MODULATION OF FEAR-RELATED BEHAVIORS BY PREFRONTAL CORTICAL GABAERGIC TRANSMISSION AND ITS RELEVANCE TO SCHIZOPHRENIA

by

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ABSTRACT

Individuals with schizophrenia are hypothesized to have a “noisy” prefrontal cortex (PFC), resulting from deficient gamma-aminobutyric acid (GABA) inhibitory neurotransmission. Given that the PFC regulates emotional functioning, it is possible that neuropathological alterations in PFC GABA function contribute to deficits in affective functioning in schizophrenia. In particular, schizophrenic patients have been suggested to apply aberrant motivational salience to cues, often being hyporesponsive to cues that predict reinforcement, and hyperresponsive to cues that do not predict reward. These types of deficits can be assessed in non-human animals using discriminative Pavlovian fear conditioning and latent inhibition paradigms. In the present study, experiments were conducted to elucidate the role of PFC GABAergic transmission in the allocation of motivational salience to conditioned stimuli. Animals were trained to lever press for sucrose reward, after which fear was assessed (using conditioned suppression of lever pressing) on one of the two aversive conditioning paradigms conducted. Saline infused control animals showed normal application of motivational salience, characterized by adaptive discrimination between aversive (CS+) and neutral cues (CS-) during discriminative Pavlovian fear conditioning, and acquired irrelevance to a stimuli that was preexposed during latent inhibition. Pharmacological blockade of GABA$_A$-receptors prior to the conditioning or test phase of the discriminative fear task eliminated the ability to behaviorally discriminate between a CS+ and CS-. Interestingly, only pre-conditioning infusions resulted in elevated fear to the CS-, suggesting that abnormal hyperresponsivity to a neutral cue is related to deficient GABA function during acquisition, but not recall. Blockade of GABA$_A$-receptors prior to conditioning had no effect on latent inhibition, but did enhance fear in animals that were non-preexposed to the conditioned stimulus. In contrast, latent inhibition was abolished following GABA-blockade prior to the test phase of the task. Taken together, these deficits suggest that PFC GABA transmission is critical for adaptive behavior following aversive conditioning. Such deficits observed in schizophrenia may be causally related to PFC GABA deficiency, predisposing these individuals to aberrant attributions of motivational salience to environmental stimuli.
PREFACE

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I. INTRODUCTION

Schizophrenia is a devastating neuropsychiatric disorder that afflicts approximately 0.5-1% of the world’s population (McGrath et al, 2008; Saha et al, 2005). Patients typically suffer from tripartite symptoms, including hallucinations and delusions (positive symptoms), affective disturbance and amotivation (negative symptoms), and executive dysfunction (cognitive symptoms). While the florid positive symptoms of the disorder are often adequately treated with antipsychotic medication that target dopamine D\textsubscript{2} receptors, cognitive and affective disturbances persist, representing a largely unmet therapeutic burden. As these symptoms are reported to be premorbid (Cannon et al, 2000; Reichenberg et al, 2010) and one of the best predictors of functional outcome (Fenton and McGlashan, 1987; Green, 1996; Nuechterlein et al, 1986), a better understanding of the neuropathophysiological mechanisms contributing to them should be developed.

Convergent evidence implicates aberrant prefrontal cortical (PFC) function in schizophrenic cognitive symptoms (Gonzalez-Burgos et al, 2011; Goto et al, 2010; Volk and Lewis, 2002). Many of the executive functions consistently reported to be deficient in individuals with schizophrenia, such as working memory, cognitive flexibility, and selective attention, are dependent on precise neuromodulatory activity within the PFC (Miller and Cohen, 2001). One such neuromodulator of critical importance to PFC function is the fast inhibitory neurotransmitter gamma-aminobutyric acid (GABA). In PFC, inhibitory interneurons release GABA and control the activity of excitatory, glutamatergic pyramidal neuron microcircuits (Freund and Katona, 2007; Freund, 2003). This inhibitory influence generates task-related oscillatory activity that allow for normal cognitive functioning (Fries, 2009). In post-mortem brain tissue of individuals with schizophrenia, numerous alterations suggestive of GABAergic...
hypofunction in sub-regions of PFC such as the dorsolateral PFC (dlPFC) have been reported (Hashimoto et al., 2003; Volk et al., 2000). These include decreases in GAD67 mRNA and protein, one of the primary synthesizing enzymes for GABA (Guidotti et al., 2000; Volk et al., 2000), parvalbumin, a calcium-binding protein specific for a fast-spiking interneuron subtype (Hashimoto et al., 2003), as well as differential expression of chloride transporters and GABA_A-receptors that control passive and active membrane properties, respectively (Arion and Lewis, 2011). Cortical network models suggest that hypofunction of enzymatic and proteomic activity associated with GABA such as those observed post-mortem in schizophrenia would result in disturbed oscillatory activity in PFC (Volman et al., 2011). Indeed, such oscillatory abnormalities are commonly observed in the gamma (30-80 Hz) frequency range in schizophrenic patients (Cho et al., 2006; Gandal et al., 2011; Haenschel et al., 2009). Activity of parvalbumin-expressing interneurons in the PFC are necessary and sufficient to generate gamma-frequency oscillatory activity, further implicating GABA dysfunction in schizophrenia (Carlén et al., 2011; Sohal, 2012). Recent investigation suggests that these GABAergic abnormalities manifest themselves particularly in deficient neurotransmission at the GABA_A-receptor subtype (Hasan et al., 2012; Takahashi et al., 2013). Thus, neuropathological GABA hypofunction may result in disturbed oscillatory activity and cortical-dependent processing in schizophrenia.

Neuropsychopharmacological research further implicates GABAergic hypofunction in schizophrenia’s behavioral and neurophysiological deficits. Antagonists at the N-methyl D-aspartate receptor (NMDAR) are known to produce a state of cortical disinhibition that preferentially decreases the activity of GABA interneurons, while increasing the activity of principle pyramidal neurons (Homayoun and Moghaddam, 2007). These compounds have been shown to produce and exacerbate cognitive, negative, and psychotic symptoms in normal and
schizophrenic individuals, respectively (Corlett et al, 2006; Krystal et al, 1994). When given to rats, NMDAR antagonists result in cognitive deficits including behavioral flexibility (Kos et al, 2011; Stefani and Moghaddam, 2005) and working memory (Jackson et al, 2004; Velázquez-Zamora et al, 2011). Preclinical administration of these compounds decreases markers of GABA function in the PFC including parvalbumin (McKibben et al, 2010; Romón et al, 2011) and alters oscillatory activity (Pinault, 2008; Saunders et al, 2012) in rodents similar to what is manifest in schizophrenia. One might anticipate that, if the ability of NMDAR antagonists to induce schizophrenia-like abnormalities is due to their ability to produce GABA hypofunction, direct reduction of activity at GABA-receptors themselves should result in a similar phenotype. Indeed, in both rats and non-human primates, pharmacological antagonism of GABA$_A$-receptors in the PFC has been shown to produce behavioral and neurophysiological changes that resemble those observed in the disorder (Enomoto et al, 2011; Paine et al, 2011; Pehrson et al, 2013; Rao et al, 2000). Performance on a delayed-response working memory task was impaired in monkeys subjected to GABA-receptor blockade in the dIPFC (Rao et al, 2000; Sawaguchi et al, 1988, 1989). In rats, the same manipulation in a homologous region of the medial PFC (mPFC), the prelimbic cortex, produces deficits in executive function including set-shifting and attention (Enomoto et al, 2011; Paine et al, 2011; Pehrson et al, 2013). This manipulation disinhibits pyramidal circuits (Paine et al, 2011; Rao et al, 2000), likely producing a decrease in cortical signal-to-noise ratio similar to what is observed in schizophrenia (Frantseva et al, 2012; Winterer et al, 2006). It is likely that GABAergic hypofunction in the PFC produces a “noisy” disinhibited cortex and cognitive deficits, deficits that can be modeled effectively using intra-mPFC GABA$_A$-receptor antagonism.
While it is becoming increasingly clear that certain cognitive deficits in schizophrenia may be related to PFC GABAergic hypofunction, less is known about the role of such neuropathology in emotional dysfunction. Emotional deficits have long been known to be present in schizophrenia. At the turn of the 20th century, both Kraepelin and Stransky posited that individuals with schizophrenia display a distinct “ataxia of the feelings”, or a disconnection between emotion and cognition (Kraepelin, 1919). They believed that this predisposes schizophrenic patients to maladaptive emotional “oscillations”, which may interfere with normal functioning. Such inappropriate emotional functioning continues to be an area of intense research in relation to schizophrenia. Recently, it has been similarly hypothesized that there exists a cognitive-emotional “disinteraction” in schizophrenia, whereby affective disturbances bias cognition, and vice versa (Anticevic and Corlett, 2012). One critical aspect of this dysfunction is the aberrant application of meaning to affective stimuli in schizophrenia. For example, compared to healthy controls, behavioral and physiological responses to reinforced stimuli are decreased in schizophrenics (Diaconescu et al, 2011; Hofer et al, 2001; Jensen et al, 2008; Roiser et al, 2012), suggesting that these individuals cannot properly utilize affective reinforcement (reward or punishment) to guide behavior. Such maladaptive behavioral responding is reflected at the neural level by inappropriate activation of cortical, limbic, and striatal regions in response to rewarded and neutral stimuli (Jensen et al, 2008; Murray et al, 2008). Regions of the PFC including the cingulate, medial and dIPFC that are critical for cognition also regulate affect and limbic reactivity (Euston et al, 2012; Pessoa, 2008), suggesting a possible overlapping neuroanatomical basis for emotional and cognitive deficits in schizophrenia. Given that schizophrenic cognitive deficits appear to be related at least in part to PFC GABAergic dysfunction, it is possible these
neuropathological changes also play a causal role in emotional disturbances, particularly the aberrant application of meaning to conditioned stimuli.

Deficits in affective functioning can be assessed in translational animal models using discriminative Pavlovian fear conditioning and latent inhibition tasks. Both of these tasks have been suggested to assess the appropriate application of affective meaning or salience to stimuli (Anticevic and Corlett, 2012; Gray and Snowden, 2005). Discriminative fear conditioning assesses the ability to adaptively respond to explicitly aversive and explicitly neutral stimuli. Healthy control individuals discriminate between an aversive conditioned stimulus (CS+) and a neutral conditioned stimulus (CS-), responding with elevated fear to the CS+ and decreased fear to the CS-. In contrast, individuals with schizophrenia consistently display deficiencies in developing such a discrimination (Jensen et al., 2008; Hofer et al., 2001). In particular, schizophrenic individuals apply elevated salience to the neutral CS- and less to the CS+, as compared to healthy individuals, resulting in stimulus generalization rather than discrimination. Similar misattribution of affective salience has been hypothesized to underlie deficits in the latent inhibition (LI). In LI, repeated non-reinforced preexposure to a stimulus can retard learning about the motivational relevance of that stimulus upon subsequent conditioning.

Typically, two groups are exposed to different stimulus contingencies, one receives repeated non-reinforced presentations of a to-be-conditioned stimulus (preexposed; PE), while one does not receive any preexposed presentations of the to-be-conditioned stimulus (nonpreexposed; NPE). Then, both groups experience a pairing of the stimulus (CS; the same stimulus that was preexposed to one group) and reinforcement, often an aversive unconditioned stimulus (e.g., footshock). PE animals acquire the irrelevance of the stimulus through the initial repeated non-reinforced presentations of the CS, and thus show less fear to the stimulus following aversive
conditioning. Individuals with acute schizophrenia typically present with disrupted LI, characterized by those in the PE condition learning the new association more quickly than control individuals (Gal et al, 2005; Gray et al, 1995; Young et al, 2005). This suggests that the capacity to learn the irrelevance of a particular stimulus is compromised, indicative of aberrant salience attribution. In addition, the NPE group essentially undergoes a mild non-discriminative fear conditioning protocol during the LI of conditioned fear (no preexposure; 3 CS+shock pairings), for which behavioral parameters such as expression and extinction of fear can be evaluated. Fear extinction is dependent on sub-regions of the PFC in both humans and rats (Delgado et al, 2008; Sotres-Bayon and Quirk, 2010), and has been shown to be disturbed in schizophrenia (Holt et al, 2009). These two tasks allow for the pre-clinical assessment of a constellation of fear-related behaviors known to be disturbed in schizophrenia, including discrimination learning, LI, and the extinction of fear conditioning.

The particular role of GABAergic transmission in the PFC during affective learning is unknown. Given that schizophrenia is characterized by affective dysfunction as well as GABAergic neuropathology in PFC, a causal role between the two may exist. Behavioral and neurophysiological deficits that are relevant to schizophrenia can be produced by blocking GABAergic neurotransmission in the mPFC of rats, enabling the study of such a potential causal relationship. The present study examined fear-behavior on translational discriminative fear conditioning and latent inhibition paradigms following timepoint-specific GABA_A-receptor antagonism.
II. MATERIALS AND METHODS

Animals

Cohorts of male Long Evans rats (Charles River Laboratories, Montreal, Canada) arrived weighing approximately 250-300g. Animals were initially group housed and provided with ad libitum access to food and water. Following a week of acclimatization to the colony, animals were stereotaxically implanted with bilateral guide cannula into the prelimbic region of mPFC (see Stereotaxic Surgery). For the remainder of the experiment, all animals were single-housed and food-restricted to approximately 90% of their free feeding (post-surgery) weight. All testing was conducted in accordance with Canadian Council on Animal Care and the Animals Care Committee of the University of British Columbia.

Stereotaxic surgery

Animals were anesthetized with a combination of ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and analgesia was administered (Anafen, 10mg/kg s.c.). Twenty-three gauge bilateral stainless steel guide cannula were aimed at the prelimbic mPFC according to the following coordinates (Paxinos and Watson, 2005) from bregma: AP: +3.2 mm; ML: ±0.7 mm; from dura: DV: -2.8, with the intraural bar set to -3.3 mm. To prevent accumulation of substance in the guide cannula, stainless steel obdurators flush with the end of the guide cannula were inserted. Rats were given approximately one week to recover from surgery before beginning operant training.

Apparatus

Behavioral testing was conducted in eight standard operant chambers (30.5 X 24 X 21 cm; Med Associates, St. Albans, VT, USA). Chambers were housed in a sound attenuating
enclosure equipped with a fan to provide ventilation and mask ambient noise. Each chamber was fitted with two retractable levers, separated on one wall of the rectangular chamber by a food receptacle, which allowed for delivery of sucrose reinforcement (45 mg pellet; BioServ, Frenchtown, NJ, USA). Above each retractable lever were situated two 100 mA cue lights. On the chamber wall opposite the food receptacle, a single 100 mA house light was situated directly next to an auditory speaker, which allowed for the delivery of auditory stimuli via a programmable audio generator (ANL-926, Med Associates). Four infrared photobeams located just above the grid floors tracked locomotor activity (number of beam breaks), while a fifth beam positioned at the food receptacle tracked food receptacle entries. Grid floors were electrified and allowed for the delivery of a scrambled footshock. Experimental data were recorded by a personal computer for analysis offline.

**Microinfusion**

To acclimatize animals to the microinfusion procedure, all animals were given two days of mock infusions 10 min prior to their final VI60 training days (see below). These consisted of obdurator removal, insertion of a mock injector flush with the end of the guide cannula, and placement in the infusion enclosure for approximately two min. All microinfusions were conducted 10 min prior to animals being placed in operant chambers. On the infusion day, animals received bilateral infusion of 0.9% saline (0.5 μl/side) or bicuculline methobromide in a 0.9% saline solution (50 ng/μl at a volume of 0.5 μl/side). Bicuculline at a dose of 50ng/μl produces cognitive and neurophysiological deficits in rats reminiscent of schizophrenia (Enomoto et al, 2011) and is orders of magnitude less concentrated than when used in studies of epileptiform activity (Schneider and De Lore Arnaiz, 2013). Infusion was conducted over 75 s,
with the injectors left in place to allow for diffusion for an additional 60 s. Microinfusion injectors extended 0.8 mm beyond the end of the guide cannula.

**Lever press training**

For all experiments, rats were initially trained to lever press for sucrose reward. Twenty-four hrs prior to their first operant training session, all rats were provided with approximately 30 sucrose pellets in their home cage to eliminate potential neophobia to the primary reinforcer. Prior to the first operant training session, 2-3 sucrose pellets were placed in the food receptacle and crushed pellet dust was placed on the left lever to eliminate neophobia in the operant chamber and facilitate lever-press acquisition. Over approximately 1-3 sessions, rats acquired a fixed ratio 1 (FR1) schedule on the left lever, with a criterion of 40 presses in 30 min. Over the next 3 days (one operant session per day), animals were trained on an increasing variable interval (VI) schedule whereby reward was provided every 15 (VI15), 30 (VI30), or 60 (VI60) s. All animals were then trained on a VI60 schedule for approximately 9 d, following which latent inhibition or discriminative fear training began. Lever pressing on a VI60 ratio engenders a high rate of lever-press responding in rats, which allows for the accurate assessment of conditioned suppression behavior (McAllister, 1997).

**Discriminative fear conditioning**

*Conditioning*

Following lever press training, rats underwent discriminative fear conditioning modeled after the protocol described by Antunes and Moita (2010; see Fig. 1a). Animals were placed in the operant chamber (no levers or house light) and initially presented twice with a 30 s neutral conditioned stimulus (CS-; 1 kHz tone + cue lights). Following these two presentations, animals were pseudorandomly presented with six more CS- presentations, and six 30 s aversive
conditioned stimulus (CS+; 9 kHz tone + flashing houselight co-terminating with 0.5mA/1 s footshock) presentations. The session ended following two additional CS+ presentations. In total, animals received eight CS- and eight CS+ presentations, with CS presentations occurring on average every three min. The particular tones associated with shock/no shock were selected because rats trained in a discriminative fear conditioning protocol tend to generalize their fear responses toward a 22 kHz tone, which corresponds to the fundamental frequency of alarm calls (Bang et al., 2008). Thus, the frequency of the CS+ was chosen to be the 9 kHz frequency to avoid biasing our results toward generalization (lack of discrimination) which may arise from auditions similar to alarm calls, triggering innate fear. In addition, these visual stimuli and order of presentation were used because pilot studies revealed that this combination of stimuli produced the most robust and reliable discriminative fear responses in control animals. The next day, animals were given a baseline VI60 session (no shocks or CSs).

**Discriminative Fear Test**

Forty-eight hrs after conditioning, rats were placed in the operant chamber and allowed to lever press for reward on a VI60 schedule for five min, after which the presentation of CSs commenced. As the rat lever pressed, the 30 s CS- was presented four times (interstimulus interval: five min), followed by four presentations of the 30 s CS+ (no shocks; interstimulus interval: five min). The main dependent variable was conditioned suppression of lever pressing during CS presentation, which was used as an index of conditioned fear. Animals naturally suppress instrumental responding following exposure to an aversively conditioned stimulus (Kamin et al., 1963). Conditioned suppression of lever pressing was calculated by taking [(A-B)/(A+B)], where A was the number of lever presses over the 30 s prior to the CS presentation,
and B was the number of lever presses during the 30 s CS presentation. Thus, a suppression ratio of 1 indicates complete suppression of lever pressing, while a ratio of 0 indicates no suppression.

*Group assignment*

We tested the effects of intra-mPFC infusions of saline or bicuculline in four groups of rats. Two groups received saline/bicuculline infusion into the mPFC prior to the conditioning phase, and were tested drug free 48 hrs later. Another two groups received saline/bicuculline infusions prior to the discriminative fear test phase. Animals were matched for the average number of lever press over the last two days of VI60 training and then assigned to one of the four groups.

*Latent inhibition*

*Conditioning*

The latent inhibition task used was adapted from McAllister (1997). Following approximately 9 d of lever press training, rats were allocated to separate groups (see below), based on the number of lever presses made over the previous two VI60 days (Fig. 1b). This experiment consisted of three primary treatment groups; 1) intra-mPFC bicuculline prior to PE or NPE/conditioning, 2) bicuculline prior to the LI test session and 3) saline infusions prior to both conditioning and test that served as controls for the other two treatment groups. Rats receiving each of these treatments were further allocated to either preexposure (PE) or nonpreexposure (NPE) conditions during the conditioning phase. Rats were run in squads of 16, and efforts were made to ensure that each experimental cohort consisted of some rats in each treatment and preexposure group. For the PE condition, rats were placed in the operant chamber (no levers or house light), and experienced 30 presentations of a 30 s compound conditioned stimulus (CS; illumination of the cue-lights + 5kHz tone). Each CS presentation was separated by 30 s. In the
NPE group, rats were placed in the operant chamber for an equivalent amount of time, but did not experience these non-reinforced CS presentations. Thirty-six min after being placed in the chamber, all animals received three aversive conditioning pairings whereby 30 s presentations of the CS co-terminated with a 0.5mA/0.5 s footshock. The next day, animals were given a baseline VI60 session.

LI Test

Forty-eight hrs after conditioning rats were placed in the operant chamber and allowed to lever press for reward on a VI60 schedule. Five min into the session, rats received the first of four 30 s CS presentations, with an intertrial interval of 5 min. Lever press suppression again served as our index of fear, and was calculated in the same manner as for discriminative fear (see above).
Figure 1. Task designs for (a) discriminative fear conditioning and (b) latent inhibition. Note that separate groups of animals were used for saline and bicuculline infusion and for pre-conditioning and pre-test infusion.
Figure 2. Infusion locations in the prelimbic mPFC for (a) discriminative fear conditioning and (b) latent inhibition tasks. Black squares denote animals that received bicuculline, open circles represent saline-infused animals. Distance anterior to bregma in mm is reflected on the left.
**Histology**

Following experimental endpoint, rats were euthanized via pressurized carbon dioxide. Brains were removed and post-fixed in a 4% formalin solution. Brains were sectioned at 50 μm after which sections were mounted on gel-coated slides. Slides with sections containing extent of cannula tracks were stained with cresyl violet, following which placements were examined under light microscope. Data from animals with placements exclusively outside the prelimbic mPFC border were removed from analyses (Fig. 2).

**Data analysis**

Discriminative fear conditioning was analyzed separately for pre-conditioning and pre-test infusions. Suppression ratios were analyzed using between/within subjects three-way ANOVAs with Treatment group (saline vs. bicuculline) as the between subjects variable, and Stimulus type (CS+ vs CS-) and stimulus number (1-4) as the within-subjects variables. Locomotion for the entire session and the rates of lever pressing during the first five min of the session were analyzed separately using t-tests to determine if there were any non-specific effects of the drug. We analyzed the rate of lever pressing during the first five min of the session as any differences between groups that emerged in the later part of the session may have been influenced by CS presentation. Follow-up simple main effects analyses were conducted with one-way ANOVAs or t-tests, where appropriate.

Conditioned suppression data from the LI experiment were analyzed using two and three-way between/within subjects ANOVAs. Based on the initial observation that the basic LI effect was observed in control animals only during the first CS presentation, a two-tiered analysis was conducted. LI was initially assessed using a two-way ANOVA examining only the first CS presentation, with Preexposure and Treatment group (bicuculline infusions prior to conditioning,
prior to test or saline infusions) as between-subjects variables. To assess further changes in fear-related behaviors that were apparent during subsequent CS presentations, a three-way ANOVA was conducted with Tone as the within-subjects variable, and Preexposure and Treatment group again serving as between-subjects variables. Locomotion and lever pressing rates during the first five min of the session were analyzed separately using one-way ANOVAs. Follow-up simple main effects analyses were conducted with one-way ANOVAs or t-tests, where appropriate.

III. RESULTS

**Discriminative fear: Pre-conditioning infusions**

Saline-treated control rats \((n = 12)\) displayed clear discrimination between the aversive CS+ and a non-aversive CS-, expressing minimal suppression of lever pressing during CS-presentations, and robust conditioned fear during subsequent presentations of the shock-associated CS+ (Fig 3a, left). In stark contrast, GABA-receptor antagonism prior to conditioning \((n = 13)\) abolished fear discrimination, with rats displaying no discernible difference in conditioned fear during presentation of either CS (Fig. 3a, right). Analyses of these data revealed a significant Treatment x Stimulus type interaction, \((F(1, 23) = 15.76, p < 0.001)\). Simple-main effects analyses confirmed that control rats displayed significantly greater levels of suppression during the CS+ vs. the CS- \((p < 0.001)\), but there was no difference on this measure for bicuculline-treated rats (n.s.). Furthermore, GABA-blockade elevated fear to the CS- \((p < 0.05, \text{Fig. 3a})\), while at the same time decreasing fear in response to the CS+ \((p < 0.001, \text{Fig. 3a})\), as compared to saline controls. There was no significant main effect of treatment, \((F(2, 43) = 2.10, \text{n.s.})\), indicating that over the entire session, rats in both groups displayed a comparable amount of conditioned suppression, although the manner in which rats distributed their fear
response to the CS+ vs CS- was radically different between groups. Similarly, there was no significant three-way interaction, \((F(3, 69) = 1.48, \text{n.s.}; \text{Fig. 3b})\). In addition, there was no effect of drug on locomotion, \((t(23) = -0.13, \text{n.s.})\), or the rates of lever pressing, \((t(23) = -0.45, \text{n.s.})\); Table 1, left columns). Collectively, these data show that reducing PFC GABA activity during acquisition of discriminative conditioned fear causes a disruption in the expression of stimulus-appropriate emotional responses, with increased fear in response to a neutral stimulus and reduced fear for an aversive one.

**Discriminative fear: Pre-test infusions**

Similar to what was observed in pre-conditioning animals, saline-treated control rats \((n = 7)\) discriminated well between an aversive CS+ and a non-aversive CS- (Fig. 3c, left). Rats that had been subjected to discriminative fear conditioning drug-free but received intra-mPFC infusions of a GABA-receptor antagonist prior to test \((n = 7)\) again displayed impaired in discrimination learning, as revealed by a significant Treatment x Stimulus type interaction, \((F(1, 12) = 5.744, p = 0.034; \text{Fig. 3c, right})\). Simple-main effects analyses again confirmed that control rats showed substantially greater suppression during CS+ vs CS- presentations \((p < 0.01; \text{Fig. 3c})\), but there was no difference on this measure in bicuculline-treated rats (n.s.). However, unlike animals treated prior to conditioning, test animals showed no elevation in responding to the CS- \((p > 0.05)\), although they did show a similar decrease in fear to the CS+ \((p < 0.05, \text{Fig 3c})\). There was no significant main effect of treatment, \((F(1, 12) = 1.83, \text{n.s.})\), or three-way interaction, \((F(1, 36) = 1.734, \text{n.s.; Fig. 3d})\). Likewise, there was no significant effect of drug treatment on locomotion, \((t(12) = -0.56, \text{n.s.})\), or on the rate of lever pressing, \((t(12) = 1.00, \text{n.s.; Table 1, right columns})\). Thus, disruption of mPFC GABA signaling during recall disrupts
discriminative control over conditioned fear responses, although in this instance, the effect is due primarily to reduced fear expression to an aversive stimulus.
Figure 3. Suppression data for animals receiving (a, b) pre-conditioning or (c, d) pre-test infusion on a discriminative fear conditioning task. (a) Saline-infused animals displayed elevated fear to the CS+ and decreased fear to a CS-, \( (p < 0.001) \). However, animals receiving bicuculline infusions prior to conditioning did not show discrimination, instead showing elevated fear to a CS-, \( p < 0.05 \), and decreased fear to a CS+, \( (p < 0.01) \). (b) The same data as in a, plotted as a function of tone number. Saline-infused animals showed discrimination across all four CS-/CS+ tones (open circles), while pre-conditioning GABA-blockade animals did not (black squares). (c) Intra mPFC infusions of bicuculline prior to the discriminative fear test also abolished discrimination between the CS+ and CS-, when compared to controls that displayed robust discrimination. (d) Same data as in c, plotted as a function of tone number. Error bars reflect +SEM. Black star denotes \( p < 0.05 \) as compared to CS-. #-symbol denotes \( p < 0.05 \) between CS- for saline and pre-conditioning GABA-blockade. *-symbol denotes \( p < 0.05 \) between CS+ for saline and GABA-blockade.
Table 1. Pre-conditioning and pre-test infusion group averages for locomotion and lever presses per min. Values displayed as means ±SEM. No significant differences were observed across groups for locomotion or lever presses per min, all p-values > 0.05.

LI

As displayed in Figure 4a and 5, control animals showed the classic LI effect, whereby PE animals (n = 8) expressed less lever press suppression than NPE (n = 8) animals. However, this effect was only apparent during the first tone presentation as the suppression in the NPE group extinguished by the second tone presentation, with there being no difference in the suppression averaged across four tones. Therefore, in order to analyze differences in LI specifically between the different treatment groups, our initial analysis focused on conditioned suppression during the first CS presentation. Analysis of these data revealed a significant main effect of Preexposure, \(F(1, 43) = 15.60, p < 0.001\), no main effect of Treatment, \(F(2,43) = 1.76, \text{n.s.}\), but importantly, also uncovered a significant Treatment by Preexposure interaction, \(F(2, 43) = 4.46, p < 0.05\). Simple-main effects analyses confirmed that NPE control rats displayed greater levels of conditioned suppression relative to PE control rats \((p < 0.05, \text{Fig. 4a})\). Similarly, rats that received infusions of the GABA-receptor antagonist prior to conditioning demonstrated LI, with NPE rats \((n = 9)\) showing more fear compared to their PE \((n = 8)\) counterparts \((p < 0.001, \text{Fig. 4b})\). In stark contrast to the other treatment groups, intra-mPFC

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<th>Pre-conditioning Infusion</th>
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<td></td>
<td>Saline</td>
<td>GABA-blockade</td>
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<tr>
<td></td>
<td>Saline</td>
<td>GABA-blockade</td>
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<tr>
<td>Locomotion</td>
<td>1372 ±160</td>
<td>1274 ±139</td>
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<tr>
<td>Lever presses</td>
<td>17 ±1</td>
<td>17 ±2</td>
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<tr>
<td>per min</td>
<td>1043 ±166</td>
<td>21 ±4</td>
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<td>1229 ±287</td>
<td>16 ±3</td>
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GABA-blockade prior to the test session completely abolished the LI effect, wherein both NPE ($n = 8$) and PE ($n = 8$) animals displayed comparable levels of conditioned fear to the first CS presentation (n.s., Fig. 4c). Thus, disruption of mPFC GABA signaling during preexposure/conditioning does not affect the acquisition of LI, but this manipulation prior to test markedly disrupted the recall of learned irrelevance.

We then conducted a second analysis comparing conditioned suppression over all four tone presentations, to ascertain whether there were any differences between groups in the subsequent expression and extinction of conditioned fear (Fig. 5). This analysis revealed a significant Treatment x Preexposure interaction, ($F(2, 43) = 3.429, p = 0.042$), although the three-way interaction was not significant, ($F(6, 129) = 0.82$, n.s.). Subsequent portioning of the two-way interaction revealed that, for PE animals, there were no differences in the levels of conditioned suppression across the treatment groups, ($F(2, 21) = 0.82$, n.s.; Fig. 5a). However, for NPE animals (i.e.; those that underwent a standard non-discriminative fear conditioning procedure), bicuculline treatment prior to the conditioning phase caused a significantly exacerbated fear response, ($F(2, 43) = 3.43, p < 0.05$), when compared to rats receiving bicuculline prior to the LI test (Tukey’s $p < 0.05$) or controls (Tukey’s $p < 0.05$), which did not differ from each other (n.s.). There was no difference in locomotion across NPE or PE conditions, or treatment groups, and no interaction (all $F$’s $< 3.9$, $p$-values $> 0.05$; Table 2). There was a slight decrease in the rates of lever pressing for rats receiving bicuculline prior the LI, ($F(2, 43) = 4.243, p = 0.021$, Tukey’s $p < 0.05$), as compared to saline-infused controls (Table 2). Collectively, these data indicate that disruption of mPFC GABA transmission during distinct phases of learning has differential effects on acquisition and LI of conditioned fear.

Reducing mPFC GABA impairs the recall of learned irrelevance, whereas disinhibition of the
mPFC during standard non-discriminative fear conditioning leads to exaggerated recall of a fear response.
Figure 4. Suppression data for animals receiving intra-mPFC saline prior to both conditioning and test phases (a) or bicuculline prior to conditioning (b) or test (c). (a) Saline-infused animals displayed a classic LI effect, with PE animals showing less fear than NPE animals during test. (b) Pre-conditioning bicuculline infusion did not alter LI expression, as the NPE group displayed elevated fear as compared to the PE group. (c) LI was abolished by pre-test intra-mPFC bicuculline, with there being no difference in fear between NPE and PE animals. All error bars reflect +SEM. * = p < 0.05 between PE and NPE groups.
Figure 5. Suppression data for animals receiving intra-mPFC infusion in the PE (a) or NPE (b) groups. (a) No difference was observed in the level of suppression for the PE groups, regardless of when animals were infused, \((F(2, 21) = 0.82, \text{n.s.})\). (b) Intra-mPFC bicuculline prior to conditioning (black squares) elevated fear expression across four tones as compared to the saline (open circles) or pre-test bicuculline (grey triangle) infused animals. All error bars reflect +SEM. * = \(p < 0.05\) between pre-conditioning bicuculline and both other groups (pre-test bicuculline and saline-infused controls).

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<tr>
<th></th>
<th>Saline</th>
<th>Pre-conditioning GABA-blockade</th>
<th>Pre-test GABA-blockade</th>
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<tbody>
<tr>
<td>Locomotion</td>
<td>926 ±124</td>
<td>1007 ±86</td>
<td>977 ±105</td>
</tr>
<tr>
<td>Lever presses per min</td>
<td>30 ±3</td>
<td>22 ± 2</td>
<td>18 ± 3*</td>
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Table 2. Locomotion and rate of lever pressing over the first five min of the test session averaged across PE/NPE group as there was no effect of task condition. Animals receiving pre-test GABA-blockade made significantly fewer lever presses per min than did saline-infused control animals. * = \(p < 0.05\) vs. saline
IV. DISCUSSION

These experiments examined the role of GABAergic transmission in the PFC on two tasks that are both reliant on the appropriate utility of affective information and deficient in schizophrenia. Pharmacological reduction in GABA signaling eliminated the ability to behaviorally discriminate between a neutral CS- and an aversive CS+. Infusion prior to conditioning induced a behavioral profile characterized by more suppression to the CS- and less suppression to the CS+. Interestingly, GABA-blockade prior to the test phase of the discriminative Pavlovian fear conditioning task resulted only in a decrease in suppression to the CS+, but no corresponding elevation in fear to the CS-. These results suggest that GABAergic signaling is necessary for acquisition and recall of discriminative conditioning, although elevations in fear to the CS- are related specifically to GABA-hypofunction during acquisition. The role of PFC GABA on learned irrelevance behavior was examined using a LI protocol. We observed a dissociation between the impact of pre-test or pre-conditioning GABA-blockade on LI performance. When infused prior to the test phase, LI was completely abolished. When infused prior to the conditioning phase, LI was intact, but fear was dramatically increased in the NPE group. Although lesion studies have suggested that the PFC does not appear to play a prominent role in LI, these results suggest that discrete portions of the task (recall) may be influenced by GABAergic hypofunction. In addition, elevated fear in the NPE group receiving GABA-blockade prior to conditioning is concordant with literature suggesting that elevated prelimbic mPFC activity exacerbates conditioned fear. We suggest that GABA dysfunction in the PFC of individuals with schizophrenia may be related to emotional perturbations, including the inappropriate use of affective information to guide behavior.
Role of the mPFC GABA in discriminative Pavlovian conditioning

Here we provide evidence that a “noisy” mPFC contributes to deficits in discrimination learning when infused prior to the conditioning or test phase of the task. These results expand upon the known role of the mPFC in Pavlovian discrimination learning. In rabbits (a mammalian species with comparable neuroanatomy to the rat), regions of the mPFC are critical for discriminative Pavlovian aversive conditioning as measured by the bradycardiac response (Gibbs and Powell, 1988; Maxwell et al, 1994) or instrumental avoidance learning (Gabriel and Orona, 1982; Orona and Gabriel, 1983). Animals conditioned to distinguish between an aversive CS+ and a neutral CS- display a characteristic decrease in heart-rate (bradycardia), a peripheral measure of autonomic fear responsivity, and rapidly acquire the ability to turn a running wheel for the cessation of an aversive stimulus. Electrophysiological recordings in animals undergoing aversive Pavlovian discrimination conditioning illustrate that separate populations of neurons exist in the mPFC which preferentially increase their firing in response to either a CS+ or CS- during aversive conditioning (Gibbs and Powell, 1991; Maxwell et al, 1994). In areas of the dorsomedial PFC including the anterior cingulate cortex, the majority of neurons respond simply to a CS+, signaling the aversive nature of the stimuli (Gibbs and Powell, 1991). In comparison, in the prelimbic cortex neuronal activity is altered in response to both the CS- and CS+, suggesting that separate neurons in the prelimbic PFC signal both the relative safety and aversion of cues (Maxwell et al, 1994). In this way, dissociable populations of neurons may encode the associative consequence of stimuli, whether they predict danger or safety.

Thus, well orchestrated activity in the mPFC is necessary for accurate discrimination between stimuli that are explicitly aversive or explicitly neutral. As GABAergic antagonism is known to increase activity of mPFC neurons following microinfusion (Paine et al, 2011; Rao et
al, 2000), it is likely that activity of neurons naturally responsive to either a CS+ or CS- are increased non-selectively. This would be expected to result in an impairment in discriminative neural activity and consequent behavior. Consistent with this interpretation, a similar lack of stimulus specific firing produced by intra-PFC GABA-antagonism in monkeys causes a loss of appropriate tuning in some neurons, and an increase in inappropriate tuning in others (Rao et al, 2000). This resulted in a subsequent decrease in GABA-mediated delay period activity during working memory (Rao et al, 2000). The authors hypothesize that cortical circuits function in such a way that tightly regulated firing occurs only to behaviorally-relevant events. It is also possible that a disorganized pattern of mPFC activity will impinge on the activity of structures downstream from the mPFC, in particular the “limbic-motor interface” in the nucleus accumbens (Mogenson et al, 1980). The mPFC densely innervates the nucleus accumbens (Vertes, 2004) and modulates neuronal activity in the region (O’Donnell et al, 1999). Glutamatergic excitatory projections from the mPFC to the nucleus accumbens are critical for behavioral responding to rewarded discriminative stimuli (Ishikawa et al, 2008). Inactivation of the prelimbic mPFC in rats eliminates discriminative neural activity in the nucleus accumbens core while reducing behavioral responding to a rewarded discriminative stimulus and increasing responding to an unrewarded stimulus (Ishikawa et al, 2008). Given these results, it is perhaps not surprising that both a decrease in activity (reversible or permanent lesion) and an increase in activity (GABA-blockade) result in a loss of behavioral discrimination. Either manipulation likely creates an aberrant pattern of neuronal activity and consequent output by the mPFC, impairing the ability to discriminate between reinforced stimuli. By tipping the delicate excitatory/inhibitory balance in the PFC towards disorganized excitation, deficits in emotionally-regulated behaviors including Pavlovian fear discrimination may result.
A similar behavioral profile was observed following pre-test intra-mPFC bicuculline infusion. This suggests that, in addition to being critical for the encoding of discriminative fear conditioning, prefrontal cortical GABAergic transmission contributes to the recall of affective associations. This recall deficit is compatible with theories suggesting that the mPFC is critical for recalling learned adaptive responses, particularly those with emotional association (Euston et al., 2012). These authors suggest that the mPFC utilizes previously learned affectively salient associations to produce favorable motivated responses. Discriminative fear conditioning requires animals to recall the association between discrete stimuli and guide their instrumental behavior according to the affective memory of each stimulus. By producing a “noisy” cortex, recall of an affectively salient stimulus such as a CS+ may be impaired, resulting in behavioral indifference towards such an aversive stimulus. In the present study, GABA-blockade prior to test exclusively decreased fear to the CS+, suggestive of such a deficit. In addition, the decrease in fear to the CS+ observed in both pre-conditioning and pre-test GABA-blockade groups may be resultant from generalization of the CS- and CS+. During test, rats initially experienced presentations of the CS- followed by presentations of the CS+. In GABA-deficient rats, a generalized memory trace of the CS+/CS- may have caused rats to extinguish their fear during the CS- presentations, resulting in less fear when exposed to the CS+.

**Role of mPFC GABA in LI**

In a separate series of experiments, rats underwent LI following intra-mPFC GABAergic antagonism. LI provides an index of learned irrelevance, whereby PE animals acquire the irrelevance of a preexposed stimulus, and thus are slower to learn further conditioning of that particular stimulus. Preexposure is less effective at retarding further conditioning in individuals with schizophrenia, particularly those that are acutely psychotic (Gal et al., 2005; Gray et al,
1995; Young et al, 2005). This procedure has been variously linked to the aberrant application of salience based on a misattribution of affective meaning to a preexposed stimuli (Gray and Snowden, 2005).

In contrast to many previous reports regarding LI, we observed an alteration in LI following mPFC manipulation, specifically GABA-blockade prior to the test phase of the task attenuated LI expression. One prominent framework for understanding the neural correlates of LI is the “switching” model posited by Weiner et al. (2009). This model emphasizes that efferent projections to the nucleus accumbens core or shell and midbrain dopamine neurons in the ventral tegmental area (VTA) regulate switching between a stimulus-reinforcement (generally, NPE) or stimulus-no event (generally, PE) pattern of responding during LI. Critical for the ability to switch to a stimulus-reinforcement response pattern is the efflux of dopamine from the VTA to the nucleus accumbens core. As lesions (Joel et al, 1997; Lacroix et al, 2000b; Schiller and Weiner, 2004) and catecholaminergic (Lacroix et al, 2000a) modulation of the prelimbic mPFC typically have no effect on LI, the region has been largely disregarded as a potential contributor to LI expression. Given that the prelimbic mPFC projects to the nucleus accumbens and VTA (Sesack and Carr, 2002; Vertes, 2004) and that stimulation of the mPFC increases DA efflux and modulates activity in the nucleus accumbens (O’Donnell et al, 1999; Taber et al, 1995), it is somewhat surprising that the balance of evidence indicates that LI is unaffected by lesions of the mPFC. However, there were a number of important differences in the present study compared to previous research that may explain the decrease in LI observed following GABA-blockade prior to test.

The majority of LI studies regarding the mPFC have utilized permanent lesions to abolish signal outflow from mPFC (Joel et al, 1997; Lacroix et al, 2000b; Schiller and Weiner, 2004).
These lesions are typically induced prior to the conditioning and preexposure phase of the task. Using such methodology, it is not possible to assess the contribution of the region to discrete task phases. In the present study, LI was intact when the pharmacological manipulation was conducted prior to the conditioning phase, but not when conducted prior to the test phase. Contributions of the mPFC may relate specifically to the recall of learned irrelevance, further suggesting that the mPFC is critical for the retrieval of affectively encoded information (Euston et al., 2012). In addition, although LI is not altered following lesion of a particular brain region, there is evidence to suggest that activation of the same brain region may result in a separate behavioral profile. For example, excitotoxic lesions of the ventral hippocampus do not alter LI expression, but pharmacological activation of the same region attenuate LI (Pouzet et al., 2004). For these reasons, it is possible that the role of mPFC activity in LI has simply been obscured by various methodological issues.

There are a number of possible explanations for the observed deficit in LI following mPFC GABA-blockade prior to test. Antagonism of GABA-receptors in the mPFC produces hyperactivity of DA neurons in the VTA, inducing a specific increase in phasic burst firing of DA neurons (Enomoto et al., 2011). Phasic activity of DA neurons is thought to underlie associative learning, in that cue-reinforcement pairings induce phasic DA neuron activity (Schultz, 2013). By disinhibiting the cortex prior to the test phase, when animals are being exposed to the CS during instrumental performance, DA neurons may increase their phasic activity, increasing DA efflux in the nucleus accumbens. In the context of the “switching” model of LI, this increase in phasic DA in the nucleus accumbens facilitates a “switch” from the stimulus-no event contingency learned during PE to a stimulus-reinforcement (CS+shock) pattern of responding characterized by fear to the PE stimuli. In the present study, this would be
manifest as elevated suppression in the PE animals, and consequently disrupted LI. Another possibility is that, rather than directly influencing mesoaccumbens DA release, mPFC GABA-blockade could monosynaptically excite downstream structures known to facilitate switching, such as the nucleus accumbens or basolateral amygdala (BLA). PFC stimulation can drive nucleus accumbens excitatory activity (O’Donnell et al, 1999), which would be expected to influence motivated behavior, for example encouraging switching behavior during LI. In addition, stimulation of the mPFC excites the majority of BLA neurons (Likhtik et al, 2005), which may then facilitate DA release and activity in the nucleus accumbens core. Finally, the mPFC is known to be involved in mnemonic functions, which can be disrupted by GABAergic antagonism (Rao et al, 2000). Similar to the effect observed during pre-conditioning GABA-blockade during discriminative Pavlovian aversive conditioning, disinhibition of the mPFC may impair the recall an affect-laden stimulus association such as that formed during preexposure. This would result in the absence of a difference in fear between the PE and NPE group, which was observed.

Our observation that mPFC GABA-blockade prior to conditioning does not impair LI is consistent with a previous report from our laboratory (Enomoto et al, 2011). GABAergic signaling in the mPFC does not seem to be critical for the ability to acquire the irrelevance of a PE stimulus, as the PE group displayed appropriate levels of suppression to the CS during test in a manner comparable to controls. However, even though LI was intact following mPFC GABA-blockade prior to conditioning, the expression of conditioned fear during test was dramatically increased in NPE animals as compared to their saline control. Animals in the NPE group can be thought of as having undergone a relatively mild non-discriminative fear conditioning protocol (no preexposure; 3 CS+shock pairings). Elevated fear following pre-conditioning GABA-
blockade in the NPE group is in agreement with the established role of the prelimbic mPFC in conditioned fear expression (Sotres-Bayon and Quirk, 2010). Neural activity in the prelimbic mPFC increases in response to a CS that predicts an aversive consequence (Burgos-Robles et al., 2009). Similarly, microstimulation of the region elevates conditioned fear (Vidal-Gonzalez et al., 2006), while inactivation impairs the expression of conditioned fear (Sierra-Mercado et al., 2011). Prelimbic mPFC activity is correlated with extinction-failure such that increased prelimbic excitation slows extinction learning (Burgos-Robles et al., 2009). By disinhibiting the prelimbic mPFC during conditioning, NPE animals may encode the CS+shock pairing more strongly than control animals, responding with elevated fear (suppression of lever pressing) during the test phase of the LI task.

These results suggest that the acquired irrelevance of a CS during a LI procedure is encoded differently from the explicit neutrality of a CS- during a discriminative fear conditioning task. In intact animals, both procedures produce a pattern of responding to the CS- or preexposed CS that reflects the relative “safety” of the cue. Control rats respond indifferently towards the CS- during discriminative fear conditioning or the preexposed shock-associated CS during LI, as reflected by no appreciable conditioned suppression. GABA-blockade during conditioning does not alter fear-related suppression to a “neutral” CS for PE animals, but increases fear towards a CS- in discriminative fear conditioning during test. This difference may reflect the relative exposure to the “neutral” CS, as PE animals experienced 30 CS exposures as compared to the eight CS- presentations during discriminative fear conditioning. This explanation is unlikely, given that saline infused animals in both tasks showed comparably minimal levels of suppression, indicating that both situations resulted in similar “safety” knowledge about the CS- or preexposed CS. One possible explanation is that GABA-blockade
resulted in generalization of fear, rather than discrimination, to the CS-/CS+. A CS- may be interpreted as more aversive due to an inability to distinguish its neutrality from the CS+. Further research should examine the impact of GABA-blockade during encoding of an explicitly neutral stimulus, without a discrimination component. Given that the majority of studies suggest no role for the prelimbic mPFC in the encoding of irrelevance in LI, the mechanisms underlying such acquired neutrality may be independent of the mPFC.

Our observation that pre-conditioning prefrontal GABA-blockade resulted in excessive fear to an aversive CS in NPE rats on a LI task but decreased fear to a CS+ on a discriminative fear task may at first seem contradictory. However, it is likely that neural activity in the mPFC is dramatically differentially impacted by the necessity of a discrimination, as compared to the relative simplicity of one exclusively aversive CS in the LI task. During discriminative fear learning, activity of separate populations of mPFC neurons likely reflect the safety of the CS- and aversive nature of a CS+ (Maxwell et al, 1994). In comparison, during aversive conditioning of a single stimulus, as occurred for the NPE group during LI, most neurons in the prelimbic mPFC become responsive to this stimulus (Baeg et al, 2001; Gilmartin and McEchron, 2005). For example, in trace fear conditioning, the vast majority of stimulus responsive neurons in the prelimbic mPFC of rats respond with excitation to an aversively conditioned CS (Gilmartin and McEchron, 2005). Artificially elevating cortical activity via GABA-antagonism may increase the activity of separate CS-/CS+ responsive neurons during discriminative fear, but will mostly increase the activity of CS responsive neurons for NPE animals during the conditioning of LI. This would be expected to lead to stronger encoding of the aversive nature of the CS in NPE animals, manifest by elevated fear and delayed extinction during the test phase. Thus, the seemingly opposing effects of mPFC GABA-blockade on fear conditioning likely reflect
fundamental differences in how neurons in the mPFC and downstream structures encode aversive versus neutral stimuli under discriminative or non-discriminative (NPE) conditions.

**Discrimination and latent inhibition deficits: Relevance for schizophrenia**

The deficits in fear-related behaviors reported here mirror affective deficits observed in schizophrenia. Elevated responding to neutral stimuli has been consistently reported in simple and discriminative paradigms. Meta-analysis suggests that, although individuals with schizophrenia display relatively intact hedonic emotional reactions, they respond with greater aversion than controls when exposed to neutral stimuli (Cohen and Minor, 2010). When simply viewing social scenes, control individuals display a pattern of subjective emotional arousal characterized by low arousal to neutral scenes and high arousal to negative scenes (Haralanova et al, 2012; Llerena et al, 2012). In comparison, schizophrenic individuals do not show this pattern, instead responding with inappropriately high arousal to neutral scenes. Such emotional appraisal deficits have been observed at the neural level, as measured by hemodynamic responses in a mPFC network (Holt et al, 2011). Holt et al. (2011) reported that, when control individuals were exposed to social sentences with neutral, positive, or negative affective valence, mPFC activity was increased in response to sentences with emotional (positive/negative) content. Patients with schizophrenia instead displayed elevated mPFC activity to non-emotional, neutral social sentences.

Elevated cortical activity and behavioral abnormalities have been observed on Pavlovian discrimination tasks similar to the one utilized in the present study. Jensen et al. (2008) trained schizophrenic patients and non-psychiatric controls to discriminate between an aversive CS+ and a neutral CS-. Patients displayed less responsivity to a CS+ and greater responsivity to a CS- compared to control individuals, as measured by galvanic skin response (GSR) and subjective
rating. This maladaptive pattern of responding has been reported in individuals high in certain schizotypal traits (Balog et al., 2013). Using the same mild conditioning protocol, Balog et al. (2013) found that high levels of the schizotypal trait “reality distortion” predicted both lower responses to a CS+ and higher responses to a CS-. Such abnormal patterns of Pavlovian conditioning are not limited to aversive stimuli, as similar deficits have been reported using an appetitive discriminative conditioning protocol (Diaconescu et al., 2011). Regions of the PFC including the right PFC and medial cingulate cortex, as well as downstream regions known to contribute to associative learning such as the ventral striatum and basolateral amygdala (BLA) are reported to be hyperactive to neutral stimulus presentations in patients, as compared to control individuals (Hall et al., 2008; Jensen et al., 2008; Murray et al., 2008). Our data suggest that elevated responding to a neutral stimulus may be related to GABAergic dysfunction specifically during acquisition. Many of the studies suggesting elevated responding to a neutral stimulus have no recall component (Jensen et al., 2008; Murray et al., 2008), indicating that the discrimination deficits in schizophrenia are related to acquisition abnormalities.

Although hyperresponsivity to neutral cue presentation has been hypothesized to underlie deficient LI in schizophrenia (Gray and Snowden, 2005), our results do not support a role for GABA transmission during preexposure/conditioning (when salience is being assigned to the CS) in LI. If GABA-hypofunction prior to preexposure/conditioning produced elevated salience to the neutral preexposed cue, one would expect these animals to show elevated fear when again exposed to the stimulus during test. Instead, pre-conditioning GABA-blockade animals showed normal LI, having learned the irrelevance of the preexposed stimulus similarly to controls. However, by blocking PFC GABA-receptors prior to the test phase of the task we produced a deficit in LI expression. These findings suggest that GABA dysfunction may not play a role in
deficient LI resultant from affective salience misattribution during preexposure. Instead, GABAergic hypofunction may produce an inability to accurately recall affective information learned during preexposure/conditioning, eliminating LI expression. Instead of decreasing LI, mPFC GABA-blockade when animals were being fear conditioned (NPE animals) increased the expression of conditioned fear during test. This deficit is reminiscent of deficient extinction learning in schizophrenia, whereby individuals do not recall extinction memories as strongly as controls, continuing to respond with elevated fear despite extinction conditioning (Holt et al, 2009, 2012). Extinction failure is correlated with ventromedial PFC (vmPFC) overactivation in schizophrenia patients, which may be related to changes in excitation/inhibition due to endemic GABAergic hypofunction.

Taken together, the results of the present study and those conducted in schizophrenia point to distributed cortico-limbic-striatal dysfunction during affective processing in schizophrenia. This circuitry has been described as being critical for the application of motivational salience to stimuli (Anticevic and Corlett, 2012; Sesack and Grace, 2010; Ventura et al, 2007). Motivational salience, or the ability of environmental cues to modulate behavior based on their association with reward or punishment, has long been hypothesized to underlie delusional states in schizophrenia, tied to the dopamine hypothesis of the disorder (Gray, 1995; Kapur, 2003). Elevated dopaminergic activity in the midbrain of individuals with schizophrenia may cause innocuous stimuli to be interpreted as salient. More recent research suggests that aberrant activity in PFC also plays a central role in accurate salience attribution (Corlett et al, 2006, 2007; Roiser et al, 2010; Schmidt and Roiser, 2009). For example, administration of an NMDAR antagonist known to produce cortical disinhibition results in disturbed reward learning and reward-related function in the prefrontal cortex (Corlett et al, 2006). Similar aberrant
Salience has been observed following NMDAR antagonist administration in rodents, with mice losing mPFC discriminative neural activity to a salient (aversive) and non-salient (neutral) tone following NMDAR-antagonist administration (Moessnang et al., 2012). Thus, GABA-hypofunction in cortical regions may influence salience attribution in two ways, directly by engaging in cognitive-associative processes, or indirectly by modulating activity of limbic and striatal regions known to be involved in salience attribution. Further research should examine whether these two pathways contribute equally to the assignment of motivational salience, or if they are in some way dissociable.

These deficits in affective processing may be particularly relevant for a number of reasons. First, it is possible that aberrant assignment of salience to environmental stimuli underlies psychotic symptoms, in particular delusions. While such symptoms are treated effectively using antipsychotics which block the dopamine D₂ receptor (Creese et al., 1996; Kegeles et al., 2008) in many cases delusional activity does not completely disappear, but instead loses its motivational relevance (Kapur, 2003). It is possible that delusions result from aberrant cortical activity, which then impinges on midbrain regions known to engage motivated behavior including the ventral striatum and VTA. An improved understanding of this residual delusion phenomenon could lead to more effective treatment of such symptoms. In addition, the interaction between affective and cognitive processes may present a significant impediment in social and cognitive functioning for individuals with schizophrenia. As previously illustrated, innocuous stimuli such as neutral sentences and pictures elicit stronger emotional reactions in individuals with schizophrenia than in non-psychiatric controls (Haralanova et al., 2012; Holt et al., 2011). Such biases in interpreting situational information will likely hamper social and occupational success, both of which are strong predictors of functional outcome (Fenton and
Mcglashan, 1987; Green, 1996). Finally, affective dysfunction have been shown to interact with cognitive functioning (Anticevic et al, 2011). During a working memory task, non-psychiatric control individuals are susceptible to emotional distracters that are aversive in nature, with relatively little distraction observed following a neutral distracter. In contrast, and in fitting with aberrant salience attribution, individuals with schizophrenia were susceptible equally to distraction from emotional and neutral distracters, coupled with aberrant neural responses in cortical and subcortical regions. To the extent that these deficits may be explained by cortical GABA-hypofunction, it is possible that enhancement of function particularly at the GABA$_A$-receptor may rescue such behaviors. Although partial agonists at the GABA$_A$-receptor have shown inconsistent efficacy for alleviating cognitive symptoms of schizophrenia (Buchanan et al, 2011; Lewis et al, 2008), it is possible that these compounds may be beneficial in treating affective dysfunction.

**Limitations**

In order to minimize the number of microinfusions (and thus potential for epileptiform activity due to kindling as well as damage from the infusion boli) required, a truncated LI protocol was used, whereby the conditioning phase consisted of both the NPE/PE phase and the aversive pairings. It is possible that mPFC GABA activity may influence pain sensitivity, thus altering LI expression through a non-paradigmatic mechanism. Our data argue against this interpretation, as animals infused with GABA-receptor antagonists show elevated fear (NPE and GABA-blockade prior to conditioning), decreased fear (CS- suppression following GABA-blockade prior to conditioning), or no alteration (PE and GABA-blockade prior to conditioning). Similarly, although infusions were aimed at the prelimbic mPFC, it is likely that some infusions at the dorsal and ventral extent of the prelimbic mPFC influenced excitability in neighboring
regions. Given that mPFC sub-regions are extensively reciprocally connected (Vertes, 2004), the impact of slight encroachment is likely minimal.

We utilized conditioned suppression of lever pressing as our proxy index of learned fear. While conditioned suppression and freezing behavior (another common indicator of fear-conditioning) are often highly correlated, double dissociations between these two behaviors have been observed in some brain regions critical for fear learning (McDannald and Galarce, 2011; McDannald, 2010). For example, lesions of the central nucleus of the amygdala, BLA, and ventrolateral periaqueductal grey impair conditioned freezing, but not conditioned suppression. However, no such dissociation has been observed following inactivation or lesion of the prelimbic mPFC (Quirk et al, 2000; Sierra-Mercado et al, 2011), suggesting that conditioned suppression is an adequate indicator of conditioned fear.

Our results suggest that PFC GABA transmission may play a role in salience attribution following aversive conditioning. Aberrant salience manifestations in schizophrenia have been observed following both appetitive (Diaconescu et al, 2011) and aversive paradigms (Jensen et al, 2008). While it is possible that the PFC contributes to the assessment of salience generally, our results are likely only applicable to aversive paradigms. Further studies utilizing appetitive conditioning procedures should be conducted to examine the potential generalizability of PFC GABA transmission in salience attribution.

**Summary and conclusions**

The present study provides evidence for the involvement of prefrontal cortical GABAergic neurotransmission in the expression and LI of conditioned fear. A prevailing view of neuropathology in schizophrenia suggests that GABAergic interneuron function is negatively impacted by disease processes, resulting in disturbances in cortical excitation/inhibition.
Correlation evidence in humans and causal evidence in other animals suggest that these deficits are related to cognitive dysfunction. The PFC regulates affective functions as well, suggesting a possible role for GABAergic transmission in this region in these deficits. Pharmacological blockade of mPFC GABA\textsubscript{A} receptors in rats recapitulated aversive discrimination deficits observed in schizophrenia, including elevated fear to a neutral stimulus and decreased fear to an aversive stimulus. The same manipulation exacerbated conditioned fear in animals having undergone a mild non-discriminative fear conditioning protocol when infused prior to conditioning, and eliminated LI when infused prior to the recall phase of the task. These deficits may be indicative of a fundamental role for normal GABA transmission within the mPFC in the development and recall of affective motivational salience, processes thought to be disturbed in schizophrenia.
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