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Astrocytes in Cocaine Addiction and Beyond

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Abstract

Drug addiction remains a key biomedical challenge facing current neuroscience research. In addition to neural mechanisms, the focus of the vast majority of studies to date, astrocytes have been increasingly recognized as an “accomplice.” According to the tripartite synapse model, astrocytes critically regulate nearby pre- and postsynaptic neuronal substrates to craft experience-dependent synaptic plasticity, including synapse formation and elimination. Astrocytes within brain regions that are implicated in drug addiction exhibit dynamic changes in activity upon exposure to cocaine and subsequently undergo adaptive changes themselves during chronic drug exposure. Recent results have identified several key astrocytic signaling pathways that are involved in cocaine-induced synaptic and circuit adaptations. In this review, we provide a brief overview of the role of astrocytes in regulating synaptic transmission and neuronal function, and discuss how cocaine influences these astrocyte-mediated mechanisms to induce persistent synaptic and circuit alterations that promote cocaine seeking and relapse. We also consider the therapeutic potential of targeting astrocytic substrates to ameliorate drug-induced neuroplasticity for behavioral benefits. While primarily focusing on cocaine-induced astrocytic responses, we also include brief discussion of other drugs of abuse where data are available.

Keywords

astrocytes; cocaine; addiction; nucleus accumbens; synaptic plasticity; synaptogenesis

Introduction

The term glia is derived from “glue” in Greek, but glia functions go far beyond simply providing structural and metabolic support to neurons. The abundance of glial cells progressively and substantially increases along both evolutionary phylogeny and brain

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complexity[1]. For example, the astrocyte-to-neuron ratio in human brains (~1–1.4:1) is higher than that in rodents (~0.4:1) or C. elegans (~0.2:1)[2–5]. While human brains have higher metabolic demands, such a high ratio would appear to be an excessive number of astrocytes for just structural and metabolic support. Indeed, astrocytes—the most prevalent type of glia—are substantially larger in human brains, with more sophisticated morphologies that contact more synapses than any other species[6, 7]. Furthermore, the propagation speed of intracellular Ca^{2+} waves in human astrocytes is ~4-fold faster than in rodents[6]. Human-originated astrocytes maintain their enhanced cellular features when engrafted and induced in the mouse brain, resulting in enhanced experience-dependent synaptic plasticity and improved learning in these chimeric mice[8]. Such results suggest that astrocytes not only participate in brain metabolism and stability, but also are key substrates defining the brain's cognitive capacity[9–13].

Astrocytes are broadly known for their role in neurotransmitter and ionic homeostasis, synaptic and neuronal modulation, and blood-brain barrier maintenance. Astrocytes exhibit a complex morphology, with primary branches extending and elongating into delicate terminal leaflets, called peripheral astrocyte processes. Such perisynaptic processes enwrap the pre- and postsynaptic components of synapses, and these interactions occur at a distance as small as 10 nm, indeed closer than the width of some synaptic clefts[14–16]. An array of neurotransmitter receptors and transporters are expressed in astrocytes, highlighting that astrocytes may respond directly to neuronal signals[17–22]. Astrocytes can release substances into the extracellular space via anion channels, unpaired hemichannels of gap junctions, transporters or exchange antiporters, and other forms of non-vesicular release[23–26]. On the other hand, components of the basic machineries that mediate Ca^{2+}-dependent vesicular release by exocytosis have been detected in astrocytes, including vesicular glutamate transporters (VGLUT1/2) and the SNARE protein cellubrevin[27]. While the exact mechanisms and physiological relevance remain under debate[28, 29], vesicular release of substances from astrocytes has been detected in many experimental conditions[30–34]. Among astrocyte-secreted factors, some are classic neurotransmitters and neuromodulators, such as glutamate and adenosine triphosphate (ATP), while others are unique to astrocytes. With these mechanisms, astrocytes are thought to reciprocally communicate with neurons and regulate synaptic transmission[35] through a structural complex, termed the "tripartite synapse" (Figure 1), composed of a presynaptic nerve terminal and a postsynaptic dendritic spine enveloped by astrocyte processes, now viewed as the basic unit of synaptic transmission[35, 36]. Increasing evidence suggests that astrocytes display significant regional heterogeneity and specificity in the brain[37], although much remains to be learned about the molecular and cellular basis, and functional consequences, of such regional differences.

Delineating the precise mechanistic underpinnings of drug addiction remains a key research goal for the neuroscience community. A defining feature of drug addiction in humans is the high rate of relapse even after prolonged drug abstinence, which is often triggered by re-exposure to environmental cues that were previously associated with drug experience. Rodent studies reveal that cue-induced cocaine seeking and relapse are mediated in part by altered glutamatergic synaptic transmission within the nucleus accumbens (NAc), a brain region that has been critically implicated in reward learning and motivated behaviors[38–41]. The vast majority of studies of cocaine's action on the brain have concentrated on neurons.

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However, increasing evidence suggests that astrocytes play a dynamic role in the formation and maintenance of maladaptive changes at glutamatergic synapses in the NAc and related limbic regions during cocaine experience, promoting cocaine seeking and relapse after drug withdrawal[42–45]. Here, we discuss cellular features of astrocytes and their involvement in addiction-related neuronal changes at the synapse and circuit levels.

Basic features of astrocytes

Ca^{2+}-mediated astrocyte activity

Astrocytes do not generate action potentials. Rather, information sensing and signaling output by astrocytes is thought to be mediated in part by intracellular Ca^{2+} transients[46, 47]. These transients can be mediated through G protein-coupled receptors (GPCRs) and their activation of IP3-activated type 2 receptors (IP3R2) on the endoplasmic reticulum (ER) of astrocytes, resulting in ER release of Ca^{2+} and elevation of intracellular Ca^{2+} levels (Figure 1)[48–52]. In addition, ionotropic receptors may mediate Ca^{2+} influx into astrocytes (Figure 1)[53, 54]. Through these various types of mechanisms, astrocytes respond to neuron-derived signaling molecules and generate several patterns of intracellular Ca^{2+} waves in a finely graded and subcellularly heterogeneous manner. In contrast to neural networks that are composed of spatially separated neurons, astrocytes can directly couple with neighboring astrocytes via gap junctions[55, 56]. In this manner, up to dozens of astrocytes are connected to form an electrically low-resistance reticular system called a syncytium. This network of coupled astrocytes is a key feature of astrocyte biology, which is found throughout the central nervous system and across many species[56, 57]. Interestingly, not all neighboring astrocytes directly couple, for example, astrocyte networks within the somatosensory cortex largely follow a single barrel[56]. Regardless, an astrocyte syncytium can cover the space of hundreds of microns in diameter with millions of synapses contained therein. Depending on the physiological condition, the Ca^{2+} waves can be restricted within one astrocyte or, alternatively, propagate to other astrocytes via the syncytium for synchronous network effects[58, 59].

Early studies focused on astrocytic Ca^{2+} activities within the cell soma, since ER storage was viewed as the predominant source of Ca^{2+} for the entire astrocyte[28, 60]. Improvements in the spatiotemporal detection of Ca^{2+} now demonstrate active Ca^{2+} signals throughout an astrocyte—with subcellular localizations and microdomains that exhibit dynamic features of these Ca^{2+} transients[29, 61, 62]. In fact, Ca^{2+} signals are detected in far distal astrocytic processes[62], and can be independent of somatic Ca^{2+} sources[61, 63].

One potential consequence of these localized Ca^{2+} events is 'gliotransmission'[61]. Ca^{2+} signaling in astrocytes is far from straightforward, however. While Ca^{2+}-induced gliotransmission and resulting synaptic regulation have been observed frequently[64–70], it has also been shown that Ca^{2+} elevations in astrocytes are not always associated with the release of gliotransmitters[71], suggesting a subtle and complex relationship between astrocytic Ca^{2+} levels and gliotransmission[28, 62, 72]. While the mechanistic link between astrocytic Ca^{2+} and gliotransmitter release has not to date been fully characterized, it is clear that the process is distinctly different from presynaptic neurotransmitter release in neurons. Nonetheless, astrocytes do exhibit diverse Ca^{2+} activities in distinctive sub-

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compartments[73–75]. The result of this activity may be the release of a variety of transmitters via several different mechanisms[23, 70, 76, 77]. Of note, a single astrocyte can release different types of gliotransmitters, which cause multi-phasic synaptic responses in nearby neurons[78]. Furthermore, the type of released gliotransmitters is correlated with the duration and frequency of neural activities nearby, suggesting that astrocytes can sense and process spatiotemporal information from neurons[78].

Reciprocal communication between astrocytes and neurons

A single mature astrocyte can contact up to 140,000 synapses in rodents, and upwards of 2 million in humans[7, 79]. Of note, the enwrapment of synapses by astrocytes is crucial for a range of astrocyte functions, including the sensing and modulation of neuronal activity, preventing neurotransmitter spillover from synapses, as well as the stabilization of synapses themselves. Furthermore, an individual astrocyte can contact synapses from multiple, different neurons, resulting in a term now coined "astrocyte-defined synaptic islands"[80]. Neurons within a synaptic island spread out up to 200 μm[81], thereby poising astrocytes to potentially contribute to the activity of neuronal ensembles.

Glutamate is an example of a neurotransmitter through which astrocytes and neurons communicate bidirectionally. Elevation of astrocytic Ca^{2+} levels can result in a SNARE protein–dependent vesicular release of glutamate[82]. Independent of vesicular release, glutamate can diffuse out from astrocytes via nonspecific ion channels upon activation of G-protein-signaling[83, 84], or be transported out of astrocytes via a cystine-glutamate exchanger[85]. Nonetheless, astrocyte-released glutamate activates extrasynaptic GluN2B-containing NMDA receptors (NMDARs) on neurons, resulting in slow inward currents that depolarize these neurons synchronously[86, 87]. Astrocyte-released glutamate may also activate pre- and postsynaptic glutamatergic receptors, influencing both presynaptic release and postsynaptic responsiveness[88, 89]. Conversely, astrocytes respond to neuronal-released glutamate via metabotropic glutamate receptors (mGluRs) or ionotropic glutamate receptors. Among the eight known mGluRs, mGluR3 and 5 have been detected in astrocytes[90, 91]. mGluR3, which is $G_{i/o}$-coupled, is expressed predominantly in adult astrocytes and is presumed to inhibit adenylyl cyclase with no direct influence on Ca^{2+} activity. Indeed, little to no Ca^{2+} activity is recorded in cortical or hippocampal astrocytes in responses to an mGluR3 agonist[92, 93]. However, a robust Ca^{2+} signal is detected in striatal astrocytes[93].

In contrast, activation of mGluR5 increases Ca^{2+} levels in astrocytes through the $G_{q/11}$-linked activation of PLCβ-IP3R2-PKC or other signaling pathways[94–96]. There has been an interesting debate related to the existence and functional importance of mGluR5 in mature astrocytes. Using immunostaining combined with electron microscopy, it was demonstrated in the hippocampus and cortex that astrocytic expression of mGluR5 decreases to very low levels after development[97]. In line with this result, mGluR5 agonists fail to induce Ca^{2+} elevations in adult astrocytes within these brain regions[93, 97]. These results suggest that mGluR5-mediated astrocytic responses decline substantially after development. However, albeit at very low levels, mGluR5 is detected in other preparations, raising the possibility that mGluR5 activity is still present, particularly in distal processes where Ca^{2+} signals may be too weak to be detected based on traditional bulk-loaded fluorescent Ca^{2+}

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dyes. Highlighting astrocyte heterogeneity and regional specificity, it is also important to note that expression of mGluR5 in astrocytes differs substantially among different brain regions[93]. Indeed, in the NAc of 2–6 weeks old mice, activation of mGluR5 initiates Ca^{2+} oscillations in astrocytes[95]. In addiction-oriented studies, administration of mGluR5 antagonists, especially via intra-NAc infusion, decreases cocaine taking during cocaine self-administration and cocaine seeking after drug withdrawal[98–101]. It is important to determine whether these behavioral responses to mGluR5 manipulations are mediated by mGluR5 located in astrocytes or neurons.

Another molecule that may act as both a neuro- and gliotransmitter is ATP[102]. ATP can be released from astrocytes through a Ca^{2+}-dependent mechanism involving SNARE proteins[103, 104]. In these experiments, a variety of stimulations that induce astrocytic Ca^{2+} waves can trigger ATP release[65, 105, 106]. In the cerebral cortex, synaptically-released glutamate induces astrocytic ATP release via activation of astrocytic NMDARs, presumably also involving Ca^{2+}-mediated processes[107, 108]. As well, astrocytic release of ATP is mediated via mechanisms independent of Ca^{2+} or SNARE proteins[109, 110]. Astrocytes respond to neuronal or astrocytic release of ATP through several purinergic P2 class ATP receptors (Figure 1). Both *in vitro* and *in vivo* data demonstrate that astrocyte-released ATP induces Ca^{2+} waves in nearby astrocytes[106, 111]. Through ATP-purinergic signaling, Ca^{2+} waves initiated in one astrocyte can propagate to nearby astrocytes[111, 112] in a regenerative and neural activity-independent manner[113]. P2Y1 receptor-mediated ATP signaling has been shown to induce glutamate release from astrocytes[114].

Once released, ATP is quickly (within 200 ms) hydrolyzed to adenosine by ectonucleotidases within the extracellular matrix[115]. Thus, in addition to ATP receptors, ATP-derived adenosine influences neural activities by binding neuronal A1 receptors (A1Rs) (Figure 1). Through A1Rs, adenosine originating from astrocytes enhances basal glutamatergic synaptic transmission and reduces the magnitude of LTP[65], effects that might be related to the action of astrocytic adenosine on synaptic expression of NMDARs[116].

Dopamine is a neuromodulator that regulates synaptic and circuit activities and has been implicated in many psychiatric disorders[117–120]. D1 and D2 dopamine receptors (D1Rs and D2Rs), which are coupled to distinct intracellular signaling cascades[121], are both detected in astrocytes[122–125]. While activation of D1Rs leads to an elevation of astrocytic levels of Ca^{2+} and activation of cAMP-PKA signaling[123, 126–129], the effects of dopamine on astrocytes also appear to be mediated by D2Rs and other mechanisms. Specifically, incubation of cultured astrocytes with dopamine induces stellation of astrocyte processes, which is only partially mediated by cAMP signaling[130]. In hippocampal brain slices, superfusion of dopamine induces a fast onset elevation of astrocytic levels of Ca^{2+}, which is followed by a more sustained decrease[131]. Application of a mixture of D1R and D2R antagonists blunts these biphasic dopamine-induced effects, while D1R antagonists alone selectively prevent the first phase elevation of astrocytic Ca^{2+} levels, suggesting D2Rs as a key in decreasing astrocytic Ca^{2+} level during the second phase following dopamine[131]. Consistent with this interpretation is the finding that application of D2R agonists decreases astrocytic levels of Ca^{2+} in PFC slices[124]. Dopamine also influences astrocytes in the ventral tegmental area (VTA) and NAc, work discussed in the next section.

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In addition to responding to dopamine, astrocytes regulate extracellular levels of dopamine via astrocytic dopamine, norepinephrine, and other transporters[132–135]. After reuptake, dopamine in astrocytes is metabolized by monoamine oxidase, and the resulting oxygen species increase intracellular levels of Ca^{2+}[136], thus providing a D1R- and D2R-independent mechanism underlying dopamine-mediated regulation of astrocyte Ca^{2+} activity. Interestingly, vesicular monoamine transporter 2 is detected in astrocytes, and genetic deletion of VMAT2 in PFC astrocytes results in impaired LTP and behavior[137, 138], suggesting the possibility that dopamine can be packaged for vesicular release in astrocytes. If this or similar astrocytic dopamine release exists, which requires confirmation, astrocytes may serve a noncanonical source of dopaminergic signaling.

Astrocytes regulate synaptic plasticity and synaptogenesis

Experience-dependent synaptic plasticity serves a key cellular mechanism for learning and memory[139–143]. In the classical Hebbian model, manipulation of pre- and postsynaptic activities alone is sufficient to induce many forms of LTP or LTD at glutamatergic synapses. However, more recent evidence suggests that astrocytes are also integral to some forms of LTP and LTD. In response to LTP induction, the astrocytic coverage of synapses increases[144]. This morphological change may effectively increase astrocyte-mediated regulation of synaptic transmission via stabilization of synapses themselves as well as via preventing neurotransmitter synaptic spillover. Additionally, direct astrocytic stimulation[66] or elevation of astrocytic Ca^{2+} levels[145] transiently increases presynaptic release probability at hippocampal glutamatergic synapses. Furthermore, when astrocytic Ca^{2+} elevation is paired with postsynaptic depolarization, the increased presynaptic release probability becomes persistent, resulting in an LTP-like effect at these synapses[145]. These results raise the important possibility that, without presynaptic conditioning, coincidental activities of postsynaptic neurons and their neighboring astrocytes are sufficient to induce synaptic plasticity. Correspondingly, electrophysiological or pharmacological suppression of astrocytic activities impairs the induction of the classic NMDAR-dependent hippocampal LTP[146, 147]. In a form of spike timing-dependent LTD (t-LTD), in which coincident postsynaptic release of endocannabinoids (eCBs) and presynaptic activation of NMDARs are essential, astrocytes serve as a lynchpin; postsynaptically-released eCBs activate CB1 receptors on adjacent astrocytes, which, in turn, release glutamate to activate presynaptic NMDARs, accomplishing the induction process[148]. Furthermore, adenosine signaling originating from astrocyte-released ATP coordinates adjacent synapses such that both monosynaptic LTP and heterosynaptic LTD are induced simultaneously[65]. These findings describe an active role of astrocytes in the induction and possibly also maintenance of Hebbian plasticity.

Synaptogenesis requires participation of both pre- and postsynaptic neurons[149, 150], with increasing evidence suggesting that this process also requires the participation of astrocytes[151]. During development, astrocytes and neurons share the same precursor cells, but astrogenesis occurs in postnatal stages, after the bulk of neurogenesis is accomplished[152–154]. Importantly, robust synaptogenesis only occurs after the emergence of astrocytes, particularly during the first 2 to 3 postnatal weeks in rodents, which is a major maturation window for astrocytes[151, 155]. Evidence suggests that astrocytes actively

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and directly contribute to both the structural and functional aspects of synaptogenesis and synapse maturation. Most prominently studied are synaptogenic factors derived from astrocytes. Neuronal cultures devoid of astrocytes form far fewer synapses than in neuron-astrocyte co-cultures[156], and those synapses appear to be functionally immature[157]. Treating these astrocyte-free cultures with astrocyte-conditioned medium induces the formation of new synapses, suggesting that astrocytes secrete soluble factors to promote synaptogenesis[158–162].

This review focuses on one set of soluble astrocyte-derived factors, thrombospondins (TSPs). For a comprehensive review of astrocytes and synaptogenesis and maturation, we refer the reader to a recent review[155]. Through activation of their neuronally-located target, α2δ−1, astrocyte-secreted TSPs promote synaptogenesis during development[158, 163]. TSPs are multimeric, multidomain glycoproteins that, by binding to proteases, cytokines, growth factors, and other components of the extracellular matrix, facilitate cell attachment, migration, cytoskeletal dynamics, and angiogenesis[164, 165]. TSP-induced synapses appear structurally normal—with pre- and postsynaptic specializations, normal amounts of presynaptically-docked vesicles, and regular dimensions of postsynaptic densities—but are postsynaptically “silent” in that they do not contain functionally stable AMPA glutamate receptors (AMPARs)[158]. This AMPAR-labile feature is consistent with immature, AMPAR-silent glutamatergic synapses found in the developing brain, some of which subsequently mature and become fully functional by recruiting AMPARs[166–169]. Of note, synapses generated in astrocyte-present neuronal cultures do express stable postsynaptic AMPARs, highlighting that astrocyte-derived signaling molecules other than TSPs are involved in synapse maturation[158].

α2δ−1, described originally as an auxiliary subunit of voltage-gated calcium channels (VGCCs) located at both axons and dendrites[170–172], has been identified as one of the key neuronal targets for TSP-mediated synaptogenesis. Overexpression of α2δ−1 augments TSP-induced synaptogenesis, while expression of a dominant-negative form of α2δ−1 impairs TSP-induced synaptogenesis[163]. Gabapentin, an α2δ−1 antagonist that is used clinically to treat chronic pain, binds to the α2 domain of α2δ−1, the same binding site as TSPs[173, 174], but the binding does not alter Ca^{2+} currents conducted by VGCCs[175]. Administration of gabapentin prevents astrocyte- and TSP-induced synaptogenesis, identifying α2δ−1 as an essential component in this developmental synaptogenic signaling[163]. However, despite its intimate relationship with VGCCs, α2δ−1-mediated synaptogenesis is independent of VGCC function[163]. The key cellular steps from TSP-α2δ−1 binding to synapse formation have not been elucidated[176]. The small GTPase Rac1 has been implicated in this process[177], and Rac1 is also known to influence synapse plasticity following cocaine exposure[178, 179]. In addition to α2δ−1, TSPs also interact with other synaptic proteins, such as neuroligins, to refine the synaptogenic process[180]. Beyond TSPs, astrocytes secrete hevin and sparc, which, among other molecules, facilitate synaptogenesis as well[160, 181, 182]. These and other results depict a critical role of astrocytes in synapse formation during development.

Cooperatively operating with synaptogenesis in forming new circuits, synapse elimination is essential for the refinement of neural circuits in the developing brain as well as remodeling

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of neural circuits and the turnover of synapses in the adult brain. Similar to microglia, astrocytes can engulf large synaptic components (i.e., synaptosomes), and direct them to lysosomes for ultimate degradation[183, 184]. A key form of such astrocyte-mediated synapse elimination is mediated by astrocyte phagocytic receptors, such MERTK (a type of protein tyrosine kinase) and MEGF10 (an epidermal growth factor-domain family protein), which can sense "eat-me" signals (e.g., phosphatidylserine) that are presented by targeted debris to execute the engulfment[185–187]. As such, this form of phagocytosis is not random, but, rather, driven by neuronal activities and mediated by precise signaling exchange between neurons and astrocytes. Astrocytes also release cytokines, such as TGF-β, which can tag synapses for microglia-mediated synapse elimination[188, 189]. Detailed discussion of these and other forms of astrocyte-mediated synapse elimination can be found in several excellent reviews[183, 184, 190].

Astrocytes in neural adaptations after exposure to drugs of abuse

Astrocytes in the VTA-NAc reward circuit respond directly to dopamine

Dopamine's involvement in the reward pathway and in substance abuse and other psychiatric disorders is well documented[117–120, 191–205]. Exposure to natural rewards or to drugs of abuse like cocaine induces bursting activity of VTA dopamine neurons, leading to a transient increase in extracellular dopamine levels in the NAc and other limbic forebrain regions[191, 192]. In contrast, withdrawal from cocaine or other drugs of abuse decreases extracellular dopamine levels in these brain regions[196–199], whereas re-exposure to the drug or to drug-predicting cues after drug withdrawal increases such levels[200–205]. It is noteworthy, as mentioned above and detailed below, that astrocytes in several brain regions associated with the reward circuitry respond directly to the dynamics of extracellular dopamine.

In vivo optogenetic stimulation of VTA dopaminergic nerve terminals within the NAc induces dopamine release and, concomitantly, increases Ca^{2+} activity in roughly half of NAc astrocytes, lasting for several seconds[206]. This dopamine-elicited astrocytic response appears to be mediated at least in part by D1Rs on astrocytes and, potentially through ATP/adenosine signaling, contributes to transient inhibition of nearby glutamatergic synaptic transmission[206]. In concert with these results, superfusion of cocaine, which elevates extracellular dopamine levels in NAc slices[207, 208], increases both the frequency and total number of astrocytic Ca^{2+} transients[209]. Paradoxically, AAV-mediated deletion of D1Rs in NAc astrocytes increases basal Ca^{2+} activity in this brain region[206]. In the VTA and surrounding ventral midbrain areas, activation of D2Rs on astrocytes revealed a blunted Ca^{2+} response[210]. These findings, coupled with distinct responses of astrocytes in hippocampus and PFC to dopamine (see above), again highlight the regional heterogeneity of astrocytes and implicate a far more complex dopamine-mediated regulation of astrocytes than previously understood.

Astrocytes in the NAc

Transcriptomic analyses[211] reveal that striatal astrocytes (encompassing NAc and dorsal striatum) express high levels of K^+ channels, setting their equilibrium membrane

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potential at ~−85 mV, which is more hyperpolarized than astrocytes in many other brain regions[212, 213]. Striatal astrocytes also express high levels of gap-junction proteins, allowing for synchronized activities among connected astrocytes[93]. Interestingly, this astrocytic syncytium can spread across brain regions such that a dye infused into a single striatal astrocyte can eventually diffuse into cortical astrocytes[214]. Basal spontaneous Ca^{2+} activities in striatal astrocytes are insensitive to TTX, suggesting that they are relatively independent of ongoing neuronal activities[93].

Striatal GABAergic medium spiny neurons (MSNs) comprise >90% of the local neuronal population and represent the sole projection neurons of this region[215]. MSNs can be divided into two subpopulations: one that expresses predominantly D1Rs and the neuropeptides dynorphin and substance P, and another that expresses D2Rs and enkephalin[216]. In the dorsal striatum, subpopulations of astrocytes show selective responsivity to the activity of either D1 or D2 MSNs[217]. Such cell-type specific neuron-astrocyte interactions may contribute to the heterogeneous responses of D1R versus D2R NAc MSNs to cocaine, such as selective generation of silent synapses in D1R MSNs after noncontingent cocaine exposure[218]. Compared to noncontingent procedures, astrocytic adaptations to contingent (volitional) exposure to cocaine are likely more complex than previously believed given that astrocytes respond both to neuronal activity and directly to dopamine. Much of this work to date has focused on dorsal striatum, with studies of NAc specifically now being a high priority.

Extinction is a training procedure in rodent models of drug relapse, in which the learned drug taking and seeking are diminished in the absence of anticipated reinforcement. Extinction requires new reinforcement learning that involves remodeling of glutamatergic transmission in the NAc[219–223]. After extinction of cocaine self-administration, NAc expression of glial fibrillary acidic protein (GFAP) as well as the overall surface area and volume of astrocytes are decreased, effects accompanied by reduced co-localization of astrocyte processes with the presynaptic nerve terminal marker, synapsin-1[224]. Importantly, without extinction training, the synapse-astrocyte proximity remains largely unchanged in the NAc after cocaine self-administration, selectively linking this form of astrocytic adaptation to extinction[225]. Furthermore, this extinction-related astrocytic adaptation is only detected in the NAc and not in the PFC or basolateral amygdala (BLA), indicating the unique response of NAc astrocytes in extinction learning[225]. Similar extinction-related changes in NAc astrocytes are also observed after extinction from methamphetamine[226] or heroin[227] self-administration. Importantly, re-exposure to heroin-associated cues after extinction transiently restores the reduced synapse-astrocyte proximity[227], suggesting a dynamic role of NAc astrocytes in response to cue-induced drug seeking after drug extinction. DREADD-mediated activation of NAc astrocytes after drug extinction decreases subsequent cue-induced cocaine[228] and methamphetamine[226] seeking, possibly through restoring astrocyte-mediated glutamate homeostasis in the NAc (see below).

Unlike psychostimulants, opioids initiate their primary pharmacological effects by activating μ opioid receptors (MORs)[229]. NAc astrocytes express MORs[230] that can be activated upon opioid administration. In acutely prepared NAc slices, superfusion of morphine increases astrocytic Ca^{2+} levels through IP3R2-mediated release from intracellular stores[231].

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Activation of MORs induces astrocytic glutamate release, which, in turn, induces NMDAR-mediated slow inward currents in nearby neurons[231]. Considering that an individual striatal astrocyte contacts ~20 neuronal somas[93], morphine-induced slow inward currents may be present throughout NAc MSNs. In drug-naïve animals, intra-NAc infusion of astrocyte-conditioned medium, which contains a variety of soluble astrocytic factors, acutely enhances the rewarding effect of morphine[232]. Furthermore, intra-NAc infusion of such media from morphine-pretreated astrocytes results in a stronger preference for drug-associated environments than infusions of media from astrocytes without morphine pretreatment[232]. Albeit preliminary, these results suggest a clear link between NAc astrocytes and opioid-induced behaviors.

Astrocytes regulate glutamate homeostasis

Cortical glutamatergic projections to the NAc regulate general reward seeking under physiological conditions[233–235]. Proper functioning of this projection is maintained, in part, by homeostatic regulation of glutamate at both synaptic and extrasynaptic sites. Disruption to this homeostasis during drug experience contributes to drug seeking and relapse[236].

A key element to this disruption is GLT-1, a glutamate transporter expressed in perisynaptic astroglial processes that contributes to >90% of glutamate reuptake[21, 22, 237–242]. Chronic exposure to either psychostimulants or opioids reduces the expression of GLT-1 in the NAc at both the mRNA and protein levels[243, 244]. Furthermore, chronic drug exposure reduces the overlap of perisynaptic astroglial processes with synaptic markers, suggesting decreased astrocytic insulation of synapses[224–227]. Both of these changes impair the clearance of synaptic glutamate, facilitating spill-over to perisynaptic regions, and establishing an inter-synaptic crosstalk[245–248] (Figure 2). Glutamatergic synaptic transmission within the NAc is pathophysiologically upregulated after withdrawal from many classes of drugs of abuse[233, 249–251]. The diminished reuptake of glutamate through GLT-1 may exacerbate this pathophysiology. Moreover, glutamate spillover can activate glutamatergic receptors such as NMDARs and mGluRs in neighboring synapses[146, 252–254], resulting in nonspecific neuronal activity not observed in drug-naïve subjects. Indeed, administration of mGluR5 antagonists decreases cocaine seeking after drug withdrawal[98–101]. Multiple pharmacological approaches that normalize GLT-1 function in cocaine-exposed animals have proven useful in reducing cue-induced reinstatement of cocaine seeking in animal models[243, 255, 256], raising the hope for treating drug relapse by targeting astrocyte-maintained glutamate homeostasis.

While astrocytic GLT-1 regulates synaptically-released glutamate, the cystine/glutamate (xCT) exchanger in astrocytes maintains ambient levels of glutamate within the extracellular space[257]. xCT functions via the exchange of one extracellular cystine for one intracellular glutamate[258]. xCT therefore contributes to the extracellular tone of glutamate, which, in turn, modulates activity of presynaptic group II mGluRs (e.g., mGluR2/3) that control levels of synaptically-released glutamate[257]. This xCT-mediated regulation of glutamate is impaired after drug experience[236]. After withdrawal from cocaine or nicotine self-administration, levels of xCT in the NAc core are reduced, contributing to the drug withdrawal-associated reduction of extracellular glutamate[85, 255, 259–261]. A basal level

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of extracellular glutamate is needed for tonic activation of presynaptic group II mGluRs (e.g., mGluR2/3) to limit glutamate release from presynaptic terminals[262]. As such, decreased levels of ambient glutamate compromises this inhibitory control of presynaptic glutamate release and promotes glutamate overflow, a cellular maladaptation that contributes to drug seeking[263] (Figure 2). In fact, astrocytic h3Mdq-DREADD-mediated reductions cocaine seeking (see below) are ameliorated with concomitant administration of mGluR2/3 antagonists[228].

A therapeutic approach to targeting xCT has also been tested with the use of N-acetylcysteine, a cysteine prodrug and substrate of xCT. Administration of N-acetylcysteine restores the compromised function of xCT following chronic cocaine exposure and decreases drug- and cue-induced relapse to cocaine seeking[259]. N-acetylcysteine also restores GLT-1 expression in the NAc and is thought to ameliorate extrasynaptic glutamate spillover following cocaine extinction and reinstatement[243]. This might be due to the restoration of plasticity at PFC-to-NAc synapses known to be impaired after cocaine self-administration[264]. Furthermore, after withdrawal from cocaine self-administration, administration of N-acetylcysteine enhances the sensitivity of rats to the punishment that induces extinction of drug seeking, suggesting multi-angled anti-relapse effects of N-acetylcysteine[265]. In early clinical studies, administration of N-acetylcysteine reduces cocaine craving and thus decreases the risk of relapse in individuals during drug abstinence[266]. Beyond cocaine, N-acetylcysteine also reduces the number of cigarettes consumed in active smokers[261] and exhibits beneficial effects on PTSD patients[267, 268].

As discussed above, by decreasing the function or protein expression of GLT-1 and xCT, cocaine experience simultaneously increases spillover of synaptically-released glutamate and decreases ambient glutamatergic tone in the NAc, both of which promote cocaine seeking[243]. This line of results raises several interesting considerations. For example, mGluR activity at cortical glutamatergic projection terminals versus dopaminergic projection terminals are linked to the effects of GLT-1 versus xCT, respectively[257, 263, 269, 270]. If both of these effects are mediated by the same astrocytes, the astrocyte microdomains or processes that are close to glutamatergic versus dopaminergic synapses are likely to operate independently. This is not surprising, as such compartmentalized responses of astrocytes to different forms of stimulation has been demonstrated[271, 272]. However, this potentially domain-oriented functioning of astrocytes indicates that a more nuanced investigation of astrocyte-neuron partnership is needed. Furthermore, decreased function of GLT-1 after extinction of cocaine self-administration increases the likelihood of glutamate spillover, resulting in increases of glutamate in neuropil. This effect may somewhat compensate for the reduction of ambient glutamate in the NAc upon diminished expression of xCT. As such, homeostatic mechanisms may exist in NAc astrocytes[43, 273], functioning in part through GLT-1 and xCT to balance synaptic versus ambient glutamate. Emerging evidence suggests that such homeostatic regulation or dysregulation between GLT-1 and xCT may be organized by cascades of epigenetic changes following extinction from cocaine self-administration[274].

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Astrocyte-mediated synaptogenesis

The neural rejuvenation hypothesis of drug addiction[275] proposes that "repeated exposure to drugs of abuse induces plasticity mechanisms that are normally associated with brain development within the brain's reward circuitry, which mediate the highly efficient and unusually stable memory abnormalities that characterize addiction." Astrocytes promote synapse formation during development, but these astrocyte-mediated synaptogenic mechanisms become much less active after postnatal week 3 in rodents when the major wave of synapse formation is accomplished[276]. Cocaine experience generates AMPAR-silent synapses in the adult NAc, and these synapses exhibit several features consistent with nascent glutamatergic synapses observed during development[277, 278]. Recent results demonstrate that cocaine-induced generation of silent synapses in the adult NAc is mediated, in part, by an astrocyte-derived developmental synaptogenic mechanism, namely, TSP-α2δ−1 signaling[209]. TSPs are abundantly expressed during early developmental stages, but decline to low levels in most brain regions during adulthood[279]. Albeit at low levels, basal expression of TSP1/2 is detected in the adult NAc, where neuronal expression of α2δ−1 remains robust at this time point[209, 280]. In acutely prepared NAc slices, superfusion of cocaine increases Ca^{2+}-mediated activities in astrocytes[209]. Within the dorsal striatum, DREADD-mediated activation of $G_{i/o}$-coupled GPCRs increases Ca^{2+} activity in astrocytes with the subsequent release of TSP1[281]. While the underlying mechanisms remain largely unclear, these studies suggest that the cocaine-induced release of TSP2 from astrocytes may reflect a direct effect of dopamine on astrocytic GPCR signaling and downstream elevations in intracellular Ca^{2+} levels. Administration of gabapentin, which as noted earlier disrupts the binding of TSP2 to α2δ−1[163, 177], prevents cocaine-induced generation of silent synapses and cocaine-induced spinogenesis in the NAc, suggesting the involvement of TSP-α2δ−1 signaling[209]. Additionally, selective knockdown of either α2δ−1 in neurons or TSP2 in astrocytes prevents cocaine-induced generation of silent synapses in the NAc, indicating that astrocyte-derived TSP2, and α2δ−1 on MSNs, form a synaptogenic signal to generate silent synapses *de novo* in response to cocaine[209], by analogy to what is observed during development[158, 163] (Figure 3). These results—in line with the neural rejuvenation hypothesis—suggest that cocaine experience utilizes astrocyte-mediated synaptogenic mechanisms to "re-develop" NAc circuits, potentially generating new connectivity patterns that support cocaine-associated memories.

After drug withdrawal, some cocaine-generated silent synapses mature by recruiting AMPARs, which may stabilize the remodeled NAc circuits and result in the consolidation of cocaine memories[179, 218, 235, 282–284]. These synapses appear to remain dynamic, as retrieval-induced destabilization and reconsolidation of cue-associated cocaine memories are influenced by the re-silencing and re-maturation of these synapses, respectively, following re-exposure to cocaine-associated cues[179, 285]. Thus, the generation of silent synapses is a key mechanism through which astrocytes can influence NAc circuits to promote cue-induced cocaine seeking. It is therefore not surprising that selectively disrupting astrocytic TSP-α2δ−1-signaling during cocaine self-administration, preventing cocaine-induced generation of silent synapses and therefore silent synapse-mediated NAc remodeling, results in both decreased cue-induced cocaine seeking after cocaine withdrawal and decreased cue-induced reinstatement of cocaine seeking after cocaine extinction[209]. Importantly, intra-

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NAc disruption of TSP-α2δ−1-signaling does not prevent animals from acquiring operant responding to cocaine in the first place[209], indicating that astrocyte-mediated synaptogenesis in the NAc is selectively involved in the formation of cue-associated cocaine memories without affecting basic instrumental learning.

In addition to synaptogenesis during cocaine exposure, TSP-α2δ−1 signaling may contribute to withdrawal-associated neural adaptations. Selectively inhibiting NAc α2δ−1 only after extinction from cocaine self-administration decreases cocaine priming-induced reinstatement of drug seeking[280]. This finding indicates that TSP-α2δ−1 signaling and presumably other astrocytic mechanisms are critically involved in neural adaptations that mediate multiple phases of drug-associated behaviors. Furthermore, chemogenetically upregulating NAc astrocyte activity after drug extinction acutely decreases subsequent cocaine[228] and methamphetamine[226] seeking, suggesting that, in addition to adaptive changes, NAc astrocytes also influence ongoing NAc circuit function that controls drug-seeking behaviors.

Concluding Remarks

Astrocytes are increasingly recognized as key players in brain function under physiological and pathophysiological conditions. We highlight some recently identified mechanisms through which astrocytes regulate synapses and neural circuits to promote addiction-related behaviors. Additionally, in cocaine-trained animals, manipulating astrocytes prevents drug-induced neural adaptations and ameliorates addiction-related behavioral abnormalities. These findings not only establish astrocytes as promising cellular targets to explore novel mechanisms underlying drug addiction, but also specify several future directions. First, a wide range of astrocytic substrates responds to neural activities and external stimuli. GLT-1 and xCT likely represent the tip of the iceberg of astrocytic substrates that undergo adaptive changes following repeated drug experience. It is important for future studies to systematically screen and identify key astrocytic substrates that are changed by drug experience to influence behaviors. Second, while this manuscript focuses on the NAc, many other brain regions are also critically involved in drug-induced behaviors. For example, similar to the NAc, cocaine self-administration increases the density of dendritic spines expressed by pyramidal neurons in the prefrontal cortex (PFC), suggesting a similar synaptogenic process[286, 287]. However, astrocytes in the NAc and PFC are highly heterogeneous, and their differential responses to cocaine are only beginning to be elucidated[225]. It is critical for future studies to characterize such regional specificity, neuronal-partner selectivity, and domain-specific specializations, and how these features influence drug-induced alterations. Third, drug exposure, withdrawal, extinction, reinstatement, and other related conditions constitute different aspects of the drug-related experience that contribute differentially to the addicted state. Astrocytes influence both synaptogenesis during drug exposure and dysregulation of glutamate homeostasis after drug withdrawal, indicating a phasic involvement of astrocytes. It is important to determine the astrocytic contributions across these phases to influence neural and behavioral consequences. The role of astrocytes in addiction is likely defined both by the direct actions of drugs of abuse on astrocytes as well as by astrocytic dysregulation that occurs as a consequence of dynamic neuron-astrocyte interactions during drug withdrawal, abstinence,

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and relapse. These divergent mechanisms underscore the need to elucidate the increasingly complex role of astrocytes in the brain under normal and pathophysiological conditions. Pursuing these and other questions will provide a new angle to understanding the cellular and circuit mechanisms underlying drug-induced behavioral abnormalities.

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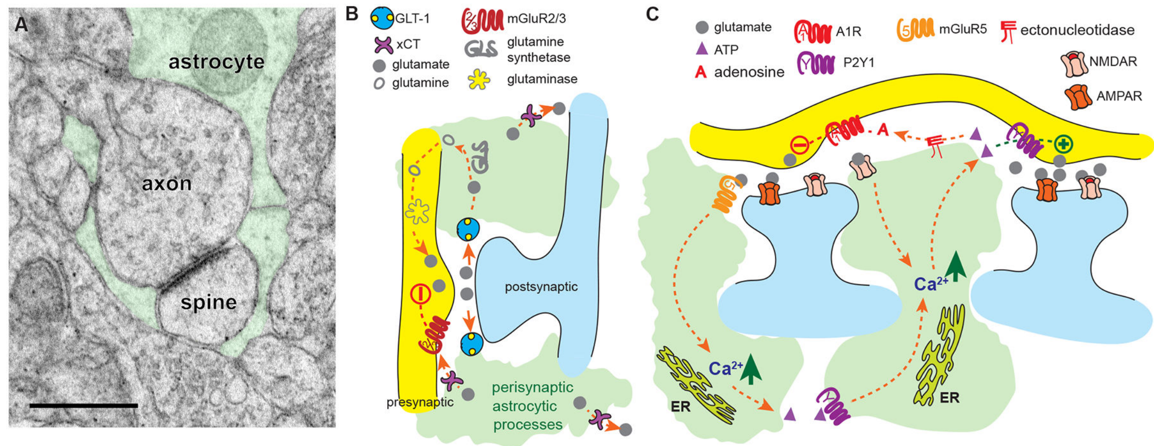

Figure 1. Astrocyte-mediated regulation of transmission at the tripartite synapse.

A Electron microscopic image showing a tripartite synapse in the NAc, in which a glutamatergic axospinous synapse is wrapped by astrocyte processes (scale bar corresponds to 0.5 μm). **B** Diagram illustrating astrocytic mechanisms of glutamate homeostasis at glutamatergic synapses. Specifically, GLT-1 on astrocytes removes synaptically released glutamate, which can be converted to glutamine within astrocytes and transported back into presynaptic terminals. xCT located at perisynaptic astrocytic processes maintains extracellular levels of ambient glutamate. **C** Diagram showing mechanisms of increasing Ca^{2+} in astrocytes, including ER-derived Ca^{2+} release or entry via ionotropic receptors, that play a key role in the signaling pathways employed by astrocytes to regulate glutamate transmission, including ATP-P2Y1, adenosine-A1R, glutamate-mGluR5, and glutamate-mGluR2/3 signaling pathways.

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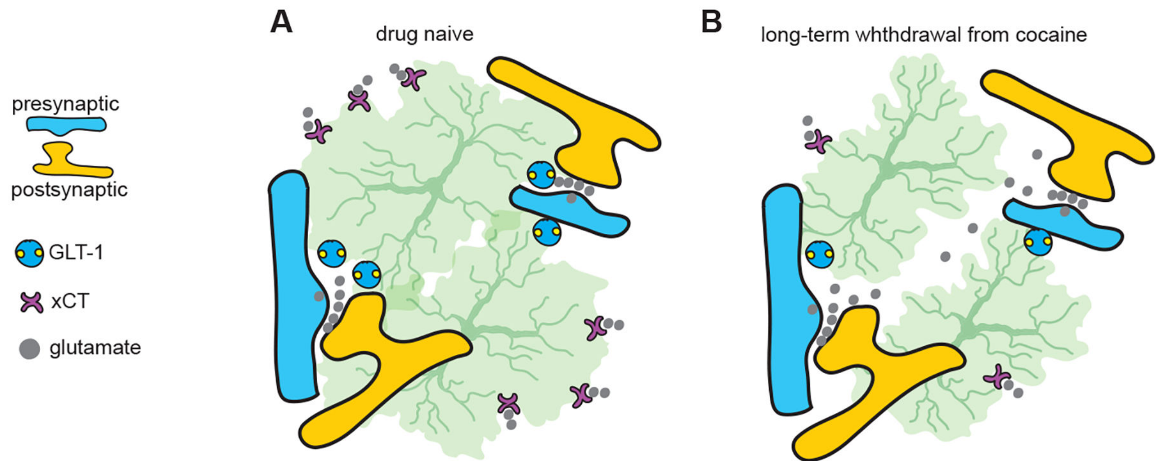

Figure 2. Dysregulation of NAc extracellular glutamate after cocaine experience.

A In drug naïve animals, GLT-1 is a key astrocytic glutamate transporter that maintains low levels of glutamate at synaptic and perisynaptic locations, while xCT is a key astrocytic glutamate antiporter that maintains ambient glutamate levels for tonic activities of mGluRs in the NAc. **B** After withdrawal from cocaine self-administration, astrocytes retract from synapses and both GLT1- and xCT-mediated regulation of glutamate is compromised, resulting in increased levels of synaptic and perisynaptic glutamate but decreased levels of ambient, extracellular glutamate.

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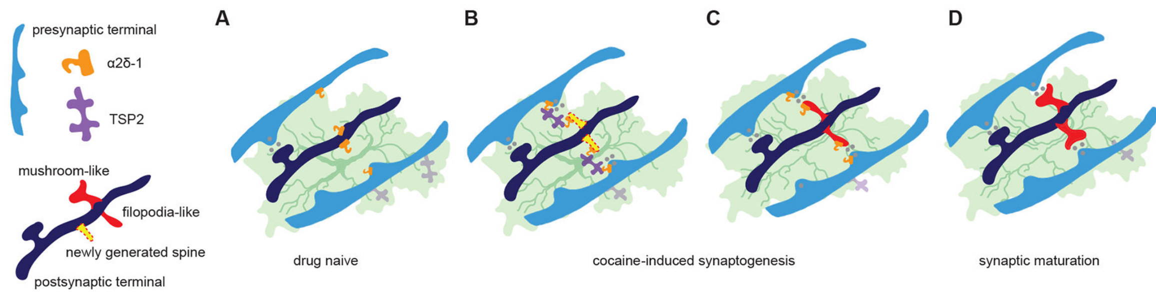

Figure 3. Astrocyte-mediated synaptogenesis after cocaine self-administration.

A Astrocytic TSP2 and neuronal α2δ−1 constitute a synaptogenic signaling pathway in the developing brain to promote formation of glutamatergic synapses. Both astrocytic TSP2 and neuronal α2δ−1 are present in the adult NAc in drug naïve animals. **B** Cocaine self-administration induces formation of nascent, AMPAR-silent synapses in the NAc, which is mediated in part by TSP2-α2δ−1 signaling. These nascent synapses are thought to have relatively immature spine morphology. **C,D** After cocaine withdrawal, some of these newly formed, immature AMPAR-silent synapses are maintained (**C**) and, by recruiting and stabilizing AMPARs, gradually transform into fully mature synapses, resulting in long-term changes of circuit connectivity (**D**).