

RESEARCH ARTICLE

Prefrontal Cortical GABA Modulation of Spatial Reference and Working Memory

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Abstract

Background: Dysfunction in prefrontal cortex (PFC) GABA transmission has been proposed to contribute to cognitive dysfunction in schizophrenia, yet how this system regulates different cognitive and mnemonic functions remains unclear.

Methods: We assessed the effects of pharmacological reduction of GABA_A signaling in the medial PFC of rats on spatial reference/working memory using different versions of the radial-arm maze task. We used a massed-trials procedure to probe how PFC GABA regulates susceptibility to proactive interference. Male rats were well-trained to retrieve food from the same 4 arms of an 8-arm maze, receiving 5 trials/day (1–2 min intervals).

Results: Infusions of the GABA_A receptor antagonist bicuculline (12.5–50 ng) markedly increased working and reference memory errors and response latencies. Similar treatments also impaired short-term memory on an 8-baited arm task. These effects did not appear to be due to increased susceptibility to proactive interference. In contrast, PFC inactivation via infusion of GABA agonists baclofen/muscimol did not affect reference/working memory. In comparison to the pronounced effects on the 8-arm maze tasks, PFC GABA_A antagonism only causes a slight and transient decrease in accuracy on a 2-arm spatial discrimination.

Conclusions: These findings demonstrate that prefrontal GABA hypofunction severely disrupts spatial reference and short-term memory and that disinhibition of the PFC can, in some instances, perturb memory processes not normally dependent on the frontal lobes. Moreover, these impairments closely resemble those observed in schizophrenic patients, suggesting that perturbation in PFC GABA signaling may contribute to these types of cognitive deficits associated with the disorder.

Keywords: GABA, prefrontal cortex, reference memory, schizophrenia, working memory

Introduction

Schizophrenia is associated with deficits in multiple domains of cognition, including working memory, attention, reasoning/problem solving, and a generally slower speed of processing (Goldberg and Weinberger, 1988; Hartman et al., 2003; Hutton et al., 2002; Marder et al., 2004; Nuechterlein et al., 2004; Keefe and Fenton, 2007; Heerey et al., 2008). These impairments often present prior to the onset of the psychotic symptoms, persist throughout the course of disease, and are predictive of long-term patient outcome (Green, 1996; Reichenberg et al., 2010). Furthermore, while currently available medications may treat

positive symptoms of schizophrenia (hallucinations, delusions), they are relatively ineffective at treating cognitive symptoms. Thus, understanding the neural basis of cognitive deficits associated with schizophrenia is critical in order to develop better treatments for this disorder.

Among the numerous neural and cellular perturbations that have been proposed to underlie aspects of schizophrenia symptomatology, several lines of evidence suggest that reduced prefrontal GABA transmission may be a key contributor to cognitive impairment associated with the disease. Alterations in the expression of

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markers of GABA function are amongst the most highly reproducible pathologies observed in post-mortem schizophrenic brains. mRNA and protein levels of the GABA synthesis enzyme glutamic acid decarboxylase-67 are consistently found to be reduced in the prefrontal cortex (PFC) of schizophrenics (Akbarian et al., 1995; Guidotti et al., 2000; Volk et al., 2000; Hashimoto et al., 2008; Thompson et al., 2009; Curley et al., 2011), potentially leading to decreased GABA synthesis and release. Optimal prefrontal GABA transmission is thought to be critical for generating neural oscillations—particularly in the gamma range—that underlie cognitive functions such as working memory (Howard et al., 2003; Gonzales-Burgos and Lewis, 2008). This cortical network activity has been shown to be suppressed in schizophrenic patients engaged in cognitive tasks (Cho et al., 2006; Minzenberg et al., 2010). Furthermore, several pharmacological and neurodevelopmental animal models of schizophrenia are also associated with disruptions in prefrontal GABA functioning (Amitai et al., 2012; Richetto et al., 2013; Thomases et al., 2013).

Although a link between prefrontal GABA hypofunction and cognitive deficits associated with schizophrenia has been proposed, until recently there has been surprisingly little preclinical research investigating the contribution of the GABA transmission to cognitive functions governed by the frontal lobes. Early reports found that GABA_A receptor antagonism within the monkey dorsolateral PFC impaired delayed alternation (Sawaguchi et al., 1988, 1989) and disturbed task-related interneuron and pyramidal cell activity (Rao et al., 2000). In keeping with these earlier findings, work by our group has shown that pharmacological reduction in prefrontal GABA_A transmission recapitulates several cognitive and behavioral aspects of schizophrenia in the rat. Infusion of the GABA_A receptor antagonist bicuculline into the prelimbic medial PFC resulted in impaired cognitive flexibility, enhanced phasic dopamine activity, and led to speed-of-processing deficits (Enomoto et al., 2011). Subsequent studies further revealed that intra-PFC infusion of low doses of GABA_A receptor antagonist also induced schizophrenia-like deficits in attention assessed with a 3- or 5-choice serial reaction-time task (Paine et al., 2011; Pehrson et al., 2013).

In contrast to the above-mentioned findings, antagonism of PFC GABA_A receptors did not impair working memory accuracy on a delayed response variant of the radial-arm maze task, although these manipulations did increase response latencies (Floresco et al., 1997; Enomoto et al., 2011). This was a surprising finding considering that working memory assessed in this manner is markedly impaired by inactivation of the medial PFC (Seamans et al., 1995; Floresco et al., 1997, 1999; Enomoto et al., 2011). In that study, it was proposed that the lack of effect of PFC GABA_A antagonism on working memory may be related to the incorporation of a relatively long delay between sample and test phases, and that responding during the test phase was self-paced. In comparison, tasks used to assess working memory capacity in patients often use shorter delay intervals and provide subjects with multiple trials in quick succession, requiring them to overcome proactive interference and discard information from previous trials (Park and Holzman, 1992; Fleming et al., 1997; Pukrop et al., 2003).

Recent studies with schizophrenic subjects have employed back-translational approaches, using virtual maze tasks similar to those used with rodents to assess cognition. In one notable study (Spieker et al., 2012), schizophrenic patients displayed marked reference and working memory deficits on a virtual version of the classic spatially-cued radial maze task initially described by Olton and Papas (1979). These findings highlight the translational relevance of using these types of tasks in combination with

preclinical animal models to probe the potential mechanisms underlying cognitive impairment in schizophrenia. As such, a primary goal of the present study was to assess the importance of GABAergic transmission in the rat medial PFC in regulating and reference/working memory using a similar radial-maze task. In so doing, we used a massed-trials variant of this task where rats were given multiple trials within a daily session. This was employed to increase cognitive load and induce proactive interference, as it requires subjects to disambiguate whether an arm was entered on the current or previous trial, which may increase increases errors on later trials (Roberts and Dale, 1980). A similar procedure has also been used in combination with a reference/working memory task using a 12-arm maze (Meck and Williams, 1999). Our expectation was that reducing PFC GABA transmission would exert a greater effect on performance on latter trials relative to the first. Subsequent experiments employed reversible inactivation of the medial PFC to assess its contribution to working/reference memory performance, and the effects of PFC GABA_A antagonism on simpler tasks assessing short-term memory and spatial discrimination abilities.

Methods

Subjects

Long Evans rats (250–300 g) were purchased from Charles River Laboratories and were initially group-housed upon arrival from the supplier. After 1 week, animals were single-housed and food-restricted to 85–90% of their free-feeding weight prior to beginning behavioral training or surgical preparations. Experiments were conducted in accordance with the Canadian Council on Animal Care and University of British Columbia Animal Care Committee.

Behavioral Procedures

All behavioral tests were conducted during the animals' light cycle. All testing was conducted on an 8-arm radial maze (see [Supplementary Materials](#) for details).

Reference/Working Memory Task

This task was initially described by Olton and Papas (1979) and assessed both long-term (reference) and short-term (working) memory. Although the term working memory has been used to describe a variety of cognitive operations related to storage and manipulation of information (Baddeley, 2012), here the term refers to processes related primarily to short-term memory, to maintain consistency with other studies using similar tasks. Rats were required to retrieve reward pellets placed in 4 of the 8 arms of the maze. The particular arms that were baited remained constant over testing for each rat, but the specific patterns were counterbalanced across rats. During training sessions, rats were allowed a maximum of 5 min to retrieve the four pellets. Errors were divided into reference memory (i.e., entering an arm that was never baited) and working memory errors (re-entering previously baited arm during a trial).

Initially, rats were given one trial per day. Once an individual rat reached a criterion of 1 or fewer errors for 3 consecutive days, subsequent sessions consisted of 5 trials per day, separated by an interval of ~1 min. Daily sessions continued until a rat again made 1 or fewer errors on the first trial of the session for 3 consecutive days, after which they underwent surgery. Following surgery, animals were re-trained, receiving 5 trials/day until they again achieved criterion performance for 3 consecutive days.

8-arm Foraging Task

Training was similar to that described above, with the primary exception that rats were required to retrieve food placed on all 8 arms. As with the reference/working memory task, rats were trained using 1 trial/day to a criterion of ≤ 1 error for 3 consecutive days, and then given 5 trials/day until achieving criterion performance during the first trial.

Spatial Discrimination

For this experiment, animals were implanted with cannulae prior to behavioral training. In this task, 5 of 8 arms of the maze were blocked using opaque Plexiglass inserts to create a T-shaped configuration, with a stem arm and 2 choice arms. For each rat, the orientation of the stem and choice arms relative to the testing room, and the location of a baited arm (left or right) remained constant across trials and training days (counterbalanced across rats). At the start of each trial, a rat was placed in the stem and required to traverse towards the baited arm, during which the latency to enter an arm was recorded. Errors were scored upon entry into the unbaited arms. Rats initially received 10 trials/day until making ≤ 2 errors (~2 days) and then received 20 trials/day until they reached criterion of ≤ 3 errors/day for 3 consecutive days.

Surgery

Upon achieving criterion performance on either the working/reference memory or 8-arm foraging task, rats were fed *ad libitum* for 2–3 days and then subjected to surgery. Rats were anesthetized with ketamine/xylazine (100/7 mg/kg) and implanted with bilateral 23-gauge stainless-steel guide cannulae that targeted the prelimbic region of the medial PFC using the following stereotaxic coordinates (flat skull: anterior/posterior = +3.4 mm, mediolateral = ± 0.7 mm from bregma, dorsoventral = -3.0 mm from dura; Paxinos and Watson, 1998). Rats were then given ~7 days to recover from surgery. Figure 1 shows the location of all acceptable infusion placements (see Supplementary Materials for details on histology).

Drugs

Reduction of PFC GABA_A transmission was achieved using the receptor antagonist bicuculline methobromide (12.5 or 50 ng in 0.5 μ l). These doses have been shown to be effective at altering forms of cognition when infused into the PFC, but are below the threshold for inducing seizures (Enomoto et al., 2011; Paine et al., 2011). In the present study, no signs of seizures, such as wet dog shakes, were observed. On rare occasions some rats displayed transient head-bobbing behavior for 10–15 minutes following bicuculline infusions, but this effect quickly dissipated over the course of testing. Previous neurophysiological studies have shown that intra-PFC infusions of 50 ng bicuculline can enhance neural activity in downstream regions for at least 2 hours (Enomoto et al., 2011), which was well within the timeframe required for rats to complete a test session. Inactivation of the PFC was achieved using a cocktail of the GABA_A agonists baclofen and muscimol (100 ng each in 0.5 μ l).

A within-subjects design was used for all experiments. On the first infusion test day, a proportion of the rats in each group received saline infusions, and the remaining rats received drugs (bicuculline or baclofen/muscimol). After the first test day, rats were retrained until they again achieved criterion performance, then received a second counterbalanced test day. Additional details on the micro-infusion procedure and data analyses are provided in the Supplementary Materials.

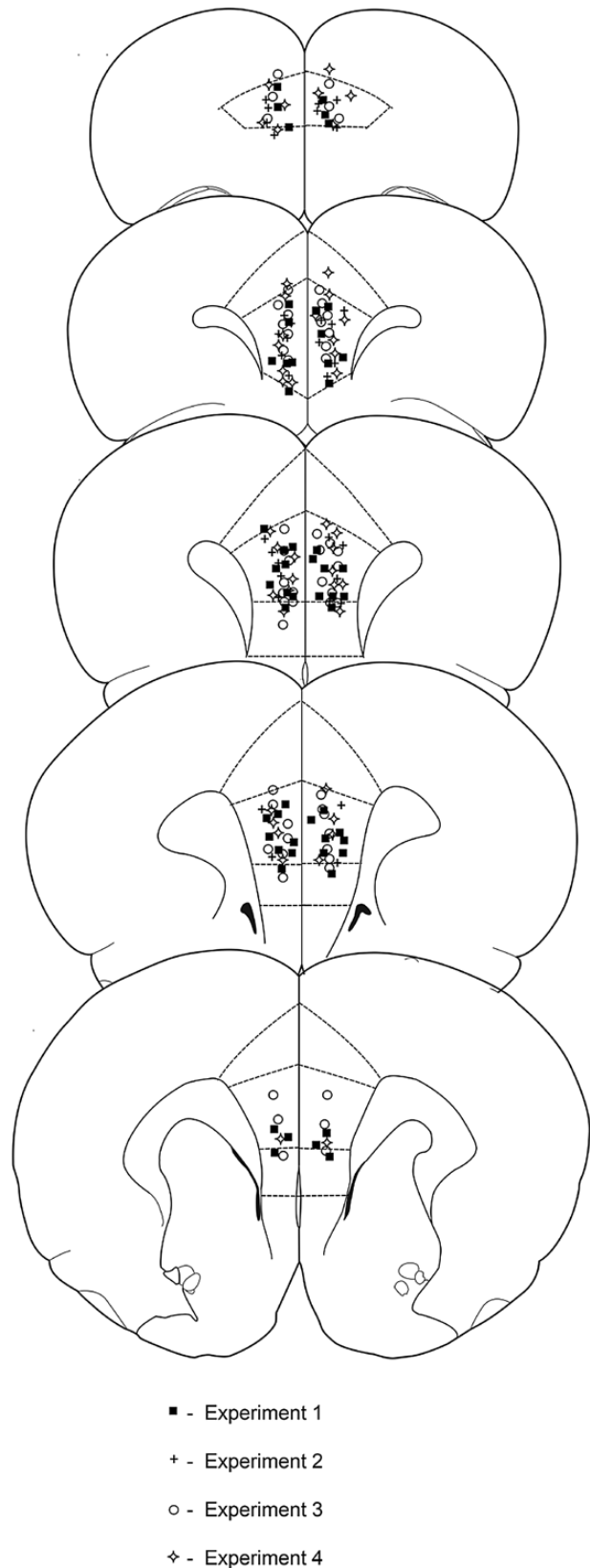


Figure 1. Location of all acceptable infusion placements within the medial PFC. Different symbols denote the locations of infusions for rats for each of the four specific experiments.

Results

Experiment 1: Reduced Prefrontal GABA_A Receptor Transmission and Reference/Working Memory Performance

Pharmacological reduction of PFC GABA_A transmission via infusion of low doses of bicuculline markedly impaired performance (Figure 2A). Analysis of the error data yielded a significant main effect of treatment ($F_{2,14} = 6.51, p < 0.05$) and a treatment \times trial interaction ($F_{2,14} = 4.34, p < 0.05$). Simple main effects analyses revealed that, contrary to our expectation, infusion of both the 12.5 and 50 ng dose increased errors ($p < 0.05$) relative to saline treatments during the first trial of the session. However, during subsequent trials, only the 50 ng dose impaired performance ($p < 0.05$). Interestingly, this analysis also revealed that under control conditions, the number of errors made during the first trial of the session did not differ from the average number of errors made over trials 2–5 ($p > 0.40, n.s.$). This indicates that any potential proactive interference induced by this massed trials procedure was insufficient to increase erroneous responding on subsequent trials. Likewise, the number of errors committed on trials 1 versus 2–5 did not differ after infusions of either dose of bicuculline (both $p > 0.50, n.s.$). Two rats not included in these analyses had placements ventral to the medial PFC, and did not display marked alterations in performance after bicuculline versus saline.

A separate analysis of reference and working memory errors across the 5 trials revealed a main effect of treatment ($F_{2,14} = 4.32, p < 0.05$) and a treatment \times error type interaction ($F_{2,14} = 3.87, p < 0.05$; Figure 2B). Breakdown of the interaction revealed that

reference and working errors were increased by both doses of bicuculline ($p < 0.05$), with the 50 ng dose causing significantly ($p < 0.05$) more reference memory errors when compared to the 12.5 ng dose. Thus, antagonism of PFC GABA_A receptors disrupted both reference and working memory performance, with the 50 ng dose having a more pronounced deleterious effect on reference memory and overall performance during the latter trials.

Reducing prefrontal GABA_A transmission also caused a dose-dependent increase in the time to initiate a trial (Figure 2C) and the average time per subsequent choice (Figure 2D). Analysis of the time to initiate data yielded a main effect of treatment ($F_{2,14} = 4.43, p < 0.05$), and a treatment \times trial interaction ($F_{8,56} = 2.46, p < 0.05$). Subsequent analysis confirmed that only the 50 ng dose increased initiation latencies, with this effect being statistically significant during the first 2 trials ($p < 0.05$). For the average time per subsequent choice, the analysis only yielded a main effect of treatment ($F_{2,14} = 8.50, p < 0.01$) but no interaction ($F_{8,56} = 1.25, n.s.$). Again, only the 50 ng dose was effective at increasing choice latencies, although the effects of this dose during the first trial were quite variable (range 26–92 s). Additionally, we observed a significant positive correlation between choice latencies and errors during the first trial ($r = +0.69, p < 0.05$).

Experiment 2: Reduced Prefrontal GABA_A Transmission and Performance of an 8-Arm Foraging Task

Experiment 1 revealed that intra-PFC GABA_A antagonism impaired both reference and working memory, with this effect manifesting during the first trial of the test session and

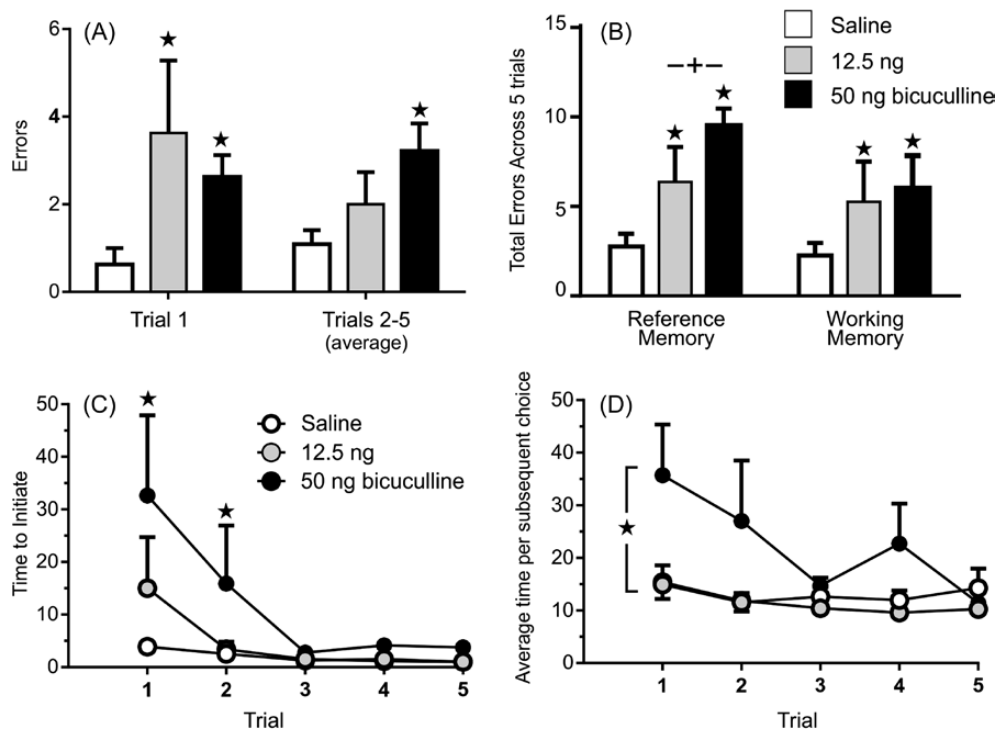


Figure 2. Prefrontal GABA_A antagonism blockade disrupts spatial reference and working memory performance on a radial maze task. For this and all other figures, values displayed are the mean \pm SEM. (A) Infusion of both a 50 and 12.5 ng dose of bicuculline increased the number of errors on Trial 1, while only the 50 ng dose increased the average number of errors on Trials 2–5. (B) Both doses of bicuculline increased the total number of reference and working memory committed over the 5 trials of a test session. However, the 50 ng dose caused significantly more reference memory errors than the 12.5 ng dose. (C) Intra-PFC infusions of 50 ng bicuculline increased the time to initiate a trial, specifically in Trials 1 and 2. (D) The 50 ng dose increased the average time per subsequent choice across all trials. * $p < 0.05$ vs saline, + $p < 0.05$ 50 ng vs 12.5 ng.

persisting over subsequent trials. Notably, under control conditions rats did not commit more errors during latter trials relative to the first, suggesting that the cognitive load of this procedure did not induce sufficient proactive interference to degrade performance. This may be related to the fact that once rats were well-trained on this task, they made only 4–6 choices per trial, whereas in previous studies this procedure used a 12-arm maze so that well-trained rats made 8–12 choices per trial (Meck and Williams, 1999). Thus, in Experiment 2 we trained a separate group on a short-term memory version of the radial maze task, wherein subjects had to visit all 8 arms of the maze in a non-repetitive manner. The expectation was that increasing the number of items to be remembered across each trial would make rats more susceptible to proactive interference and promoting more errors during latter trials. This in turn would enable us to better evaluate how PFC GABA transmission may modulate cognitive performance when levels of proactive interference were higher. In this experiment, we only tested the effect of the 50 ng dose of bicuculline, as this dose was more effective at impairing performance in Experiment 1.

Similar to Experiment 1, intra-PFC bicuculline produced a profound impairment in performance of this task, which emerged on the first trial. Analysis of the error data revealed a significant main effect of treatment on the total number of errors ($F_{1,5} = 33.40, p < 0.01$; Figure 3A). There was no significant treatment \times trial interaction ($F_{1,5} = 0.001, n.s.$; Figure 3B), nor was there a significant main effect of trial ($F_{1,5} = 0.52, n.s.$). However, a direct comparison of the number of errors committed after control treatments on trials 1 versus 2–5 revealed that rats made significantly more errors in later trials in comparison

to the first ($F_{1,5} = 10.00; p < 0.05$). This indicates that the use of a massed trials procedure with this task was sufficient to induce proactive interference. However, this effect was occluded by the substantial increase in errors after bicuculline treatments. One rat not included in these analyses had placements in the medial orbitofrontal cortex and also exhibited impairments on this task.

Reducing prefrontal GABA_A transmission again increased the time taken to initiate each subsequent choice (Figure 3C and D). A repeated measures ANOVA revealed a significant treatment \times trial interaction on the time to initiate a trial ($F_{4,20} = 2.98, p < 0.05$). Simple main effects analysis revealed that time to initiate was increased only on the first trial ($p < 0.01$). For average subsequent choice time, a main effect of treatment and treatment \times trial interaction were observed ($F_{1,5} = 18.13, p < 0.01$; $F_{4,20} = 19.15, p < 0.001$): bicuculline increased choice latencies on trials 1–3 ($p < 0.05$). Again, the effects of bicuculline on this measure during the first trial were highly variable (range 20–73 s). However, unlike what was observed in Experiment 1, here we observed a significant negative correlation between choice latencies and errors during the first trial ($r = -0.75, p < 0.05$). Thus, even though reducing PFC GABA signaling impaired accuracy and increased choice latencies on two separate radial maze tasks, the relationship between these two effects was not consistent across experiments. Collectively, the results of this experiment demonstrate that reducing PFC GABA transmission also impairs short-term memory assessed with a radial maze procedure, but this effect did not appear to be driven by an increased susceptibility to proactive interference.

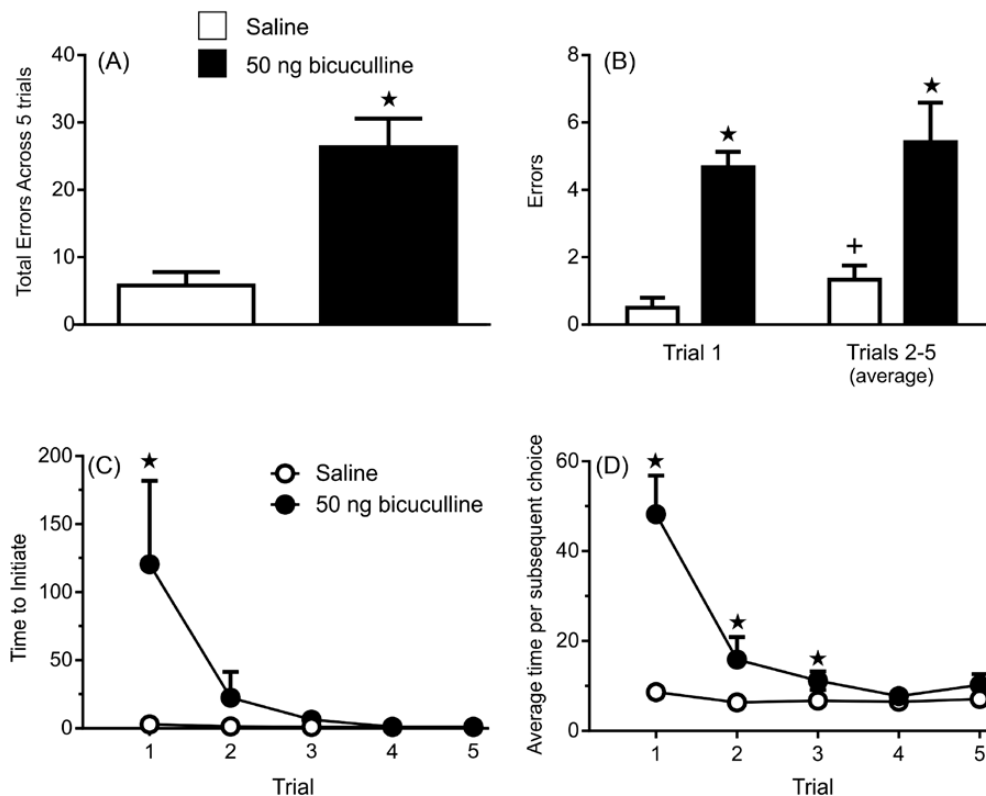


Figure 3. Prefrontal GABA_A receptor antagonism disrupts short-term memory on an 8-arm foraging task. (A) Intra-PFC infusion of 50 ng bicuculline increased the total number of errors committed across the 5 trials of a test session. (B) These treatments increase the number of errors made during for Trial 1 and subsequent errors during Trials 2–5. Under control conditions, rats made significantly more errors during Trials 2–5 relative to the first trial, indicative of an increase proactive interference during the latter trials. (C) Infusion of bicuculline increased the time to initiate during the first trial of a test session. (D) These treatments increased the average time per subsequent choice during Trials 1–3. * $p < 0.05$ vs saline, + $p < 0.05$ Trials 1 vs 2–5 for saline condition.

Experiment 3: PFC Inactivation and Reference/Working Memory Performance

Reducing PFC GABA_A transmission markedly impaired both reference and working/short-term memory, assessed with two different radial-arm maze tasks. These findings raise the question of the normal contribution of the PFC in facilitating search behavior guided by reference/working memory. Previous studies have shown that reversible inactivation of the medial PFC does not affect continuous foraging on a radial-arm maze task similar to the one used in Experiment 2 (Seamans et al., 1995). In comparison, large aspiration lesions of the medial PFC that destroy multiple subregions induce relatively transient deficits in acquiring or re-learning reference/working memory versions of this task (Kolb et al., 1983; Kesner et al., 1987). What was unclear is how acute and more circumscribed suppression of neural activity within the medial PFC would affect reference/working memory performance using a massed-trials version of this task. Thus, we assessed the effects of reversible inactivation of the medial PFC on performance of the same task used in Experiment 1 in a separate group of well-trained rats.

In stark contrast to the effects of prefrontal GABA_A receptor antagonism, PFC inactivation had no effect on performance of the task (total errors main effect of treatment, $F_{1,7} = 1.10$, n.s.; treatment x trial interaction, $F_{1,7} = 1.58$, n.s.; Figure 4A). No difference was observed in the number of errors made over trials 2–5 in comparison to trial 1 for animals receiving infusions of either saline or inactivation, indicating lack of the proactive interference effect ($F_{1,7} = 0.35$, n.s.). PFC inactivation did not alter the number of reference and working memory errors committed over the 5 trials (all $F_s < 3.2$, n.s.). Thus, PFC inactivation had no overall effect on working and reference memory performance (Figure 4B).

PFC inactivation produced no main effect on the time taken to initiate a trial ($F_{1,7} = 3.01$, n.s.), but did reveal a significant treatment x trial interaction on the time to initiate ($F_{4,28} = 5.39$, $p < 0.01$). Subsequent analysis revealed that PFC inactivation caused a modest, but significant ($p < 0.05$) increase in the time taken to initiate at the start of the first trial (Figure 4C). PFC inactivation did not affect the average time taken per subsequent choice (main effect of treatment, $F_{1,7} = 2.36$, n.s.; treatment x trial interaction, $F_{4,28} = 0.54$, n.s.; Figure 4D). Thus, PFC inactivation had little impact on speed of processing times.

Experiment 4: Reduction of Prefrontal GABA_A Transmission and Spatial Discrimination

The impairments induced by prefrontal GABA_A antagonism in Experiments 1 and 2 prompted us to explore whether these effects reflected a more general disruption in the ability to discriminate between different maze arms. In so doing, rats were well trained on a simple left/right discrimination task, whereby one of two arms of the maze were consistently baited and rats merely had to discriminate between them and enter one of them to obtain food. In this experiment, we again only tested the 50 ng dose of bicuculline.

Infusions of bicuculline had no overall effect on accuracy in the spatial discrimination task across 20 trials, as the analysis revealed no significant main effect of treatment ($F_{1,6} = 0.36$, n.s.; Figure 5A). However, a significant treatment x trial block interaction was observed ($F_{4,24} = 4.57$, $p < 0.01$). This effect was driven by a slight increase in errors committed during the first four trials of the session, with rats making ~1 more error after bicuculline treatment relative to their performance on saline.

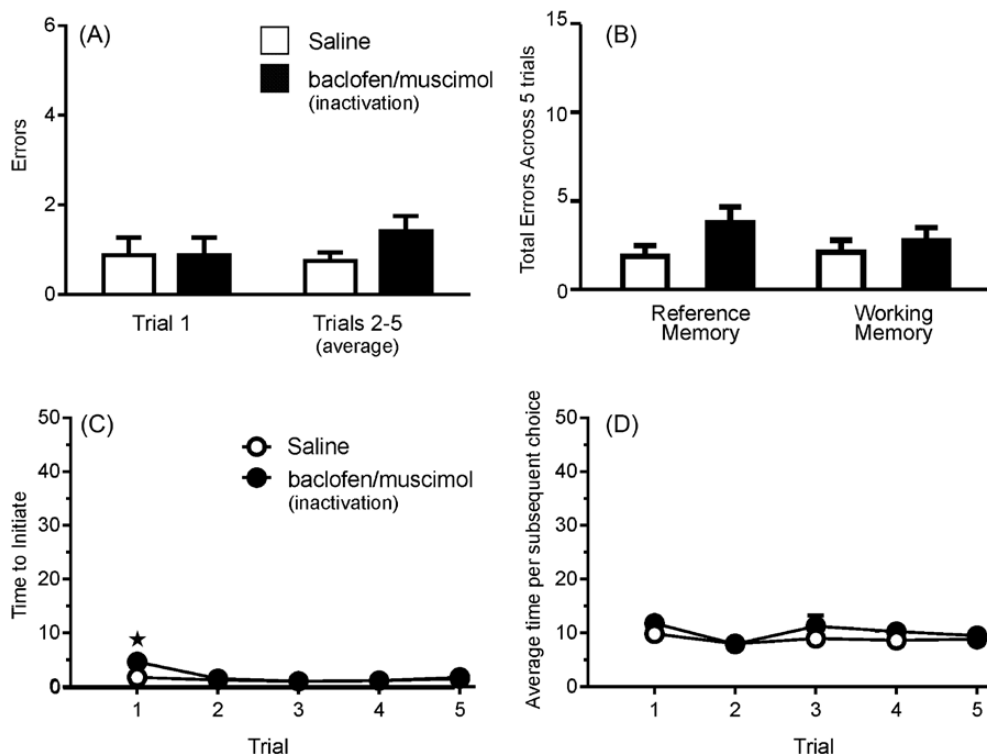


Figure 4. PFC inactivation does not affect spatial reference/working memory performance. (A) Inactivation of the medial PFC with infusion of the GABA agonists baclofen and muscimol had no effect on the number of errors on Trial 1 or the average number of errors on Trials 2–5. (B) PFC inactivation did not alter on the total number of reference or working memory errors relative to saline. (C) Inactivation of the PFC increased the latency to initiate a trial during the first trial of a test session, but did not affect the average time per subsequent choice (D). * $p < 0.05$ vs saline.

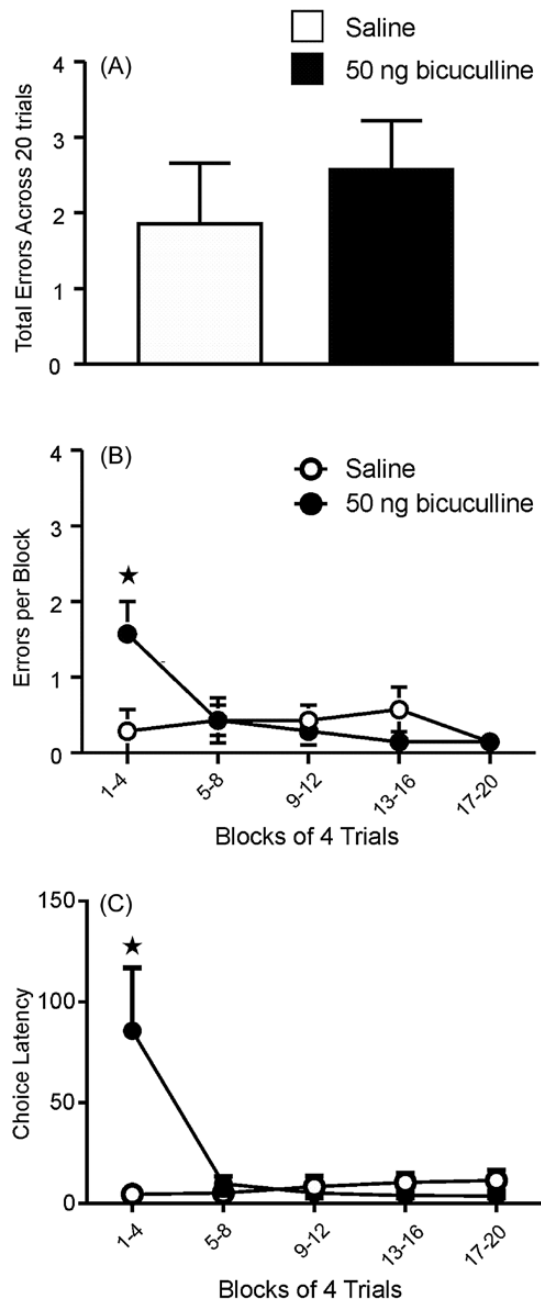


Figure 5. Prefrontal GABA_A receptor antagonism and performance of a 2-arm spatial discrimination. (A) Intra-PFC infusion of 50 ng bicuculline did not alter the total number of errors committed across the 20 trials of a spatial discrimination test session. (B) These treatments did cause a slight increase in errors made during the first 4 trials, but this effect disappears during the latter trials of a test session. (C) Choice latencies were also increased during the first 4 trials. * $p < 0.05$ vs saline.

However, this effect was transient, as no differences in accuracy were observed during the latter trials (Figure 5B). Data from 3 rats excluded from analysis for inaccurate placements did not appear to exhibit impairments on either the early or later trials.

Bicuculline infusion did not cause an overall change in the amount of time required to complete a session (Figure 5C; $F_{1,6} = 2.39$, n.s.). However, a significant interaction between treatment and trial block was revealed ($F_{4,24} = 8.001$, $p < 0.001$). This

interaction was driven by an increase in response times in the first block of trials, but not on subsequent trials (Figure 5D). Thus, aside from a slight and transient increase in errors committed at the start of the test session, reducing PFC GABA transmission did not impair performance of a relatively simple spatial discrimination.

Discussion

The primary findings of the present study are that reducing GABA_A receptor activity in the medial PFC results in a profound impairment in search behavior guided by spatial working, short-term, and/or reference memory. The effects on search accuracy were accompanied by increases in choice latencies. However, decreasing PFC GABA_A transmission did not markedly impair performance of a 2-arm spatial discrimination, suggesting that impairments observed in the other experiments were unlikely to be the result of non-specific deficits in motor, motivational, or spatial discrimination processes. Thus, these results suggest that intact PFC GABA transmission is essential for using spatial information to guide search behavior in complex environments, and disinhibition of the medial PFC can lead to pronounced impairments in these functions.

Prefrontal GABA Signaling and Reference/Working Memory

A key question we attempted to address in this study is whether prefrontal GABA transmission facilitates memory performance when information about previous choices may interfere with ongoing behavior (i.e., conditions that incur proactive interference). This hypothesis was based in part on the idea that if PFC GABA aids in filtering out irrelevant information, then memory functioning under conditions that provide interference effects from previous choices may be more susceptible to disruption by reduced PFC GABA signaling. To this end, we used a variation of the radial arm maze procedure, where multiple trials were presented with a short inter-trial delay requiring them to discard information from previous trials and focus on information relevant to the current trial. Previous studies using similar procedures have shown that well-trained rats typically make more errors during later trials relative to the first trial of a daily session (Roberts and Dale, 1980). However, with the reference/working memory task used in Experiment 1, this effect was not observed under control conditions. Moreover, PFC GABA antagonism did not preferentially increase errors during the latter trials, but instead caused a considerable increase in errors apparent on the first trial of a session. The lack of a proactive interference effect in Experiment 1 prompted another experiment, where we increased cognitive load by using a short-term memory version of the task with 8 arms baited. Here, we did observe an increase in errors in later trials relative to the first under control conditions. Yet, reducing prefrontal GABA signaling did not appear to exacerbate this effect, as these treatments again increased errors during the first trial of the session that persisted over subsequent trials. Thus, although intact prefrontal GABA transmission may be essential for efficient search guided by spatial reference or working memory, reducing this activity does not appear to increase sensitivity to proactive interference.

Disinhibition of the PFC increased both reference and working memory errors. Indeed, animals were equally likely to enter an arm that had never been baited as they were to re-enter arms visited previously during a trial. The neural substrates that underlie learning of this task have been studied in considerable

detail. Pre-training lesions of the hippocampus and medial thalamus impair both reference and working memory (Olton and Papas, 1979; Stokes and Best, 1990; Pothuizen et al., 2004). In comparison, lesions to the dorsal striatum or nucleus accumbens shell selectively impair reference and working memory, respectively (Packard and White, 1990; Jongen-Rêlo et al., 2003). The observation that reducing PFC GABA activity in well-trained rats non-selectively disrupted both reference (Experiment 1) and working memory performance (Experiments 1 and 2) suggests that these deficits may not reflect an impairment in mnemonic processing per se. Rather, we interpret these findings to indicate that disinhibition of the medial PFC disrupts the utilization of information processed by short- and long-term memory systems to implement an efficient search strategy.

In contrast to the effects of PFC GABA antagonism on the 8-arm maze tasks, similar treatments did not impair overall performance on a simpler 2-arm spatial discrimination. Bicuculline infusions slightly reduced accuracy early in the test session (~1 more error), but this effect quickly dissipated as trials progressed. These treatments may have caused a transient disorientation that interfered with approach towards the baited arm on the first few trials. The relatively muted nature of this effect contrasts with the pronounced impairment in performance during the more complex maze tasks that persisted over the entire course of the test sessions. Importantly, because impairments on the 2-arm spatial discrimination task did not persist across the session, it is unlikely that the deficits in performance observed in the other experiments are attributable to a general disruption in the ability to discriminate between arms or motivational processes.

One of the more remarkable features of the present findings is that, although disinhibition of the PFC impaired radial maze performance, suppression of PFC neural activity with GABA agonists had no effect on search accuracy guided by reference/working memory. Similarly, previous studies have shown that medial PFC inactivation does not disrupt performance of a short-term memory version of the 8-arm maze task similar to the one used here, although these manipulations do impair performance when a delay is inserted between choices (Seamans et al., 1995). As such, the present findings demonstrate that memory processes not normally dependent on the PFC may nevertheless be disrupted by disinhibitory increases in PFC activity. The prefrontal cortex sends excitatory projections to the dorsomedial striatum, the nucleus accumbens, and the midline thalamic nuclei, and also indirect input to the hippocampus via the entorhinal cortex (Sesack et al., 1989; Sesack and Pickel, 1992; Brog et al., 1993; Prasad and Chudasama, 2013). These regions have been shown to play an essential role in enabling efficient search behavior, guided by spatial reference and/or working memory (Olton and Papas, 1979; Becker et al., 1980; Schacter et al., 1989; Stokes and Best, 1990; Seamans et al., 1995; Floresco et al., 1997; Jongen-Rêlo et al., 2003; Pothuizen et al., 2004). Given these anatomical considerations, it is possible that impairments in search behavior, induced by PFC disinhibition, may be driven by aberrant patterns of excitatory outflow to downstream structures. This in turn may interfere with normal patterns of neural activity in these regions that regulate memory processes used to guide behavior. As such, the present findings highlight that impairments in cognition arising from a disinhibited or “noisy” PFC may not always reflect local perturbations of task-related neural activity, but also result from a cacophonous influence that PFC projections exert over mnemonic-related activity in downstream brain regions.

PFC GABA Transmission and Choice Latencies

In addition to disrupting efficient search behavior, antagonism of prefrontal GABA_A receptors consistently increased the latency to initiate choices. This observation mirrors previous studies from our laboratory, in which pharmacological reduction in PFC GABA signaling increased choice latencies on a delayed response variant of the radial maze task (Enomoto et al., 2011). However, in the same study we observed that similar treatments actually increased spontaneous locomotion measured in activity chambers (Enomoto et al., 2011). Thus, it is unlikely that increased choice latencies reflect reduced locomotion function. Unfortunately, we were unable to quantify changes in activity patterns induced by bicuculline infusions. Anecdotally, following these treatments, rats were active within the center of the maze, but were much slower to move from the center and initiate their first choice. Furthermore, upon entering an arm, they spent a disproportionate amount of time on it following reward retrieval before initiating another choice. We interpret these effects to reflect reduced speed-of-processing, in that rats were more hesitant to initiate specific choices, rather than reducing overall levels of activity. It is notable that the effect of intra-PFC bicuculline on choice latencies was most prominent during the earlier trials, whereas the increase in errors persisted across all trials of the test session. This suggests that the pharmacokinetics of the effects of these treatments on these two measures (accuracy vs choice latencies) differ considerably. Furthermore, these findings, in addition to the fact that increased choice latencies during the first trial showed opposing relationships to the number of errors committed in Experiments 1 and 2, suggest that speed of processing deficits induced by PFC disinhibition may not be related to impairments in performance accuracy.

On the other hand, inactivation of the PFC, which did not affect performance accuracy, caused a relatively modest increase in the latency to initiate responding on the first trial, and did not affect subsequent choice latencies. In comparison, similar treatments have been reported to increase choice latencies and impair performance during tests of working memory capacity (Davies et al., 2013). Thus it appears that the effects of PFC inactivation on speed of processing are dependent on whether this region actually contributes to task performance.

Implications for Schizophrenia

Converging evidence from post-mortem and electrophysiological studies of schizophrenic brains, as well as findings from animal models of the disorder, suggest schizophrenia is associated with disruptions in GABAergic transmission in the frontal lobes (Akbarian et al., 1995; Gonzales-Burgos and Lewis, 2008; Amitai et al., 2012; Richetto et al., 2013; Thomases et al., 2013). These pathophysiological alterations may contribute to cognitive impairments associated with the disease. A primary goal of the present study was to examine how reductions in prefrontal GABA signaling may affect cognitive and mnemonic functions that are impaired in schizophrenia to provide translational evidence that may support this assertion. In this regard, the findings of a recent study by Spieker and colleagues (2012) are particularly striking when viewed in light of the present findings. In that study, the authors employed a back-translational approach, wherein schizophrenic patients performed a virtual version of the reference/working memory radial-arm maze task nearly identical to the one used in the present study. When compared to healthy controls, patients showed reduced efficiency and accuracy of search, with increased numbers of

both reference and working memory errors and time required to complete each trial. In a similar vein, schizophrenic patients performing the CANTAB spatial working memory search task showed an increased tendency to return to locations which had already been reinforced during a previous searching sequence (Pantelis et al., 1997). Notably, these deficits were only observed when subjects had to search amongst 4–8 locations, but not fewer locations. The constellation of effects across these two studies was nearly identical to what we observed following pharmacological reductions in prefrontal GABA signaling. The remarkable qualitative similarities between these two sets of findings suggest that diminished PFC GABA neurotransmission may be a key contributing factor to the impairments in reference and working memory observed in schizophrenia.

Deficits in goal-directed navigation in schizophrenia have also been observed using other paradigms, such as virtual versions of the Morris water maze task and way-finding in other virtual environments (Hanlon et al., 2012; Zawadzki et al., 2013). Previous work suggests that spatial deficits in schizophrenia are a result of deficient allocentric navigation and formation of cognitive maps based on external cues, rather than egocentric strategies used to navigate to a target. With respect to the present study, prefrontal GABA antagonism produced profound deficits in more complicated 4- or 8-choice versions of the radial-arm maze task, where the subject must rely on external cues to navigate through the maze environment. While impairments in spatial search observed in schizophrenics have typically been thought to reflect deficits in hippocampal functioning, the present findings suggest that deficient prefrontal GABA signaling may also contribute to these types of abnormalities.

To summarize, the present findings highlight how optimal GABA signaling within the PFC is essential for efficient performance of cognitive tasks guided by spatial working and reference memory. Decreasing prefrontal GABA_A transmission impaired search behavior on a radial maze in a manner that was nearly identical to those observed in schizophrenic patients. Moreover, these treatments impaired performance of cognitive tasks that are not normally mediated by the PFC, suggesting that a hyperactivity of the frontal lobes may interfere with cognitive/mnemonic processes mediated by other circuits. These data complement a growing body of preclinical literature implicating intact PFC GABA signaling in the mediation of numerous domains of cognition, and indicating that disinhibition of the PFC can disrupt cognitive and affective dysfunction that in many instances is qualitatively similar to what is observed in schizophrenia (Japha and Koch, 1999; Enomoto et al., 2011; Paine et al., 2011; Piantadosi and Floresco, 2014). In turn, these data provide additional support for the idea that dysfunction in GABA transmission in the frontal lobes may contribute to cognitive dysfunction in schizophrenia, and further suggest that treatments designed to normalize inhibitory signaling may provide some therapeutic benefits for ameliorating these symptoms.

Supplementary material

For supplementary material accompanying this paper, visit <http://www.ijnp.oxfordjournals.org>

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References

- Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE, Jones EG (1995) Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. *Arch Gen Psychiatry* 52:258–266.
- Amitai N, Kuczenski R, Behrens MM, Markou A (2012) Repeated phencyclidine administration alters glutamate release and decreases GABA markers in the prefrontal cortex of rats. *Neuropharmacology* 62:1422–1431.
- Baddeley, AD (2012). Working memory: theories, models, and controversies. *Ann Rev Psychol* 63:1–29.
- Becker, JT, Walker, JA, and Olton, D. (1980) Neuroanatomical bases of spatial memory. *Brain Res* 200:307–320.
- Brog JS, Salyapongse A., Deutch AY, Zahm, DS (1993) The patterns of afferent innervation of the core and shell in the “accumbens” part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol* 338:255–278.
- Cho RY, Konecky RO, Carter CS (2006) Impairments in frontal cortical gamma synchrony and cognitive control in schizophrenia. *Proc Natl Acad Sci USA* 103:19878–19883.
- Curley AA, Arion D, Volk DW, Asafu-Adjei JK, Sampson AR, Fish KN, Lewis DA (2011) Cortical deficits of glutamic acid decarboxylase 67 expression in schizophrenia: clinical, protein, and cell type-specific features. *Am J Psychiatry* 168:921–929.
- Davies DA, Molder JJ, Greba Q, Howland JG (2013) Inactivation of medial prefrontal cortex or acute stress impairs odor span in rats. *Learn Mem* 20:665–669.
- Enomoto T, Tse MT, Floresco, SB (2011) Reducing prefrontal gamma-aminobutyric acid activity induces cognitive, behavioral, and dopaminergic abnormalities that resemble schizophrenia. *Biol Psychiatry* 69:432–441.
- Fleming K, Goldberg TE, Binks S, Randolph C, Gold JM (1997) Visuospatial working memory in patients with schizophrenia. *Biol Psychiatry* 41:43–49.
- Floresco SB, Seamans JK, Phillips AG (1997) Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. *J Neurosci* 17:1880–1890.
- Floresco SB, Braaksma DN, Phillips AG (1999) Thalamic-cortical-striatal circuitry subserves working memory during delayed responding on a radial arm maze. *J Neurosci* 19:11061–11071.
- Goldberg TE, Weinberger DR (1988) Probing prefrontal function in schizophrenia with neuropsychological paradigms. *Schizophr Bull* 14:179–183.
- Gonzalez-Burgos G, Lewis DA (2008) NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. *Schizophr Bull* 38:950–957.
- Green MF (1996) What are the functional consequences of neurocognitive deficits in schizophrenia? *Am J Psychiatry* 153:321–330.
- Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, Grayson DR, Impagnatiello F, Pandey G, Pesold C, Sharma R,

- Uzunov D, Costa E (2000) Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch Gen Psychiatry* 57:1061–1069.
- Hanlon FM, Houck JM, Klimaj SD, Caprihan A, Mayer AR, Weisend MP, Bustillo JR, Hamilton DA, Tesche, CD (2012) Frontotemporal anatomical connectivity and working-relational memory performance predict everyday functioning in schizophrenia. *Psychophysiology* 49:1340–1352.
- Hartman M, Steketee MC, Silva S, Lanning K, McCann H (2003) Working memory and schizophrenia: evidence for slowed encoding. *Schizophr Bull* 59: 99–113.
- Hashimoto T, Bazmi HH, Mirnics K, Wu Q, Sampson AR, Lewis DA (2008) Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. *Am J Psychiatry* 165:479–489.
- Heerey EA, Bell-Warren KR, and Gold JM (2008) Decision-making impairments in the context of intact reward sensitivity in schizophrenia. *Biol Psychiatry* 64:62–69.
- Howard MW, Rizzuto DS, Caplan JB, Madsen JR, Lisman J, Aschenbrenner-Scheibe R, Schulze-Bonhage A, Kahana MJ (2003) Gamma oscillations correlate with working memory load in humans. *Cereb Cortex* 13:1369–1374.
- Hutton SB, Murphy FC, Joyce EM, Rogers RD, Cuthbert I, Barnes TR, McKenna PJ, Sahakian BJ, Robbins TW (2002) Decision making deficits in patients with first episode and chronic schizophrenia. *Schizophr Res* 55:249–257.
- Japha K, Koch M (1999) Picrotoxin in the medial prefrontal cortex impairs sensorimotor gating in rats: reversal by haloperidol. *Psychopharmacol (Berl)* 144:347–354.
- Jongen-Rêlo AL, Kaufmann S, Feldon J (2003) A differential involvement of the shell and core subterritories of the nucleus accumbens of rats in memory processes. *Behav Neurosci* 117:150–168.
- Keefe RS, Fenton WS (2007) How should DSM-V criteria for schizophrenia include cognitive impairment? *Schizophr Bull* 33:912–920.
- Kesner RP, DiMattia BV, Crutcher KA (1987) Evidence for neocortical involvement in reference memory. *Behav Neural Biol* 47:40–53.
- Kolb B, Sutherland RJ, Whishaw, IQ (1983) A comparison of the contributions of the frontal and parietal association cortex to spatial localization in rats. *Behav Neurosci* 97:13–27.
- Marder SR, Fenton W, Youens K (2004) Schizophrenia, IX: Cognition in schizophrenia-the MATRICS initiative. *Am J Psychiatry* 161:25.
- Meck WH, Williams CL (1999) Choline supplementation during prenatal development reduces proactive interference in spatial memory. *Dev Brain Res* 118:51–59.
- Minzenberg MJ, Firl AJ, Yoon JH, Gomes GC, Reinking C, Carter CS (2010) Gamma oscillatory power is impaired during cognitive control independent of medication status in first-episode schizophrenia. *Neuropsychopharmacology* 35:2590–2599.
- Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK (2004) Identification of separable cognitive factors in schizophrenia. *Schizophr Res* 72:29–39.
- Olton DS, Papas BC (1979) Spatial memory and hippocampal function. *Neuropsychologia* 17:669–682.
- Packard MG, White NM (1990) Lesions of the caudate nucleus selectively impair “reference memory” acquisition in the radial maze. *Behav Neural Biol* 53:39–50.
- Paine TA, Slipp LE, Carlezon WA Jr. (2011) Schizophrenia-like attentional deficits following blockade of prefrontal cortex GABAA receptors. *Neuropsychopharmacology* 36:1703–13.
- Pantelis C, Barnes TR, Nelson HE, Tanner S, Weatherley L, Owen AM, Robbins TW (1997) Frontal-striatal cognitive deficits in patients with chronic schizophrenia. *Brain* 120:1823–1843.
- Park S, Holzman PS (1992) Schizophrenics show spatial working memory deficits. *Arch Gen Psychiatry* 49:975–982.
- Paxinos G, Watson C (1998) *The Rat Brain in Stereotaxic Coordinates*, 3rd ed. Orlando, FL: Academic Press.
- Pehrson AL, Bondi CO, Totah NK, Moghaddam B (2013) The influence of NMDA and GABA(A) receptors and glutamic acid decarboxylase (GAD) activity on attention. *Psychopharmacology (Berl)* 225:31–39.
- Piantadosi PT, Floresco SB (2014) Prefrontal cortical GABA transmission modulates discrimination and latent inhibition of conditioned fear: relevance for schizophrenia. *Neuropsychopharmacology* 39:2473–2484.
- Pothuizen HH, Zhang WN, Jongen-Rêlo AL, Feldon J, Yee BK (2004) Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: a within-subject, within-task comparison of reference and working spatial memory. *Eur J Neurosci* 19:705–12.
- Prasad JA, Chudasama, Y (2013) Viral tracing identifies parallel synaptic pathways to the hippocampus. *J Neurosci* 33:8494–8503.
- Pukrop R, Matuschek E, Ruhrmann S, Brockhaus-Dumke A, Tendlolker I, Bertsch A, Klosterkötter J (2003) Dimensions of working memory dysfunction in schizophrenia. *Schizophr Res* 62:259–268.
- Rao SG, Williams GV, Goldman-Rakic PS (2000) Destruction and creation of spatial tuning by disinhibition: GABA(A) blockade of prefrontal cortical neurons engaged by working memory. *J Neurosci* 20:485–494.
- Reichenberg A, Caspi A, Harrington H, Houts R, Keefe RS, Murray RM, Poulton R, Moffitt TE (2010) Static and dynamic cognitive deficits in childhood preceding adult schizophrenia: a 30-year study. *Am J Psychiatry* 167:160–169.
- Richetto J, Calabrese F, Meyer U, Riva MA (2013) Prenatal versus postnatal maternal factors in the development of infection-induced working memory impairments in mice. *Brain Behav Immun* 33:190–200.
- Roberts WA, Dale RH (1980) Remembrance of places lasts: Proactive inhibition and patterns of choice in rat spatial memory. *Learn Motiv* 12:261–281.
- Sawaguchi T, Matsumura M, Kubota K (1988) Delayed response deficits produced by local injection of bicuculline into the dorsolateral prefrontal cortex in Japanese macaque monkeys. *Exp Brain Res* 75:457–469.
- Sawaguchi T, Matsumura M, Kubota K (1989) Delayed response deficit in monkeys by locally disturbed prefrontal neuronal activity by bicuculline. *Behav Brain Res* 31:193–198.
- Schacter GB, Yang CR, Innis NK, Mogenson GJ (1989) The role of the hippocampal-nucleus accumbens pathway in radial-arm maze performance. *Behav Res* 494:339–349.
- Seamans JK, Floresco SB, Phillips AG (1995) Functional differences between the prelimbic and anterior cingulate regions of the rat prefrontal cortex. *Behav Neurosci* 109:1063–1073.
- Sesack SR, Deutch AY, Roth RH, Bunney BS (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J Comp Neurol* 290:213–242.
- Sesack SR, Pickel VM (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J Comp Neurol* 320:145–160.

- Spieker EA, Astur RS, West JT, Griego JA, Rowland LM (2012) Spatial memory deficits in a virtual reality eight-arm radial maze in schizophrenia. *Schizophr Res* 135:84–89.
- Stokes KA, Best PJ (1990) Mediodorsal thalamic lesions impair “reference” and “working” memory in rats. *Physiol Behav* 47:471–476.
- Thomas DR, Cass DK, Tseng KY (2013) Periadolescent exposure to the NMDA receptor antagonist MK-801 impairs the functional maturation of local GABAergic circuits in the adult prefrontal cortex. *J Neurosci* 33:26–34.
- Thompson M, Weickert CS, Wyatt E, Webster MJ (2009) Decreased glutamic acid decarboxylase(67) mRNA expression in multiple brain areas of patients with schizophrenia and mood disorders. *J Psychiatr Res* 43:970–977.
- Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA (2000) Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. *Arch Gen Psychiatry* 57:237–245.
- Zawadzki JA, Girard TA, Foussias G, Rodrigues A, Siddiqui I, Lerch JP, Grady C, Remington G, Wong AH (2013) Simulating real world functioning in schizophrenia using a naturalistic city environment and single-trial, goal-directed navigation. *Front Behav Neurosci* 7:180. doi:10.3389/fnbeh.2013.00180