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Cocaine-related Behaviors in Mice with Deficient Gliotransmission

Jill R. Turner¹, Laurel Ecke^{1,2}, Lisa Briand¹, Phil Haydon³, and Julie A. Blendy^{*,1}

¹Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104

²Neuroscience Graduate Group, University of Pennsylvania, Philadelphia, PA 19104

³Department of Neuroscience, Tufts University School of Medicine, Boston, MA

Abstract

Rationale—Astrocytes play an integral role in modulating synaptic transmission and plasticity, both key mechanisms underlying addiction. However, while astrocytes are capable of releasing chemical transmitters that can modulate neuronal function, the role of these gliotransmitters in mediating behaviors associated with drugs of abuse has been largely unexplored.

Objectives—The objective of the present study was to utilize mice with astrocytes that lack the ability to release chemical transmitters to evaluate the behavioral consequence of impaired gliotransmission on cocaine-related behaviors. These mice have previously been used to examine the role of gliotransmission in sleep homeostasis, however no studies to date have utilized them in the study of addictive behaviors.

Methods—Mice expressing a dominant-negative SNARE protein selectively in astrocytes (dnSNARE mice) were tested in a variety of behavioral paradigms examining cocaine-induced behavioral plasticity. These paradigms include locomotor sensitization, conditioned place preference followed by cocaine-induced reinstatement of CPP, and cocaine self-administration followed by cue-induced reinstatement of cocaine-seeking behavior.

Results—Wildtype and dnSNARE mice demonstrated no significant differences in the development or maintenance of locomotor sensitization. While there were non-significant trends for reduced CPP following a low dose of cocaine, drug induced reinstatement of CPP is completely blocked in dnSNARE mice. Similarly, while dnSNARE mice demonstrated a non-significant trend toward reduced cocaine self-administration compared to wildtype mice, dnSNARE mice do not demonstrate cue-induced reinstatement in this paradigm.

Conclusions—Gliotransmission is necessary for reinstatement of drug-seeking behaviors by cocaine or associated cues.

Keywords

astrocytes; cocaine; gliotransmission; reinstatement; conditioned place preference; self-administration; locomotor sensitization

INTRODUCTION

A national survey on drug use and health reported there were 1.5 million current cocaine users in the United States last year (SAMHSA 2011), representing a significant health

*Corresponding Author Telephone: 215-898-0730, Fax: 215-573-2041, blendy@mail.med.upenn.edu.

burden. Furthermore, there are no FDA approved medications to treat cocaine dependence, and a better understanding of the molecular mediators of cocaine addiction and relapse is necessary in order to develop a successful therapeutic. Addiction to cocaine and other drugs of abuse is defined behaviorally as a chronically relapsing brain disease characterized by compulsive drug use in the face of negative consequences (Koob and Volkow 2010). Although many of the brain regions critical in mediating cocaine addiction and relapse have been identified (Kalivas and McFarland 2003), the molecular underpinnings of this phenomenon remain elusive. It is known that behavioral manifestations associated with chronic drug use are paralleled by long-term adaptations in the brain. In particular, chronic exposure to psychomotor stimulants like cocaine and amphetamine produce adaptations in brain circuitry similar to those associated with long-term plasticity.

Neuronal contribution to the synaptic plasticity resulting from repeated cocaine exposure has been the focus of much drug addiction research in the past decades (for review, see (Bowers et al. 2010)). However, recent studies have illustrated an important role for astrocytes in the control of synaptic transmission (Haydon et al. 2009), which could impact behavioral responses to drugs of abuse. Astrocytes contribute to the maintenance of synaptic transmission in multiple ways. For example, they clear synaptic transmitters from the cleft via glial transporters and can recycle glutamate through a glutamine intermediate (Halassa and Haydon 2010). In addition to such supportive functions, recent studies have shown that Ca^{2+} signals in astrocytes can stimulate synaptic plasticity via active release of chemical transmitters, such as glutamate, D-serine, and ATP (Halassa et al. 2007). Furthermore, the innate properties of astrocytes, such as prolonged gliotransmission in response to a brief stimulus (D'Ascenzo et al. 2007) and the ability to generate neuronal synchrony (Fellin et al. 2006; Halassa et al. 2007), place them in a prime position to influence large populations of neurons and contribute to synaptic plasticity.

Continued drug use induces adaptive changes in the central nervous system, leading to drug dependence (Koob and Kreek 2007). These long-term adaptations in cellular signaling mechanisms are likely part of the maintenance of drug dependence. It is unknown how gliotransmission influences these long-term alterations. However, chronic exposure to drugs of abuse, including cocaine and morphine, lead to reactive astrocytosis and altered glial fibrillary acidic protein (GFAP) expression (Beitner-Johnson et al. 1993; Bowers and Kalivas 2003), suggesting that astrocytes are involved in the brain's response to drugs of abuse. One functional astrocytic response that has received considerable attention is the role of astrocytic cystine/glutamate transporters in cocaine-mediated behaviors. Withdrawal from repeated cocaine self-administration results in reduced extracellular glutamate levels due to decreased function of these cystine/glutamate transporters (Baker et al. 2003; Madayag et al. 2007). Correlated with this loss of glutamate is enhanced sensitivity to drug reinstatement following re-exposure to cocaine. Furthermore, pharmacological stimulation of the cystine/glutamate transporter causes glutamate release from astrocytic stores, which restores extracellular glutamate to normal levels and prevents cocaine-primed reinstatement (Baker et al. 2003). Thus, astrocytes may contribute to the pathology associated with withdrawal following chronic use of drugs of abuse. It is unknown, however, whether active release from astrocytic vesicular stores can impact cocaine-mediated behaviors. Until recently, it was impossible to differentiate the impact of neurotransmission versus gliotransmission on the acute and long-term effects of cocaine. The generation of a mouse line with normal neurotransmission but deficient gliotransmission (dnSNARE mice) has now allowed us to characterize the behavioral consequence of impaired gliotransmission on cocaine-mediated behaviors. dnSNARE mice have previously been used to examine the role of gliotransmission in sleep homeostasis (Halassa et al. 2009); however, no studies to date have utilized them to study mechanisms associated with cocaine-induced behavioral responses. In the present study we characterized dnSNARE mice in several well-validated behavioral

models of drug addiction. The progressive enhancement of locomotor stimulatory effects of cocaine, or behavioral sensitization, can occur after a single injection and be maintained for several months (Eisener-Dorman et al. 2011; Phillips et al. 1998). These sensitizing properties of cocaine have been proposed to play an important role in the persistence of drug-seeking behavior (Kalivas and Stewart 1991; Robinson and Becker 1986). Likewise, conditioned place preference (CPP), which models the conditioned reinforcing effects of drugs, relies upon Pavlovian conditioning to associate neutral environmental cues with the rewarding effects of cocaine (Tzschentke 2007). The preference that develops for the cocaine-paired environment can then be extinguished and reinstated by either an acute injection of the drug itself or exposure to a stressor (Itzhak and Martin 2002; Kreibich and Blendy 2004). In addition, drugs of abuse are readily self-administered intravenously by animals, and cocaine self-administration in mice can closely model human patterns of drug abuse (Caine et al. 1999). Similarly to CPP, re-exposure to cocaine or cocaine-paired stimuli in animals whose cocaine administration behavior has been extinguished will robustly reinstate drug seeking as measured by increased responding on a previously cocaine-paired lever. These reinstatement models are thought to model drug craving and relapse. While it is difficult to find a single animal model that mimics the entire syndrome of addiction, the characterization of dnSNARE mice in several behavioral paradigms that cross domains (i.e., locomotor sensitization, the development and reinstatement of cocaine CPP, drug self-administration and reinstatement of cocaine self-administration) will help clarify a role for gliotransmission in processes underlying drug addiction.

METHODS

Subjects

Generation of dominant-negative SNARE mice (dnSNARE) has been described previously (Pascual et al. 2005). For these experiments, dnSNARE mice were obtained by crossing two different mouse lines: GFAP.tTA, in which the expression of the tet-off tetracycline transactivator (tTA) is driven by the human astrocyte-specific GFAP promoter, and tetO.dnSNARE, in which the dnSNARE domain of the vesicle protein synaptobrevin II and the reporter enhanced green fluorescence protein (EGFP) are coexpressed under the control of the tetO promoter. In these studies we wanted to evaluate constitutive loss of gliotransmission. 12- to 16-week-old male and female dnSNARE mice and littermate controls (mice lacking both transgenes) were maintained on a standard laboratory chow during all experiments to allow expression of the dnSNARE protein throughout the lifetime of the mouse. Transgenic mice were backcrossed onto C57BL/6J mice for over 10 generations. All mice had *ad libitum* access to food and water in their holding cages. Lights were maintained on a 12 h light/dark cycle, with all behavioral testing performed during the light portion of the cycle (lights on 7:00 A.M. to 7:00 P.M.). All experiments were conducted according to the National Institutes of Health guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. The investigators were blind to the genotype and/or drug treatment of mice during behavioral testing. dnSNARE and wild-type genotypes were confirmed by PCR following behavioral tests. A total of 138 mice were used in these experiments. Each experiment utilized individual cohorts of animals in a between-subjects design and all experiments were counter-balanced across genotypes and sexes.

Drugs

Cocaine hydrochloride was obtained from NIDA Drug Supply (Research Triangle Park, NC). Saline (0.9%) was obtained from Sigma Aldrich, St Louis, MO. Drugs were dissolved in saline and injected in a volume of 0.1ml/10g of body weight.

Locomotor Sensitization

Locomotor activity in response to intraperitoneal drug administration was analyzed in a “home cage” activity monitoring system (MedAssociates, St. Albans, VT) as previously described (Mackler et al. 2008; Mague et al. 2009; Walters and Blendy 2001). Briefly, a novel cage identical to the home cage (28.9 cm × 17.8 cm × 12 cm) was placed in a photo-beam frame (30 cm × 24 cm × 8 cm) with sensors arranged in an 8-beam array strip. To avoid effects of novelty during testing, mice were injected i.p. with saline (0.9% sodium chloride) and individually placed in the recording cages for 3 days prior to drug administration. Mice were injected with either saline or cocaine (5.0, 10.0, or 20.0 mg/kg) on day 4 and injections were repeated once a day for 4 days (days 4–7, cocaine). Beam break data were recorded for 60 min. All mice remained in their home cages with no drug administration during the development of sensitization. To evaluate the expression of cocaine-induced sensitization, all mice were tested on day 21 with an i.p. injection of a challenge dose of cocaine (5, 10, or 20 mg/kg). Number of subjects in each treatment: 5mg/kg, n=6–8; 10mg/kg, n=7–8; 20mg/kg, n=6–7.

Conditioned Place Preference

Place-conditioning boxes consisted of two chambers (20 × 20 × 20 cm), one with stripes on the wall and a metal grid floor and the other with gray walls and plastic flooring. A partition with an opening separated the two chambers in each box, but allowed access to either side of the chamber. This partition was closed off during the pairing days. The conditioned place preference paradigm, including extinction and reinstatement, was performed as previously described (Kreibich and Blendy 2004; Walters and Blendy 2001). Number of wildtype and dnSNARE mice in each CPP treatment: 5mg/kg, n=9–10; 10mg/kg, n=8; 20mg/kg, n=12.

Mouse Self-Administration

Operant Food Training—Prior to catheterization, mice were trained to perform an operant response for sucrose pellets. Although prior food training can lead to cross-sensitization, mice will acquire cocaine self-administration more quickly and at a higher rate with this prior food training (Fowler and Kenny 2011; Ozburn et al. 2012; Thomsen and Caine 2007). Briefly, mice were placed in operant conditioning chambers (Med-Associates) and trained to spin a wheel manipulandum to receive a sucrose pellet (45 mg Noyes pellet) on an FR1 schedule of reinforcement. A 12-second compound cue consisting of light presentation above the active wheel and a 2,900 Hz tone occurred concurrent with each pellet administration. Additionally, there was a 20-second time-out period following each pellet administration, during which time the house light remained off. Mice were allowed to self-administer a maximum of 50 pellets per 60 min operant session. During the food self-administration phase, mice were food restricted to no less than 90% of their free feeding weight. No mice had to be excluded due to issues with the food restriction. Mice received 10 training sessions. Two wildtype and one mutant mouse did not distinguish between the active and inactive wheel (>60% active responding) and were excluded from further study.

Jugular Catheterization Surgery—Prior to surgery, mice were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine (i.p.). An indwelling silastic catheter was placed into the right jugular vein and sutured in place. The catheter was then threaded subcutaneously over the shoulder blade and was routed to a mesh backmount platform (Strategic Applications, Inc) that secured the placement. Animals that did not fully recover from jugular catheterization surgery were not used in the cocaine self-administration studies (4 wildtype mice and 2 mutant mice). Catheters were flushed daily with 0.1 ml of an antibiotic (Timentin, 0.93 mg/ml) dissolved in heparinized saline. The catheters were sealed with plastic obturators when not in use.

Cocaine Self-administration, Extinction and Reinstatement—After surgery, mice were allowed 3–4 days to recover before beginning behavioral testing. Mice were tested for cocaine self-administration behavior in 2-hour sessions (6 days per week) in the same chamber used for sucrose pellet self-administration. During testing, a quarter turn of a wheel (FR1) on the same wheel used for sucrose training now delivered an intravenous cocaine injection (0.5 mg/kg/infusion). Each session began with a single response-noncontingent infusion of cocaine accompanied by the presentation of the tone and light cue. A compound cue stimulus consisting of a cue light above the active wheel, a 2,900 Hz tone, and house light off was concurrent with each injection, followed by an additional 8 s time-out when responding had no programmed consequences and the house light remained off. Following 9 days of cocaine self-administration, a dose response analysis was performed. Animals were given two days of cocaine self-administration at either a low dose (0.25mg/kg/inf) or a high dose (1mg/kg/inf), followed by two days at the training dose. They then received two days of cocaine self-administration at the dose that was not tested previously. The doses were counterbalanced such that half the mice received the low dose first and half got the high dose. Following the dose response testing, cocaine-seeking behavior was extinguished by replacing the cocaine with 0.9% saline and removing the cue presentation. Daily 2h extinction sessions continued until animals reached a criterion less than 20% of self-administration responses on the two final days of self-administration (at the training dose of 0.5mg/kg/inf). The day after animals reached the extinction criterion, they had a cue-induced reinstatement session. During this session, the light and tone cues were presented non-contingently for 20 seconds every 2 minutes during the first 10 minutes of the session. After this time period, the cues were presented contingent with operant responding, just as was done during the cocaine self-administration phase. During the reinstatement session, animals received saline infusions following responses on the active wheel. During the cocaine self-administration phase, the patency of each animal's catheter was tested once every 5 days with 1mg–2mg of ketamine (i.v.). Animals failing to exhibit a response to the ketamine were excluded from the study (1 wildtype mouse & 1 dnSNARE mutant mouse). Number of wildtype and dnSNARE mice at each self-administration stage: food self-administration - wildtype, n=9, dnSNARE, n=12; cocaine self-administration - wildtype, n=5, dnSNARE, n=10; cocaine dose-response - wildtype, n=5, dnSNARE, n=9; cue-induced reinstatement - wildtype, n=4, dnSNARE, n=9.

Behavioral Data Analysis

Data were analyzed with GraphPad Prism 5.0 using repeated measures 2-way ANOVA with time and treatment as factors. *Post hoc* analyses for all behavioral experiments were conducted using the Bonferroni multiple comparison *post hoc* test. Significance thresholds were set at $p < 0.05$.

RESULTS

The Development and Expression of Locomotor Sensitization in dnSNARE Mice

To determine whether gliotransmission is involved in locomotor sensitization, wildtype and dnSNARE mice were tested in this paradigm. Data shown in Figure 1 demonstrate that cocaine-treated dnSNARE mice were not distinguishable from cocaine-treated wildtype mice and exhibited normal development of cocaine sensitization at all doses tested. At the 5mg/kg dose, there was a significant effect of day and treatment, as well as an interaction [Fig. 1A; main effect of day, $F(7,140)=29.66$, $p < 0.0001$; main effect of treatment, $F(3,140)=9.65$, $p < 0.0005$; interaction, $F(21,140)=5.42$, $p < 0.0001$]. Bonferroni post-hoc analysis indicated that cocaine-treated wildtype mice were significantly different from their saline-treated wildtype controls on days 5–7 ($p < 0.01$), while cocaine-treated dnSNARE mice were significantly different from their saline treated dnSNARE controls on day 4 and 7

($p < 0.05$). At the 10mg/kg dose, there was a significant effect of day and treatment and a significant interaction [Fig. 1B; main effect of day, $F(7,182)=83.84$, $p < 0.0001$; main effect of treatment, $F(3,182)=44.78$; $p < 0.0001$; interaction, $F(21,182)=18.32$, $p < 0.0001$]. Post-tests indicate that both cocaine-treated wildtype mice as well as cocaine-treated dnSNARE mice were significantly different from their respective saline-treated controls on days 4–7 ($p < 0.001$). At the 20mg/kg dose, a significant effect of day and treatment was observed in addition to a significant interaction [Fig. 1C; main effect of day, $F(7,154)=120.2$, $p < 0.0001$; main effect of treatment, $F(3,154)=26.61$, $p < 0.0001$; interaction, $F(21,154)=14.22$, $p < 0.0001$]. At this dose as well, post-hoc analyses indicated that both wildtype and dnSNARE mice treated with cocaine were significantly different from their respective saline-treated controls on days 4–7 ($p < 0.001$). No differences were observed between cocaine-treated wildtype and cocaine-treated dnSNARE mice at any doses during development of cocaine sensitization. Following a challenge dose of cocaine on day 21 (Challenge Day), there was a significant effect of prior cocaine treatment in both groups relative to their controls, but no significant genotype differences were observed at any dose of cocaine.

Development and Reinstatement of Conditioned Place Preference in dnSNARE Mice

To determine whether gliotransmission played a role in the development of conditioned place preference or the ability of a cocaine prime to reinstate this preference following an extinction period we tested wildtype and dnSNARE in the CPP paradigm. Mice were first tested at three doses of cocaine (5, 10, and 20 mg/kg) to examine whether there were dose-dependent differences in their development of CPP. There was a significant effect of day at all cocaine doses [Fig. 2B; main effect of day, $F(1,106)=45.32$, $p < 0.0001$], indicating significant cocaine conditioned place preference. Though a trend toward reduced preference scores was observed in the dnSNARE mice at the lowest dose of cocaine, there was no significant effect of genotype, nor an interaction of day and genotype, at any dose ($p = 0.6$).

In addition, we performed cocaine CPP extinction and reinstatement studies in the 10mg/kg dose group to evaluate whether cocaine-induced reinstatement was impaired in dnSNARE mice. These experiments reveal both a significant effect of day as well as a significant interaction of day and treatment [Fig. 3; main effect of day, $F(3,112)=5.75$, $p < 0.002$; interaction, $F(9,112)=3.39$, $p = 0.001$]. Post-hoc Bonferroni analyses indicate that both wildtype mice ($p < 0.05$) and dnSNARE mice ($p < 0.05$) demonstrated a statistically significant conditioned place preference to 10mg/kg cocaine compared to their respective saline-treated controls. No significant differences between groups were observed on extinction day. However, wildtype mice that had previously been conditioned with cocaine showed a statistically significant preference for the drug-paired side following drug-induced reinstatement compared to their wildtype saline controls ($p < 0.05$). In contrast, cocaine-treated dnSNARE mice were not significantly different from their dnSNARE saline-treated controls on reinstatement day, but did significantly differ from their cocaine-treated wildtype littermates ($p < 0.01$).

Cocaine Self-administration and Reinstatement of Cocaine Seeking in dnSNARE Mice

To determine if gliotransmission is involved in cocaine self-administration or in the ability of cocaine-associated cues to reinstate this behavior following an extinction period, we evaluated cocaine self-administration behaviors in dnSNARE mice. Wildtype and dnSNARE mice were first trained to respond for food pellets. The number of pellets earned and active responses during each food training session were measured. Both groups exhibited stable responding for food and earned 20 or more pellets after 5 days of training. No differences were seen between the two groups in the number of pellets earned, active response rate or percent active responding (Figure 4A). Following 10 sessions of operant

food-reinforced responding, mice began cocaine self-administration. Both wildtype and dnSNARE mice increased their operant responding for cocaine over the 9 self-administration sessions, despite concurrent extinction of food responding. Although dnSNARE mice showed a trend toward a lower number of infusions on days 5–9, statistical analyses indicated no differences between the groups in the number of infusions earned over the 9 sessions (Fig. 4B). During the dose response phase of the experiment, a two-way repeated measures ANOVA indicated a significant effect of dose as well as an interaction between genotype and dose [Fig. 4C; main effect of dose, $F(2,24)=44.28$, $p<0.0001$; interaction, $F(2,24)=3.615$, $p=0.04$]. However, post-hoc Bonferroni pair-wise comparisons did not quite reach significance for the two lower doses ($p=0.10$ for the low dose & $p=0.13$ for the middle dose). Following the operant cocaine-reinforced responding phase, mice underwent extinction training and no differences were seen in the rate of extinction or the time to meet the extinction criterion (Fig 4D; average days to extinction criterion= 7.92, $SD= 3.3$). However, during the cue-induced reinstatement session, dnSNARE mice exhibited significantly lower levels of active responding compared to wildtype mice [Fig. 4D; main effect of test, $F(1,11)=19.80$, $p=0.001$; main effect of genotype, $F(1,11)=9.23$, $p=0.011$; interaction, $F(1,11)=7.78$, $p=0.018$]. It is important to note that there was no difference between the groups in the total cocaine intake over the course of self-administration (wildtype = 136.4 mg/kg; mutant = 107.1 mg/kg).

DISCUSSION

The aim of the current study was to examine whether active release of gliotransmitters plays a role in the behavioral effects of cocaine. Utilization of dnSNARE mice with selective impairment in gliotransmission allowed us to determine whether gliotransmitter release plays an important role in cocaine sensitization, reward and reinforcement, as well as reinstatement by measuring locomotor activation, conditioned place preference (CPP), and self-administration in these mice.

Our findings suggest that gliotransmission may have an effect on the initial reinforcing effects of low doses of cocaine. dnSNARE mice showed normal development of cocaine sensitization at multiple doses (5 mg/kg, 10 mg/kg, and 20 mg/kg). In the CPP paradigm, however, there appeared to be a non-significant trend toward reduced CPP scores at the lowest dose of cocaine (5 mg/kg), while conditioned preference scores were not observably different at either the moderate (10 mg/kg) or high (20 mg/kg) dose of cocaine. While conditioned place preference allows us to assay the conditioned rewarding effects of cocaine, the mouse operant self-administration model provides us with a means to more directly measure drug taking and seeking. In this paradigm, dnSNARE mice displayed significantly reduced responding during the dose response phase of cocaine self-administration. However, despite a significant overall effect of dose, post-hoc tests for each individual doses were not significant. The lack of a statistically significant effect in the CPP and self-administration paradigms at these lower doses of cocaine are most likely driven by substantial variability observed within the groups at these doses. Future studies utilizing significantly greater number of animals would be needed to conclusively determine whether gliotransmission plays any role in the initial reinforcing effects of cocaine at the lower doses. However, this modest effect in acquisition of reward is consistent with the finding that acute cocaine does not induce the astrocytic calcium signals that underlie gliotransmitter release (D'Ascenzo et al. 2007). The initial rewarding effects of cocaine are well associated with dopamine release from the ventral tegmental area to regions including the amygdala, prefrontal cortex, and nucleus accumbens (NAc) (Pierce and Kumaresan 2006; Wise 2004; Wise and Bozarth 1987). As cocaine experience becomes more chronic, however, glutamatergic signaling begins to play a more important role. For example, changes in synaptic plasticity occurring following chronic exposure to drugs of abuse involve

alterations in glutamatergic signaling (Lee and Dong 2011; Mameli et al. 2009; Schmidt and Pierce 2010; Stuber et al. 2010). Furthermore, glutamate release in the nucleus accumbens is necessary for both cocaine- and cue-induced reinstatement (Backstrom and Hyttia 2007; Cornish and Kalivas 2000; Di Ciano and Everitt 2001; McFarland et al. 2003). Thus, astrocytic control of glutamatergic signaling during abstinent periods may critically impact reinstatement of drug-seeking behaviors.

In fact, statistically significant differences were observed between dnSNARE and wildtype mice during reinstatement of cocaine-related behaviors. For example, dnSNARE mice fail to reinstate following a priming injection of cocaine in a CPP paradigm. Furthermore, we show that these animals also fail to reinstate drug seeking following a cue presentation in a self-administration paradigm. When interpreting these latter results, however, it is important to address the potential confound of prior food self-administration in this study. While we, and others, utilize food self-administration prior to cocaine self-administration in mice (Alsio et al. 2011; Briand et al. 2012; Fowler and Kenny 2011; Ozburn et al. 2012; Thomsen and Caine 2007), recent studies demonstrated how difficult food responding can be to extinguish in the continued presence of food-paired cues (Thomsen and Caine 2011; Xi et al. 2011). Because our experimental design incorporates the same light/tone cues during the food and cocaine self-administration phases, we cannot separate out the contribution of food reinforcement from cocaine reinforcement during cue-induced reinstatement. Thus, subjects used in these studies have experience with extinction learning when their cocaine reinforced responding was extinguished. This history of extinction learning is likely to have influenced subsequent extinction and reinstatement behavior following cocaine self-administration since cue responding is maintained for weeks following extinction of food self-administration (Thomsen and Caine 2011).

Because of the methodological limitations that prior food self-administration presents, we cannot unequivocally state that cocaine is the only reinforcer that drives reinstatement in our study and that prior food extinction did not influence cocaine extinction behavior. However, our convergent reinstatement data from two independent models (CPP and self-administration), which utilized different methods of testing drug reward and differing modes of reinstatement, offers strong evidence that gliotransmission plays an important role in processes underlying reinstatement of conditioned reward and cocaine seeking.

One potential way gliotransmission may be involved in reinstatement is by amplifying the incoming glutamate signal that is responsible for this behavior. Glutamate afferents to the NAc activate mGluR5 receptors on astrocytes, which potentiate further glutamate release (D'Ascenzo et al. 2007). This astrocytic glutamate can then activate extrasynaptic NMDA receptors on medium spiny neurons (MSNs), increasing action potential firing of these neurons (D'Ascenzo et al. 2007). Furthermore, systemic and intra-accumbens administration of mGluR5 antagonists attenuate cocaine reinstatement (Backstrom and Hyttia 2007; Kumaresan et al. 2009), and these drugs are most likely blocking receptors on astrocytes as well as neurons. Therefore, abolishing glutamate release from astrocytes may blunt stimulus-induced increases in NAc glutamate and in turn blunt reinstatement of cocaine CPP and self-administration.

While changes in neuronal glutamate signaling may underlie the impaired reinstatement behavior observed in dnSNARE mice, the causes of these alterations are not known. These deficits may be due to a lack of glutamate or D-serine release from astrocytes or could instead be due to a lack of ATP release, which is converted to adenosine in the extracellular space and might indirectly decrease expression and functioning of NMDA receptors. Indeed, previous studies have demonstrated that the baseline deficits in NMDA receptor expression observed in dnSNARE mice can be rescued by an adenosine A1 receptor agonist and can be

mimicked in wildtype mice by an A1 receptor antagonist (Deng et al. 2011). Thus, in future studies it is important to determine which gliotransmitter is playing an active role in reinstatement.

Alternatively, gliotransmission may contribute to the cocaine-induced changes in synaptic plasticity that occur following chronic drug administration. Repeated cocaine exposure and subsequent withdrawal has been shown to upregulate surface expression of NMDA receptor subunits (Huang et al. 2009; Schumann and Yaka 2009) and induce long-term potentiation (LTP) in the nucleus accumbens (Moussawi et al. 2009). This cocaine-induced LTP prevents further synaptic plasticity from occurring and is believed to enhance relapse vulnerability; if this deficit is restored, reinstatement is prevented (Moussawi et al. 2009).

Gliotransmission has been shown to regulate synaptic transmission and plasticity under normal physiological conditions (Halassa et al. 2007; Panatier et al. 2006). For instance, astrocytes have the ability to modulate NMDA receptor function, and consequently NMDA receptor-dependent synaptic plasticity, via release of glutamate and D-serine (D'Ascenzo et al. 2007; Yang et al. 2003). Mice with impaired gliotransmission demonstrate decreased surface levels of NMDA receptor subunits NR2A and NR2B in the cortex at baseline as well as reduced synaptic NMDA receptor currents (Deng et al. 2011; Fellin et al. 2009). Thus the NMDA receptor-dependent changes in synaptic plasticity caused by cocaine exposure may also be impaired in these dnSNARE mice. It is possible that under normal circumstances, gliotransmission contributes to these cocaine-induced changes in synaptic plasticity; by impairing gliotransmission, these changes do not occur and reinstatement is prevented.

This study is the first to demonstrate a direct role of gliotransmission in cocaine mediated behavioral responses. Specifically, these data highlight the necessity of gliotransmission during processes underlying reinstatement of conditioned reward and cocaine seeking. Future experiments identifying the molecular mechanisms underlying this astrocytic contribution to reinstatement may offer new insights into the cellular mechanisms driving cocaine-related behaviors, particularly those involved in relapse. Thus, astrocytes and gliotransmission potentially represent a novel target for therapeutic intervention in the clinical population.

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Figure 1. Development and expression of locomotor sensitization in dnSNARE mice
Cocaine locomotor sensitization to 5mg/kg (A), 10 mg/kg (B), and 20 mg/kg (C) cocaine in wildtype (closed symbols) and dnSNARE (open symbols) mice. Cocaine wildtype mice significant relative to wildtype saline control: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Cocaine dnSNARE significant relative to dnSNARE saline control: &, $p<0.05$; &&&, $p<0.001$. Significant effects of previous drug treatment regardless of genotype on challenge day: ^, $p<0.05$; ^^^, $p<0.001$. Number of subjects in each treatment: 5mg/kg, $n=7-8$; 10mg/kg, $n=7-8$; 20mg/kg, $n=6-7$.

Figure 2. Development of conditioned place preference in dnSNARE mice

(A) Side preferences of wildtype and dnSNARE mice in the CPP chamber. (B) Conditioned place preference scores for cocaine-treated wildtype (solid bars) and dnSNARE (striped bars) mice at the 5mg/kg, 10mg/kg, and 20mg/kg doses. Significant effect of day: ^^^, $p < 0.001$. Number of wildtype and dnSNARE mice in each treatment: 5mg/kg, $n = 9-10$; 10mg/kg, $n = 8$; 20mg/kg, $n = 12$.

A.

PHASE	PRE	S	C	S	C	S	C	S	C	TEST	S	S	S	S	S	S	S	TEST	REINSTATEMENT
DAY	1									10								25	26

B. **Figure 3. Reinstatement of conditioned place preference in dnSNARE mice**

(A) Experimental paradigm for conditioned place preference and reinstatement. PRE: Preconditioning day 1. Day 2–9: S- saline, C- cocaine (10 mg/kg, i.p.). TEST: Test on day 10 to confirm CPP. Day 11–24: saline injections in conditioning boxes to extinguish behavior. TEST: Test on day 25 to confirm CPP is extinguished. REINSTATEMENT: Reinstatement of CPP on day 26 via an acute i.p. injection of 10mg/kg cocaine. (B) Conditioned place preference scores of wildtype and dnSNARE mutant mice following cocaine (10 mg/kg) conditioning, extinction, and reinstatement. Cocaine wildtype mice significant relative to wildtype saline control on same testing day: *, $p < 0.05$. Cocaine dnSNARE significant relative to dnSNARE saline control on same testing day: &, $p < 0.05$. Wildtype cocaine group significantly different from dnSNARE cocaine group: ##, $p < 0.01$. Number of subjects in each treatment: $n = 8$.

A. **B.** **C.** **D.**

Figure 4. dnSNARE mice exhibit decreased cocaine self-administration at low doses and decreased cue-induced reinstatement of cocaine seeking
 Food self-administration (A) and cocaine self-administration (B) behavior in wildtype (open symbols) and dnSNARE (closed symbols) mice. (C) Cocaine self-administration dose response in wildtype (open symbols) and dnSNARE (closed symbols) mice. (D) Active responses following extinction and cue-induced reinstatement in wildtype and dnSNARE mice. dnSNARE group significantly different from wildtype control on RI test day: ###, $p < 0.001$. Number of wildtype and dnSNARE mice at each self-administration stage: (A, food self-administration) wildtype, $n = 9$, dnSNARE, $n = 12$; (B, cocaine self-administration) wildtype, $n = 5$, dnSNARE, $n = 10$; (C, cocaine dose-response) wildtype, $n = 5$, dnSNARE, $n = 9$; (D, cue-induced reinstatement) wildtype, $n = 4$, dnSNARE, $n = 9$.