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Astrocytic control of synaptic transmission and plasticity: a target for drugs of abuse?

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Drug addiction is a brain disease characterized by abnormal behavioral responses, which includes, but is not limited to, compulsive drug use in the face of negative consequences. These behaviors develop over time following repeated exposure to a drug of abuse. The persistence of drug addiction throughout the lifetime of an individual suggests that exposure to drug results in long-term adaptations in the brain. While alterations in neural processes have been the focus of much research in the past decades, the roles of astrocytes and the process of gliotransmission in the underlying plasticity associated with drug addiction is not well understood. In this review we will discuss the recently identified active roles for astrocytes in the control of synapses and discuss the potential role for glia in mediating certain responses to drugs of abuse through actions on synapses and synaptic plasticity. In addition to discussing studies performed using mammalian systems we will also highlight studies performed using *Drosophila* that provide direct experimental support for a role of glial cells in mediating behavioral responses to drugs of abuse. Studies in both flies and mammals also suggest connections between the circadian system and responses to drugs of abuse. Finally, we discuss the expression by astrocytes of three metabotropic receptors, metabotropic glutamate receptor 5 (mGluR5), GABA_B and CB-1, and discuss their involvement in substance abuse.

The importance of synaptic plasticity in VTA and mediation of cocaine's effects

The mesolimbic dopamine neurons are part of a well-defined pathway involved in reward processing and prediction. This pathway consists primarily of dopamine fibers arising in the ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAc). A large body of evidence has implicated these neurons in drug taking and relapse phenomenon (Corrigall et al., 1992; Phillips et al., 2003) (see recent reviews (Salamone et al., 2005; Wise, 2004) on the role of dopamine on motivation) and almost all drugs of abuse, including alcohol, nicotine, delta-9-tetrahydrocannabinol (THC), heroin and cocaine, can produce elevated dopamine levels in the nucleus accumbens (Di Chiara and Imperato, 1988; Koob and Bloom, 1988; Pontieri et al., 1996; Wise and Bozarth, 1987). While the neurochemistry and circuitry of drug addiction is well described, it is clear that behaviors associated with compulsive drug use

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and high rates of relapse during periods of abstinence require molecular and cellular adaptations that extend beyond the initial interactions of addictive drugs in the nervous system.

Addiction is associated with long-term behavioral changes produced by repeated exposure to drugs of abuse. In particular, chronic exposure to psychomotor stimulants like cocaine and amphetamine produce adaptations in brain circuitry similar to those associated with long-term plasticity (Kauer and Malenka, 2007). Alterations in the electrophysiology of neurons in the mesolimbic dopamine reward pathway has been observed following exposure to a variety of drugs of abuse. Specifically, enhanced excitatory synaptic transmission, as evidenced by an increase in glutamate receptor activation, occurs in VTA dopamine neurons following exposure to cocaine, nicotine, and alcohol (Saal et al., 2003; Ungless et al., 2001; Liu, Pu and Poo, 2005).

Of particular interest is the role of neuronal NMDA receptors for the cocaine-dependent induction of synaptic plasticity and behaviors. Cocaine administration leads to both an enhancement of excitatory synaptic transmission onto dopaminergic (DA) neurons of the VTA (Saal et al., 2003; Ungless et al., 2001) and behaviorally to conditioned place preference. Injection of NMDA receptor antagonists into the VTA prevents both cocaine-induced synaptic plasticity and conditioned place preference (Harris et al., 2004; Harris and Aston-Jones, 2003; Kalivas and Alesdatter, 1993). Given that one critical role for astrocytes is in the control of NMDA receptor function (Panatier et al., 2006), there is the clear potential for these glial cells to contribute to the regulation of synaptic plasticity and thereby responses to drugs of abuse.

The Tripartite Synapse

Astrocytes have privileged access to synapses and we are beginning to learn that as a consequence they are able to regulate both the pre- and postsynaptic terminal of excitatory and inhibitory synapses. Because of the reciprocal signaling that can occur between astrocytes and synaptic terminals, these structures have been termed the 'Tripartite Synapse' (Araque et al., 1999). Astrocytes play a variety of roles in the regulation of synaptic transmission: they clear synaptic transmitters from the cleft through the activity of transporters and can recycle glutamate through a glutamine intermediate to the synaptic terminal where it plays important roles in both excitatory and inhibitory synaptic transmission. In addition to such supportive functions astrocytes release chemical transmitters that modulate neuronal function. Astrocytes release glutamate (D'Ascenzo et al., 2007; Fellin et al., 2004; Jourdain et al., 2007; Parri et al., 2001), the NMDA receptor co-agonist D-serine (Mothet et al., 2000; Mothet et al., 2005; Wolosker et al., 1999), and ATP (Cotrina et al., 1998; Cotrina et al., 2000; Guthrie et al., 1999), which after hydrolysis to adenosine is responsible for an adenosine 1 (A1) receptor-mediated presynaptic inhibition of excitatory synaptic transmission (Haydon, 2001; Volterra and Meldolesi, 2005).

In this review, we discuss some general examples of astrocyte-neuronal interactions in the regulation of synaptic transmission as well as specific examples of reciprocal signaling between neurons and glia in the mesolimbic pathway.

Astrocytic glutamine synthetase is critical for GABAergic synaptic transmission

Glutamine synthetase is an astrocyte specific enzyme in the brain with roles in ammonium detoxification as well as in converting glutamate to glutamine as a renewable source of chemical transmitter. Inhibition of glutamine synthetase has dramatic effects on GABAergic synaptic transmission. Inhibition of either glutamine synthetase or of the neuronal glutamine

transporter led to a reduction in the amplitude of evoked IPSCs under conditions of moderate stimulation (Liang et al., 2006). Exogenous glutamine reversed the decrement resulting from glutamine synthetase blockade. Experiments performed using minimal stimulation and with low affinity GABA receptor antagonists demonstrated that the decrement in inhibitory transmission resulted from reduced filling of vesicles with GABA showing that the pool of presynaptic GABA relies on glutamine derived from synaptically associated astrocytes (Liang et al., 2006). Whether GABAergic medium spiny neurons of the nucleus accumbens rely on astrocyte-derived glutamine, remains to be determined.

Astrocytes encode information in IP₃-dependent Ca²⁺ signals

For much of the last century we underappreciated the variety and complexity of the signaling roles of the astrocyte. Though important studies were performed showing astrocytes play essential roles in metabolic support of neurons (Tsacopoulos and Magistretti, 1996) the apparent lack of excitability of these glial cells led to their being cast into the shadows rather than being at the forefront of investigation. However, with the development of optical methods – inexpensive digital cameras, confocal and later 2-photon microscopy together with the development of fluorescent indicators of Ca²⁺ – we now understand that though electrically inexcitable, the astrocyte exhibits biochemical excitability that is manifest in terms of Ca²⁺ oscillations (Wang et al., 2006).

Initial cell culture studies showed that astrocytes express receptors for neurotransmitters (e.g. glutamate) and that their activation elicits Ca²⁺ oscillations that can propagate from one astrocyte to another (Cornell Bell et al., 1990; Guthrie et al., 1999; Porter and McCarthy, 1997). Later studies have shown that the stimulation of presynaptic axons activate astrocytic Ca²⁺ signals (Porter and McCarthy, 1996). For example, stimulation of the Schaffer collateral elicits astrocytic Ca²⁺ elevations in hippocampal slice preparations and more recently the activation of glutamatergic afferents that innervate the nucleus accumbens leads to astrocytic Ca²⁺ signals (D'Ascenzo et al., 2007). (A discussion of astrocytic signaling in the nucleus accumbens is provided beneath.) Convincing demonstrations of neuronal activation of glial Ca²⁺ signals has been provided by examples in the retina and barrel cortex where natural sensory stimuli have been shown to elicit astrocytic Ca²⁺ signals: optical activation of photoreceptors elicits Ca²⁺ signals in retinal Muller glia (Newman, 2005) and movement of a whisker, in addition to activation of cortical pyramidal neurons, stimulates Ca²⁺ signals in neighboring astrocytes (Wang et al., 2006).

Astrocytes express a plethora of receptors for chemical transmitters (Porter and McCarthy, 1997; Verkhratsky et al., 1998; Verkhratsky and Kettenmann, 1996). Many of these receptors are metabotropic receptors, although ionotropic may also be expressed. Activation of presynaptic afferents frequently engages metabotropic receptors to stimulate astrocytic Ca²⁺ signals. Such receptors include metabotropic glutamate receptor 5 (mGluR5) as well as metabotropic receptors activated by purines (i.e. P₂Y₁). These receptors couple through phospholipase C to cause an elevation of diacylglycerol and inositol trisphosphate (IP₃). In turn IP₃ gates IP₃-regulated channels expressed in IP₃-sensitive Ca²⁺ stores to cause an elevation of cytosolic Ca²⁺. In addition to mGluR5 and P₂Y₁ astrocytes have been shown to express receptors for most chemical transmitters with some evidence suggesting that dopamine receptors might be expressed in these glia (Khan et al., 2001).

Astrocytic Ca²⁺ signals lead to the release of chemical transmitters

A breakthrough discovery in this field was the cell culture observation that Ca²⁺ signals in astrocytes stimulated neuronal Ca²⁺ elevations (Nedergaard, 1994; Parpura et al., 1994) hinting at the potential for a dialog between neurons and glia. Ca²⁺ elevations were shown to be both necessary and sufficient for the release of chemical transmitter from astrocytes (Parpura and

Haydon, 2000). Similar processes were documented in acutely isolated brain slice preparations: glutamate-mediated astrocyte-to-neuron signaling was observed in thalamus (Parri et al., 2001), hippocampus (Angulo et al., 2004; Fellin et al., 2004), cortex (Ding et al., 2007; Halassa et al., 2007) then later in the nucleus accumbens (D'Ascenzo et al., 2007). For example, ligands that induce astrocytic Ca^{2+} signals, or photolysis of caged Ca^{2+} that had been selectively loaded into astrocytes, caused slow, NMDA receptor-mediated neuronal currents (D'Ascenzo et al., 2007; Fellin et al., 2004). Additionally, experimentally induced Ca^{2+} signals within astrocytes can evoke a modulation of the frequency of miniature synaptic currents (Fiacco and McCarthy, 2004; Liu et al., 2004) showing that astrocytes have the potential to regulate synaptic transmission.

Astrocytes regulate neuronal NMDA receptors and synaptic plasticity

Glutamate is not the only chemical transmitter that has been shown to be released from these glial cells. Astrocytes express serine racemase which converts L- to D-serine (Mothet et al., 2005; Panatier et al., 2006; Schell et al., 1995). D-serine is released from astrocytes in response to elevated internal Ca^{2+} and in the extracellular space D-serine acts as a co-agonist of the NMDA receptor (Mothet et al., 2000). Indeed, in several brain regions D-serine is the endogenous ligand for the glycine-binding site of the NMDA receptor (Mothet et al., 2000). In the supraoptic nucleus the availability of astrocytic D-serine is critical for controlling NMDA receptor activity and as a consequence determines whether stimuli that induce synaptic plasticity lead to long-term potentiation (LTP) or long-term depression (LTD) (Panatier et al., 2006). The ability of astrocytes to control synaptic plasticity raises the possibility that they mediate or modulate the responses to drugs of abuse when considering the plastic events known to occur in the mesolimbic pathway

The potential for astrocytes regulating synaptic transmission and plasticity has been particularly well documented in studies performed by Araque's group where they have studied the ability of astrocytes to regulate plasticity of the Schaffer collateral CA1 synapse. Photolytic elevation of Ca^{2+} levels in astrocytes, induces the release of glutamate from astrocytes and lead to a long term facilitation of the excitatory synapses in this pathway (Perea and Araque, 2007). Additionally, this newly identified pathway for glial regulation of synaptic plasticity is mediated through the activation of mGluR5, which as will be discussed beneath, is an important receptor for mediating certain behavioral effects of drugs of abuse. Therefore, the astrocyte has the potential to promote synaptic plasticity.

Astrocyte-specific inducible transgenic animals reveal roles for gliotransmission

Although astrocytes can release gliotransmitters and they are able to modulate neuronal function it has been difficult to determine their roles in neuronal networks and in mediating behaviors because it has been experimentally challenging to selectively inhibit the process of gliotransmission. Although several pathways for the release of gliotransmitters have been identified, there is general consensus that under physiological external Ca^{2+} levels, and in response to elevated internal Ca^{2+} signals, astrocytes can release transmitters via an exocytotic mechanism (Volterra and Meldolesi, 2005). For example, the Ca^{2+} -dependent release of D-serine, glutamate and ATP have been shown to be attenuated by clostridial toxins that proteolytically cleave proteins essential for exocytosis (Araque et al., 2000; Bezzi et al., 2004; Coco et al., 2003; Jourdain et al., 2007; Mothet et al., 2005). Based on this, as well as other evidence, we developed a molecular genetic strategy to inhibit regulated exocytosis of gliotransmitters so that it would be possible to selectively perturb gliotransmission and determine consequences for synaptic transmission, plasticity and behavior.

Exocytosis relies on the formation of a ternary complex termed the SNARE complex. Three proteins form this complex: a vesicular SNARE protein (e.g. synaptobrevin or VAMP), a membrane protein (syntaxin) and a membrane associated protein (in neurons, SNAP-25 and astrocytes SNAP-23). The ternary complex is formed between SNARE domains of each of the proteins. Exocytosis is inhibited when the formation of this complex is prevented. Two strategies can be used to inhibit exocytosis – the use of clostridial toxins which cleave individual SNARE proteins, or the expression of dominant negative SNARE domains that compete with endogenous proteins and prevent the formation of the ternary complex. To develop an inducible astrocyte-specific transgenic animal with impaired gliotransmission we conditionally expressed a dominant negative SNARE domain in astrocytes (Pascual et al., 2005). Although there are numerous membrane trafficking pathways in a cell, each requires distinct SNARE proteins (Chen and Scheller, 2001). Therefore the expression of the dnSNARE of synaptobrevin II is highly specific for the regulated release pathway.

Using these transgenic animals we discovered the absence of extracellular adenosine, which tonically inhibits synaptic transmission through activation of presynaptic A1 receptors (Pascual et al., 2005). We showed that astrocytes release ATP which is hydrolyzed in the extracellular space to adenosine which in turn inhibits synaptic transmission. Given that adenosine receptors form heteromultimers with DA receptors it will be intriguing to determine whether the manipulation of gliotransmission and thereby of extracellular adenosine impacts dopamine responses in the reward circuitry.

To summarize the studies we have reported in the preceding discussion, it is now clear that astrocytes can modulate synapses and promote synaptic plasticity, and more recent studies have shown the requirement for gliotransmission in controlling A1 receptor-dependent regulation of synaptic transmission. With the introduction of additional molecular genetic strategies it will become possible to determine the roles of each gliotransmitter in the control of brain function and behavior, as well as to ask whether these pathways are utilized to mediate responses to drugs of abuse, or alternatively whether it would be possible to therapeutically target astrocytes to activate/inhibit their signaling systems in order to modify neural adaptations to drugs of abuse.

Astrocytes are plastic and respond to drugs of abuse

A classical property of astrocytes is their ability to become 'reactive' in pathological states. For example, in Parkinson's disease, Alzheimer's disease and epilepsy, the expression of the astrocyte specific protein glial fibrillary acidic protein (GFAP) is known to increase (Miller, 2005). The functional consequences of changes in protein expression are not known. However, it is worth noting that exposure to drugs of abuse such as cocaine and morphine lead to reactive astrocytosis and altered GFAP expression (Bowers and Kalivas, 2003; Narita et al., 2006a; Narita et al., 2006b; Song and Zhao, 2001). Clearly then, the astrocyte is part of the brain's response to drugs of abuse, but studies have not addressed the functional role of these changes in glial protein expression.

One area of considerable interest is the role of astrocytic cystine/glutamate transporters that are altered following cocaine exposure (Baker et al., 2002; Baker et al., 2003; Kalivas et al., 2003). Following withdrawal from repeated cocaine self-administration, extracellular glutamate levels fall due to reduced function of the astrocytic cystine/glutamate transporters. Correlated with this loss of glutamate is sensitivity to drug relapse following a re-exposure to cocaine. To test the involvement of this pathway in susceptibility to drug relapse Kalivas' group provided exogenous cystine, to stimulate cystine uptake and glutamate release, and showed that cocaine-primed drug-seeking behavior was abolished (Baker et al., 2003). Thus initial drug

exposure can change protein expression in astrocytes and the activity of glial transporters which can have critical consequences on drug-seeking behavior.

Genetic studies reveal connections between glia, circadian behavior and the response to drugs of abuse

Despite the correlation between reactive astrogliosis and exposure to drugs of abuse there has been little experimental evaluation of astrocyte functions in the brain's response to cocaine, apart from that cited above. The fruit fly *Drosophila* provides an outstanding genetic platform for studying the roles of glial cells in behavior and the response to drugs of abuse. We therefore discuss this system as it indicates that there are additional important roles for glia in mediating the brain's responses to drugs of abuse.

Importantly, the fly nervous system – composed of approximately $2-3 \times 10^5$ neurons (in the brain, thoracic ganglia and abdominal ganglia) and an equivalent number of glial cells – contains astrocyte-like cells and other types of glia. Previous reviews have discussed *Drosophila* glial cell differentiation (Jones, 2005), the similarities among vertebrate and insect glial cell subtypes (Freeman and Doherty, 2006) and the roles of glia in neuronal development (Freeman, 2006; Parker and Auld, 2006). In short, fly glia can be categorized into several subtypes, based on the location in the developing or adult nervous system. Simplistically, glia of the adult nervous system fall into 4 major categories: cortex, neuropil, surface, and peripheral. Of interest for this review, cortex glia, which are associated with neuronal cell bodies, are morphologically similar to mammalian astrocytes and like astrocytes are resident in the spaces between neurons. However, little is known about glia-neuron communication in the adult fly brain.

Fly glia and behavior

Countless studies, too numerous to cite, have analyzed the neuronal cell groups and circuitries required for the execution of specific *Drosophila* behaviors. However, until recently, glial cell functions have largely been ignored in the analysis of *Drosophila* behavior. The analysis of glial cells in behavior has been made possible by the many studies of glial cell development and glia-neuron interactions in the developing nervous system (Freeman, 2006; Jones, 2005; Parker and Auld, 2006). Such studies have defined subtypes of glia in the fly nervous system (Beckervordersandforth et al., 2006; Hartenstein et al., 1998) and provided genetic tools and reagents for analyzing glial cell function in adult behavior. In the past several years, a number of studies have suggested or demonstrated roles for *Drosophila* glial cells in the regulation of neuronal excitability, other physiological responses, and behaviors including those relevant for understanding responses to drugs of abuse and circadian timing. Due to space limitations, we do not discuss all these studies but instead refer the reader to the papers describing the work (Bainton et al., 2005; Comas et al., 2004; Grosjean et al., 2008; Richardt et al., 2002; Rival et al., 2006; Sanchez et al., 2006; Suh and Jackson, 2007; Walker et al., 2006; Yuan and Ganetzky, 1999). Instead, we highlight recent work on fly glial cell functions as related to circadian behavior and responses to drugs of abuse. We also discuss possible mechanistic connections between the circadian system and the machinery regulating drug responses.

Flies and drugs of abuse

A number of studies have examined responses of flies to ethanol and cocaine with the goal of defining the cellular and molecular bases of sensitivity, addiction and tolerance to the drugs. The work in this area has been well reviewed (Wolf and Heberlein, 2003), with many studies indicating a remarkable similarity between the responses of mammals and flies to such drugs

of abuse. Like mammals, flies can develop tolerance to ethanol and there is evidence that dopamine and cAMP signaling pathways can modulate effects of the drug. Similarly, the fly dopamine (dDAT) and serotonin (dSERT) transporters are thought to be targets of cocaine action (Corey et al., 1994; Demchyshyn et al., 1994; Porzgen et al., 2001; Wu and Gu, 2003) and flies with reduced Type II PKA activity show resistance to effects of the drug and lack sensitization to it (Park et al., 2000). Consistent with a role for aminergic systems in the action of cocaine, the ectopic expression of inhibitory and stimulatory $G\alpha$ subunits in dopaminergic and serotonergic neurons affects drug sensitivity in predictable ways and blocks sensitization to the drug (Li et al., 2000). It is also known that overexpression of the fly monoamine vesicular transporter, which is localized to dopaminergic and serotonergic neurons, decreases sensitivity to cocaine while stimulating locomotor activity and courtship behavior (Chang et al., 2006). Finally, a recent report indicates that flies lacking the trace amines tyramine and octopamine are hypersensitive to cocaine (Hardie et al., 2007). Thus, there are obvious parallels between flies and mammals with regard to the cellular and molecular mechanisms regulating responses to drugs of abuse, making *Drosophila* an excellent genetic model for studies in this area.

Fly glia and cocaine sensitivity

The most direct evidence that glia are required for responses to drugs of abuse comes from studies of so-called *Drosophila moody* mutants, which exhibit increased sensitivity to cocaine and nicotine but reduced sensitivity to ethanol (Bainton et al., 2005). The *moody* locus encodes two distinct G protein-coupled receptor (GPCR) isoforms which are localized to surface glia of the fly's brain, cells known to have an important role in regulating the insect blood-brain barrier (BBB). The two *Moody* isoforms have distinct and complementary functions in determining normal cocaine sensitivity. However, they are redundant for BBB function; i.e., *moody* mutants lacking one isoform have normal BBB function but abnormal cocaine sensitivity. This indicates that the cocaine phenotype of *moody* mutants is not a consequence of an altered BBB, but rather depends on another function of *Moody* proteins in regulating cocaine sensitivity. Interestingly, the expression patterns of *Moody* and another glial protein called *Ebony* (see next section) may overlap in a subset of glial cells. *Moody* is localized within surface glia and other glial cells which contain the glial-specific transcription factor *Repo*. Similarly, all *Ebony*-containing glia are *Repo* positive.

Drosophila Ebony and the glial regulation of circadian behavior

Until recently, there was no direct evidence that glia participate in the circadian control of behavior. Work from one of our labs (FRJ) has recently documented a role for a glial-specific protein known as *Ebony* in circadian control (Suh and Jackson, 2007). Null mutants for the *ebony* gene have altered circadian locomotor activity rhythms although the neuronal circadian oscillator appears to be intact. Surprisingly, *Ebony* is localized to ~400 adult fly glial cells, although the glial subtype has not been defined. Remarkably, the expression of wild-type *Ebony* in glial cells is completely sufficient to restore normal behavior to mutants.

The *ebony* gene encodes β -alanine synthetase (BAS), an enzymatic activity required for conjugating β -alanine to several different amines including dopamine (Richardt et al., 2003). *Ebony* protein show circadian changes in abundance with peaks occurring at the beginning of the day (times of high aminergic activity), consistent with the idea that *Ebony* is required for terminating amine action; the products of *Ebony* – N- β -alanine amines – might directly drive locomotor activity (as gliotransmitters) or be required for the recycling of neuronal amine neurotransmitters; the latter function is similar to the proposed role of astrocytes in terminating glutamate action and recycling glutamate, via glutamine, to synaptic terminals. Genetic interaction studies suggest that N- β -alanine dopamine and dopaminergic pathways are relevant for the circadian function of *Ebony* (Suh and Jackson, 2007). Given the evidence that

dopaminergic signaling and glia can regulate the fly's responses to drugs of abuse (Bainton et al., 2000; Bainton et al., 2005), it may be worthwhile to examine the possibility that the Ebony glial cells may help modulate neuronal responsiveness to such drugs.

Circadian clocks and drugs of abuse

Studies of both flies and mammals suggest that circadian systems and drug responses may utilize shared molecular mechanisms. The earliest genetic studies in this area showed that cocaine sensitization is eliminated in mutants of 4 different *Drosophila* circadian genes (*per*, *Clock*, *cycle* and *doubletime*) and the mutants also fail to show the normal cocaine induction of Tyrosine Decarboxylase activity (Andretic et al., 1999). Those studies did not identify the cell types that mediate this abnormal response to cocaine. Given the known role of Repo-positive fly glial cells in regulating responses to cocaine and the presence of both the Moody transporter (Bainton et al., 2005) and clock proteins in such glia (Suh and Jackson, 2007), it is a distinct possibility that circadian genes function in glia to regulate cocaine sensitization.

Similar to fly circadian mutants, the mouse *mPer1* mutant lacks cocaine sensitization whereas *mPer2* mice show a hypersensitized cocaine response (Abarca et al., 2002). Consistent with the altered cocaine responses, *mPer1* mice exhibit a lack of cocaine response in conditioned place preference (CPP) tests (i.e., cocaine is not rewarding), whereas *mPer2* mice show a strong response to the rewarding properties of cocaine. Interestingly, there is diurnal variation in cocaine responses and CPP, with stronger responses at ZT4 than at ZT12, indicating a clock regulation of the behavior.

In contrast, mouse mutants of the *Clock* gene show enhanced cocaine-induced sensitization and are more sensitive to the rewarding effects of cocaine in a CPP paradigm (McClung et al., 2005). The expression of *Clock* in dopaminergic (DA) neurons and other cell types of the VTA, a critical area for reward, together with changes in DA neuronal firing rates and bursting in the *Clock* mutant, suggest that the cocaine phenotypes of the mutant result from altered DA neurotransmission. As *Clock* is probably expressed in neurons and glia, it remains a possibility that changes in glial cell physiology contribute to the mutant phenotypes.

Given that studies using flies have shown the importance of glia in mediating responses to cocaine it is therefore worthwhile to consider whether receptor systems that are known to be involved in mediating mammalian responses to drugs of abuse are expressed in glia and whether by regulating the tripartite synapse they have the potential to impact neuronal excitability, synaptic transmission and/or synaptic plasticity. Consequently, we use the remainder of this review to discuss three receptor systems and their roles in glial cells.

mGluR5 activates astrocytic Ca^{2+} signaling and gliotransmission in the nucleus accumbens

mGluR5 is a critical receptor for mediating locomotor effects of cocaine. In mGluR5^{-/-} mice, or in mice treated with the mGluR5 antagonist MPEP, cocaine administration fails to cause drug seeking behavior (Chiamulera et al., 2001; Tessari et al., 2004). It is well known that astrocytic Ca^{2+} signaling can be activated through mGluR5 in cortex and hippocampus (D'Ascenzo et al., 2007; Ding et al., 2007; Fellin et al., 2004). Given the importance of mGluR5 in drug-seeking behaviors we therefore asked whether astrocytes of the nucleus accumbens respond to mGluR5 activation with Ca^{2+} signals.

Whole-cell patch clamp recordings performed in the nucleus accumbens showed that astrocytes resident within this nucleus exhibit the classical properties of astrocytes: they express GFAP, have a low input resistance, large negative resting potential, a linear I/V relationship and are

connected by gap junctions. Additionally, numerous ligands evoked Ca^{2+} signals in these glial cells: ATP, the GABA_B agonist baclofen, and the class I metabotropic glutamate receptor agonist, DHPG (D'Ascenzo et al., 2007). Additionally, the stimulatory effect of DHPG was attenuated by the mGluR5 specific antagonist MPEP (D'Ascenzo et al., 2007). When taken together with immunocytochemistry showing that mGluR5 is expressed by astrocytes we can therefore conclude that in addition to neuronal actions, mGluR5 acts through glia.

We and numerous other laboratories have shown that astrocytes release glutamate in response to a Ca^{2+} signal (Araque et al., 1999; Haydon, 2001; Volterra and Meldolesi, 2005). We confirmed a similar process in the nucleus accumbens and that this glial glutamate acts on neurons through extrasynaptic NMDA receptors (D'Ascenzo et al., 2007). Given that glutamatergic neurons innervate the GABAergic medium spiny neurons (MSN) of the nucleus accumbens we therefore asked whether astrocytes might be activated by afferent stimulation and provide a feed-forward excitation to MSNs. Stimulation of glutamatergic afferents with a brief theta burst stimulation evoked Ca^{2+} signals in astrocytes that were sustained for tens of minutes beyond the initial stimulus. Moreover, coincident with this period of elevated astrocytic Ca^{2+} signals were repetitive glial-dependent excitation of the MSN that was mediated by postsynaptic NMDA receptors. We next asked whether mGluR5 was required for the activation of the glial-feed-forward excitation of MSNs. Addition of the mGluR5 antagonist, MPEP, reduced the ability of afferent stimulation to stimulate astrocytic Ca^{2+} signals and inhibited the corresponding glial-dependent excitation of the MSN.

Although we have not determined whether the mGluR5-dependent excitation of the astrocyte is required to mediate responses of drugs of abuse, that this newly identified pathway is present provides a new signaling system to evaluate and to ask whether drugs of abuse act in part through gliotransmission. Additionally, given that astrocytes have been shown to be capable of regulating synaptic plasticity through an mGluR5-dependent mechanism (Perea and Araque, 2007) the door is open to elucidate the relative roles of presynaptic, postsynaptic and astrocytic receptors in mediating responses to substances including cocaine.

GABA_B receptors are expressed by neurons and astrocytes

GABA_B receptors mediate the majority of slow prolonged inhibition in the CNS. These G-protein coupled receptors act to decrease neuronal activity via the inhibition of voltage-gated Ca^{2+} channels and the activation of inwardly rectifying K^+ channels (GIRKs); these effects are dependent upon Gav/o hetero-trimeric G-proteins (Bowery and Enna, 2000). GABA_B receptor activation inhibits adenylyl-cyclase, resulting in reduced efficacy of PKA signaling pathways together with activity of mitogen-stimulated protein kinase (MAP) but increased intracellular Ca^{2+} levels via phospholipase C and store operated channels (Bettler and Tiao, 2006; Bowery and Enna, 2000). Two GABA_B receptor “subunits,” GABA_{BR1} and R2 , are found in mammals. These receptors are members of the serpentine class C G-protein coupled receptor (GPCR) superfamily. In contrast to other GPCRs, functional GABA_B receptors are composed of R1/R2 heterodimers as demonstrated by biochemical and physiological analysis. Gene knock out has also revealed that the R1 and R2 sub-units are critical components of all neuronal receptor sub-types; their individual deletion completely abolishes the expression of functional GABA_B receptors throughout the brain (Bettler and Tiao, 2006; Bowery and Enna, 2000).

GABA_B agonists and newly developed receptor allosteric modulators has been demonstrated to block the rewarding effects of cocaine in both humans and animals (Roberts, 2005; Shoptaw et al., 2003; Slattery et al., 2005). Moreover GABA_B receptor agonists have also been reported to block the rewarding properties of nicotine, heroin and sensitization to amphetamine (Akhondzadeh et al., 2000; Paterson et al., 2005; Spano et al., 2007; Zhou et al., 2004). However

it remains to be established if these effects of GABA_B receptor agonists on reward are mediated by neuronal and/or astrocytic populations of GABA_B receptors.

While it is accepted that GABA_B receptors play a critical role in regulating neuronal signaling; however, their expression in the brain is not restricted solely to neurons. We know that both GABA_BR1 and R2 subunits are abundantly expressed by astrocytes and other types of glia (Charles et al., 2003; Fraser et al., 1994). For example these receptors subunits are expressed in astrocytic processes surrounding symmetric and asymmetric synapses in the CA1 sub-field of the hippocampus. While much more must be done to explore the functional significance of astrocytic GABA_B receptors and their possible roles in gliotransmission, it is interesting to note that activation of astrocytic GABA_B receptors has been shown to induce plasticity of inhibitory synaptic transmission (Kang et al., 1998) and glial GABA_B receptors mediate heterosynaptic depression in the hippocampus (Serrano et al., 2006).

Astrocytic CB-1 receptors mediate a neuron-to-glial signaling pathway

Although the majority of studies of CB-1 receptors in the nervous system have focused on synaptic actions, it has recently been demonstrated that astrocytes can express CB-1 receptors and as a consequence mediate a non-neuronal pathway of neuronal excitation (Navarrete and Araque, 2008). Neuronal depolarization is known to cause the generation of endocannabinoids which can act on synaptic receptors to modulate synaptic transmission. However, Araque's group made the surprising observation that neuronal depolarizations evoked astrocytic Ca²⁺ signals that were attenuated by CB-1 receptor antagonists. As already discussed in relation to mGluR5-dependent activation of astrocytic Ca²⁺ signals, the consequence of CB-1-dependent astrocytic Ca²⁺ signals is the release of the gliotransmitter glutamate which excites neighboring neurons through non-synaptic NMDA receptors. The demonstration of this novel CB-1-dependent excitation of the astrocyte raises the potential that astrocytes contribute to cannabinoid-related behavioral responses.

Historically, evaluation of dopamine and its primary target neurons, the medium spiny neurons of the nucleus accumbens have dominated addiction research. However, given the persistent and long-lasting nature of this disease, it is clear that a broader scope of investigation is necessary to fully understand underlying mechanisms. There is significant evidence demonstrating that astrocytes play active roles in the regulation of synaptic transmission and synaptic plasticity. Given the importance of these plastic events in mediating actions of drugs of abuse, together with the availability of molecular genetic strategies to manipulate each component of the tripartite synapse, we are now poised to determine the relative roles of gliotransmission and synaptic transmission in mediating adaptations of the brain which underlie the addictive actions of substances of abuse.

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