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The Opioid-Addicted Tetrapartite Synapse

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Abstract

Opioid administration in preclinical models induces long-lasting adaptations in reward and habit circuitry. The latest research demonstrates that in the nucleus accumbens opioid-induced excitatory synaptic plasticity involves pre- and post-synaptic elements, as well as adjacent astroglial processes and the perisynaptic extracellular matrix. Here we outline opioid-induced modifications within each component of the tetrapartite synapse and provide a neurobiological perspective on how these adaptations converge to produce addiction-related behaviors in rodent models. By incorporating changes observed at each of the excitatory synaptic compartments into a unified framework of opioid-induced glutamate dysregulation, we highlight new avenues for restoring synaptic homeostasis that might limit opioid craving and relapse vulnerability.

Keywords

opioid; presynapse; postsynapse; extracellular matrix; astroglia; plasticity

INTRODUCTION

Opioid addiction is a chronic disorder, characterized by compulsion to use drugs and a propensity to relapse, even after the acute opioid withdrawal syndrome has elapsed (1). Remarkably, 80% of heroin users report early use of opioid analgesics (2), supporting the notion that opioid exposure produces long-lasting brain adaptations that can lead to compulsive drug use and seeking. Such brain adaptations manifest in clinical symptoms including impaired cognition (3), deficits in learning, memory, and attention (4), and increased impulsivity (5, 6). These impairments are thought to arise from pathological disruptions in prefrontal and subcortical circuits that contribute to an uncontrollable desire to use opioids (craving), and can escalate drug use and increase the likelihood of relapse (6–8). An important consequence of these adaptations is that abstinent heroin users exhibit motivational bias toward heroin-related stimuli (9, 10), and elevated cue-reactivity in humans is linked to higher self-reported craving (11). These cognitive changes in opioid

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users are long-lasting with studies pointing to drug craving and associated persistent deficits in decision-making in former opioid users after nearly two years of abstinence (12). Available medications poorly address the enduring pathophysiology that causes opioid associated environmental stimuli or stress to trigger relapse in humans.

PRECLINICAL MODELS OF OPIOID ADDICTION

Linking brain cellular pathophysiology with symptoms of human opioid use disorder (OUD) requires preclinical animal models that mimic behavioral phenotypes observed in humans with OUD. For example, rats receiving chronic non-contingent administration of opioids exhibit deficits in learning and memory tasks (13–16) and opioid use and abstinence in rodents involves development of social withdrawal (17), anxiety- and depression-like behaviors (18, 19), vulnerability to stressors (20), and decreased motivation for natural rewards (21), similar to symptoms observed in OUD (3, 4, 22–26). In self-administration models of OUD, rodents perform operant tasks to receive intravenous opioid infusions, often paired with a conditioning stimulus (cue). Additionally, punishment or aversive/threatening cues can be incorporated into operant training to assess an animal's loss of motivation to acquire the drug reward (27). Animals typically undergo a withdrawal phase after self-administration with or without extinction training, before undergoing stimulus-induced opioid seeking, at which point a relapse-like state is provoked by exposure to a drug-associated context or cue, an unconditioned stressor, or a priming opioid injection. Self-administration models can also include measures of use escalation, withdrawal, extinction learning, and relapse, thus incorporating many features of OUD (28, 29). In addition, rodents can be trained to self-administer natural rewards, like sucrose, and neuroadaptations occurring during self-administration, extinction or reinstated seeking of natural rewards can be compared to those observed in animals trained to self-administer opioids (30). In this review, we focus on the pathophysiology induced by non-contingent and self-administered opioids, and cue-induced opioid seeking, where the neurobiological adaptations are not confounded by acute drug pharmacology.

Although dopamine strongly contributes to the reinforcing properties of all drugs of abuse including opioids (reviewed in (31)), a robust literature implicates dysregulated homeostasis at glutamatergic synapses in OUD-associated cognitive impairments and cue reactivity (i.e. craving) (32, 33). For example, glutamate levels measured by magnetic resonance spectroscopy in reward-associated brain regions in opioid-dependent users correlate positively with measures of impulsivity (34), and opioid-conditioned cues evoke craving in parallel with fMRI measures of increased activity in cortico- and amygdalo-striatal glutamatergic circuits (35). In this review, we explore the preclinical data that identify the cellular underpinnings of these of OUD-induced glutamatergic adaptations found in human imaging studies.

THE TETRAPARTITE GLUTAMATERGIC SYNAPSE

Glutamatergic neurotransmission subserves cognitive functions, including learning and memory (36, 37), and glutamatergic plasticity contributes to addiction- and relapse-related behaviors (38). Although the contribution of glutamatergic plasticity to addiction has been

more thoroughly characterized in models of psychostimulant use (reviewed in (39)), important roles have been discovered for glutamatergic plasticity in many aspects of opioid addiction, including opioid-related learning (40, 41), somatic withdrawal symptoms (42, 43), and motivated drug seeking (44). In fact, adaptations shared between the two drug classes, particularly during cued seeking, point to the possibility of a common addiction cellular pathophysiology. However, there we will also point out instances where adaptations are different between psychostimulants and opioids, and bring particular focus to opioid-induced excitatory synaptic adaptations in the nucleus accumbens (NA, Figure 1A), a brain region widely identified as critical for generating motivated behaviors, including opioid use and seeking (45). The glutamatergic cortical and subcortical NA afferents are, in part, topographically organized and make differential contributions to the shell (NAshell) and core (NAcore) subcompartments of the NA (Figure 1A). Research from different laboratories tends to focus on either the NAshell or NAcore, and we will note distinct impacts by opioids on glutamate homeostasis in the two subcompartments. Figure 1A illustrates the general circuitry where glutamatergic synapses undergo enduring and transient plasticity after opioid extinction and during provoked opioid seeking, respectively.

Opioid-induced excitatory synaptic plasticity within the NA involves adaptations at multiple levels, including, but not limited to presynaptic transmitter release (44), the relative abundance and composition of post-synaptic receptors (46), and astroglial regulation of glutamate uptake and release (47, 48). More recently, a fourth synaptic compartment was found to be modified by opioid use, the perisynaptic extracellular matrix (ECM) (49). The ECM is a proteinaceous structure that functions as a signaling domain to regulate post-synaptic plasticity (50). Considered together, these four synaptic compartments (the canonical pre- and postsynapse, astroglial perisynaptic processes and the ECM) comprise the *tetrapartite synapse* (51) (Figure 2) regulate classic forms of pre- and postsynaptic plasticity, such as long-term depression and potentiation (52). More recently, the tetrapartite synapse as an integrated unit has been shown to regulate the maladaptive plasticity produced by repeated use of many addictive drugs, including opioids (53). Below we outline the opioid-associated neuroadaptations in each of the four components, and then integrate these data back into the synaptic tetrapartite structure.

PRE-SYNAPTIC PLASTICITY

Glutamatergic afferents to the accumbens regulate reinstated opioid seeking (45, 53) and inactivation of prelimbic cortical afferents to NAcore disrupts heroin seeking, as well as the increase in extracellular glutamate produced during heroin-primed reinstatement (44). The increase in glutamate release evoked during heroin reinstatement can be blocked by stimulating presynaptic metabotropic glutamate receptor2/3 (mGluR2/3), which reduces presynaptic glutamate release probability (54, 55). Moreover, deletion of mGluR2 in transgenic rats enhances heroin intake during self-administration, potentiates morphine-induced analgesia and augments naloxone-precipitated withdrawal symptoms (56). mGluR2/3 is $G_{i\alpha}$ -coupled and signaling through $G_{i\alpha}$ -coupled receptors is negatively regulated by activator of G-protein signaling 3 (AGS3). Down-regulating AGS3 in NAcore, but not NAshell, potentiates mGluR2/3 signaling and decreases reinstated heroin seeking (57, 58). Akin to mGluR2/3, presynaptic cannabinoid1 (CB1) receptors regulate glutamate

release probability, and intra-accumbens administration of AM251, a CB1 receptor antagonist, diminishes the duration required to extinguish morphine conditioned place preference (CPP), as well as the duration of morphine-induced reinstatement of CPP (59). In addition, the CB1 receptor antagonist, AM4113, dose-dependently suppresses heroin self-administration (60). Taken together, these data demonstrate that regulating glutamate presynaptic release probability in the NAc core inhibits opioid-seeking.

In addition to presynaptic regulation of glutamate release, neuroadaptations in the excitability of glutamatergic neurons projecting to the accumbens can also regulate opioid use and seeking. For example, the firing frequency in glutamatergic afferents from the amygdala, but not PFC is increased after chronic morphine treatment (61). There are two primary subtypes of projection neurons (medium spiny neurons, MSNs) in NA that express D1- or D2-dopamine receptors (62). In general, plasticity in excitatory signaling within the NA onto D1-MSNs is associated with enhanced opioid seeking (63, 64), while signaling at D2-MSNs triggers extinction-related behaviors (65). Within the NAc shell there is increased glutamate release probability at synapses on D1-MSN, while at D2-MSN synapses there is decreased release probability after repeated non-contingent morphine (66). Opioid self-administration also increases glutamatergic release probability on D1-MSNs (67). Alternatively, morphine withdrawal increases c-Fos expression in paraventricular thalamic (PVT) projections to medial NAc shell (68). Specifically, PVT to NAc shell D2-MSNs show increased postsynaptic currents that are normalized by depotentiating optogenetic photostimulation of PVT terminals synapsing onto D2 MSNs (68). In a μ -opioid receptor (MOR)-null transgenic mouse, targeted rescue of MOR only in striatal D1 MSNs restores morphine-induced CPP (69). Similarly, remifentanyl self-administration reduces sensitivity of D1-, but not D2 MSNs, to MOR modulation in NAc shell. While these studies provide insight into cell- and pathway-specific alterations associated with opioid exposure in NAc shell (Figure 1B), little is known about such adaptations in the NAc core, or how such changes in release probability are affected by extinction and reinstatement.

POST-SYNAPTIC PLASTICITY

Spine morphology

On GABAergic MSNs the principal post-synaptic computational units are dendritic spines (70). Long-term opioid use affects postsynaptic glutamatergic plasticity and disrupting post-synaptic plasticity is sufficient to attenuate reinstated seeking induced by opioid-associated cues or opioid prime (44, 46). Opioid administration in preclinical models produces actin-dependent morphological plasticity in dendritic spines (46), including in spine head diameter, density, and neck length, all of which indicate opioid-induced changes in spine signaling capacity (71, 72). Early reports using Golgi staining found that both contingent and non-contingent repeated morphine administration produce wide-spread, enduring reductions in spine density in the NAc shell (73). More recent 3-dimensional reconstruction of dye-filled MSNs reveals that heroin self-administration decreases spine head diameter, as well as impairs LTP and LTD in PFC projections to the NAc core (46, 74). The thinner spines observed after 14d of extinction from heroin exhibited increased surface expression of GluN2B and reduced AMPA/NMDA ratio, measures indicating blunted capacity for

synaptic plasticity (46). These adaptations are necessary for reinstated drug seeking initiated by heroin prime, and primed drug seeking transiently potentiates glutamatergic inputs to MSNs, as indicated by increased spine density, spine head diameter, and field EPSCs (46).

Receptor composition and density

In addition to morphological plasticity, opioid administration causes functional plasticity of excitatory synapses due to composition and abundance of post-synaptic receptor insertion. As mentioned above, morphological adaptations in NAc core spines during heroin extinction are associated with increased surface expression of post-synaptic GluN2B and reduced AMPA/NMDA receptor expression (46). Thus, an important mechanism for opioid-induced post-synaptic adaptations involves receptor internalization or downregulation on spines (46), and changes in density or composition of receptors at dendritic spines modulate synaptic output (75). For instance, ionotropic glutamate receptors on neurons in the central amygdala diffuse to dendritic compartments more proximal to the soma after non-contingent morphine administration, facilitating signal propagation (76). Such changes are expected to impact dendritic processing of glutamatergic and GABAergic inputs.

NMDA glutamate receptors (NMDARs) are critically involved in linking glutamate transmission with synaptic plasticity and behavior (77) and in regulating learning and memory processes (78). NMDA receptors are implicated in a number of opioid-mediated addictive behaviors including self-administration (79, 80), extinction learning (81), naloxone-precipitated withdrawal severity (82), drug-related contextual learning (83), and the aversion experienced during abstinence (81). Moreover, NMDARs are involved in the LTP and LTD disruption observed after heroin extinction, which is required for reinstated seeking (74). Specifically, heroin self-administration produces enduring upregulation of NMDAR containing GluN2B subunits, and selective blockade of GluN2B in NAc core prevents reinstated heroin seeking (46).

AMPA receptors (AMPAARs) are activated at resting membrane potential and are primary mediators of postsynaptic transmission in NA (84). GluA1 and GluA2 AMPAR subunits undergo dynamic activity-dependent trafficking at synapses (85), and induce changes in spine morphology and synaptic strength (86). GluA1 surface expression is increased by experience-dependent plasticity, facilitated by the fact that homomeric GluA1 AMPARs are permeable to Ca^{2+} (87, 88), leading to increased channel conductance, LTP, and synaptic strength (89, 90). AMPAR composition and trafficking is highly responsive to opioid administration (66, 91). For instance, repeated non-contingent morphine increases surface expression GluA2-lacking AMPARs in hippocampal synapses, limiting the capacity of these synapses to undergo LTD (92). Subsequent studies demonstrated that context-dependent behavioral sensitization to morphine is blocked by disrupting GluA1-phosphorylation in the hippocampus (93), because activity-dependent phosphorylation of GluA1 is linked with its expression at the synaptic surface (94). Heroin-associated cues produce similar downregulation of GluA2 in the infralimbic region of the PFC, which signals to the NAc shell (Figure 1A). However, in the absence of increased GluA1 and/or GluA3 expression, as observed in the hippocampus after non-contingent morphine (92), the overall loss of synaptic AMPARs results in synaptic depression that is necessary for cue-induced

reinstatement of seeking (95). Consistent with these findings are data showing that potentiation of AMPARs in the PFC and NAc shell facilitate extinction learning and suppress cued reinstatement (96), while glutamate release and AMPAR stimulation mediate reinstated seeking in NAc core (44).

Cell subtype specificity

How the postsynaptic adaptations described above segregate according to expression of either D1 or D2 receptors is under active investigation. High levels of NMDARs in the absence of abundant AMPARs (low AMPA/NMDA) are consistent with silent synapses that cannot overcome the NMDAR Mg^{2+} block needed to generate EPSCs (97, 98). Psychostimulants promote new synapse generation on D1-MSNs in the NAc shell that are silenced when the drug is no longer available due to enhanced expression of GluN2B-containing NMDARs and reduced AMPA/NMDA (99). During reinstatement, shuttling of Ca^{2+} -permeable AMPARs from extrasynaptic sites to synapses rapidly un-silences synapses on D1-MSNs and produces LTP (99, 100). In contrast, synaptic silencing in the NAc shell following repeated opioid treatment induces endocytosis of AMPARs on D2 MSNs, weakening spine structure and synaptic strength (Figure 1B) (46, 99). As a consequence there is a relative increase in synaptic strength on D1- versus D2-MSNs that promotes opioid seeking (66, 99). Whether a similar pattern involving post-synaptic potentiation after psychostimulants and suppressed inhibition after opioids occurs in NAc core is unknown.

ASTROCYTES

Glutamate transport and release

Substantial data support a role for astrocytes in regulating synaptic structure and function (101, 102). Astroglial modulation of synaptic excitation occurs via glutamate uptake and release that regulates the balance between synaptic and extrasynaptic glutamate (referred to as glutamate homeostasis (33, 103)). Astrocytes and their perisynaptic astroglial processes (PAPs) express glutamate receptors (104, 105), and GLT-1, the principal glutamate transporter that recovers ~90% of synaptically released glutamate (Figure 2) (106). GLT-1, which is expressed largely on PAPs, and PAP motility regulate access of synaptically released glutamate to the extrasynaptic space and facilitate efficient excitatory synaptic transmission (101).

Akin to other addictive drugs (48), contingent heroin administration reduces astroglial expression of GLT-1 in NAc core (47, 107) (Figure 2C). The enduring down-regulation of GLT-1 after heroin extinction impairs clearance of synaptically released glutamate in NAc core, as shown by increased activation of extrasynaptic NMDARs (47, 107). Down-regulated GLT-1 leads to elevated extracellular glutamate during reinstated seeking for opioids and other addictive drugs measured using *in vivo* microdialysis or glutamate biosensors (44, 108, 109). The mechanisms triggering GLT-1 downregulation are unclear, although dysregulated signaling via astroglial glutamate receptors may contribute since stimulation of astroglial mGluR3 increases GLT-1 protein expression (105) (Figure 2C). Thus, it seems likely that the opioid-associated reductions in activity of glutamatergic afferents to the accumbens discussed above contribute to downregulating GLT-1. It should

be noted that it is not known whether GLT-1 is downregulated during the opioid self-administration or withdrawal phases of operant training.

Although cue-induced glutamate release in the NAc core is largely TTX sensitive and thus of presynaptic origin (44), astrocytes can also tune presynaptic transmission through astroglial glutamate release directly onto presynaptic mGluR2/3 autoreceptors (110). Increasing astroglial glutamate release onto mGluR2/3 during cue-induced cocaine reinstatement reduces lever pressing and points to an important role for the cystine-glutamate antiporter in regulating reinstated drug seeking (110, 111) (Figure 2). The antiporter imports cystine in a 1:1 exchange for glutamate released extracellularly, which maintains glutamatergic tone on release-regulating presynaptic mGluR2/3 and facilitates glutathione synthesis (112, 113). Interestingly, in contrast with psychostimulant use (114, 115), surface expression of the catalytic subunit of the antiporter, xCT is increased in NA after heroin self-administration and extinction (47) (Figure 2). Since activating the antiporter with N-acetylcysteine reduces reinstated heroin and cocaine seeking (116, 117), it is interesting to speculate that the heroin-associated increase and cocaine-associated decrease in xCT on NAc core PAPs may selectively modulate D2- or D1-MSNs (118), respectively, akin to the different drug-dependent effects on MSN excitability discussed above.

In addition to cystine-glutamate exchange, other mechanisms of glutamate release by astrocytes have been reported (119), but none have yet been found to contribute to glutamate plasticity observed after extinction from opioids or during reinstated seeking. For instance, while stimulating astroglial MORs increases glutamate transmission in hippocampus via the glutamate channel TREK-1 (120), this channel is also downregulated after chronic opioid use (121).

Morphological plasticity

Based on the importance of glutamate uptake in cue-induced reinstatement of opioid seeking and the relatively high rates of GLT-1 surface diffusion (122), we began investigating the synaptic proximity of astroglial processes after heroin self-administration. Astroglial retraction from the synapse occurs after extinction from cocaine self-administration (123, 124). Similarly, after heroin extinction the synaptic proximity of the astroglial surface and immunoreactive GLT-1 is reduced (unpublished observations) (Figure 2C). Thus, in addition to reduced uptake of synaptically released glutamate by down-regulated GLT-1, a diminished diffusion barrier resulting from astroglial synaptic retraction may contribute to the spillover of synaptic glutamate during reinstated heroin seeking, akin to what has been observed after extinction from cocaine self-administration (44, 125). Four studies support this possibility. Down-regulated GLT-1 enhances extrasynaptic stimulation of mGluR5 and GluN2b and promotes cocaine seeking (125, 126), inhibiting glutamate uptake via intracerebroventricular administration of the GLT-1 antagonist TBOA enhances acquisition of morphine place preference (42), and activating GLT-1 using MS-153 after chronic morphine treatment attenuates the severity of naloxone-precipitated withdrawal (127).

Recently, we found that heroin-associated cues rapidly and transiently increase the proximity of astroglial peripheral processes with the synapse (unpublished observations; Figure 2D) and the transient re-association of the astroglial surface with NAc core synapses

during heroin reinstatement appears to be compensatory. When the re-association is prevented by knockdown of the actin binding protein ezrin, which is selectively expressed in PAPs (128), cue-induced heroin seeking was potentiated (unpublished observations). Taken together, the enduring reductions in GLT-1 expression and changes in synaptic proximity of astrocyte processes after heroin extinction, along with their morphological plasticity during cue exposure demonstrate a critical role for astrocytes in opioid seeking.

Interestingly, different striatal astrocytes respond selectively to synaptic activity on either D1- or D2-MSNs (129). Thus, the broad distribution of astrocytes with varying degrees of morphological plasticity during reinstated seeking (unpublished observations) may reflect selective changes in synaptic proximity to one or the other MSN subtype. Selective adaptations in GLT-1 expression and astrocyte morphological plasticity in the vicinity of D1 versus D2 synapses would be expected to produce opposite outcomes on opioid seeking, and is an intriguing possibility consistent with the aforementioned selective effects of chronic opioids on D1- versus D2-MSN excitability and morphology.

EXTRACELLULAR MATRIX

The extracellular matrix (ECM) is a proteinaceous network comprised primarily of glycoproteins and proteoglycans (130, 131). It serves as a structural scaffold that tethers neurons and glia via interactions with cellular adhesion molecules (CAMs), such as integrins (53), and constitutes a signaling domain when catalytically activated (130). Global knockdown of neuronal CAMs reduces morphine place preference, supporting ECM involvement in opioid reward (132). ECM degradation by matrix metalloproteinases (MMPs) permits neuronal and astroglial morphological adaptations that contribute to synaptic plasticity (50). MMPs are zinc-dependent endopeptidases that digest ECM proteins to facilitate synaptic remodeling, specifically morphological changes in dendritic spines and trafficking of NMDARs and AMPARs into the synaptic membrane (133, 134). Given MMP involvement in synaptic reconfiguration, their role in pathological addiction-related behaviors has been explored for over a decade (135). MMP-2 and MMP-9 are in the gelatinase family, and have been investigated for their role in drug extinction and reinstatement using *in vivo* zymography, an assay in which FITC-quenched gelatin acts as a substrate for proteolytic cleavage by activated MMP-2 and 9 (49, 50). Our lab showed that MMP-9 activity transiently increases during cue-induced heroin reinstatement (49), which is required for reinstated cocaine seeking and the transient synaptic potentiation in NAc core associated with reinstated seeking (49). Since cued heroin seeking also potentiates NAc core MSNs, MMP-9 involvement in heroin seeking seems probable. Recently, we discovered that cue-induced heroin seeking is associated with active MMP-2,9 gelatinolytic puncta selectively around D1-MSNs compared to saline controls and heroin extinguished groups (unpublished observations). These observations fit within the canonical perspective that activating D1 MSNs promotes motivated behaviors (63, 64, 136). Further studies investigating the strength of specific glutamatergic inputs onto D1 MSNs during cued heroin seeking may provide evidence of a relationship between pre- and postsynaptic activity as they relate to MMP induction.

Chronic morphine treatment activates MMP-9 in spinal cord, which contributes to its antinociceptive effects (137) and attenuates symptoms of morphine withdrawal (138). MMP-9 can be activated by nitrosylation and MMP-9 activation in both spinal cord and NA by opioids or opioid-associated cues results from increased nitric oxide synthesis. Moreover, MMP-9 activation in spinal cord and NA signals into cells via $\beta 1$ - and $\beta 3$ -integrin, respectively (138, 139), and in NA this signaling is necessary for reinstated cocaine seeking (139). Following heroin extinction, the perineuronal net (PNN) comprised of ECM proteins is reduced in mPFC and NA (140). Interestingly, cued-heroin seeking restores PNN protein expression, and a broad-spectrum MMP inhibitor attenuates cued heroin seeking (140). The apparent paradox of increasing ECM proteins simultaneous with increasing MMP-9 is possible because PNN proteins are not substrates for MMP-9 catalytic activity (141).

NORMALIZING ABERRANT TETRAPARTITE SYNAPTIC HOMEOSTASIS IN OPIOID ADDICTION

The studies outlined above demonstrate enduring adaptations in each of the four synaptic compartments in NA following repeated non-contingent or self-administered opioids, and opioid seeking is associated with rapid and transient plasticity within each compartment. In some instances the adaptations in one compartment have been experimentally linked to adaptations in other compartments (Figure 2). This is most clear with astroglial retraction and downregulated GLT-1, which are necessary for presynaptic glutamate spillover, induction of MMP activity, and morphological and physiological postsynaptic potentiation. Although much work remains to fully characterize how the tetrapartite synaptic compartments interact to regulate plasticity, it is clear that each of the four compartments are altered by opioid use and that these alterations regulate the initiation and intensity of opioid-seeking in rodent models of OUD.

Our developing understanding of tetrapartite synaptic physiology and pathophysiology has led to novel drug development strategies targeted to adaptations in each tetrapartite compartment. In the presynaptic compartment, the most tractable possibility is using mGluR2/3 agonists to reduce opioid seeking, although concerns have been raised due to parallel decreases in seeking of natural rewards. Promising postsynaptic receptor pharmacological manipulations include blocking mGluR5, which prevents conditioned morphine reward (142). Additionally, intracerebroventricular NMDAR and AMPAR antagonism during context extinction impairs morphine-induced reinstatement of CPP and increases NA c-fos expression (143–145). Also, heroin cue-induced and primed reinstatement and accompanying increases in spine density in NAc core are impaired by ifenprodil, a GluN2B antagonist (46).

Several studies focus on reversing glutamate spillover following chronic opioid use by restoring function and/or expression of GLT-1 or the cystine-glutamate antiporter with N-acetylcysteine (NAC) or ceftriaxone, and thereby reducing heroin cue and prime reinstatement (107, 117, 146). Other compounds such as propentofylline (methylxanthine adenosine uptake inhibitor) and clavulanic acid (β -lactam antibiotic) enhance expression of GLT-1 and inhibit morphine place preference (147, 148). It remains unknown whether these

astroglial-targeting restorative agents are effective at preventing changes in astroglial synaptic proximity that contribute to cued opioid seeking. Finally, therapeutics based on opioid-induced adaptations in the ECM seem furthest from development given the difficulties encountered in clinical trials using MMP-9 inhibitors in treating neuroinflammation (149).

CONCLUSIONS

This review makes clear that understanding the molecular mechanisms underpinning how the four tetrapartite compartments interact to regulate one another is necessary for understanding normal synaptic plasticity, as well as the aberrant physiology induced by opioids. The preclinical data justify this research direction as a potential source of novel pharmacotherapies for OUD. However, there is need for a larger research effort into fundamental mechanisms of tetrapartite synaptic integration before we will likely be able to develop comprehensive biological rationales for reversing opioid-induced tetrapartite pathophysiology as a means to control OUD.

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Figure 1.

(**A**) Glutamatergic afferents from cortical and subcortical brain regions converge onto nucleus accumbens (NA, with core and shell subcompartments indicated). Following repeated exposure to opioids, these projections undergo maladaptive plasticity contributing to aberrant drug seeking after periods of extinction (Figure 2). (**B**) Schematic outlining classic D1 vs D2 dichotomy with D1 medium spiny neurons (MSN) firing predominating during opioid seeking, while D2 MSNs are activated by extinction and the aversion associated with opioid withdrawal. Bullets indicate cell-type specific observations discussed in the text. Prefrontal cortex (PFC) subdivided into prelimbic (PL) and infralimbic (IL) with dashed line; hippocampus (HPC), paraventricular thalamus (PVT), and amygdala (AMY).

Figure 2.

Tetrapartite synaptic structure and regulation of synaptic glutamate homeostasis by astroglial processes. Tetrapartite synaptic structure demonstrated in an electron micrograph from the mouse hippocampus (A, scale bar= 200nm). Presynaptic afferent (blue) with synaptic vesicles and mitochondrion. Post-synaptic terminal (purple), with electron-dense post-synaptic density. Nearby astroglial perisynaptic processes (PAP, green) surround the synaptic cleft. The perisynaptic extracellular matrix (orange) serves as a signaling medium between all cellular components of the synapse. (B) Under normal physiological conditions, GLT-1

(blue) on PAPs is responsible for the majority of glutamate (small gray circles) uptake. Stimulation of astroglial mGluR3 (orange) has been linked to upregulating GLT-1 (105). Astroglial glutamate release through the cystine-glutamate antiporter (brown) can modulate release at glutamatergic terminals by stimulating presynaptic mGluR2/3 (orange) that negatively regulate presynaptic glutamate release probability. (C) After extinction from heroin or cocaine self-administration, NAc core astrocytes retract from the synapse, reducing their capacity for glutamate uptake through GLT-1 (blue). Downregulated expression of GLT-1 may result in part from reduced stimulation of astroglial mGluR3 (orange). Astroglial retraction may also disrupt presynaptic tone on mGluR2/3 via cystine-glutamate exchange. (D) During 15 minutes of cued heroin seeking, astroglial processes exhibit increased synaptic proximity as well as increased surface expression of GLT-1, both of which serve to attenuate seeking behavior by hindering synaptic glutamate spillover into the extracellular space. Micrograph in (A) was generated by A.K at the Electron Microscopy Resource Center at The Rockefeller University.