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ORIGINAL INVESTIGATION

Acute elevations of brain kynurenic acid impair cognitive flexibility: normalization by the alpha7 positive modulator galantamine

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Abstract
Rationale Cognitive deficits represent a core symptom cluster in schizophrenia (SZ) that is predictive of outcome but not effectively treated by current antipsychotics. Thus, there is a need for validated animal models for testing potential pro-cognitive drugs.
Objective As kynurenic acid levels are increased in prefrontal cortex (PFC) of individuals with SZ, we acutely increased brain levels of this astrocyte-derived, negative modulator of alpha7 nicotinic acetylcholine receptors (α7nAChRs) by administration of its bioprecursor kynurenine and measured the effects on extracellular kynurenic acid and glutamate levels in PFC and also performance in a set-shifting task.
Results Injections of kynurenine (100 mg/kg, i.p.) increased extracellular kynurenic acid (1,500%) and decreased glutamate levels (30%) in PFC. Kynurenine also produced selective deficits in set-shifting. Saline- and kynurenine-treated rats similarly acquired the compound discrimination and intra-dimensional shift (saline, 7.0 and 6.3 trials, respectively; kynurenine, 8.0 and 6.7). Both groups required more trials to acquire the initial reversal (saline, 15.3; kynurenine, 22.2). Only kynurenine-treated rats were impaired in acquiring the extra-dimensional shift (saline, 8.2; kynurenine, 21.3). These deficits were normalized by administering the α7nAChR positive allosteric modulator galantamine (3.0 mg/kg, i.p) prior to kynurenine, as trials were comparable between galantamine + kynurenine (7.8) and controls (8.2). Bilateral local perfusion of the PFC with galantamine (5.0 μM) also attenuated kynurenine-induced deficits.
Conclusions These results validate the use of animals with elevated brain kynurenic acid levels in SZ research and support studies of drugs that normalize brain kynurenic acid levels and/or positively modulate α7nAChRs as pro-cognitive treatments for SZ.

Keywords Cognition · Schizophrenia · Animal model · Kynurenine · Glutamate

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Introduction

Deficits in executive function represent a core symptom cluster in schizophrenia (SZ) (Keefe 2007; Kerns et al. 2008; Nuechterlein et al. 2004; 2009). Such impairments can present prior to the onset of psychotic episodes and persist throughout the course of the disease (Gold 2004; Heinrichs 2005). Moreover, the severity of the cognitive deficits represents the best predictor of long-term functional outcome and social rehabilitation (Chen et al. 2005; Prouteau et al. 2005). While recent clinical trials suggest modest pro-cognitive effects of atypical antipsychotics (Potkin et al. 2001; Rollnik et al. 2002) and nicotinic-based pharmacotherapies (Buchanan et al. 2008; Olincy et al. 2006), there are currently no drug treatments that effectively alleviate the cognitive impairments present in SZ. Thus, the development of validated animal models that can serve as an empirical platform to test novel, cognition-enhancing adjunctive medications has become a major research focus.

Several of the most-limiting cognitive deficits seen in SZ (i.e., attention, working memory, and cognitive flexibility) are functions known to depend, in part, upon the integrity of the prefrontal cortex (PFC; Floresco et al. 2009; Goto et al. 2010; Kehagia et al. 2010; Volk and Lewis 2010). More specifically, intact cholinergic (Parikh and Sarter 2008), glutamatergic (Egerton et al. 2005; Stefani et al. 2003), and dopaminergic (Gruber et al. 2010; Robbins and Arnsten 2009) transmission within the PFC is necessary for the expression of one or more of these cognitive constructs. While the pathophysiology of SZ is complex, heterogeneous, and extends beyond the PFC, post-mortem and neuroimaging studies have revealed marked dysregulations in prefrontal cholinergic (Mathew et al. 2007; Scarr et al. 2009), glutamatergic (Bauer et al. 2008; Volk and Lewis 2010; Woo et al. 2008), GABAergic (Gonzalez-Burgos et al. 2010; Volk and Lewis 2010; Volk et al. 2010), and dopaminergic (Guillin et al. 2007; Winterer and Weinberger 2004) transmission. Thus, abnormalities in the information processing functions mediated by these transmitter systems may contribute significantly to the cognitive deficits seen in SZ.

Another well-established pathophysiology in SZ, which may cause an exacerbation of each of the neurotransmitter-specific dysregulations described above, is the disruption of the kynurenine pathway of tryptophan degradation (Erhardt et al. 2001; Miller et al. 2006; Schwarcz et al. 2001; Wonodi et al. 2011). L-Kynurenine ("kynurenine"), a pivotal metabolite of this pathway, enters the brain from the circulation and is then rapidly accumulated by astrocytes (Gal and Sherman 1980; Speciale et al. 1989). Kynurenine is then transaminated within astrocytes, mainly by kynurenine aminotransferase II (KAT II), to form kynurenic acid (Guidetti et al. 2007). Subsequently, upon release into the extracellular space, physiological levels of kynurenic acid antagonize alpha7 nicotinic acetylcholine receptors (α7nAChRs) via a negative modulation of an allosteric binding site (Hilmas et al. 2001; Lopes et al. 2007). By inhibiting cortical α7nAChRs, which are frequently located on presynaptic terminals (Livingstone et al. 2010), kynurenic acid tonically modulates cortical levels of ACh, glutamate, and dopamine. Moreover, elevated levels of kynurenic acid, produced by systemic or local administration of kynurenine, further reduce basal or stimulated levels of these transmitters (Konradsson-Geuken et al. 2009, 2010; Wu et al. 2006; Zmarowski et al. 2009).

As kynurenic acid levels are elevated in the cortex (Schwarcz et al. 2001) and cerebrospinal fluid (Erhardt et al. 2001) of patients with SZ, animal studies in which brain kynurenic acid levels are raised experimentally have significant heuristic value. Indeed, acute systemic administration of kynurenine, which increases brain kynurenic acid levels (Swartz et al. 1990), results in marked cognitive deficits in adult rodents that resemble the cognitive constructs disrupted in SZ. For example, this treatment in rats produces impairments in auditory sensory gating (Erhardt et al. 2004; Shepard et al. 2003) and spatial working memory (Chess et al. 2007).

The present study was designed to determine the effects of peripheral kynurenine application on performance in an attentional set-shifting task (Birrell and Brown 2000; Tait and Brown 2008). This paradigm recreates cognitive operations required to perform the Wisconsin Card Sort Task (Pantelis et al. 1999) and the intra- and extra-dimensional (ID/ED) shift of the Cambridge Neuropsychological Test Automated Battery (Barnett et al. 2010)—two well-established tasks that reveal impaired cognitive flexibility in individuals with SZ. Successful performance in these tasks requires normal prefrontal function (Birrell and Brown 2000; Lie et al. 2006; Robbins 2007). Our results, which include microdialysis data monitoring the extracellular levels of kynurenic acid and glutamate in the PFC, revealed that kynurenine administration adversely affects attentional set-shifting. Using galantamine application within the PFC as an experimental tool, we also provide evidence that this task-impairing effect is critically dependent on prefrontal α7nAChRs.

Materials and methods

Animals

Male Wistar rats (250–350 g; Charles River, Wilmington, MA, USA) were used in all experiments. The animals were maintained on a 12:12-h light/dark cycle (lights on 0600 hours) in temperature- and humidity-controlled, AAALAC-approved animal facilities. Rats were individually housed in plastic cages lined with corncob bedding (Harlan Teklad, Madison, WI, USA) with ad libitum access to water. Animals used in attentional set-shifting experiments were food deprived to 85% of baseline weight prior to training and testing; all other animals had access to food ad libitum. All procedures were approved by the Institutional Animal Care and Use Committees of The Ohio State University and the University of Maryland School of Medicine, in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

L-Kynurenine sulfate ("kynurenine") and galantamine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were purchased from a variety of commercial suppliers and were of the highest purity available.

Microdialysis (experiment 1)

Rats were anesthetized using chloral hydrate (360 mg/kg, i.p.) and placed in a stereotaxic frame. A guide cannula (0.65 mm,

o.d.; Carnegie Medicin, Stockholm, Sweden) was positioned unilaterally in the PFC (AP, 3.2 anterior to bregma; L, ±0.6 from the midline; DV, 2.0 mm below the dura) and anchored to the skull with screws and dental cement. On the following day, a CMA 10 microdialysis probe (membrane length, 2 mm; Carnegie Medicine) was inserted through the guide cannula and perfused with Ringer solution (in mM—NaCl, 144; KCl, 4.8; $MgSO_4$, 1.2; $CaCl_2$, 1.7; and pH 6.7) at a rate of 1 μl/min.

Samples were collected every 30 min. After the establishment of stable baselines (four consecutive samples that did not differ by more than 10%) for kynurenic acid and glutamate, animals received an i.p. injection of saline (0.9%, control), kynurenine (100 mg/kg), or kynurenine (100 mg/kg) + galantamine (3 mg/kg). Thirty-minute fractions were then collected for an additional 6 h.

Quantification of kynurenic acid and glutamate Kynurenic acid and glutamate were measured in the same microdialysis samples by high-performance liquid chromatography with fluorescence detection.

For kynurenic acid determination, 15 μl of the dialysate were directly applied to a 3-μm C_{18} reverse phase column (80×4.6 mm, ESA; Bedford, MA, USA). Kynurenic acid was isocratically eluted at a flow rate of 1 mL/min, using a mobile phase containing 200 mM zinc acetate and 5% acetonitrile, pH 6.2. In the eluate, kynurenic acid was detected fluorimetrically (excitation wavelength of 344 nm and emission wavelength of 398 nm; Perkin Elmer Series 200, Beaconsfield, UK). The retention time was approximately 5.0 min (Swartz et al. 1990).

The other 15 μl of the sample were used for the determination of glutamate. Briefly, 30 μl of *o*-phthalaldehyde/β-mercaptoethanol were added, and the mixture was applied to a reverse phase column (C_{18}, 5 μm; 250× 4.6 mm; Thermo Electron Corporation, Waltham, MA, USA) perfused at a flow rate of 1 ml/min with a gradient consisting of 1% 2 M sodium acetate/7.5% acetonitrile (A) and 30% acetonitrile, 30% methanol (B) (3 min—A, 88% and B, 12%; 2 min—A, 80% and B, 20%; 5 min—A, 0% and B, 100%; 2 min—A, 80% and B, 20%; and 3 min—A, 88% and B, 12%). In the eluate, glutamate was detected fluorimetrically (excitation wavelength of 390 nm and emission wavelength of 460 nm; Perkin Elmer Series 200). The retention time was approximately 4.5 min (Shank et al. 1993).

Set shifting behavior (experiments 2 and 3)

Experiment 2: systemic administration of kynurenine and galantamine Prior to testing in an attentional set-shifting task, animals were assigned to one of four groups. The first and second groups received a systemic injection of saline (1 ml/kg, i.p.) or kynurenine (100 mg/kg, i.p.), respectively, 45 min prior to task onset. The third group received an injection of galantamine (3 mg/kg, i.p.) 50 mins prior to task onset, followed 5 minutes later by an injection of saline. The fourth group received an injection of galantamine (3 mg/kg, i.p.) 50 min prior to task onset, followed 5 min later by an injection of kynurenine (100 mg/kg, i.p.).

Experiment 3: effects of intra-PFC galantamine on systemic kynurenine administration Microdialysis probes were inserted into bilateral cannulae in the PFC (same coordinates as in experiment 1; probes were placed at opposing 20° angles to accommodate the dual cannulae head stages), and either artificial cerebrospinal fluid (aCSF; containing NaCl (126.5 mM), $NaHCO_3$ (27.5 mM), KCl (2.4 mM), Na_2SO_4 (0.5 mM), KH_2PO_4 (0.5 mM), $CaCl_2$ (1.1 mM), $MgCl_2$ (0.8 mM), and dextrose (1 mM)) or galantamine (5 μM) was perfused bilaterally for 1 h prior to the onset of testing in the attentional set-shifting task and continued throughout the session. Fifteen minutes after the onset of the local perfusion (45 min prior to testing), animals received an injection of either saline or kynurenine (100 mg/kg, i.p.). While experiment 1 utilized Ringer's solution for dialysis perfusion and experiment 3 utilized aCSF, our previous study (Zmarowski et al. 2009) demonstrated that the effects of kynurenine on kynurenic acid and glutamate levels is unaffected by the choice of Ringer's or aCSF as the dialysis perfusion fluid.

Apparatus Rats were trained to dig in terra cotta pots (4-in. diameter) to retrieve a sugary cereal reward. The outer surface of the pots was covered with cloth, the inside filled with a digging medium, and each pot was scented with a distinctive odor. The testing chamber consisted of a wooden box covered in self-adhesive contact paper, in which one third was divided lengthwise to create two-choice chambers.

Training All training and testing procedures were modeled after those used by Birrell and Brown (2000). Animals were food deprived to ~85% of baseline weight to ensure sufficient motivation to perform. For approximately 1 week prior to testing, rats were habituated to the testing chamber for 20 min per day, with no discriminatory stimuli or rewards available. Starting 4 days prior to testing (i.e., day 0), the animals were placed in the testing environment for 20 min with a pot filled with pine shavings and baited with several pieces of cereal reward. The next day (day 1), the rats were trained to dig in the pot to retrieve the reward until they successfully retrieved ten consecutive rewards. During the intertrial interval, the rat was separated from the choice chambers by a moveable barrier. The onset of each trial was signaled by the removal of the barrier to allow

access to the pots. On day 2, rats were trained to discriminate between two pots that differed across one of the possible stimulus dimensions (odor, texture, and digging medium), until they successfully chose the "correct" exemplar for ten consecutive trials under each stimulus dimension.

Testing On day 3, rats were tested in seven separate discriminations. In each case, they were required to select the "correct" pot by choosing between two exemplars of the relevant dimension (i.e., jasmine vs. gardenia odors). The list of potential discriminations, presented as sets of discriminanda used for different stages of the task, is presented in Table 1. For the first four trials of every stage, animals were allowed to explore the alternate pot following an incorrect choice. These were termed, as is customary with the digging task, "exploration trials." After these first four exploration trials, the trial was terminated after the animal chose a pot by digging, regardless of whether the choice was correct or not. For each stage of the task, testing continued until the rat reached a criterion of six consecutive correct choices (exploration trials were included in the total trials to criterion). In the simple discrimination (SD), the pots differed in only one of the three stimulus dimensions. In the compound discrimination (CD), the pots differed across two dimensions, one relevant and one irrelevant, but the correct exemplars were the same as those used in the SD. For all reversal stages (REV), the rats were required to learn that the previously incorrect exemplar was now correct and that the previously reinforced exemplar was now incorrect. For the intra-dimensional shift (ID) stage, all new exemplars were introduced, but the same stimulus dimension was reinforced. For the extra-dimensional shift (ED) stage, all new exemplars were introduced again, but this time the previously irrelevant dimension was now reinforced. The order of stages was the same for all animals, but the stimulus dimensions used for each rat were counterbalanced such that, within drug conditions, each dimension was represented at each stage of the task. In general, rats completed all stages of the task within 90–150 min (depending upon error rate).

Statistical analysis

Biochemical experiments (experiment 1) Absolute values (fmol/15 μl) of basal extracellular kynurenic acid and glutamate (collections 1–4) were compared using one-way repeated measures analysis of variance (ANOVA) for differences as a function of drug condition. In the absence of significant differences in basal levels, subsequent drug effects were expressed as a percentage of the average baseline for the drug treatment group and compared using a series of two-way ANOVAs (drug condition and time as factors). Post hoc comparisons, using dependent *t* tests, were conducted comparing each post-baseline (collections 5–15) value to the last baseline (collection 4).

Behavioral experiments (experiments 2 and 3) An overall two-way ANOVA (drug condition as a between subject factor; stage of task as a within subject factor) was conducted on the number of trials necessary to reach criterion. Following a significant interaction, one-way ANOVAs were performed to determine between-drug group effects on each stage of the task. In all ANOVAs, the Huynh–Feldt correction was utilized to reduce type I errors associated with repeated measures ANOVAs (Vasey and Thayer 1987). In addition, paired samples *t* tests were performed to compare CD vs. REV1 and ID vs. ED within each drug group.

Table 1 Perceptual dimensions and specific stimuli used in the attentional set-shifting task

Dimension	Training set	Set 1	Set 2	Set 3
Odor	Lavender	Cinnamon	Gardenia	Rose
	Raspberry	Patchouli	Jasmine	Lilac
Medium	White paper	Light foam	Plastic beads	Plastic buttons
	Green paper	Dark foam	Metallic beads	Gold buttons
				Corncob bedding
				Gravel
Texture	Paper	Velour fabric	Felt fabric	Yarn
	Parafilm	Cotton fabric	Cotton fabric	Cotton fabric

Each testing session used three sets of digging pots that were used for clusters of stages in the task. Specifically, one set was used for the single discrimination (SD), compound discrimination (CD), and the first reversal (REV1). Another set was used for the intra-dimensional shift (ID) and second reversal (REV2). A third set was used for the extra-dimensional shift (ED) and the third reversal (REV3). The assignment of sets of stimuli to stage clusters was randomized among rats

Results

Experiment 1: kynurenine-induced changes in cortical kynurenic acid and glutamate

The effects of systemic administration of saline, kynurenine, or kynurenine + galantamine on prefrontal levels of kynurenic acid and glutamate are illustrated in Fig. 1. Basal extracellular concentrations of kynurenic acid and glutamate, not corrected for recovery from the microdialysis probe, were 37.4±0.5 fmol/15 µl (=2.5 nM) and 31.5±0.6 pmol/15 µl (=2.1 µM), respectively. Comparisons of *basal* levels of kynurenic acid or glutamate among the three drug groups revealed no significant differences (all P values, >0.05); thus, subsequent analyses were conducted on levels expressed as a percent change from these baseline values. As expected, an overall ANOVA revealed that systemic administration of the drugs produced marked, time-dependent changes ($F_{28,\ 154}$=28.39; P<0.001) in prefrontal kynurenic acid (Fig. 1 (top)). Relative to the stable levels seen following saline, kynurenine led to significant elevations in kynurenic acid levels in PFC ($F_{14,\ 98}$=42.29; P<0.001) that became evident 30 min after administration (i.e., collection 5), began to decline after 2.50 h but remained elevated throughout the dialysis session. The combination of kynurenine (100 mg/kg, i.p) and galantamine (3 mg/kg, i.p.) also increased kynurenic acid levels relative to those seen following saline control injections (drug, $F_{14,\ 98}$=57.81; P< 0.001). Finally, the addition of galantamine had no effect on kynurenine's ability to elevate cortical kynurenic acid levels ($F_{14,\ 56}$=74.51; P<0.001; Fig. 1 (top)).

With regard to glutamate, an overall ANOVA revealed that systemic administration of the test compounds produced marked, time-dependent changes ($F_{28,\ 154}$=3.20; P< 0.001) in prefrontal glutamate levels (Fig. 1 (bottom)). Kynurenine decreased glutamate levels, relative to those seen in saline controls ($F_{14,\ 98}$=4.00; P<0.001). Post hoc comparisons indicated that levels remained significantly reduced in collections 5–9 and then returned to basal values. In contrast to the lack of an effect on kynurenic acid levels (Fig. 1 (top)), the combination of kynurenine (100 mg/kg, i.p)+galantamine (3 mg/kg, i.p.) blocked kynurenine-induced reduction in glutamate levels ($F_{14,\ 56}$= 1.09; P=0.89). In fact, there was no significant difference in glutamate levels between saline and the kynurenine + galantamine condition (drug, $F_{1,\ 7}$=1.90; P=0.21; Fig. 1 (bottom)).

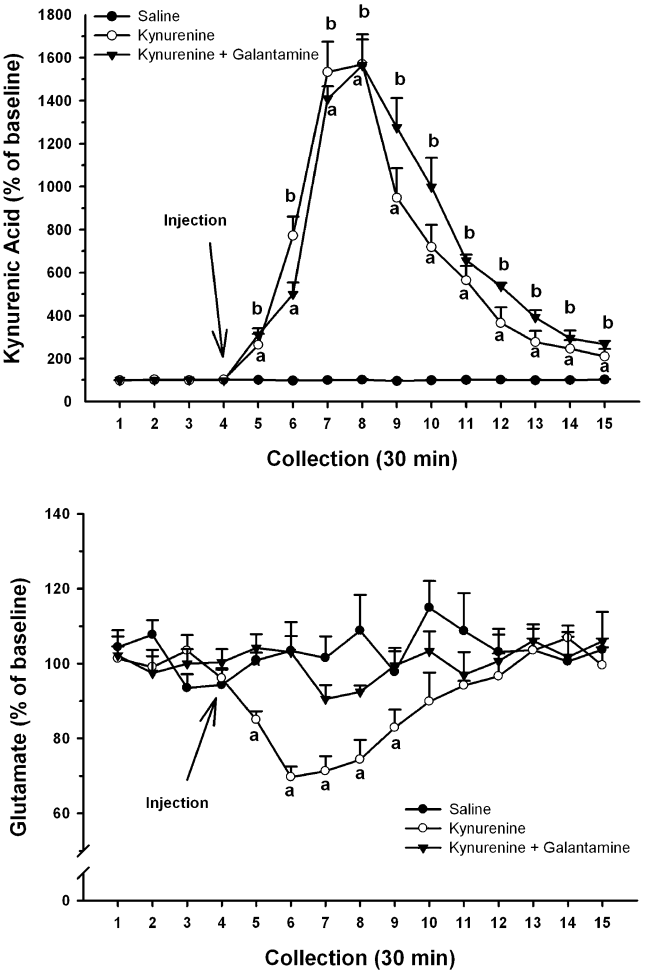

Fig. 1 *Top*, effects of systemic saline, kynurenine (100 mg/kg, i.p.), or a combined administration of kynurenine (100 mg/kg, i.p.)+ galantamine (3 mg/kg, i.p.) on extracellular levels of kynurenic acid in the PFC (n=5 rats). Saline injections did not affect kynurenic acid levels. Administration of kynurenine or kynurenine + galantamine led to similar, marked increases in kynurenic acid levels in the first post-injection collection, reaching a maximum 2 h later, and approaching baseline levels by the end of the microdialysis session. (*Bottom Panel*) Effects of systemic saline, kynurenine (100 mg/kg, i.p.), or a combined administration of kynurenine (100 mg/kg, i.p.)+galantamine (3 mg/kg, i.p.) on extracellular levels of glutamate in the PFC (n=5 rats). Glutamate levels were determined in the same microdialysate samples as kynurenic acid above. Saline injections did not affect glutamate levels. Glutamate levels following kynurenine administration resulted in a mirror image profile to that seen for kynurenic acid (*top*), decreasing in the first collection interval, reaching a nadir 90 min later, and returning to baseline levels 2.50 h after injection. However, pretreatment with galantamine eliminated the kynurenine-induced reduction in prefrontal glutamate levels. *a*, *b* Significantly different from levels during the last baseline (collection 4), just prior to drug injection (P values of <0.05). See text for absolute baseline concentrations

Experiment 2: kynurenine-induced changes in set-shifting behavior

The effects of systemic administration of saline, kynurenine (100 mg/kg, i.p.), kynurenine + galantamine (3 mg/kg, i.p.), and saline + galantamine on performance in an attentional set-shifting task are summarized in Fig. 2. An overall ANOVA revealed significant effects of drug ($F_{3,\ 18}$=23.54; P<0.001), stage of task ($F_{6,\ 108}$=19.94; P<0.001), and a

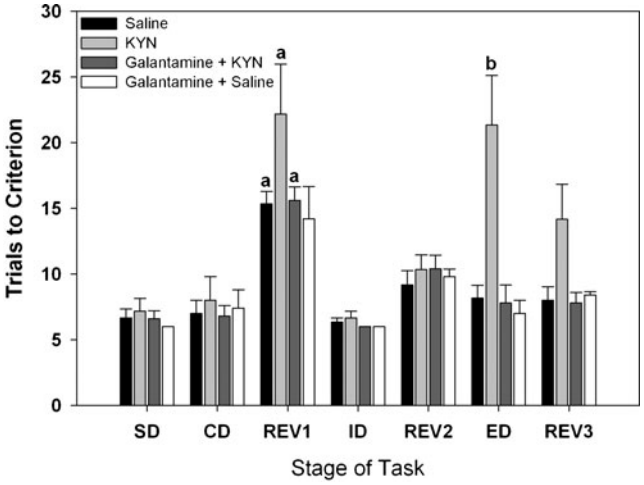

Fig. 2 Mean trials to criterion (±SEM) for drug treatment groups in various stages of the attentional set-shifting task. Animals from each treatment group were tested in each of the stages of the task in the order indicated. All groups of rats readily acquired the single (SD) and compound (*CD*) discriminations. As expected, each group required more trials to learn the initial reversal (*REV1*). Each group demonstrated comparable abilities to form an attentional set, as evidenced by the rapid acquisition of an intra-dimensional shift (*ID*) to a novel stimulus. Rats treated with kynurenine (*KYN*; 100 mg/kg, i.p.) exhibited marked deficits in the ability to make an extra-dimensional shift (*ED*), and this deficit was normalized following pretreatment with galantamine (3 mg/kg, i.p.; n=5–6 rats/drug group). *a* Significantly different, within treatment group, from the trials to criterion for the CD stage; *b* significantly different, within treatment group, from the trials to criterion for the ID stage (all P values, <0.05)

drug-by-stage interaction ($F_{18,\ 108}$=2.56; P=0.01) on the number of trials to reach criterion. Relative to saline-treated controls, rats given kynurenine had no difficulties acquiring the initial discriminations (SD and CD) or performing an intra-dimensional shift (ID; all P values, >0.05). As anticipated, significantly more trials were required to reach criterion during the initial reversal (REV1), relative to the CD, in each group (both P values, <0.05). Importantly, however, kynurenine-treated rats, but not controls, exhibited marked deficits, relative to their rapid acquisition of the ID, on learning the extra-dimensional shift (ED; P=0.01).

A series of one-way ANOVAs was conducted to reveal drug effects on the REV1 and ED stages of the task. There were no significant between-group differences in the trials to criterion in REV1 (all P values, >0.05; Fig. 2). In contrast, kynurenine-treated rats required more trials to complete the ED stage than did saline controls ($F_{1,\ 11}$= 11.45; P=0.007). Rats treated with kynurenine also required more trials to reach criterion in the ED stage than did those that received kynurenine + galantamine ($F_{1,\ 10}$= 9.73; P=0.012). In fact, the addition of galantamine normalized the performance of kynurenine-treated rats, in the ED stage, to levels seen in control rats, as evident by the comparable trials to criterion between the kynurenine + galantamine and saline groups (P=0.83).

Experiment 3: effects of prefrontal galantamine on kynurenine-induced deficits

The photomicrograph in Fig. 3 depicts a representative bilateral placement of microdialysis probes for the delivery of aCSF or galantamine into the PFC. To accommodate the bilateral perfusions in experiment 3, guide cannulae were implanted at opposing 20° angles (see figure caption for more detail). The 200-μm extension of the cannula guides shown here places the membrane tips of the microdialysis probes into the prelimbic region of the PFC. The coordinates for these placements were also selected to result in probe termination in prefrontal regions comparable to those sampled following the unilateral implantation of microdialysis probes in experiment 1 (placements not shown). Only animals with verified placements within the PFC were used in these studies.

The effects of intra-PFC perfusions of aCSF or galantamine (5 μM) on the task performance of saline controls and rats treated with systemic kynurenine (100 mg/kg, i.p.) are summarized in Fig. 4. An overall ANOVA revealed significant effects of drug ($F_{3,\ 16}$=7.55; P=0.002), stage of task ($F_{6,\ 96}$=20.52; P<0.001), and a drug-by-stage interaction ($F_{18,\ 96}$=2.97; P=0.004) on the number of trials to reach criterion. Consistent with the data summarized in Fig. 2, kyurenine-treated rats given a control infusion of aCSF into the PFC exhibited no problems in acquiring the task (SD and CD) in a fashion similar to that seen in saline-

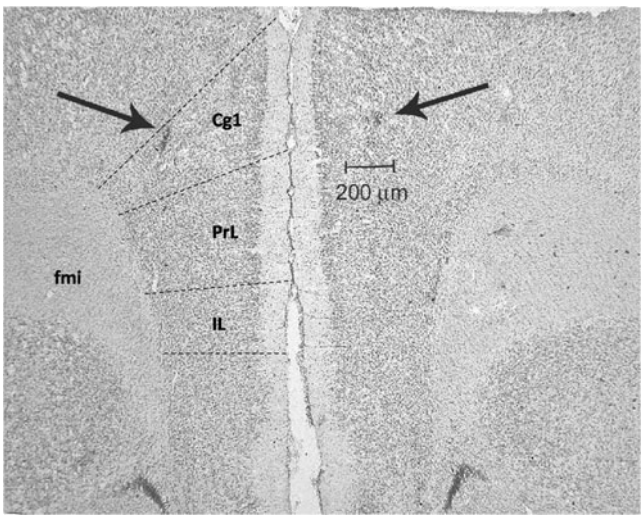

Fig. 3 Representative photomicrograph showing bilateral placements of guide cannulae for microdialysis probes that were used for the local perfusion of aCSF or galantamine into the medial PFC (experiment 3). The left hemisphere placement depicts the tract of a cannula (*arrow*) implanted at a 20° rostral angle, while the right hemisphere placement depicts the tract implanted at a 20° caudal angle (*arrow*). Note: the left and right guide cannulae terminated approximately 200 μm posterior and anterior, respectively, to this section; thereby placing both dialysis membranes in the pre-limbic region of the PFC. Abbreviations: *Cg1* cingulate cortex, *PrL* pre-limbic, *IL* infra-limbic, *fmi* forceps minor

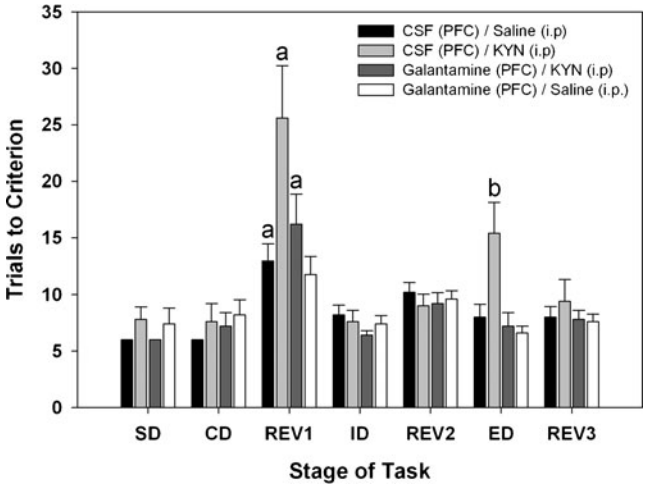

Fig. 4 Mean trials to criterion (±SEM) for drug treatment groups in various stages of the attentional set-shifting task. The four drug conditions consisted of an intra-PFC infusion of either aCSF ("CSF") or galantamine (5 μM) for 1 h prior to the onset of testing and, 15 min after the start of the perfusion, an i.p. injection of either saline or kynurenine (*KYN*; 100 mg/kg). The results were strikingly similar to those seen in Fig. 2. Neither local perfusion of aCSF nor the delivery of galantamine to saline-treated controls affected the results seen in experiment 2 (Fig. 2). All groups of rats readily acquired the single (*SD*) and compound (*CD*) discriminations but required more trials to learn the initial reversal (*REV1*). Each group demonstrated comparable abilities to form an attentional set, as evidenced by the rapid acquisition of an intra-dimensional shift (*ID*) to a novel stimulus. Finally, kynurenine-treated rats exhibited marked deficits in the ability to make an extra-dimensional shift (*ED*), and this deficit was normalized following the intra-PFC perfusion of galantamine (n=5 rats/drug group). *a* Significantly different, within treatment group, from the trials to criterion for the CD stage; *b* significantly different, within treatment group, from the trials to criterion for the ID stage (all P values, <0.05)

treated controls (both P values, >0.05). They also had little difficulty learning the ID shift, reaching criterion within a control-like number of trials (P>0.05). As seen in experiment 2, both groups of rats took longer to acquire the REV1 than either the CD or ID stages of the task (all P values, <0.05), yet only the aCSF/kynurenine group exhibited deficits in learning the ED shift (P=0.02).

Bilateral infusions of galantamine did not affect the task performance of saline-treated control rats with the exception that the typical increase in trials necessary to complete the first reversal (REV1), although elevated, was no longer significantly higher than trials required for the CD stage (P>0.05). In contrast, intra-cortical galantamine eliminated the characteristic deficit in ED shifting seen in the aCSF/kynurenine group ($F_{1,9}$=7.64; P=0.025) and in the kynurenine group from experiment 2 (Fig. 2), rendering the rats' performance comparable to that seen in the aCSF/saline control group ($F_{1,9}$=0.23; P=0.64). The characteristic reversal deficit (REV1) was still observed in the galantamine/kynurenine group, although there was a non-significant trend towards a reduction in the number of trials necessary to reach criterion in this stage (P=0.08).

Discussion

The experiments in this study were designed to explore the effects of acute elevations of brain levels of the α7nAChR negative modulator kynurenic acid (through administration of its bioprecursor kynurenine) on attentional set-shifting behavior. The results yielded several important observations. First, systemic administration of kynurenine produced marked, time-dependent increases in kynurenic acid levels in PFC with a corresponding phase of decreased extracellular glutamate levels. Second, kynurenine-induced changes in glutamate, but not in kynurenic acid, were blocked by pretreatment with the α7nAChR positive modulator galantamine—demonstrating that kynurenine's effects on glutamate were mediated by kynurenic acid's actions at the α7nAChR. Third, systemic administration of kynurenine produced selective impairments in the initial reversal and extra-dimensional stages of the set-shifting task. Finally, these kynurenine-induced deficits were normalized by either systemic or local, intra-PFC perfusion of galantamine—indicating that antagonism of α7nAChRs within the PFC, and associated reductions in prefrontal glutamate, are necessary for the detrimental effects of systemic kynurenine on cognitive flexibility. The discussion that follows focuses on the information processing demands of the stages of the set-shifting task, potential chemo-anatomical mechanisms associated with kynurenine's impairing effects on the ability to learn a reversal and an extra-dimensional shift, the therapeutic actions of galantamine, and the implications of these results for the pathophysiology and treatment of SZ.

Stages of the set-shifting task and their cognitive components

The set-shifting task utilized in these experiments and others (Birrell and Brown 2000; McGaughy et al. 2008; Tait and Brown 2008) is based on the ID/ED shifts used in tasks with primate and human subjects, including SZ patients (Barnett et al. 2010). This test of cognitive flexibility involves multiple stages designed to assess the integrity of specific components of cognition. The animal's ability to learn a response rule to solve a two-choice discrimination is assessed in CD, and the data indicate that kynurenine treatment does not interfere with the animals' general ability to acquire discriminations using the dimensions of texture, digging material, and odor as relevant stimuli. Thus, impairments in subsequent phases of the task (i.e., REV1 and ED) cannot be attributed to an inability to

solve two-choice discriminations or to an insufficient motivation to perform. Initial reversal learning (REV1) represents a class of behavioral flexibility that requires the subject to switch stimulus-reinforcement associations within one of the stimulus dimensions used in this task. Reversal learning required more trials to criterion than did any other stage of the task, and this was seen both in controls and in kynurenine-treated rats. However, kynurenine-treated animals exhibited even more difficulty learning the initial reversal, often choosing the previously rewarded condition. The ability to form an attentional set enables the rat to apply the reinforcement history of one element of a perceptual dimension (i.e., odor/gardenia) to a totally novel stimulus (i.e., jasmine) within that same odor dimension, and this was tested in ID. The data reveal that treatment with kynurenine did not disrupt the ability to form an attentional set and to acquire shifts within that original dimension. A demanding form of behavioral flexibility in this task is the ability to switch attentional sets from one perceptual dimension to another (i.e., ED). This requires the animal to actively inhibit responding to a recently rewarded stimulus dimension (i.e., odor) and, at the same time, to acquire a novel discrimination involving the formation of a new attentional set to a completely different stimulus dimension (i.e. texture) and one that has recently been irrelevant in solving the discriminations. Unlike controls, kynurenine-treated rats consistently exhibited marked deficits in the trials required to make this ED shift.

Unlike other tasks measuring behavioral flexibility (cf. Floresco et al. 2006; Stefani et al. 2003; Stefani and Moghaddam 2005), the digging task does not allow one to characterize the errors as perseverative vs. non-perseverative. Subjects are presented with *novel* exemplars at the beginning of the ID and ED shift stages. While the lack of prior exposure to the particular exemplars provides great confidence in the formation of attentional sets, it also precludes defining the source of error as perseverative responding according to a previous strategy as the stimuli driving the previously relevant responses are no longer present.

Chemo-anatomical mediation of cognitive flexibility

Activity within subregions of the PFC is vital for the expression of attentional set-shifting behavior (Birrell and Brown 2000; Dalley et al. 2004; Dias et al. 1996; Ragozzino et al. 1999; Stefani and Moghaddam 2005). Recent studies have suggested a clear dissociation between the ventral regions of the medial PFC (mPFC) and the orbitofrontal cortex (OFC) of the rat in mediating the two task components (REV1 and ED) most affected by the kynurenine treatment in the present study. Thus, the OFC is necessary for the expression of reversal learning, whereas the mPFC is critical for attentional set-shifting (Dalley et al. 2004; Ghods-Sharifi et al. 2008; McAlonan and Brown 2003; Rygula et al. 2010). Decreases in cortical cholinergic transmission impair serial reversal learning (Cabrera et al. 2006; Robbins and Roberts 2007). Moreover, we recently reported that intra-mPFC infusions of the cholinergic antagonists mecamylamine or scopolamine lead to deficits in the REV1, but not ED, stages of our task (Brooks et al. 2010). These data are relevant to the present experiments, because elevations of cortical kynurenic acid have been shown to decrease stimulated levels of prefrontal ACh (Zmarowski et al. 2009). Whether this mediation by cholinergic transmission is due to processes intrinsic to the mPFC or reflects links between the mPFC and the OFC remains to be determined.

Neuronal activity within the mPFC is critical for the performance of extra-dimensional shifts in a variety of species (for reviews, see Dalley et al. 2004; Robbins and Arnsten 2009). The evidence clearly indicates the necessity of NMDA receptor activation for such performance (Brooks et al. 2010; Dalton et al. 2011; Darrah et al. 2008; Pedersen et al. 2009; Stefani et al. 2003). There is also evidence that activity at metabotropic glutamate receptors (mGlu3 and mGlu5) can modulate performance in set-shifting tasks (Baune et al. 2010; Stefani and Moghaddam 2010). The ability of kynurenine to decrease basal levels of prefrontal glutamate (Fig. 1 (bottom)) and to impair the ED shift in our subjects (Fig. 2) is consistent with this literature. Moreover, the fact that galantamine blocked kynurenine's effect on glutamate levels (Fig. 1 (bottom)) and that its systemic or intra-cortical administration normalized performance in the ED stage of the task (Figs. 2 and 4) strongly supports a role of prefrontal glutamatergic transmission for extra-dimensional shifting.

In addition to glutamate, dopaminergic systems have also been linked to the ability to acquire an ED shift. Prefrontal dopamine, via D1 receptor activation, is necessary for normal performance in an attentional set-shifting task (Floresco et al. 2006). Other studies report a role for dopamine receptors in the dorsal (Ragozzino et al. 2002) and ventral striatum (Floresco et al. 2006; Haluk and Floresco 2009) in the mediation of set-shifting. The modulatory role of cortical and striatal dopamine on set-shifting is consistent with observations that systemic administration of kynurenine reduces basal levels of extracellular dopamine in these regions (Wu and Schwarcz, unpublished observations; cf. also Rassoulpour et al. 2005).

Validity of the model and relevance to schizophrenia

Elevations in brain kynurenic acid and the induction of executive function deficits represent an animal model of SZ

with significant face, construct, and predictive validity. The model is predicated on the well-established dysregulations in the kynurenine metabolic pathway seen in SZ (see Wonodi and Schwarcz 2010, for review). For example, expression of tryptophan 2,3-dioxygenase is increased in the frontal cortex of individuals with SZ (Miller et al. 2004), yielding enhanced production of the bioprecursor kynurenine. At the same time, the SZ brain shows reduced expression and activity of the enzyme kynurenine 3-monooxygenase (Sathyasaikumar et al. 2010; Wonodi et al. 2011), biasing kynurenine degradation toward kynurenic acid synthesis. As a result, kynurenic acid levels are elevated in cortex (Schwarcz et al. 2001) and cerebrospinal fluid (Erhardt et al. 2001). Importantly, this increase may in turn trigger dysregulations in several neurotransmitter systems (Konradsson-Geuken et al. 2010; Rassoulpour et al. 2005; Wu et al. 2010; Zmarowski et al. 2009) believed to be impaired in SZ (Guillin et al. 2007; Hyde and Crook 2001; Javitt 2007; Krystal et al. 1999).

Numerous studies have identified kynurenic acid-induced cognitive deficits in animals that share constructs with parallel tasks in the clinical population. Elevated kynurenic acid levels have been shown to disrupt auditory sensory gating (Shepard et al. 2003), pre-pulse inhibition (Erhardt et al. 2004; Nilsson et al. 2006), contextual discriminations (Chess et al. 2009), and spatial working memory (Chess et al. 2007; Pocivavsek et al. 2011). The present study extends this syndrome of cognitive deficits to impairments in attentional set-shifting, a task frequently used to assess cognitive flexibility in animals (Birrell and Brown 2000; Robbins 2007; Tait and Brown 2008) and patients (Barnett et al. 2010; Pantelis et al. 1999).

Finally, both the neurochemical and cognitive impairments produced by kynurenine were alleviated by systemic administration or intra-PFC perfusion of the α7nAChR positive modulator galantamine. While galantamine also inhibits the activity of acetylcholinesterase (AChE; Lilienfeld 2002), we have previously shown (Wu et al. 2010) that the classic AChE inhibitor donepezil (Shigeta and Homma 2001) does not reproduce the ability of galantamine to block kynurenine's effects on prefrontal glutamate levels (Fig. 1, bottom panel). We are currently determining whether donepezil, like galantamine, would attenuate kynurenine-induced deficits in set-shifting. At this point in time, our results lend further support to the conclusion that the effects of kynurenine are mediated via negative modulation of the α7nAChR (Lopes et al. 2007) and that activity at this receptor subtype is necessary for normal reversal learning and extra-dimensional shifts in this cognitive flexibility task.

The pro-cognitive effects of galantamine in this animal model are consistent with recent clinical studies suggesting some potential of the drug as an adjunctive treatment in SZ (Buchanan et al. 2008; Schubert et al. 2006; but see Lindenmayer and Khan 2011). Collectively, these data suggest that strategies designed to normalize the brain levels of kynurenic acid and/or to provide positive modulation/partial agonism of α7nAChRs may represent a rational approach to the development of cognition-enhancing adjunctive medications for the treatment of SZ.

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