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Reduction in Phencyclidine Induced Sensorimotor Gating Deficits in the Rat Following Increased System Xc − Activity in the Medial Prefrontal Cortex

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Abstract

Rationale: Aspects of schizophrenia, including deficits in sensorimotor gating, have been linked to glutamate dysfunction and/or oxidative stress in the prefrontal cortex. System x_c^-, a cystine- glutamate antiporter, is a poorly understood mechanism that contributes to both cellular antioxidant capacity and glutamate homeostasis.

Objectives: Our goal was to determine whether increased system x_c^- activity within the prefrontal cortex would normalize a rodent measure of sensorimotor gating.

Methods: In situ hybridization was used to map mRNA expression of xCT, the active subunit of system x_c^-, in the prefrontal cortex. Prepulse inhibition was used to measure sensorimotor gating; deficits in prepulse inhibition were produced using phencyclidine (0.3–3 mg/kg, sc). N-acetylcysteine (10–100 μM) and the system x_c^- inhibitor (*S)*-4-carboxyphenylglycine (CPG, 0.5 μM) were used to increase and decrease system x_c^- activity, respectively. The uptake of ^{14}C-cystine into tissue punches obtained from the prefrontal cortex was used to assay system x_c^- activity.

Results: The expression of xCT mRNA in the prefrontal cortex was most prominent in a lateral band spanning primarily the prelimbic cortex. Although phencyclidine did not alter the uptake of ^{14}C-cystine in prefrontal cortical tissue punches, intra-prefrontal cortical infusion of N-acetylcysteine (10–100 μM) significantly reduced phencyclidine- (1.5 mg/kg, sc) induced deficits in prepulse inhibition. N-acetylcysteine was without effect when co-infused with CPG (0.5 μM), indicating an involvement of system x_c^-.

Conclusions: These results indicate that phencyclidine disrupts sensorimotor gating through system x_c^- independent mechanisms, but that increasing cystine-glutamate exchange in the prefrontal cortex is sufficient to reduce behavioral deficits produced by phencyclidine.

Keywords: schizophrenia, prefrontal cortex, prepulse inhibition, phencyclidine, sensorimotor gating, glutamate, system x_c^-, nonvesicular, cystine-glutamate antiporter

Introduction

Abnormal activity within the prefrontal cortex has been linked to schizophrenia and may contribute to aspects of the disease that are only marginally responsive to existing antipsychotic medications (Berman et al. 1986; Garey et al. 1998; Glantz and Lewis 2000; Lieberman et al. 2005; Rajkowska et al. 1998). Dysregulation of excitatory signaling has been widely linked to deficits in prefrontal cortical activity observed in schizophrenia (Bunney and Bunney 2000;

Javitt et al. 2011; Moghaddam and Javitt 2012), although the nature of the potential pathological changes are not clear. Reduced, increased, or de-synchronized excitatory activity in the prefrontal cortex has been proposed to occur in schizophrenia (Franzen and Ingvar 1975; Ingvar and Franzen 1974; Jackson et al. 2004; Moghaddam and Adams 1998; Potkin et al. 2009). Identifying the cellular basis of prefrontal deficits in more detail, especially as it relates to excitatory signaling, will advance our understanding of the neurobiology of schizophrenia and possibly reveal novel therapeutic targets.

The equivocal nature of excitatory signaling in relation to abnormal prefrontal cortical activity may stem from critical gaps in our understanding of glutamate. Although often described as the primary excitatory neurotransmitter in the brain (Coyle and Puttfarcken 1993; Marino et al. 2001), we are only now unmasking how the many components of this complex network of receptors, membrane-expressed enzymes, transporters, and release mechanisms function in an integrated manner to regulate the neurotransmitter actions of this amino acid. Extant data indicate the existence of multiple glutamate release mechanisms by astrocytes (Araque et al. 2000; Bezzi et al. 1998; Parpura et al. 1994; Pasti et al. 2001; Ye et al. 2003) that may independently maintain glutamate concentrations in distinct compartments (e.g., synaptic and extrasynaptic; Bridges et al. 2012). If this is the case, a hypo- and hyper-glutamatergic state may exist in these separate compartments.

System x_c^- is an example of a poorly understood regulator of glutamate homeostasis that may exert profound control over multiple aspects of synaptic function (Baker et al. 2002; Moran et al. 2005; Moussawi et al. 2009; Moussawi et al. 2011). Originally identified over thirty years ago as a sodium-independent glutamate transporter (Bannai and Kitamura 1980), system x_c^- functions as a cystine-glutamate antiporter or exchanger that couples the uptake of one molecule of extracellular cystine to the release of one molecule of intracellular glutamate (Bannai 1986; Piani and Fontana 1994). Nonvesicular glutamate release by system x_c^- regulates synaptic activity by stimulating extrasynaptic receptors including inhibitory group II metabotropic glutamate receptors (Baker et al. 2002; Kupchik et al. 2011; Moran et al. 2005; Pow 2001), which is thought to then inhibit synaptic neurotransmitter release (Battaglia et al. 1997; Moran et al. 2005). Thus, manipulations of system x_c^- activity could differentially impact levels of extracellular glutamate in extrasynaptic and synaptic compartments. An important question to be addressed is whether increased system x_c^- activity in the prefrontal cortex is

sufficient to manipulate behaviors thought to stem from excitatory signaling within the prefrontal cortex, especially since extant data suggest that system x_c^- activity in the prefrontal cortex may be altered in schizophrenia (Baker et al. 2008; Do et al. 2000; Raffa et al. 2011).

The purpose of the current set of studies was to determine whether system x_c^- activity in the prefrontal cortex may influence sensorimotor gating, in part because it may be altered in schizophrenia as a result of abnormal excitatory signaling in the prefrontal cortex (Koch and Bubser 1994; Kumari et al. 2008; Majic et al. 2011; Swerdlow et al. 2001; Wilmsmeier et al. 2010). Sensorimotor gating refers to the ability of the central nervous system to regulate the capacity of sensory information to influence behavior (Braff and Geyer 1990; Graham 1975; Swerdlow et al. 1986). It is often assessed in rodents using prepulse inhibition, which involves measuring the inhibition of a startle response when the onset of the startle-eliciting auditory stimuli is indicated to the subject by a preceding, relatively mild acoustic stimulus. Attempts to reveal the neural basis of sensorimotor gating deficits in schizophrenia often involve the use of non-competitive NMDA receptor antagonists, such as phencyclidine, to disrupt prepulse inhibition of an acoustic startle response (Braff et al. 1978; Geyer et al. 2001; Swerdlow et al. 2006).

Materials and Methods

Animals and Surgeries

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 300–400 grams were individually housed in a temperature controlled room with a 12-h light/dark cycle with food and water *ad libitum*. The housing conditions and care of the rats were in accordance with the Animal Welfare Act, and all procedures were approved by the Marquette University IACUC Committee. The total number of rats used in these experiments was 172; each rat was tested only once. Rats used in the microdialysis studies were anesthetized using pentobarbital (50 mg/kg, IP) with atropine sulfate (1 mg/kg, IP) pretreatment to limit tracheobronchial secretions. One pair of bilateral guide cannula (20 gauge, 14 mm; Plastics One, Roanoke, VA) were implanted using coordinates (+3.1 mm anterior, ±1.0 mm mediolateral to Bregma, and −0.75 mm ventral from the surface of the skull at a 6° angle from vertical) derived from Paxinos and Watson (Paxinos and Watson 1986). Following surgery, rats were provided acetaminophen (480 mg/L) in their drinking water for 2 days and were given at least six days to recover prior to testing.

In situ *hybridization*

Brains were rapidly removed and frozen in optimal cutting temperature solution (OCT, Sakura, Torrance, CA) in a dry ice and ethanol bath and then cut into 12 μm coronal sections. Standard *in vitro* transcription methods were used to generate riboprobes against xCT (Choi, Milwaukee, WI) which was subsequently diluted in hybridization cocktail (Amresco, Solon, OH) and tRNA. Sections were hybridized overnight at 55°C in a mixture of tRNA, formamide, dextran, NaCl, EDTA, Denhardt's solution and ^{33}P-labeled riboprobe. After hybridization, slides were rinsed in 2× SSC buffer (pH 7.0). They were treated with RNase A in a 0.5 M sodium chloride, 10 mM Tris, 1 mM EDTA buffer for 30 min at 37 °C and then washed in the same buffer without RNase A for 30 min at 37 °C. Slides were stringently washed in 0.5× SSC for 30 min at 6 °C and then exposed to autoradiographic film (3 days) and then subsequently coated with Kodak autoradiographic emulsion NTB (Rochester, NY) and exposed for 9 days to produce silver grains. Following standard autoradiography development, NTB emulsion-dipped sections were counterstained with 0.5% cresyl violet. Photodocumentation of silver grains was achieved using dark field microscopy (Axioskop-2, Zeiss, Thornwood, NY) and Axiovision image analysis software (Zeiss, Thornwood, NY).

$[^{14}C]$Cystine Uptake

Rats were decapitated and the brains were rapidly extracted and cut into 1 mm coronal slices using a brain matrix. Tissue punches (1.25 mm diameter, Stoelting, Wood Dale, IL) were collected from the medial prefrontal cortex and incubated at 37°C for approximately 30 minutes on a nylon bolting cloth platform, submerged beneath 2 mm of standard buffer (Lobner and Lipton 1993); the standard buffer contained 124 mM NaCl, 3.0 mM KCl, 1.4 mM KH_2PO_4, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 2.4 mM $CaCl_2$, and 4 mM glucose, equilibrated with 95% O_2, 5% CO_2; pH was 7.4. After a 30 min wash, the tissue was incubated for 10 minutes in standard buffer containing phencyclidine (0 or 3 μM) or the system x_c^- inhibitor (*S*)-4-carboxyphenylglycine (CPG; 0 or 1 mM). ^{14}C-cystine (0.03 μ Ci/ml; PerkinElmer, Boston, MA) and DL-*threo*-β-Benzyloxyaspartic acid (10 μM; to prevent the uptake of cystine via X_{AG}) was then added to the incubation buffer for ten minutes. Next, the tissue punches were washed 4 times in ice cold standard buffer and dissolved in 250 μl of 1% sodium dodecyl sulfate (SDS). One aliquot (100 μl) was used to measure ^{14}C-cystine uptake by scintillation counting; a second aliquot (100 μl) was used to assess protein content by the BCA method. Cystine uptake (CPM/μg protein)

was normalized to the vehicle group run concurrently (i.e., in the same plate).

In Vivo Drug Treatments

Phencyclidine (0–3 mg/kg; NIDA Drug Supply Program, Research Triangle, NC) was dissolved in isotonic saline. N-acetylcysteine (0–100 µM, Sigma Chemical Co., St Louis, MO) and CPG (0.5 µM; Tocris-Cooksin, Ellisville, MO) was dissolved in microdialysis buffer.

Prepulse Inhibition

Rats were placed on a platform in a sound attenuating chamber (27.62×35.56×49.53cm); Hamilton Kinder, CA) that rested on a motion sensing plate. A matching session was conducted to determine the magnitude of the startle response for each rat. This session consisted of a five minute habituation period followed by 20 trials; 17 trials involved the presentation of a single auditory stimulus (pulse stimulus; 50 dB above the 60 dB background noise, 40 ms duration) and three trials in which a prepulse stimulus (12 dB above background, 20 ms duration) was presented 100 ms before the pulse stimulus. Rats were then assigned into the various treatment groups based on the magnitude of their startle response. At least one day later, an experimental session was conducted to assess sensorimotor gating. On this day, rats received a 5 or 10 min habituation period followed by 58 discrete trials; eight background trials with only background noise, 26 trials with only the pulse stimulus (50 dB above background), and 24 trials with the pulse stimulus being preceded by one of three prepulse stimuli (2, 6, or 15 dB above background). The percent of prepulse inhibition was determined as 100-(average prepulse startle response/average startle stimulus alone)*100.

In Vivo *Microdialysis*

Microdialysis probes, constructed as previously described (Baker et al. 2002). The active membrane, approximately 1 mm in total length, began 1 mm beyond the most distal aspect of the guide cannula to minimize the influence of tissue damaged by the guide cannula to our measures. Dialysis buffer (5 mM glucose, 140 mM NaCl, 1.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 0.15% phosphate buffer saline, pH 7.4) was pumped through the probes at a rate of 1 µl/min. Drugs were administered as described below for each experiment. Reverse dialysis was used for local infusions since it permits delivery of a low

concentration of a drug (especially when considering an average recovery rate of 7% that is typical of our probes) across time as well as across the dorsal-ventral axis of the prefrontal cortex.

Histology

Rats included in the microdialysis studies were given an overdose of pentobarbital (60 mg/kg, IP), and the brains were fixed by intracardiac infusion of 0.9% saline followed by 2.5% formalin solution. Brains were removed and stored in 2.5% formalin for at least seven days prior to sectioning. The tissue was then blocked and coronal sections (100 μM) were cut and stained with cresyl violet to verify probe placements. Rats determined to have misplaced guide cannula were excluded from all behavioral analyses.

Statistics

Primary comparisons involving only two groups (i.e., Exps 1 and 5) were analyzed using an independent-samples T-test. Primary comparisons involving more than two groups were analyzed using ANOVA with drug treatment (i.e., phencyclidine, N-acetylcysteine) as between-subjects factors and prepulse intensity as a repeated measure. The presence or absences of CPG was used as an additional between subjects variable used in experiment 4. Significant interactions and main effects were further analyzed using Tukey HSD ($p < 0.05$).

Design of experiment 1

This experiment was designed to characterize system x_c^- in the prefrontal cortex. First, we used *in situ* hybridization to determine the distribution of mRNA of xCT, the active subunit for system x_c^-. Next, we measured ^{14}C-cystine uptake in *ex vivo* tissue punches obtained from the prefrontal cortex in the absence or presence of the system x_c^- inhibitor CPG to confirm the existence of cystine-glutamate exchange by system x_c^-.

Design of experiment 2

The purpose of this experiment was to select a dose of phencyclidine for subsequent experiments. Rats received an acute injection of phencyclidine (0–3 mg/kg, SC) ten minutes before being placed in the startle chamber. Testing was conducted as described above.

Design of experiment 3

This experiment was designed to determine the impact of increased cystine-glutamate exchange on phencyclidine-induced deficits in prepulse inhibition. To do this, we reverse dialyzed the cysteine prodrug N-acetylcysteine (0–100 µM) directly into the prefrontal cortex for one hour prior to and after phencyclidine (0 or 1.5 mg/kg, SC) administration. One hour after phencyclidine, the probes were removed and rats underwent prepulse inhibition testing as described above.

Design of experiment 4

This experiment was designed to verify that N-acetylcysteine antagonizes phencyclidine-induced deficits in prepulse inhibition by increasing system x_c^- activity in the prefrontal cortex. To do this, the system x_c^- inhibitor CPG (0 or 0.5 µM), was infused into the prefrontal cortex. Twenty minutes later, N-acetylcysteine (0 or 30 µM) was added to the dialysis buffer such that CPG and N-acetylcysteine were co-infused until prepulse inhibition testing. One hour after CPG and N-acetylcysteine treatment began, phencyclidine was administered (0–1.5 mg/kg, SC). One hour post phencyclidine, the probes were removed and rats underwent prepulse inhibition testing as described above.

Design of experiment 5

This experiment was used to determine whether phencyclidine directly influences the rate of cystine-glutamate exchange by system x_c^-. To do this, we measured the uptake of ^{14}C-cystine uptake in prefrontal cortical tissue punches incubated in the absence (N = 9) or presence (N = 7) of phencyclidine (3 µM). The concentration of phencyclidine chosen exceeds estimates of NMDA receptor affinity for this compound by 3–15 fold (Morris et al. 2005).

Results

System x_c^- appears to be differentially expressed across subregions of the prefrontal cortex. The distribution of xCT mRNA within the prefrontal cortex reveals a particularly prominent lateral band of xCT mRNA contained primarily in the prelimbic cortex (see Figure 1). The uptake of ^{14}C-cystine by tissue punches obtained from this subregion of the prefrontal cortex was significantly reduced by the

system x_c^- inhibitor CPG ($t_{(14)}$=12.4, p<0.001) revealing the existence of functional cystine-glutamate exchange by system x_c^- in this region.
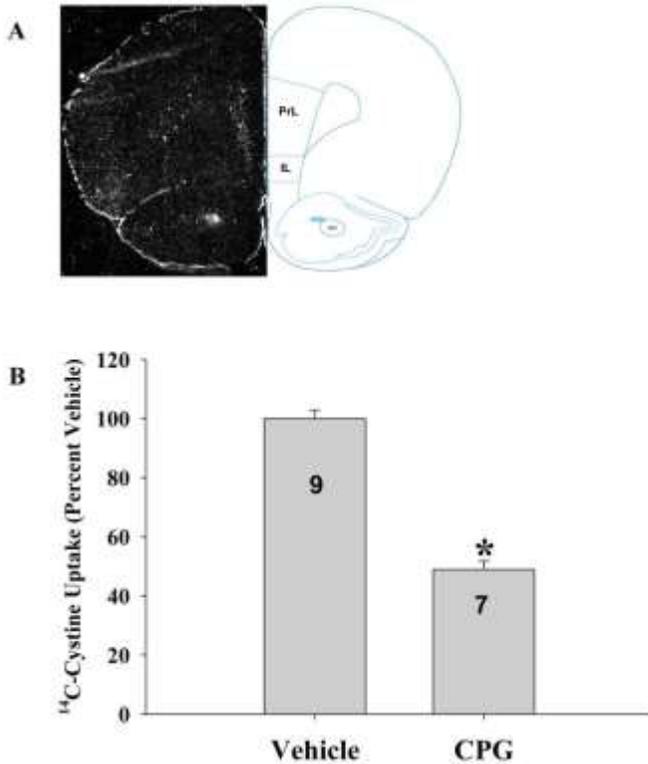

Fig. 1 Cystine-glutamate exchange by system x_c^- activity is present in the prefrontal cortex. **(a)** A representative coronal section displaying the expression pattern of xCT mRNA via *in situ* hybridization (left, dark field photomicrograph). The right illustration, adapted from (Paxinos and Watson 1998), indicates that xCT mRNA is primarily expressed in prelimbic region of the prefrontal cortex (PrL) with minimal expression in the infralimbic cortex (IL). **(b)** The uptake of ^{14}C-cystine by prefrontal cortical tissue punches in the absence or presence of the system x_c^- inhibitor (*S*)-4-carboxyphenylglycine (CPG). The number of subjects for each group is listed in the bar graph. * indicates a difference from controls (t-test, *p* < 0.05).

Phencyclidine produced a dose-dependent impairment in prepulse inhibition of the acoustic startle reflex (see Figure 2). An ANOVA with drug treatment as a between subjects variable and prepulse intensity as a repeated measure yielded a significant interaction ($F_{(12,102)}$=5.77, p<0.001). Analysis of the simple main effects revealed a significant main effect of drug treatment at prepulse intensities of six ($F_{(6,57)}$=9.20, p<0.001) and 15 dB ($F_{(6,57)}$=14.79, p<0.001), but not two dB ($F_{(6,57)}$=0.40, p=0.88). Post hoc analyses indicated that phencyclidine produced deficits in prepulse inhibition at doses exceeding 1 mg/kg (Tukey, p<0.05). A main effect of phencyclidine treatment ($F_{(6,57)}$=5.80, p<0.001) on startle magnitude was obtained. Post-hoc analysis revealed that phencyclidine dose-

dependently (1.25, 2.0 mg/kg) altered startle magnitude (Table 1; Tukey, p<0.05).
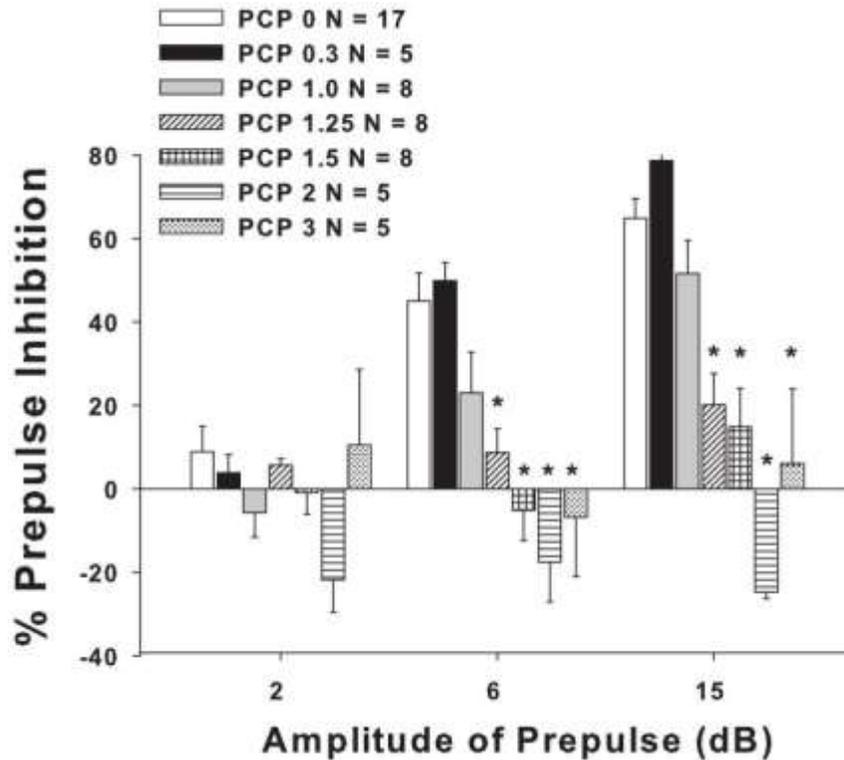

Fig. 2 Phencyclidine produces a dose-dependent disruption of prepulse inhibition. The data is expressed as the mean (+ SEM) percent inhibition of the startle response to a loud auditory stimulus when preceded by a mile-modest, nonstartle-eliciting auditory cue ranging between 2–15 dB. Behavior depicted was obtained from rats that had received an acute injection of phencyclidine (PCP, 0–3 mg/kg; SC) ten minutes prior to testing. * indicates a difference from controls receiving 0 mg/kg phencyclidine (Tukey HSD, P<0.05).

Table 1 Startle magnitude for each experimental group in each behavioral experiment

Exp 2	PCP 0	PCP 0.3	PCP 1.0	PCP 1.25	PCP 1.5	PCP 2.0	PCP 3.0
	1.82±0.19	1.58±0.47	2.19±0.35	3.37±0.53*	3.18±0.31	3.61±1.77*	1.06±0.32
Exp 3	NAC 0/PCP 0	NAC 0/PCP 1.5	NAC 10/PCP 1.5	NAC 30/PCP 1.5	NAC 100/PCP 1.5		
	1.63±0.28	1.63±0.34	1.99±0.33	1.92±0.59	1.75±0.26		
Exp 4a	CPG 0/NAC 0/PCP 0	CPG 0/NAC 0/PCP 1.5	CPG 0/NAC 30/PCP 1.5				
	1.63±0.29	2.87±0.62	2.31±0.54				
Exp 4b	CPG 0.5/NAC 0/PCP 0	CPG 0.5/NAC 0/PCP 1.5	CPG 0.5/NAC 30/PCP 1.5				
	1.75±0.27	2.43±0.33	2.20±0.53				

PCP phencyclidine, *NAC* N-acetylcysteine, *CPG* (S)-4 carboxyphenylglycine
*p<0.05, compared to PCP 0

Figure 3a depicts the impact of N-acetylcysteine infused into the medial prefrontal cortex via reverse dialysis on phencyclidine-induced

deficits in sensorimotor gating. An ANOVA with drug treatment as a between subjects variable and prepulse intensity as a repeated measure yielded a significant interaction ($F_{(8,54)}$=2.26, p<0.05). Analysis of the simple main effects revealed a significant effect of drug at every prepulse intensity (2 dB: $F_{(4,31)}$=4.12, p = 0.01; 6 dB: $F_{(4,31)}$=10.70, p<0.001; 15 dB: $F_{(4,31)}$=7.15, p<0.001). Post hoc analyses indicated that phencyclidine-induced impairment of sensorimotor gating was attenuated by N-acetylcysteine at every prepulse intensity (figure 4; Tukey, p<0.05). There were no significant differences in the startle amplitude between the groups included in this study (ANOVA: $F_{(4,33)}$= 0.21, p>0.05, table 1). The subregion of the prefrontal cortex targeted in the microdialysis studies is depicted in Figure 3b.
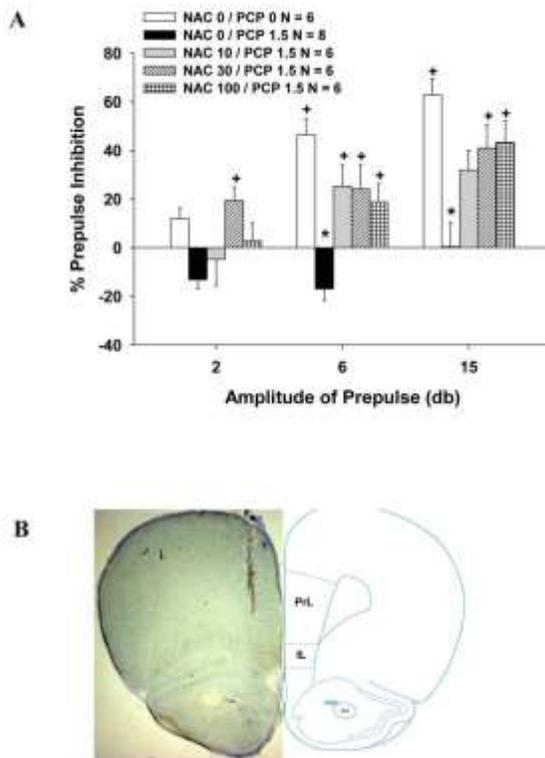
Fig. 3 N-acetylcysteine infused into the prefrontal cortex antagonizes phencyclidine-induced deficits in prepulse inhibition. **(a)** The mean (+ SEM) prepulse inhibition displayed by rats receiving N-acetylcysteine (NAC, 0 – 100 μM) into the prefrontal cortex followed 1 hour later by an acute injection of phencyclidine (0 or 1.5 mg/kg, SC). * indicates a significant difference from control rats receiving only saline (NAC 0/PCP 0; Tukey HSD, *p* < 0.05). + indicates a significant difference from rats receiving only phencyclidine (NAC 0/PCP 1.25; Tukey HSD, *p* < 0.05). **(b)** The microdialysis probes were placed primarily in the prelimbic cortex (PrL), although aspects of the infralimbic (IL) may have been perfused as well. A representative coronal section illustrates the placement of a microdialysis probe. The tract created extends approximately 2 mm from the guide cannula with sampling only occurring from the

most ventral 1 mm because this is the portion of the probe containing the dialysis membrane.
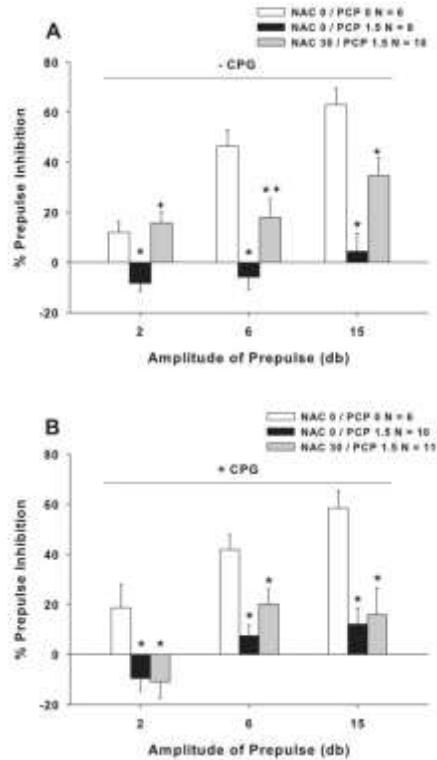

Fig. 4 N-acetylcysteine targets system x_c^- to antagonize deficits in prepulse inhibition produced by phencyclidine. The data is expressed as mean (+ SEM) prepulse inhibition displayed by rats receiving intra-prefrontal N-acetylcysteine (30 μM) (a) in the absence or (b) or presence of the system x_c^- inhibitor CPG (0.5 μM) co-infused into the prefrontal cortex. CPG was infused alone for 20 min, N-acetylcysteine was then added to the microdialysis buffer for 60 min prior to the injection of phencyclidine (1.5 mg/kg, SC). **(a and b)** * indicates a significant difference from respective vehicle controls (i.e., rats receiving NAC 0/PCP 0; Tukey HSD, $p < 0.05$). + indicates a significant difference from respective phencyclidine controls (NAC 0/PCP 1.5; Tukey HSD, $p < 0.05$).

Figure 4 demonstrates that N-acetylcysteine antagonizes phencyclidine-induced deficits in prepulse inhibition by increasing cystine-glutamate exchange from system x_c^-. An ANOVA with drug treatments (ie, N-acetylcysteine and PCP treatment) as between subjects variables and prepulse intensity as a repeated measure produced a significant interaction between CPG treatment and drug treatment ($F_{(1,46)} = 4.57$, p<0.05). To further deconstruct the interaction, we examined the simple main effect of drug treatment at each level of the CPG variable. In the absence of CPG, an ANOVA with drug treatment as a between subjects variable and prepulse intensity as a repeated measure yielded a significant interaction between these variables ($F_{(4,42)} = 3.01$, p<0.05). Analysis of the simple main effects

revealed a significant main effect of drug treatment at every prepulse intensity (2 dB: $F_{(2,23)}$= 12.41, p<0.001); 6 dB $F_{(2,23)}$= 13.82, p<0.001; 15 dB $F_{(2,23)}$= 12.35, p<0.001). Post hoc analyses indicated that N-acetylcysteine antagonized phencyclidine-induced deficits in prepulse inhibition when tested in the absence of CPG (figure 4a; Tukey, p<0.05). In the presence of CPG, a two-way ANOVA including treatment (N-acetylcysteine and phencyclidine treatments) as the between subjects variable and prepulse intensity as the repeated measures yielded a significant main effect of treatment ($F_{(2,24)}$= 10.35, p<0.001) in the absence of a significant interaction between treatment and prepulse intensity ($F_{(4,48)}$= 1.91, p>0.05). Post hoc analyses indicated that N-acetylcysteine failed to alter phencyclidine-induced deficits in prepulse inhibition when tested in the presence of CPG (Figure 4b; Tukey, p>0.05). There were no significant differences in the startle amplitude between the groups included in this study ($F_{(2,23)}$= 1.10, p>0.05; $F_{(2,26)}$= 0.49, p>0.05; table 1).

A comparison of ^{14}C-cystine uptake by tissue punches obtained from the prefrontal cortex did not reveal an effect of phencyclidine ($t_{(25)}$=1.21, p>0.05). The mean values (± SEM) for vehicle and phencyclidine-treated tissue punches were 100±9.9 and 83±9.2, respectively. These data indicate that glutamate dysfunction produced by phencyclidine does not likely involve altered activity of system x_c^- in the prefrontal cortex.

Discussion

Abnormal glutamatergic signaling particularly in cortical structures has been linked to negative symptoms and cognitive deficits present in schizophrenia (Bunney and Bunney 2000; Deserno et al. 2012; Goghari et al. 2010; van Veelen et al. 2010). As a result, identifying cellular mechanisms capable of regulating glutamate signaling may advance our understanding of the neurobiological basis of schizophrenia and facilitate the development of pharmacotherapies. System x_c^-, which exchanges extracellular cystine for intracellular glutamate (Bannai 1986; Bridges 2011), is a poorly understood regulator of glutamate homeostasis that may exert profound control over multiple aspects of synaptic function (Baker et al. 2002; Moran et al. 2005; Moussawi et al. 2009; Moussawi et al. 2011) and may be altered in schizophrenia (Baker et al. 2008; Bridges et al. 2012). The primary finding of the present report is that infusion of the cysteine prodrug N-acetylcysteine into the prefrontal cortex attenuates phencyclidine-induced deficits in sensorimotor gating and this effect is dependent upon intact system x_c^- activity. Collectively, these data illustrate the capacity of system x_c^- to regulate a complex behavior

involving the prefrontal cortex. Below, we discuss the potential relevance of this data set to our understanding and potential treatment of schizophrenia.

In the current study, infusion of N-acetylcysteine into the prefrontal cortex antagonized phencyclidine-induced deficits in prepulse inhibition. The effects of N-acetylcysteine on prepulse inhibition are likely due to increased activity of system x_c^- in the prefrontal cortex, particularly within the prelimbic region of this structure. To establish this, we first verified the existence of system x_c^- in the prefrontal cortex by demonstrating the presence of mRNA for xCT, the active subunit of system x_c^-, and functional cystine-glutamate exchange in prefrontal cortical tissue. Next, we found that reverse dialysis of N-acetylcysteine directly into this region antagonized phencyclidine-induced deficits in prepulse inhibition, an effect not observed when the system x_c^- inhibitor CPG was co-infused. This finding is similar to our previous observations that N-acetylcysteine required intact system x_c^- activity to alter multiple behaviors including phencyclidine-induced deficits in working memory (Baker et al. 2008; Kau et al. 2008). Interestingly, this could stem from either the release of glutamate or the increase in intracellular thiols expected from increased system x_c^- activity (Baker et al. 2002; Bridges et al. 2012; Shimosato et al. 1995), which is expressed on astrocytes (Pow 2001). It is important to note that studies demonstrate the possibility that glutathione, a thiol that is dependent upon the transport of cystine for de novo synthesis, is capable of functioning as a signaling molecule (Janaky et al. 1999; Ogita et al. 1995), and has been shown to influence excitatory signaling (Baker et al. 2002; Moran et al. 2005). In either case, the data are consistent with the conclusion that astrocytes may be key in regulating the activity of the prefrontal cortex in a manner that alters complex behaviors. Interestingly, activation of astrocytes has recently been shown to synchronize the firing large numbers of neurons through a glutamate-dependent manner (Angulo et al. 2004; Poskanzer and Yuste 2011). Thus, an important area of future research is the degree to which system x_c^- may be involved in these phenomena, especially since the origin of the glutamate may be nonvesicular (Angulo et al. 2004).

The present data may also have implications for our understanding and treatment of schizophrenia. Phencyclidine-induced deficits in prepulse inhibition has been used to study the neural basis of sensorimotor gating deficits observed in schizophrenia as well and to screen putative therapeutics that may exert antipsychotic effects (Geyer et al. 2001; Swerdlow et al. 2008). However, the constructive

validity of the phencyclidine model is questionable given that there are likely to be critical distinctions between the basis of abnormal behaviors produced by acute administration of phencyclidine and a chronic illness such as schizophrenia. In addition, the predictive validity of this model may also be questioned given that a number of currently used antipsychotics fail to alter phencyclidine-induced deficits in numerous behaviors, including prepulse inhibition. To this latter point, however, the inability of most currently used antipsychotics to significantly improve prepulse inhibition deficits produced by phencyclidine may parallel the poor efficacy of these medications in treating numerous symptoms associated with schizophrenia, particularly those thought to involve, at least in part, the prefrontal cortex such as sensorimotor gating and cognitive deficits (Japha and Koch 1999; Kumari et al. 2008; Swerdlow et al. 2001). In support, clozapine, which appears unique from other antipsychotics in that it significantly reduces sensorimotor gating and cognitive deficits in schizophrenia (Oranje et al. 2002), also has been shown to attenuate phencyclidine-induced deficits in prepulse inhibition (Bakshi et al. 1994; Geyer et al. 2001; Li et al. 2011), but see (Cilia et al. 2007; Schwabe et al. 2005).

An important question is the possibility that altered system x_c^- function may be evident in schizophrenia, and, if so, whether any potential disruption might impact the therapeutic utility of this target. In the current study, we did not find evidence that the psychotomimetic phencyclidine disrupted system x_c^- activity, although, as discussed above, there likely are key distinctions between abnormal behaviors produced by schizophrenia and acute phencyclidine administration. Further, we cannot rule out the possibility that phencyclidine alters system x_c^- activity under certain conditions (e.g., in vivo) or in a tissue-specific manner (e.g. cell type or brain region). Regardless, in schizophrenia, glutathione levels in the dorsolateral prefrontal cortex are significantly reduced (Do et al. 2000), which may indicate reduced system x_c^-. The reason for this is that intracellular cystine availability is thought to be the rate-limiting step in the synthesis of this key antioxidant (Kranich et al. 1998; Sagara et al. 1993). Perhaps as a result of the decrease in glutathione, the protein levels of xCT, the active subunit for system x_c^-, is significantly increased in the dorsolateral prefrontal cortex in post-mortem tissue obtained from individuals afflicted with schizophrenia relative to controls (Baker et al. 2008). In support, decreased glutathione levels has been shown to be sufficient to up-regulate system x_c^- (Seib et al. 2011). At the very least, these studies indicate that stimulation of system x_c^- is a potential therapeutic target for schizophrenia, which has been verified in a clinical trial (Berk et al. 2008).

Because system x_c^- is at the interface between oxidative stress and excitatory signaling, it is unclear how N-acetylcysteine reduces behavioral and neurochemical measures in rodents that may be relevant for schizophrenia (Baker et al. 2008; Chen et al. 2010; das Neves Duarte et al. 2012) or the actual symptoms of the disease in man (Berk et al. 2008; Berk et al. 2011). Specifically, system x_c^- couples the uptake of extracellular cystine to the release of intracellular glutamate (Baker et al. 2002; Bannai 1984; Bridges 2011; Cho and Bannai 1990). Evidence implicating the glutamate component of cystine-glutamate exchange include observations that system x_c^- regulates multiple aspects of synaptic activity including extracellular glutamate levels, activation levels of glutamate receptors, and synaptic plasticity (Baker et al. 2002; Kupchik et al. 2011; Moran et al. 2005; Moussawi et al. 2009; Moussawi et al. 2011). In addition, astrocytes have been shown to synchronize the activity of large numbers of neurons through a glutamate-dependent mechanism (Angulo et al. 2004; Poskanzer and Yuste 2011). The potential significance of the latter point is evident from estimates that human cortical astrocytes may contact millions of neurons (Oberheim et al. 2006). Thus, increased glutamate release from system x_c^- may reverse several cellular events linked to schizophrenia ranging from NMDA receptor hypofunction to desynchronized firing of pyramidal neurons in the cortex.

Evidence implicating the cystine-component of cystine-glutamate exchange in the therapeutic effects of N-acetylcysteine stems largely from observations that 1) schizophrenia is associated with an imbalance in levels of antioxidants such as glutathione, the synthesis of which is at least partially dependent on system x_c^- as described above, and reactive oxygen species, or 2) depletion of glutathione in rodents recreates aspects of schizophrenia (Cabungcal et al. 2007; Gysin et al. 2011; Kulak et al. 2012; Steullet et al. 2006). In support, a decrease in glutathione inversely correlates with the severity of negative symptoms (Matsuzawa et al. 2008). Surprisingly, however, N-acetylcysteine has been shown to restore numerous interesting neurochemical abnormalities in glutathione-deficient mice without restoring glutathione levels (das Neves Duarte et al. 2012).

Interestingly, the cystine and glutamate components of system x_c^- may converge on key cellular events thought to underlie aspects of schizophrenia, as demonstrated by the observation that depletion of glutathione is sufficient to produce NMDA receptor hypoactivity (Steullet et al. 2006). Collectively, these studies indicate that the study of system x_c^- may have profound impact on the integration of important components of neurophysiology such as excitatory signaling

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and oxidative stress that are often studied in isolation as well as our ability to understand and treat debilitating CNS disease states such as schizophrenia.

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