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Inhibition of GABA synthesis in the prefrontal cortex increases locomotor activity but does not affect attention in the 5-choice serial reaction time task

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Abstract

Attention deficits are a core cognitive symptom of schizophrenia; the neuropathology underlying these deficits is not known. Attention is regulated, at least in part, by the prefrontal cortex (PFC), a brain area in which pathology of γ -aminobutyric acid (GABA) neurons has been consistently observed in post-mortem analysis of the brains of people with schizophrenia. Specifically, expression of the 67-kD isoform of the GABA synthesis enzyme glutamic acid decarboxylase (GAD67) is reduced in parvalbumin-containing fast-spiking GABA interneurons. Thus it is hypothesized that reduced cortical GABA synthesis and release may contribute to the attention deficits in schizophrenia. Here the effect of reducing cortical GABA synthesis with L-allylglycine (LAG) on attention was tested using three different versions of the 5-choice serial reaction time task (5CSRTT). Because 5CSRTT performance can be affected by locomotor activity, we also measured this behavior in an open field. Finally, the expression of Fos protein was used as an indirect measure of reduced GABA synthesis. Intra-cortical LAG (10 μ g/0.5 μ l/site) infusions increased Fos expression and resulted in hyperactivity in the open field. Intra-cortical LAG infusions did not affect attention in any version of the 5CSRTT. These results suggest that a general decrease in GABA synthesis is not sufficient to cause attention deficits. It remains to be tested whether a selective decrease in GABA synthesis in parvalbumin-containing GABA neurons could cause attention deficits. Decreased cortical GABA synthesis did increase locomotor activity; this may reflect the positive symptoms of schizophrenia.

Keywords

attention; schizophrenia; GABA; 5-choice serial reaction time task; locomotor activity; prefrontal cortex

1.0 Introduction

Cognitive deficits, which span multiple domains including attention, are a core feature of schizophrenia (<http://www.nimh.nih.gov/publicat/schizosph.cfm>) and are largely refractory to currently available antipsychotic treatments. Moreover these deficits predict the ability of afflicted individuals to integrate into society (Green et al., 2004). Thus, understanding the

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biological bases of the attention deficits in schizophrenia may lead to the development of more efficacious treatments for this devastating disorder.

Pathology in cortical γ -aminobutyric acid (GABA) neurons is one of the most reliable abnormalities found in post-mortem analyses of the schizophrenic brain (reviewed in Lewis et al., 2012). Both mRNA and protein expression of the 67-kilodalton isoform of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD67) are reduced in the dorsolateral prefrontal cortex (PFC) of individuals with schizophrenia (Curley et al., 2011; Hashimoto et al., 2003; Volk et al., 2000) suggesting a reduction in GABA synthesis and release in this brain area. Additional changes within the cortical GABA system support the notion that reduced GAD67 expression results in decreased cortical GABA synthesis: both the mRNA and protein expression of the GABA reuptake transporter (GAT1) are reduced in the dorsolateral PFC (Woo et al., 1998; Pierri et al., 1999; Volk et al., 2001) and post-synaptic GABA_A receptor α 2-subunit expression is increased in the dorsolateral PFC (Volk et al., 2002; Beneyto et al., 2011). These abnormalities may reflect compensatory changes aimed to mitigate the effects of reduced GABA release.

The aforementioned changes in GAD67 appear to be most prominent in a select-subset of GABA neurons, those containing the calcium binding protein parvalbumin (PV) (see Lewis et al., 2012 for review). PV-containing GABA neurons are fast-spiking and synapse on the soma and axon initial segment of excitatory pyramidal neurons (Markram et al., 2004). These neurons are hypothesized to underlie the generation of gamma oscillations (Gonzalez-Burgos and Lewis, 2008); a form of neural synchrony evoked during performance on cognitive tasks (Gruber et al., 1999; Steinmetz et al., 2000). People with schizophrenia do not adequately modulate gamma oscillations during tasks of attention and working memory and perform poorly on these tasks (Cho et al., 2006; Basar-Ergolu et al., 2007; Basar-Ergolu et al., 2009; Minzenberg et al., 2010). Combined these data may be interpreted to suggest that decreases in cortical GABA synthesis may contribute to the cognitive deficits observed in schizophrenia.

To date, the cognitive effects of selectively decreasing cortical GABA synthesis have not been characterized. There are, however, numerous findings within the preclinical literature that support the hypothesis that a reduction in cortical GABA synthesis contributes to cognitive deficits similar to those observed in schizophrenia. First, gestational exposure to the toxin methylazoxymethanol acetate (MAM), a well characterized rat model of schizophrenia, causes long-term reductions in cortical PV expression; this is associated with both decreased gamma band oscillations and impaired performance on cognitive tasks (Lodge et al., 2009; Featherstone et al., 2007). Second, chronic NMDA receptor antagonist administration reduces cortical expression of GAD67 and PV in rats (Behrens et al., 2007; Amitai et al., 2012); this is associated with impairments in attention (Amitai et al., 2007; Paine et al., 2009) and working memory (e.g., Daamgard et al., 2010). Finally, blockade of cortical GABA_A receptors impairs attention (Paine et al., 2011), behavioral flexibility (Enomoto et al., 2011) and working memory (Sawaguchi et al., 1989). Together these findings provide indirect support for the hypothesis that decreased GABA synthesis causes cognitive deficits similar to those seen in schizophrenia.

The goal of the current experiment was to directly test whether reducing GABA synthesis within the PFC causes attention deficits. GABA synthesis was inhibited by intra-cortical infusions of L-allylglycine (LAG), a drug that reduces GABA synthesis when administered systemically (Horton et al., 1978) and intra-cranially (Cunha et al., 2010). Visuospatial attention was measured using three different versions of the 5-choice serial reaction time task (5CSRTT), a rodent task **based on Leonard's 5-choice serial reaction time task used**

to study attention in humans (Robbins, 2002). Because performance on the 5CSRTT can be affected by changes in locomotor activity, this was also assessed.

2.0 Materials and Methods

2.1 Rats

Forty-six male Sprague-Dawley rats born at Oberlin College were used. Rats were maintained on a 14-h/10-h light-dark cycle (lights on at 0700h) and were group housed until post-natal day (PND) 55; during this time they had unlimited access to food (Purina Rat Chow) and water. On PND 55 rats used in the 5CSRTT experiments were housed in pairs (until the time of surgery at which point they were housed singly) and those used in the locomotor activity test were housed singly. Once in their new housing conditions rats were food restricted to ~85% of their free feeding weight; rats were fed after daily training sessions. Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy Press, 1996) and Oberlin College policies.

2.2 Drugs

L-Allylglycine (LAG) was purchased from Sigma-Aldrich (St. Louis MO) and dissolved in physiological saline (0.9%).

2.3 Surgery

Rats were anesthetized with sodium pentobarbital (65 mg/kg, IP) and implanted with bilateral guide cannulae (26-gauge; Plastics One, Roanoke, VA) aimed at the border of infralimbic (IL) and prelimbic cortex (PrL) cortices (relative to bregma: AP = + 2.8, ML = ± 0.75, DV = -1.8 mm from dura [Paxinos and Watson, 2009]). Skull screws and dental acrylic secured the guide cannulae in place. Obturators and injector needles (33-gauge) extended 1.5 mm below the guide cannulae.

2.4.1 The Standard 5-Choice Serial Reaction Time Task (5CSRTT)—Rats were trained on the 5CSRTT as described previously (Paine et al., 2011). Sessions started with the delivery of 1 food pellet (45-mg, Bio-Serv, Frenchtown NJ); the first trial commenced upon pellet retrieval. A nose poke into the magazine initiated the first 5-sec inter-trial interval (ITI) and illumination of the house light. At the end of the ITI, a 1.0-sec light stimulus was presented at the rear of one of the five stimulus locations (apertures). Rats had up to 5 sec (limited hold) to make a response. A response in the illuminated aperture (correct response) triggered delivery of 1 food pellet and illumination of the magazine light, which remained illuminated for 5 sec following pellet delivery. Nose pokes in the remaining apertures were considered incorrect responses and triggered a 5-sec time-out (TO) during which the house light was extinguished. Similarly, failure to respond during the limited hold (i.e., an omission) triggered a 5-sec TO. The subsequent trial was automatically initiated at the end of the TO period or the limited hold (correct responses). Responses occurring during the ITI were considered premature responses and also triggered a 5-sec TO; the same trial was automatically re-started at the end of the TO period. Responses occurring during the TO period had no programmed consequences. Sessions ended after 90 trials or 30 min. Performance measures of interest were: % accuracy ((correct responses/[correct + incorrect responses])*100), % omissions ([omissions/trials completed]*100), premature responses, magazine entries, correct response latency (the time from the stimulus onset to a correct response) and reward latency (the time from a correct response to the collection of the food). Subjects were considered to have acquired the task when their accuracy was greater than 60% (chance performance in this test is 20%) and omissions were fewer than 20% for 5 consecutive days. Upon reaching criterion performance, the rats underwent surgery to implant guide cannulae; following surgery rats were housed singly.

Rats (n=23) were allowed to recover for 5–7 days and then were re-stabilized for a minimum of 5 days prior to drug testing. To habituate rats to the infusion procedure they were first infused with vehicle (VEH, 0.9% physiological saline); data from this session was discarded. On test days, rats were infused with LAG (0, 1.25, 2.5, 5.0 or 10.0 $\mu\text{g}/0.5\text{ }\mu\text{l}/\text{side}$) 25 min prior to testing. The dose and time of testing was based on Cunha et al. (2010) who found maximal behavioral effects of LAG infusions between 15–45 min following infusions into the periaqueductal grey area. Half of the rats received the drug doses in an ascending order and half of the rats received the drug dose in a descending order. There was a minimum of two drug-free days between infusions.

2.4.2 Short Stimulus Duration 5CSRTT—After completion of the dose response curve on the standard version of the 5CSRTT a subset of rats (n=9) were tested on a version of the 5CSRTT in which the stimulus duration was shortened to 0.5-sec; all of the other aspects of the task were unchanged from the standard task. Each rat was tested on this version of the task twice: once following an LAG infusion (10.0 $\mu\text{g}/0.5\text{ }\mu\text{l}/\text{side}$) and once following a vehicle infusion (0.0 $\mu\text{g}/0.5\text{ }\mu\text{l}/\text{side}$). Rats were trained on the standard version of the task between drug test sessions. Dose order was counterbalanced across rats.

2.4.3 Long Stimulus Duration 5CSRTT—A separate group of rats (n=12) was trained upon a simplified version of the 5CSRTT. Training was similar to the standard 5CSRTT with the exception that the stimulus duration was never shorter than 5-sec (i.e., the duration of the limited hold). Once performance on this task stabilized, rats were implanted with guide cannula (see above), allowed to recover for 1 week and then restabilized on the task for at least 5 days. After behavior was stable rats were first habituated to the infusion procedure by giving them a vehicle infusion; data from this session was discarded. Each rat was then tested on two occasions: once following an LAG infusion (10.0 $\mu\text{g}/0.5\text{ }\mu\text{l}/\text{side}$) and once following a vehicle infusion (0.0 $\mu\text{g}/0.5\text{ }\mu\text{l}/\text{side}$). Dose order was counterbalanced across rats.

2.4.4 Continuous Reinforcement Task—Rats trained and tested using the long stimulus duration 5CSRTT were then retrained on the continuous reinforcement task. In this task all 5 apertures are illuminated for the duration of the limited hold (5-sec) and a response in *any* aperture resulted in the delivery of a sugar pellet reward. Once performance was stable, each rat was then tested on two occasions: once following an LAG infusion (10.0 $\mu\text{g}/0.5\text{ }\mu\text{l}/\text{side}$) and once following a vehicle infusion (0.0 $\mu\text{g}/0.5\text{ }\mu\text{l}/\text{side}$). Dose order was counterbalanced across rats.

2.5 Locomotor Activity in an Open Field

Locomotor activity was recorded in automated (43.2 $\times$ 43.2 cm) activity chambers (MED Associates, St. Albans, VT). On ~PND 60, rats (n=11) were implanted with guide cannulae (see above) and allowed at least one week to recover. Each locomotor activity session consisted of a 30 min habituation period and a 30 min test period. After the 30 min habituation period the rat was removed from the chamber and administered a drug infusion. The rat was returned to its home cage for 25 min and then put in the activity chamber for the 30 min test period. Activity was assessed twice for each rat, once when VEH (0.5 $\mu\text{l}/\text{side}$) was infused and once when LAG (10 $\mu\text{g}/0.5\text{ }\mu\text{l}/\text{side}$) was infused. The order of vehicle versus drug administration was counterbalanced across rats. Activity sessions were separated by at least 3–4 drug-free days.

2.6 Histology

Following the last test session rats were anesthetized with sodium pentobarbital (100 mg/kg, IP) and transcardially perfused with 0.9% NaCl followed by 4% paraformaldehyde.

Following perfusions, brains were removed, post-fixed for 24 h and then cryoprotected in 30% sucrose prior to slicing on a microtome. Sections (40 μm) were mounted on slides, stained with cresyl violet and used to assess cannulae placements.

2.7 Fos Immunohistochemistry

In order to confirm that LAG infusions decreased GABA synthesis, a subset of rats ($n=14$) tested in the standard 5CSRTT was perfused 120 min following infusions of LAG (10 $\mu\text{g}/0.5 \mu\text{L}/\text{side}$) or VEH. Briefly, endogenous peroxidase activity was quenched by incubation in 0.3% H_2O_2 and then non-specific binding was blocked by a 2 h incubation in AB media (0.3% Triton X-100, 2% normal goat serum (Invitrogen, Carlsbad, CA) and 1% bovine serum albumin (Sigma) in 0.01 M Tris buffered saline). Sections were then incubated overnight with a polyclonal rabbit anti-c-Fos antibody (PC38T, Calbiochem, La Jolla, CA; 1:10,000 diluted in AB media). The following day sections were washed, incubated for 1 h at room temperature in biotinylated goat anti-rabbit immunoglobulin G secondary antibody (Vector Laboratories, Burlingame, CA; 1:200 diluted in AB media) and then incubated with avidin-biotin-peroxidase complex (Vectastain ABC Elite kit; Vector Laboratories) for 30 min at room temperature. Finally, sections were visualized using 0.05% 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 (Vector Laboratories) for 10 min. Rinsing in Tris buffered saline terminated the reaction. The number of Fos immunopositive cells was counted using ImageJ software by an unbiased observer. Cells were counted at 40X magnification.

2.8 Statistical Analyses

5CSRTT data were analyzed using separate one-way repeated measures ANOVAs with Dose as the within-subjects factor or with paired samples t-tests. Total locomotor activity was analyzed using a 2-way repeated measures ANOVA with Condition (VEH, LAG) and Session (Habituation, Test) as within-subjects factors. Locomotor activity data across the habituation and test periods were analyzed separately using 2-way repeated measures ANOVAs with Condition (VEH, LAG) and 5-min Block (1–6) as within-subjects factors. Significant effects were further analyzed using an estimated marginal means procedure with a least significant differences correction. The number of Fos immunopositive cells was analyzed using independent samples t-tests.

3.0 Results

3.1 5-Choice Serial Reaction Time Task

3.1.1 Histology—Of the 23 rats trained on the standard 5CSRTT, performance of 4 failed to re-stabilized following surgery. These rats were not used in behavioral tests, but were included in the analysis of Fos expression. 2 rats were excluded from the statistical analysis ($n=1$ from the Fos expression experiment and $n=1$ from the short stimulus duration experiment) due to an inaccurate cannulae placement. Cannulae placements were accurate for a total of 17 rats tested in the standard 5CSRTT (see Figure 1A).

Of the 12 rats trained on the long stimulus duration and continuous reinforcement task tasks, 1 rat was excluded from statistical analysis due to an inaccurate cannulae placement. Cannulae placements were accurate for a total of 11 rats (see Figure 1B).

3.1.2 Fos Immunohistochemistry—LAG (10 $\mu\text{g}/0.5 \mu\text{L}/\text{side}$) significantly increased the number of Fos immunoreactive cells in both the prelimbic ($t(11) = 3.39$, $P < 0.01$) and infralimbic cortices ($t(11) = 2.99$, $P < 0.05$, see Figure 2). These data confirm that the highest dose of LAG increased cortical activation suggesting that LAG effectively decreased GABA synthesis.

3.1.3 Standard 5CSRTT—LAG infusions significantly affected omissions ($F(4,64) = 15.65$, $P < 0.01$; see Figure 3B). A low dose of LAG (1.25 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$) significantly decreased omissions ($P < 0.05$), while a high dose of LAG (10.0 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$) significantly increased omissions relative to vehicle ($P < 0.01$).

Premature responses were significantly increased by LAG infusions ($F(4, 64) = 3.98$, $P < 0.01$; see Table 1). LAG (2.5 and 5.0 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$) increased premature responses relative to vehicle ($P < 0.05$).

Magazine entries were significantly affected by LAG infusion ($F(4, 64) = 4.07$, $P < 0.01$; see Table 1), but no dose was significantly different from vehicle infusions (all $P > 0.05$).

The reward retrieval latency was significantly increased by LAG infusions ($F(4, 64) = 4.55$, $P < 0.01$; see Figure 3C); LAG (10 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$) increased the reward retrieval latency relative to vehicle ($P < 0.5$).

Neither accuracy of responding nor the correct response latency were affected by LAG infusions (both $F < 2.34$, both $P > 0.05$; see Figure 3A and Table 1).

3.1.4 Short Stimulus Duration 5CSRTT—When the stimulus duration was shortened to 0.5 sec LAG infusions significantly increased omissions ($t(7) = 3.38$, $P < 0.05$; see Figure 3E). All other performance measures were not affected by LAG infusions ($t < 1.58$, all $P > 0.05$, see Figure 3 and Table 2).

3.1.5 Long Stimulus Duration 5CSRTT—When the stimulus duration was lengthened to 5 sec LAG infusions significantly increased the number of omissions ($t(10) = 3.27$, $P < 0.01$; see Figure 3H), the reward retrieval latency ($t(10) = 2.41$, $P < 0.05$; see Figure 3I) and the correct response latency ($t(10) = 2.38$, $P < 0.05$; see Table 2). All other performance measures were not affected by LAG infusions ($t(10) < 1.58$, all $P > 0.05$, see Table 2).

3.1.6 Continuous Reinforcement Task—LAG infusions did not significantly affect performance on the continuous reinforcement task (all $t(10) < 2.10$, all $P > 0.05$, see Table 3). There was however a trend for LAG to increase omissions ($t(10) = 2.10$, $P = 0.06$).

3.2 Locomotor Activity

Three rats were excluded from the data analysis; 2 rats were excluded because of inaccurate cannulae placements and 1 rat was excluded because data were >3 standard deviations above the mean following an LAG infusion. A total of 8 rats were used for statistical analysis (see Figure 4).

Rats infused with LAG (10 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$) exhibited significantly more overall activity than rats infused with saline ($F(1,7) = 15.02$, $P < 0.05$, see Figure 5A). There was no effect of session (habituation or test) or a significant session $\times$ drug interaction (both $F < 3.7$, $P > 0.05$) on the total amount of activity exhibited.

Over the habituation session, prior to drug infusions, activity decreased ($F(5, 35) = 26.37$, $P < 0.05$, see Figure 5B), but there was no effect of drug or a block $\times$ drug interaction (both $F < 1.0$, $P > 0.05$). Activity was higher in the first trial block compared to all the other trial blocks (all $P < 0.05$).

During the test session, following infusions, there was a significant block $\times$ drug interaction ($F(5,35) = 2.80$, $P < 0.05$, see Figure 5B). Saline-infused rats exhibited more activity in block 1 than they did in all subsequent blocks (all $P < 0.05$). LAG-infused rats exhibited more

activity in block 1 than in block 2 ($P < 0.05$); activity in blocks 3–6 was not different than in block 1 (all $P > 0.05$). Moreover, LAG-infused rats exhibited more activity than saline-infused rats in blocks 3, 5 and 6 (all $P < 0.05$).

4.0 Discussion

The goal of the current experiment was to determine if blocking cortical GABA synthesis was sufficient to cause attentional deficits similar to those observed in schizophrenia. L-allylglycine is a potent and selective GAD inhibitor (Horton et al., 1978). Indeed, we observed a significant increase in Fos expression 120 min following LAG infusions into the PFC. Fos expression is increased following neuronal activation (Guzowski et al., 2005); this result indirectly suggests that GABA synthesis and release were decreased by LAG. At a dose that significantly increased Fos expression, intra-cortical infusions of LAG significantly increased locomotor activity in an open field, but did not significantly affect attention in the 5CSRTT.

4.1 Effects of LAG on behavior

The 5CSRTT is a complex operant conditioning task designed to measure attention and impulse control (Robbins, 2002). Measures of attention in the 5CSRTT include accuracy of responding and omissions (missed trials). In the current experiment, intra-cortical LAG infusions did not affect accuracy of responding in any version of the 5CSRTT, but did increase omissions in all versions of the 5CSRTT. Although it is possible that increased omissions could reflect impaired attention, several observations suggest that, at least in this case, they do not. First, when the standard 5CSRTT was altered so as to increase or decrease the attentional demands (i.e., altering the stimulus duration), the number of omissions following LAG infusions did not systematically vary with task difficulty. Importantly, the task difficulty manipulations did result in systematic changes in both the accuracy of responding and the number of omissions following saline (vehicle) infusions. Second, there was a trend for LAG to increase omissions in the continuous reinforcement task. In this task, all 5 stimulus lights are illuminated and the rats have up to 5 sec to respond in any aperture to receive a reward, thus the attentional demands of this task were minimal. It is therefore unlikely that the increase in omissions caused by LAG infusions in the continuous reinforcement task reflects an attention impairment.

Increased omissions in the 5CSRTT can also reflect a decrease in motivation or altered locomotor activity (Robbins, 2012; Paine et al., 2011). Additional measures of motivation in the 5CSRTT include the reward retrieval latency and the number of food-seeking behaviors (i.e., magazine entries) (Paine et al., 2011; Nemeth et al., 2010; Robbins, 2002). Pre-feeding rats, thereby decreasing motivation to respond for food reward, causes the reward retrieval latency to increase and causes the number of magazine entries to decrease (Nemeth et al., 2010 and unpublished observations). In the current experiment, LAG infusions significantly increased the reward retrieval latency on two versions of the 5CSRTT (and non-significantly increased it on the continuous reinforcement task). Furthermore, LAG infusions non-significantly decreased the number of magazine entries on all versions of the 5CSRTT (but not the continuous reinforcement task). Combined these data indicate that LAG infusions may have a tendency to decrease motivation to respond and that this decrease in motivation could contribute to the increase in omissions observed. Because the effects of LAG infusions on measures of motivation were modest at best, future research is needed to definitively determine if intra-cortical LAG infusions decrease motivation.

Alternatively, the increase in omissions observed following intra-cortical LAG infusions may have resulted from the observed increase in locomotor activity in the open field. Such an increase in locomotor activity interfered may have interfered with the rats ability to

respond in the 5CSRTT, or otherwise engage in the task. Moreover, it is reasonable to speculate that hyperactivity would equally impair performance on all versions of the 5CSRTT (irrespective of the attentional demands), as was observed in the current experiment. Thus, we speculate increased locomotor activity contributed, at least in part, to the increase in omissions and that omissions were not increased as result of an attention deficit per se.

Although we did not observe an attentional deficit following intra-cortical LAG infusions, we did observe an increase in premature responses following infusions of moderate doses of LAG. Premature responses measure impulse control or response inhibition (Paine et al., 2011; Robbins, 2002); another cognitive function that is impaired in schizophrenia (Hughes et al., 2012; D'Souza et al., 2012; Bellgrove et al., 2006). Moreover, acute administration of an NMDA receptor antagonist (another animal model of schizophrenia) also increases premature responses (i.e., acute NMDA receptor antagonist administration; Paine et al., 2009; Amitai et al., 2007; Murphy et al., 2005). Thus, although the data from the current experiment do not support the hypothesis that GABA dysfunction contributes to the attention deficits in schizophrenia, they do provide evidence that disrupted cortical GABA transmission could contribute to other cognitive deficits in schizophrenia.

Surprisingly, and counter to our hypothesis, we observed that a low dose of LAG (1.25 µg/0.5 µl/side) significantly decreased omissions. The decrease in omissions may reflect a cognitive enhancing effect of LAG (i.e., improved attention) or may reflect an increase in motivation to respond (e.g., Paine et al., 2011; Nemeth et al., 2010; Robbins, 2002). Because this low dose of LAG was only tested in the standard variants of the 5CSRTT, more research is required to determine if low doses of LAG do indeed have cognitive enhancing effects.

4.2 LAG-induced Hyperactivity – *reflecting a positive symptom of schizophrenia?*

Hyperactivity, particular after amphetamine administration or NMDA receptor antagonism, is frequently used as an indirect measure of the positive symptoms of schizophrenia (Jones et al., 2010). In the current experiment we observed a significant increase in locomotor activity following LAG infusions, which may, similarly, reflect the positive symptoms of schizophrenia. Consistent with this idea, GABA_A receptor blockade significantly increases locomotor activity, an effect that is potentiated by amphetamine administration (Enomoto et al., 2011).

The positive symptoms of schizophrenia are hypothesized to result from excessive dopamine (DA) release in the striatum (Howes and Kapur, 2009). It is possible that the hyperactivity induced by the blockade of cortical GABA synthesis increases DA release in the striatum. The PFC sends dense glutamatergic projections to the DA cell bodies in the ventral tegmental area (VTA) (Sesack and Pickle, 1992). Both electrical stimulation of the PFC and GABA_A receptor blockade in the PFC increase burst firing of putative VTA DA neurons (Enomoto et al., 2011; Murase et al., 1993). Similarly, both electrical and chemical stimulation of the PFC increase synaptic DA in the nucleus accumbens, one target area of VTA DA neurons (Taber et al., 1995; Murase et al., 1993). Thus, decreasing inhibition of the PFC may similarly increase striatal DA release. Future research will address whether the hyperactivity caused by reduced GABA synthesis can be enhanced by dopamine agonists such as amphetamine and attenuated by dopamine antagonists such as haloperidol.

4.3 Role of cortical GABA in attention

Surprisingly, and contrary to our hypothesis, inhibition of GABA synthesis with LAG did not affect visuospatial attention as measured by the 5CSRTT. This was especially surprising because we have previously found that blocking GABA_A receptors within the same brain

area impairs attention in the 5CSRTT (Paine et al., 2011). In addition cortical GABA_A receptor blockade impairs attentional set-shifting (Enomoto et al., 2011) and causes working memory deficits (Sawaguchi et al., 1989). Combined these observations suggest that decreasing GABA transmission within the PFC impairs cognitive function; which was not observed in the current experiment. However, the blockade of GABA synthesis with LAG likely results in decreased synaptic transmission at both GABA_A and GABA_B receptors. GABA_B receptors are widely distributed in the brain, including in the PFC, and are located on both pre- and post-synaptic membrane (Bowery, 2006). Interestingly, administration of a GABA_B receptor antagonist improves cognitive performance in both humans and mice (see Froestl et al., 2004 for review). It is possible that the decreasing GABA synthesis reduces activation of GABA_B receptors—an effect that may enhance attention. Thus, reduced activation of GABA_B receptors may be protective against the attention deficit caused by decreased GABA_A receptor activation.

Alternatively, it is possible that the 5CSRTT is not sufficiently sensitive to detect an attentional deficit resulting from impaired cortical GABA synthesis. Although we increased the difficulty of the task by decreasing the stimulus duration, the basic format of the task was the same: rats were required to monitor a visual array only for the presentation of a brief visual target (Robbins, 2002; Paine et al., 2011). Other tasks of attention have different response requirements, which may make them more cognitively demanding. For example, the 5 choice continuous performance task requires rats to withhold responses to a particular target (Barnes et al., 2012; reviewed in Lustig et al., 2012) and the sustained attention task (SAT) requires rats to monitor a panel for either the presence or absence of a signal (Martinez et al., 2008; reviewed in Lustig et al., 2012). Future research is needed to address whether disrupting cortical GABA function would affect performance on these tasks of attention.

4.4 Role of GAD in the cognitive deficits in schizophrenia

The lack of attention deficits following inhibition of PFC GABA synthesis calls into question the hypothesis that decreases in GABA synthesis contribute to the cognitive deficits in schizophrenia. This hypothesis is based upon the observation that expression of the GABA synthesizing enzyme, GAD67, is reduced in PV-containing GABA neurons (Curley et al., 2011; Hashimoto et al., 2003; Volk et al., 2000) suggesting a reduction in cortical GABA synthesis in people with schizophrenia. This interpretation is supported by the observations that GABA reuptake transporters are reduced (Woo et al., 1998; Pierri et al., 1999; Volk et al., 2001) and the expression of the GABA_A receptor $\alpha 2$ -subunit is increased (Volk et al., 2002; Beneyto et al., 2011) in the same brain areas that GAD67 is decreased. PV-containing GABA neurons are purported to contribute to the generation of gamma oscillations, a form of neural synchrony evoked during cognitive tasks (Gonzalez-Burgos and Lewis, 2008). Notably, people with schizophrenia do not adequately evoke gamma oscillations during tasks of attention or working memory and perform worse on these tasks than controls (Cho et al., 2006; Basar-Eroglu et al., 2007; Basar-Eroglu et al., 2009; Minzenberg et al., 2010, for review see Gandal et al., 2012).

Although the results of the current experiment undermine the hypothesis that decreased GABA synthesis contributes to attention deficits in schizophrenia, there are several factors that limit the extent to which these findings can be applied to the clinical condition. First, in the current experiment inhibition of GABA synthesis was not restricted to PV-containing neurons in the PFC. Rather, GABA synthesis was likely inhibited in all varieties of cortical GABA neurons. Such widespread reductions in GABA synthesis may lead to non-specific changes in behavior (i.e., locomotor activity) that could differ from the changes in behavior that might occur if GABA synthesis was reduced in a select subset of neurons (i.e., PV-containing GABA neurons). Thus, it remains possible that reducing GABA synthesis

selectively in PV-containing interneurons could cause attentional deficits. Second, LAG inhibits both the 65 kDa form of GAD (GAD65) and GAD67—leading to significant reductions in cortical GABA concentrations (Horton et al., 1978). Although GAD67 is responsible for synthesizing the majority of GABA in the brain (Asada et al., 1997), GAD65 is hypothesized to be responsible for the synthesis of GABA for neurotransmission (Buddhala et al., 2009; Soghomonian et al., 1998). It is unclear whether a selective decrease in cortical GAD67 (or GAD65) would result in an attentional deficit. There is some evidence to suggest that GAD65 and GAD67 may play different roles in cognitive function. For example, decreasing GAD65 function in the nucleus accumbens impairs attention, but decreasing GAD67 function does not affect attention (Miner and Sarter, 1999). Third, reductions in cortical GABA levels have not been observed during in vivo measurements of the brains of people with schizophrenia (Goto et al., 2010; Kegeles et al., 2012; Tayoshi et al., 2010); implying that subtle changes in the functionality of GABA neurons, rather than systemic changes in GABA release, may contribute to the cognitive deficits in schizophrenia. Finally, GABA synthesis was inhibited acutely in the current experiment, rather than chronically as would occur in schizophrenia. It is also possible that neural adaptations to chronically reduced GABA synthesis (e.g., changes in GAT1 or GABA_A receptor expression or changes in synaptic connections) could be contributing to the cognitive deficits in schizophrenia.

4.5 Summary and Conclusions

The goal of the current experiment was to determine if acutely inhibiting cortical GABA synthesis was sufficient to cause attentional deficits similar to those observed in schizophrenia. Although we did not observe attentional deficits following GABA synthesis inhibition, we used a technique that non-specifically decreased GABA synthesis. In schizophrenia, only a subset of cortical GABA neurons appears to be affected and a more targeted approach to decreasing GABA synthesis may lead to a different end result. Inhibition of GABA synthesis did cause a significant increase in locomotor activity. In other pharmacological models increased locomotor activity is often used as an indicator of the positive symptoms of schizophrenia. Future research will be aimed at determining whether hyperactivity caused by decreased PFC GABA is mediated by increases in subcortical DA.

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Highlights

- Decreasing cortical GABA synthesis does not affect attention and may reduce motivation
- Decreasing cortical GABA synthesis increases impulsive behavior and locomotor activity
- Reduced cortical GABA synthesis may underlie some schizophrenia-like cognitive deficits

Fig 1.

Schematic drawing showing the location of injector tips of rats tested in the standard 5CSRTT (A, n=22) and long stimulus duration 5CSRTT (B, n=11). Rats were excluded (not shown) if their tips were not within the boundary of either the prelimbic (PrL) or infralimbic (IL) cortex. Numbers on the left indicate location forward from bregma. Adapted from Paxinos and Watson (2009).

Fig 2.

Effect of L-allylglycine (LAG) infusions on Fos immunoreactivity in the medial prefrontal cortex. A) LAG (10 μ g/0.5 μ L/side; LAG) increased the number of Fos immunopositive cells in both the prelimbic (PrL) and infralimbic (IL) cortices compared to vehicle (VEH). B) Schematic of the PFC approximating the field of view in Panels C–D (Paxinos and Watson, 2009). C) Fos expression following a VEH infusion. D) Fos expression following an LAG infusion. All infusions were administered 120 min before the rats were killed.

Fig 3.

Effects of L-allylglycine (LAG) on performance on variants of the 5-choice serial reaction time task (5CSRTT). A–C) Performance on the standard 5CSRTT (1.0-sec stimulus duration). D–F) Performance on the short stimulus duration 5CSRTT (0.5-sec stimulus duration); a variant of the task in which the attentional demands are increased. G–I) Performance on the long stimulus duration 5CSRTT (5.0-sec stimulus duration); a variant of the task in which the attentional demands are decreased. LAG was infused 25 min prior to testing. * $P < 0.05$, ** $P < 0.01$ compared to vehicle (0.0 µg/0.5 µL/side).

Fig 4.

Schematic drawing showing the location of injector tips of rats tested in the locomotor activity task (n=8). Rats were excluded (not shown) if their tips were not within the boundary of either the prelimbic (PrL) or infralimbic (IL) cortex. Numbers on the left indicate location forward from bregma. Adapted from Paxinos and Watson (2009).

Fig 5.

Effects of L-allylglycine (LAG) infusions on locomotor activity. Rats were habituated to the locomotor activity chamber for 30-min, infused with LAG (10.0 μ g/0.5 μ L/side) or vehicle (0.5 μ L/side) and returned to their home cage for 25 min; they were then returned to the locomotor activity chamber for a 30 min test session. A) Total activity was increased on the test day rats were infused with LAG compared to the day they were infused with vehicle. B) Locomotor activity decreased across the habituation session. Following saline infusions a similar pattern emerged; activity was highest on the first block to relative to the other trial blocks. Following LAG infusions, activity decreased from block 1 to block 2 and then increased. Moreover, LAG-infused rats exhibited more activity relative to VEH-infused rats on blocks 3, 5 and 6. * $P < 0.05$ compared to VEH, ^ $P < 0.05$ compared to Block 1.

Table 1

Effects of LAG infusions on performance on the standard 5CSRTT

Measure	0.0	1.25	2.5	5.0	10.0
Premature Responses	24.59 ± 3.03	28.53 ± 4.73	35.12 ± 5.21 *	34.71 ± 4.61 *	19.76 ± 3.03
Magazine Entries	221.1 ± 29.84	250.6 ± 32.2	250.5 ± 30.9	225.8 ± 29.3	182.5 ± 22.7
Correct Latency (sec)	0.76 ± 0.04	0.77 ± 0.03	0.77 ± 0.03	0.83 ± 0.05	0.85 ± 0.04

Note: Drug was infused in a volume of 0.5 μ L/site at the dose indicated; infusions occurred 25 min prior to testing on the standard version of the 5CSRTT.

* $P < 0.05$ compared 0.0.

Table 2

Effects of LAG infusions on performance on variants of the standard 5CSRTT

Measure	Short Stimulus Duration		Long Stimulus Duration	
	0.0	10.0	0	10.0
Premature Responses	58.44 ± 2.98	58.64 ± 2.94	90.21 ± 1.78	90.00 ± 3.01
Magazine Entries	164.3 ± 22.4	152.9 ± 32.0	167.2 ± 22.5	142.9 ± 19.9
Correct Latency (sec)	0.80 ± 0.07	0.86 ± 0.08	1.39 ± 0.13	1.79 ± 0.10 *

Note: Drug was infused in a volume of 0.5 µL/side at the dose indicated; infusions occurred 25 min prior to testing. In the short stimulus duration task the aperture light was illuminated for 0.5 sec. In the long stimulus duration task the aperture light was illuminated for 5 sec.

* $P < 0.05$ compared 0.0.

Table 3

Effects of LAG infusions on performance on the continuous reinforcement 5CSRTT

Measure	0.0	10.0
Total Responses	81.64 ± 2.58	70.18 ± 5.56
Omissions	4.02 ± 1.39	17.90 ± 6.38
Premature Responses	49.82 ± 8.43	47.36 ± 8.27
Magazine Entries	176.6 ± 22.3	196.8 ± 26.1
Correct Latency (sec)	0.93 ± 0.08	1.13 ± 0.12
Reward Latency (sec)	2.66 ± 0.36	3.12 ± 0.65

Note: Drug was infused in a volume of 0.5 μ L/side at the dose indicated; infusions occurred 25 min prior to testing. In the continuous reinforcement task all aperture lights were illuminated for 5 sec and a response in any aperture resulted in the delivery of a sugar pellet reward. No significant differences were observed (all $P > 0.05$).