block stress-induced reinstatement of heroin seeking in a self-administration model (Tobin et al. 2013). Within the CeA, dopaminergic inputs are also required for the acquisition and expression of opioid memories. D1 or D2 receptor agonists applied locally to the CeA potentiate, whereas antagonists

block, morphine CPP (Rezayof et al. 2002; Zarindast et al. 2003).

Dopamine acting on D1 and D2 receptors in the hippocampus also mediates the acquisition, expression, and reinstatement of morphine CPP (Rezayof et al. 2003; Hu et al. 2014; Khakpour-Taleghani et al. 2015; Wang et al. 2019). Although recent work indicates that the locus coeruleus sends a more prominent dopamine projection to the HPC than the VTA (Takeuchi et al. 2016), hippocampal D1 receptors are required for the potentiation of morphine CPP by NMDAR activation in the VTA, indicating that at least the acquisition of morphine CPP depends on VTA-HPC dopamine projections (Hu et al. 2014).

The mesolimbic dopamine projection to the NAc regulates both opioid reward and reinforcement. Heroin self-administration activates VTA-NAshell projecting neurons, and increases dopamine levels in the NAshell (Corre et al. 2018). Consistent with a role in opioid reward, lesioning VTA-NAshell dopamine projections increases morphine intake (Gao et al. 2013). Dopamine signaling in the NAc is also necessary for the reinstatement of opioid seeking. Lesioning the VTA-NAshell projection or blocking NAshell D1 receptors after withdrawal from self-administration reduces morphine seeking after forced abstinence (Gao et al. 2013). Dopamine in the NAshell is also required for stress-induced reinstatement and context-induced renewal of heroin seeking (Bossert et al. 2007; Tobin et al. 2013). By contrast, NAc core dopamine signaling is required for cue-induced reinstatement of heroin seeking after extinction (Bossert et al. 2007) and for the potentiation of heroin seeking by stress following forced abstinence (D'Cunha et al. 2017). Dopamine in the dorsal striatum (most likely originating from the substantia nigra) is also required for opioid seeking after abstinence and the contextual renewal of heroin seeking, and this is mediated by both D1 and D2 receptors (Bossert et al. 2009; Gao et al. 2013).

CONCLUSION

In this review, we summarize the main mechanisms by which glutamatergic neurotransmis-

sion drives the primary rewarding effects of opioids, and the glutamate systems that are responsible for the formation and expression of opioid memories. Given that glutamate is the main excitatory neurotransmitter in the brain, and that glutamatergic synaptic plasticity underlies memory formation, it is not surprising that glutamate is of critical importance for opioid memories. However, the specific mechanisms by which opioids alter the functioning of glutamatergic synapses provides important insights in the neuronal substrates that underlie opioid addiction. For instance, persistent opioid-induced changes in the synaptic density and subcomposition of two main major ionotropic glutamate receptors likely contributes to the long-term changes in the functioning of the reward system after opioid exposure. The selective increase in the expression of GluN2B-containing NMDAR, and GluA2-lacking AMPAR at glutamatergic synapses fundamentally changes the function of these synapses, and this may underlie the persistent vulnerability to relapse when these synapses are reactivated by opioid reminder cues. Dysfunction in the regulation of glutamate release by type II metabotropic glutamate receptors and glutamate uptake by glial cells may additionally contribute to this enduring vulnerability. Further elucidating the mechanisms by which memories for opioid reward and withdrawal are coded in the glutamatergic synapses in cortical and allocortical circuits and their projections to the basal ganglia will also provide important information about how opioid memories might be manipulated or weakened to prevent relapse. An exciting recent discovery is that, at least in prefrontal circuits, drug use and extinction memories may be stored in distinct neuronal ensembles, which could enable the selective targeting of opioid memories while leaving other memories intact. For instance, disrupting the glutamatergic receptor mechanisms that are required for opioid memory reconsolidation may prove useful for reducing drug craving. Therapies that recruit glutamatergic plasticity and strengthen the formation and consolidation of extinction memories could also be a fruitful approach for reducing relapse. Although much work remains to be done in

dissecting the neural circuits and synaptic mechanisms that underlie opioid memories, major progress has been made in the last two decades in understanding the mechanisms by which these memories are coded in glutamatergic synapses and systems. The studies reported here strongly suggest that therapies that target glutamatergic systems will be effective treatments for opioid addiction.

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