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ACUTE ELEVATIONS OF BRAIN KYNURENIC ACID IMPAIR COGNITIVE FLEXIBILITY: NORMALIZATION BY THE ALPHA 7 POSITIVE MODULATOR GALANTAMINE

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Abstract

Rationale—Cognitive deficits represent a core symptom cluster in schizophrenia (SZ) that is predictive of functional outcome, but not effectively treated by current antipsychotics. Thus, there is a need for the development of validated animal models for testing potential cognition-enhancing drugs.

Objective—As kynurenic acid (KYNA) levels are increased in the prefrontal cortex (PFC) of individuals with SZ, we acutely increased brain levels of this astrocyte-derived, negative modulator of the alpha7 nicotinic acetylcholine receptor ($\alpha 7$ nAChR) by administration of its bioprecursor kynurenine (KYN) and measured the effects on KYNA and glutamate levels in PFC as well as performance in a set-shifting task.

Results—Injections of KYN (100 mg/kg, i.p.) increased extracellular KYNA levels (1500% of baseline) and decreased glutamate levels (70% of baseline) in PFC. KYN also produced marked deficits in set-shifting. Saline- and KYN-treated rats similarly acquired the compound discrimination and the intra-dimensional shift (mean trials to criterion, saline: 7.0 and 6.3, respectively; KYN: 8.0 and 6.7). Both groups required more trials to acquire the initial reversal (saline: 15.3; KYN: 22.2). However, KYN-treated rats exhibited severe deficits in acquiring the extra-dimensional shift (saline: 8.2; KYN = 21.3). These deficits were normalized by an acute injection of the $\alpha 7$ nAChR positive allosteric modulator galantamine (3.0 mg/kg, i.p) 5 min prior to KYN (100 mg/kg, i.p.). Thus, trials to criterion were comparable between galantamine + KYN (7.8) and controls (8.2). Finally, the KYN-induced impairment requires antagonism of prefrontal $\alpha 7$ nAChRs, as bilateral local perfusion of galantamine (5.0 μ M) attenuated the effects of systemic KYN.

Conclusions—These results validate the use of animals with elevated brain KYNA levels in SZ research and support continued studies on drugs that normalize brain KYNA levels and/or positively modulate $\alpha 7$ nAChRs as pro-cognitive adjuncts for treating SZ.

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Keywords

cognition; schizophrenia; animal model; kynurenine; glutamate

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, 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 & Lewis, 2010). More specifically, intact cholinergic (Parikh & Sarter, 2008), glutamatergic (Egerton et al., 2008; Stefani et al., 2003), and dopaminergic (Gruber et al., 2010; Robbins & Arnsten, 2009) transmission within the PFC are 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 & Lewis, 2010; Woo et al., 2008), GABAergic (Gonzalez-Burgos et al., 2010; Volk & Lewis, 2010; Volk et al., 2010), and dopaminergic (Guillin et al., 2007; Winterer & 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 (Stone et al., 2007), enters the brain from the circulation and is then rapidly accumulated by astrocytes (Gál and Sherman, 1980; Speciale et al., 1989). Kynurenine is transaminated within astrocytes, mainly by kynurenine aminotransferase II (KAT II), to form kynurenic acid (KYNA) (Guidetti et al., 2007). Subsequently, upon release into the extracellular space, physiological levels of KYNA antagonize $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChRs) via a negative modulation of an allosteric binding site (Hilmas et al., 2001; Lopes et al., 2007). By inhibiting cortical $\alpha 7$ nAChRs, which are frequently located on presynaptic terminals (Livingstone et al., 2010), KYNA tonically modulates cortical levels of ACh, glutamate, and dopamine. Moreover, elevated levels of KYNA, 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 KYNA 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 KYNA levels are

raised experimentally have significant heuristic value. Indeed, acute systemic administration of kynurenine, which increases brain KYNA 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 & Brown, 2000; Tait & Brown, 2008). This paradigm recreates cognitive operations required to perform the Wisconsin Card Sort Task (Pantellis et al., 1999) and the intra- and extra-dimensional (ID/ED) shift of the Cambridge Neuropsychological Test Automated Battery (CANTAB; 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 & Brown, 2000; Lie et al., 2006; Robbins, 2007). Our results, which include microdialysis data monitoring the extracellular levels of KYNA 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 $\alpha 7$ nAChRs.

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 12:12h light/dark cycle (lights on 0600 h) 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 (Carnegie Medicin, membrane length: 2 mm) was inserted through the guide cannula and perfused with Ringer solution (in mM: NaCl, 144; KCl, 4.8; MgSO₄, 1.2; CaCl₂, 1.7; pH 6.7) at a rate of 1 μ l/min.

Samples were collected every 30 min. After the establishment of a stable baseline (2 h), animals received an i.p. injection of either kynurenine (100 mg/kg), galantamine (3 mg/kg), or kynurenine (100 mg/kg) + galantamine (3 mg/kg). 30-min fractions were then collected for an additional 6 h.

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

For KYNA determination, 15 μ l of the dialysate were directly applied to a 3 μ m C₁₈ reverse phase column (80 $\times$ 4.6 mm, ESA, Bedford, MA, USA). KYNA 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, KYNA was detected fluorimetrically (excitation wavelength: 344 nm, emission wavelength: 398 nm; Perkin-Elmer Series 200, Beaconsfield, UK). The retention time was approximately 5.0 min (Swartz et al., 1990).

The other 15 μ l of the sample were used for the determination of glutamate. Briefly, 30 μ l of o-phthalaldehyde/ β -mercaptoethanol (OPA) were added, and the mixture was applied to a reverse phase column (C₁₈, 5 μ m; 250 $\times$ 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 %, B: 12 %; 2 min: A: 80%, B 20%; 5 min; A: 0%, B: 100%; 2 min: A: 80%, B: 20%; 3 min: A: 88%, B: 12%). In the eluate, glutamate was detected fluorimetrically (excitation wavelength: 390 nm; emission wavelength: 460 nm; Perkin Elmer Series 200). The retention time was approximately 4.5 min (Shank et al., 1993).

Set Shifting Behavior (Experiments 2 & 3)

Experiment 2: systemic administration of kynurenine and galantamine—Prior to testing in an attentional set-shifting task, animals were placed in 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. The third group received an injection of saline followed 5 minutes later by an injection of galantamine (3 mg/kg, i.p.). The fourth group received an injection of galantamine (3 mg/kg, i.p.) followed 5 minutes 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 cannulae in the PFC (see above), and either artificial cerebrospinal fluid [aCSF; containing NaCl (126.5 mM), NaHCO₃ (27.5 mM), KCl (2.4 mM), Na₂SO₄ (0.5 mM), KH₂PO₄ (0.5 mM), CaCl₂ (1.1 mM), MgCl₂ (0.8 mM) and dextrose (1 mM)], or galantamine (5 μ M) was perfused bilaterally for 1 h prior to the onset of testing in the attentional set-shifting task and continued throughout the session. Fifteen min after the onset of the local perfusion, animals received an injection of either saline or kynurenine (100 mg/kg, i.p.).

Apparatus: Rats were trained to dig in terra cotta pots (4" 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 one 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 10 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 2 pots that differed across one of the possible stimulus dimensions (odor, texture, and digging medium), until they successfully chose the 'correct' exemplar for 10 consecutive trials under each stimulus dimension.

Testing: On Day 3, rats were tested in 7 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 each stage of the task, testing continued until the rat reached a criterion of 6 consecutive correct choices. 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.

Statistical Analysis

Biochemical experiments (Experiment 1): Absolute values (fmoles/15 μ l) of basal extracellular KYNA 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. *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 & 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 & Thayer, 1987). In addition, paired samples t-tests were performed to compare CD vs. REV1 and ID vs. ED within each drug group.

RESULTS

Experiment 1: Kynurenine-induced changes in cortical KYNA and glutamate

The effects of systemic administration of kynurenine or kynurenine + galantamine on prefrontal levels of KYNA and glutamate are illustrated in Figure 1. Basal concentrations of KYNA and glutamate, not corrected for recovery from the microdialysis probe, were 37.4 ± 0.5 fmoles/15 μ l (= 2.5 nM) and 31.5 ± 0.6 pmoles/15 μ l (= 2.1 μ M), respectively. Comparisons of basal levels of KYNA or glutamate between the two drug groups revealed no significant differences (all P's > 0.05), thus, subsequent analyses were conducted as a percent change from these baseline values. As expected, systemic administration of

kynurenine (100 mg/kg, i.p.) produced marked, time-dependent and reciprocal changes in prefrontal KYNA and glutamate levels (Figure 1, top panel). With respect to KYNA, kynurenine led to significant elevations in KYNA levels in PFC ($F_{14,56} = 63.31$, $P < 0.001$) that became evident 30 min after administration (i.e. collection 5), began to decline after 2 ½ hr, but remained elevated throughout the dialysis session. With regard to glutamate, kynurenine resulted in a decrease in glutamate levels ($F_{14,56} = 10.66$, $P < 0.001$) that remained significantly reduced in collections 5–9 and then returned to basal values.

The combination of galantamine (3 mg/kg, i.p.) and kynurenine (100 mg/kg, i.p.) differentially impacted kynurenine-induced changes in KYNA (DRUG, $F_{1,8} = 1.52$, $P = 0.25$) and glutamate levels (DRUG, $F_{1,8} = 11.82$, $P = 0.009$; Figure 1, bottom panel). One-way ANOVAs revealed that galantamine + kynurenine had no effect on kynurenine's ability to elevate cortical KYNA levels ($F_{14,56} = 74.51$, $P < 0.001$); however, the drug combination blocked kynurenine-induced reduction in glutamate levels ($F_{14,56} = 1.09$, $P = 0.89$).

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

The effects of systemic administration of kynurenine (100 mg/kg, i.p.) or kynurenine + galantamine (3 mg/kg, i.p.) on performance in an attentional set-shifting task are summarized in Figure 2. An overall ANOVA revealed significant effects of DRUG ($F_{1,18} = 23.54$, $P < 0.001$), STAGE OF TASK ($F_{6,108} = 19.94$, $P < 0.001$) and a 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, CD) or making the intra-dimensional shift (ID; all P 's > 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 's < 0.05). Importantly, kynurenine-treated rats, but not controls, exhibited marked deficits, relative to their rapid acquisition of the ID, on learning the extra-dimensional shift (ED; $P = 0.01$).

Pretreatment of saline controls with galantamine did not affect performance on any stage of the task (all P 's > 0.30). In contrast, the addition of galantamine normalized the performance of kynurenine-treated rats to levels seen in control rats. This was most apparent in the significant reduction, relative to kynurenine-treated rats, in the trials to criterion during the ED stage of the set-shifting task ($P = 0.01$). There was also a trend toward a reduction in the trials needed to complete the initial reversal (REV1; $P = 0.06$).

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

The photomicrograph in Figure 3 depicts a representative bilateral placement of microdialysis probes for the delivery of aCSF or galantamine into the PFC. The coordinates for these placements were also selected to overlap with those used for the unilateral implantation of microdialysis probes in Experiment 1 (placements not shown). To accommodate the bilateral perfusions in Experiment 3, guide cannulae were implanted at opposing 20° angles (see caption for more detail). Only animals with verified bilateral placements within the PFC were used in these studies.

The effects of intra-PFC perfusions of aCSF or galantamine (5 μ M) on the task performance of saline controls and rats treated with systemic kynurenine (100 mg/kg, i.p.) are summarized in Figure 4. An overall ANOVA revealed significant effects of DRUG ($F_{1,16} = 7.55$, $P = 0.002$), 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 Figure 2, kynurenine-treated rats given a control infusion of aCSF into the PFC exhibited no problems in acquiring the task (SD, CD) in a fashion similar to that seen in saline-treated controls (both P 's > 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 initial reversal (REV1) than either the CD or ID stages of the task (all P 's < 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 extra-dimensional shifting (ED) seen in the aCSF/kynurenine group and in the kynurenine group from Experiment 2 (Figure 2), rendering the rats' performance comparable to that seen in various control groups. 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 $\alpha 7$ nAChR negative modulator KYNA (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 KYNA levels in PFC with a corresponding phase of decreased extracellular glutamate levels. Second, kynurenine-induced changes in glutamate, but not in KYNA, were blocked by pretreatment with the $\alpha 7$ nAChR positive modulator galantamine – demonstrating that kynurenine's effects on glutamate were mediated by KYNA's actions at the $\alpha 7$ nAChR. 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 $\alpha 7$ nAChRs 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 chemoanatomical 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 & Brown, 2000; McGaughy et al., 2008; Tait & 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, ED) cannot be attributed to an inability to solve two-choice discriminations or to an insufficient motivation to perform. Initial reversal learning (REV1) is a rudimentary form of behavioral flexibility that requires the subject to switch stimulus-reinforcement associations within one of the stimulus dimensions used in this task. Reversal learning typically requires more trials to reach criterion, relative to the initial discrimination, 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. The most 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.

Chemoanatomical mediation of cognitive flexibility

Activity within subregions of the PFC is vital for the expression of attentional set-shifting behavior (Birrell & Brown, 2000; Dalley et al., 2004; Dias et al., 2007; Ragozzino et al., 1999; Stefani & 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 (REV, 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 & Brown, 2003; Rygula et al., 2010). Decreases in cortical cholinergic transmission impair serial reversal learning (Cabrera et al., 2006; Robbins & Roberts, 2007). Moreover, we have recently reported that intra-mPFC infusions of the cholinergic antagonists mecamylmine 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 KYNA have been shown to decrease basal and 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 & 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). The ability of kynurenine to decrease basal levels of prefrontal glutamate (Figure 1, top) and to impair the ED shift in our subjects (Figure 2) is consistent with this literature. Moreover, the fact that galantamine blocked kynurenine's effect on glutamate levels (Figure 1, bottom) and that its systemic or intra-cortical administration normalized performance in the ED stage of the task (Figures 2 and 4) strongly supports a role of prefrontal glutamatergic transmission for extra-dimensional shifting.

Additional neurotransmitter systems have been linked to the ability to acquire an ED shift. Thus, prefrontal dopamine, via D1 receptor activation, is necessary for normal performance in an attentional set-shifting (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 & 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). Finally, a recent study suggests that reduced prefrontal GABA activity is associated with impairments in set-shifting, but not reversal, behavior (Enomoto et al., 2011). Given the presence of $\alpha 7$ nAChRs on cortical GABAergic neurons (Lubin et al., 1999) as well as on terminals of

excitatory glutamatergic inputs to these interneurons (Gioanni et al., 1999; Vidal & Changeux, 1993), it might be expected that kynurenine administration also reduced GABA receptor activity in our experiments.

Validity of the model and relevance to schizophrenia

Elevations in brain KYNA 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 & Schwarcz, 2010 for review). For example, expression of tryptophan 2,3-dioxygenase (TDO) 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 (KMO) in SZ (Sathyaikumar et al., 2010; Wonodi et al., 2011), biasing kynurenine degradation toward KYNA synthesis. As a result, KYNA 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 & Crook, 2001; Javitt, 2007; Krystal et al., 1999).

Numerous studies have identified KYNA-induced cognitive deficits in animals that share constructs with parallel tasks in the clinical population. Elevated KYNA 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). 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 (Birrel & Brown, 2000; Robbins, 2007; Tait & 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 $\alpha 7$ nAChR positive modulator galantamine. These results lend further support to the conclusion that the effects of kynurenine are mediated via negative modulation of the $\alpha 7$ nAChR (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 the some potential of the drug as an adjunctive treatment in SZ (Buchanan et al., 20008; Schubert et al., 2006; but see Lindenmayer & Khan, 2011). Collectively, these data suggest that strategies designed to normalize the brain levels of KYNA and/or to provide positive modulation/partial agonism of $\alpha 7$ nAChRs may represent a rational approach to the development of cognition-enhancing adjunctive medications for the treatment of SZ.

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REFERENCES

- Barnett JH, Robbins TW, Leeson VC, Sahakian BJ, Joyce EM, Blackwell AD. Assessing cognitive function in clinical trials of schizophrenia. *Neuroscience and Biobehavioral Reviews*. 2010; 34:1161–1177. [PubMed: 20105440]

- Bauer D, Gupta D, Haroutunian V, Meador-Woodruff JH, McCullumsmith RE. Abnormal expression of glutamate transporter and transporter interacting molecules in prefrontal cortex in elderly patients with schizophrenia. *Schizophrenia Research*. 2008; 104:108–120. [PubMed: 18678470]
- Birrell J, Brown V. Medial frontal cortex mediates perceptual attentional set shifting in the rat. *Journal of Neuroscience*. 2000; 20:4320–4324. [PubMed: 10818167]
- Brooks, JM.; Thomsen, MS.; Mikkelsen, JD.; Sarter, M.; Bruno, JP. 2010 Neuroscience Meeting Planner. Society for Neuroscience; San Diego, CA: 2010. Attentional set-shifting deficits in a neurodevelopmental animal model of schizophrenia – Reversal with an alpha7 nAChR agonist. 368.08. Online
- Buchanan RW, Conley RR, Dickinson D, Ball MP, Feldman S, Gold JM, McMahon RP. Galantamine for the treatment of cognitive impairments in people with schizophrenia. *American Journal of Psychiatry*. 2008; 165:82–89. [PubMed: 17986678]
- Cabrera SM, Chavez CM, Corley SR, Kitto MR, Butt AE. Selective lesions of the nucleus basalis magnocellularis impair cognitive flexibility. *Behavioral Neuroscience*. 2006; 120:298–306. [PubMed: 16719694]
- Chen EY, Tam DK, Dunn EL, Miao MY, Yeung WS, Wong CK, Chan WF, Tang WN. Neurocognitive and clinical predictors for vocational outcome following first episode schizophrenia: A 3 year prospective study. *Schizophrenia Bulletin*. 2005; 31:320–320.
- Chess AC, Landers AM, Bucci DJ. L-kynurenine treatment alters contextual fear conditioning and context discrimination but not cue-specific fear conditioning. *Behavioral Brain Research*. 2009; 201:325–331.
- Chess AC, Simoni MK, Alling TE, Bucci DJ. Elevations of endogenous kynurenic acid produce spatial working memory deficits. *Schizophrenia Bulletin*. 2007; 33:797–804. [PubMed: 16920787]
- Dalley JW, Cardinal RN, Robbins TW. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neuroscience and Biobehavioral Reviews*. 2004; 28:771–784. [PubMed: 15555683]
- Dalton GL, Ma LM, Phillips AG, Floresco SB. Blockade of NMDA GluN2B receptors selectively impairs behavioral flexibility but not initial discrimination learning. *Psychopharmacology*. 2011 Epub ahead of print.
- Darrah JM, Stefani MR, Moghaddam B. Interaction of N-methyl-D-aspartate and group 5 metabotropic glutamate receptors on behavioral flexibility using a novel operant set-shift paradigm. *Behavioral Pharmacology*. 2008; 19:225–234.
- Dias R, Robbins TW, Roberts AC. Dissociation in prefrontal cortex of affective and attentional shifts. *Nature*. 1996; 380:69–72. [PubMed: 8598908]
- Egerton A, Reid L, McKerchar CE, Morris BJ, Pratt JA. Impairment in perceptual attentional set-shifting following PCP administration: a rodent model of set-shifting deficits in schizophrenia. *Psychopharmacology*. 2005; 179:77–84. [PubMed: 15682304]
- Enomoto T, Tse MT, Floresco SB. Reducing prefrontal gamma-aminobutyric acid activity induces cognitive, behavioral, and dopaminergic abnormalities that resemble schizophrenia. *Biological Psychiatry*. 2011; 69:432–441. [PubMed: 21146155]
- Erhardt S, Blennow K, Nordin C, Skogh E, Lindstrom LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neuroscience Letters*. 2001; 313:96–98. [PubMed: 11684348]
- Erhardt S, Schwieler L, Emanuelsson C, Geyer M. Endogenous kynurenic acid disrupts prepulse inhibition. *Biological Psychiatry*. 2004; 56:255–260. [PubMed: 15312813]
- Floresco SB, Magyar O, Ghods-Sharifi S, Vexelman C, Tse MT. Multiple dopaminergic receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. *Neuropsychopharmacology*. 2006; 31:297–309. [PubMed: 16012531]
- Floresco SB, Zhang Y, Enomoto T. Neural circuits subserving behavioral flexibility and their relevance to schizophrenia. *Behavioural Brain Research*. 2009; 204:396–409. [PubMed: 19110006]
- Gál EM, Sherman AD. L-kynurenine: its synthesis and possible regulatory function in brain. *Neurochemical Research*. 1980; 5:223–239. [PubMed: 6154900]

- Ghods-Sharifi S, Haluk DM, Floresco SB. Differential effects of inactivation of the orbitofrontal cortex on strategy set-shifting and reversal learning. *Neurobiology of Learning and Memory*. 2008; 89:567–573. [PubMed: 18054257]
- Gioanni Y, Rougeot C, Clarke PBS, Lepou C, Thierry M, Vidal C. Nicotinic receptors in the rat prefrontal cortex: Increase in glutamate release and facilitation of mediodorsal thalamocortical transmission. *European Journal of Neuroscience*. 1999; 11:18–30. [PubMed: 9987008]
- Gold JM. Cognitive deficits as treatment targets in schizophrenia. *Schizophrenia Research*. 2004; 72:21–28. [PubMed: 15531404]
- Gonzalez-Burgos G, Hashimoto T, Lewis DA. Alterations of cortical GABA neurons and network oscillations in schizophrenia. *Current Psychiatry Reports*. 2010; 12:335–344. [PubMed: 20556669]
- Goto Y, Yang CR, Otani S. Functional and dysfunctional synaptic plasticity in prefrontal cortex: roles in psychiatric disorders. *Biological Psychiatry*. 2010; 67:199–207. [PubMed: 19833323]
- Gruber AJ, Calhoon GG, Shusterman I, Schoenbaum G, Roesch MR, O'Donnell P. More is less: a disinhibited prefrontal cortex impairs cognitive flexibility. *Journal of Neuroscience*. 2010; 30:17102–17110. [PubMed: 21159980]
- Guidetti P, Hoffman GE, Melendez-Ferro M, Albuquerque EX, Schwarcz R. Astrocytic localization of kynurenine aminotransferase II in the rat brain visualized by immunocytochemistry. *Glia*. 2007; 55:78–92. [PubMed: 17024659]
- Guillin O, Abi-Dargham A, Laruelle M. Neurobiology of dopamine in schizophrenia. *International Review of Neurobiology*. 2007; 78:1–9. [PubMed: 17349856]
- Haluk DM, Floresco SB. Ventral striatal dopamine modulation of different forms of behavioral flexibility. *Neuropsychopharmacology*. 2009; 34:2041–2052. [PubMed: 19262467]
- Heinrichs RW. The primacy of cognition in schizophrenia. *American Psychologist*. 2005; 60:229–242. [PubMed: 15796677]
- Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits $\alpha 7$ nicotinic receptor activity and increases non- $\alpha 7$ nicotinic receptor expression: physiopathological implications. *Journal of Neuroscience*. 2001; 21:7463–7473. [PubMed: 11567036]
- Hyde TM, Crook JM. Cholinergic systems and schizophrenia: primary pathology or epiphenomena? *Journal of Chemical Neuroanatomy*. 2001; 22:53–63. [PubMed: 11470554]
- Javitt DC. Glutamate and schizophrenia: phencyclidine, N-methyl-D-aspartate receptors, and dopamine-glutamate interactions. *International Review of Neurobiology*. 2007; 78:69–108. [PubMed: 17349858]
- Keefe RSE. Cognitive deficits in patients with schizophrenia: Effects and treatment. *Journal of Clinical Psychiatry*. 2007; 68:8–13. [PubMed: 18284272]
- Kehagia AA, Murray GK, Robbins TW. Learning and cognitive flexibility: frontostriatal function and monoaminergic modulation. *Current Opinion in Neurobiology*. 2010; 20:199–204. [PubMed: 20167474]
- Kerns JG, Nuechterlein KH, Braver TS, Barch DM. Executive functioning component mechanisms and schizophrenia. *Biological Psychiatry*. 2008; 64:26–33. [PubMed: 18549874]
- Konradsson-Geuken A, Gash CR, Alexander KA, Pomerleu F, Huettl P, Gerhardt GA, Bruno JP. Second-by-second analysis of $\alpha 7$ nicotine receptor regulation of glutamate release in the prefrontal cortex of awake rats. *Synapse*. 2009; 63:1069–1082. [PubMed: 19637277]
- Konradsson-Geuken A, Wu HQ, Gash CR, Alexander KS, Campbell A, Sozeri Y, Pellicciari R, Schwarcz R, Bruno JP. Cortical kynurenic acid bi-directionally modulates prefrontal glutamate levels as assessed by microdialysis and rapid electrochemistry. *Neuroscience*. 2010; 169:1848–1859. [PubMed: 20600676]
- Krystal JH, D'Souza DC, Petrakis IL, Belger A, Berman RM, Charney DS, Abi-Saab W, Madonick S. NMDA agonists and antagonists as probes of glutamatergic dysfunction and pharmacotherapies in neuropsychiatric disorders. *Harvard Review of Psychiatry*. 1999; 7:125–143. [PubMed: 10483932]
- Lie CH, Specht K, Marshall JC, Fink GR. Using fMRI to decompose the neural processes underlying the Wisconsin Card Sorting Test. *Neuroimage*. 2006; 30:1038–1049. [PubMed: 16414280]

- Lindenmayer JP, Khan A. Galantamine augmentation of long-acting injectable risperidone for cognitive impairments in chronic schizophrenia. *Schizophrenia Research*. 2011; 125:267–277. [PubMed: 20850275]
- Livingstone PD, Dickinson JA, Srinivasan J, Kew JN, Wonnacott S. Glutamate-dopamine crosstalk in the rat prefrontal cortex is modulated by $\alpha 7$ nicotinic receptors and potentiated by PNU-120596. *Journal of Molecular Neuroscience*. 2010; 40:171–176.
- Lopes C, Pereira EFR, Wu HQ, Pranik P, Njar V, Schwarcz R, Albuquerque EX. Competitive antagonism between the nicotinic allosteric potentiating ligand galantamine and kynurenic acid at $\alpha 7^*$ nicotinic receptors. *Journal of Pharmacology and Experimental Therapeutics*. 2007; 322:48–58. [PubMed: 17446300]
- Lubin M, Erisir A, Aoki C. Ultrastructural immunolocalization of the $\alpha 7$ nAChR subunit in guinea pig medial prefrontal cortex. *Annals of the New York Academy of Sciences*. 1999; 868:628–632. [PubMed: 10414345]
- Mathew SV, Law AJ, Lipska BK, Davila-Garcia MI, Zamora ED, Mitkus SN, Vakkalanka R, Straub RE, Weinberger DR, Kleinman JE, Hyde TM. $\alpha 7$ nicotinic acetylcholine receptor mRNA expression and binding in postmortem human brain are associated with genetic variation in neuregulin 1. *Human Molecular Genetics*. 2007; 16:2921–2932. [PubMed: 17884806]
- McAlonan K, Brown VJ. Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behavioural Brain Research*. 2003; 146:97–103. [PubMed: 14643463]
- McGaughy J, Ross RS, Eichenbaum H. Noradrenergic, but not cholinergic, deafferentation of prefrontal cortex impairs attentional set-shifting. *Neuroscience*. 2008; 153:63–71. [PubMed: 18355972]
- Miller CL, Llenos IC, Dulay JR, Barillo MM, Yolken RH, Weis S. Expression of the kynurenine pathway enzyme tryptophan 2,3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. *Neurobiology of Disease*. 2004; 15:618–629. [PubMed: 15056470]
- Miller CL, Llenos IC, Dulay JR, Weis S. Upregulation of the initiating step of the kynurenine pathway in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder. *Brain Research*. 2006; 1073–1074:25–37.
- Nilsson LK, Linderholm KR, Erhardt S. Subchronic treatment with kynurenine and probenecid: effects on prepulse inhibition and firing of midbrain dopamine neurons. *Journal of Neural Transmission*. 2006; 113:557–571. [PubMed: 16082514]
- Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK. Identification of separable cognitive factors in schizophrenia. *Schizophrenia Research*. 2004; 72:29–39. [PubMed: 15531405]
- Nuechterlein KH, Luck SJ, Lustig C, Sarter M. CNTRICS final task selection: Control of attention. *Schizophrenia Bulletin*. 2009; 35:182–196. [PubMed: 19074499]
- Olinicy A, Harris JG, Johnson LL, Pender V, Kongs S, Allensworth D, Ellis J, Zerbe GO, Leonard S, Stevens KE, Stevens JO, Martin L, Adler LE, Soti F, Kern WR, Freedman R. Proof-of-concept trial of an $\alpha 7$ nicotinic agonist in schizophrenia. *Archives of General Psychiatry*. 2006; 63:630–638. [PubMed: 16754836]
- Pantelis C, Barber FZ, Barnes TR, Nelson HE, Owen AM, Robbins TW. Comparison of set-shifting ability in patients with chronic schizophrenia and frontal lobe damage. *Schizophrenia Research*. 1999; 37:251–270. [PubMed: 10403197]
- Parikh V, Sarter M. Cholinergic mediation of attention – contributions of phasic and tonic increases in prefrontal cholinergic activity. *Annals of the New York Academy of Sciences*. 2008; 1129:225–235. [PubMed: 18591483]
- Pedersen CS, Goetghebuer P, Dias R. Chronic infusion of PCP via osmotic mini-pumps: a new rodent model of cognitive deficit in schizophrenia characterized by impaired attentional set-shifting (ID/ED) performance. *Journal of Neuroscience Methods*. 2009; 185:66–69. [PubMed: 19761795]
- Potkin SG, Fleming K, Jin Y, Gulasekaram B. Clozapine enhances neurocognition and clinical symptomatology more than standard neuroleptics. *Journal of Clinical Psychopharmacology*. 2001; 21:479–483. [PubMed: 11593072]
- Prouteau A, Verdoux N, Briand C, Lesage A, Lalonde P, Nicole L, Reinhartz D, Stip E. Cognitive predictors of psychosocial functioning outcome in schizophrenia: A follow-up study of subjects

- participating in a rehabilitation program. *Schizophrenia Research*. 2005; 77:343–353. [PubMed: 16085207]
- Ragozzino ME, Ragozzino KE, Mizumori SJY, Kesner RP. The role of the dorsomedial striatum in behavioral flexibility for response and visual cue discrimination learning. *Behavioral Neuroscience*. 2002; 116:105–115. [PubMed: 11898801]
- Ragozzino ME, Wilcox C, Raso M, Kesner RP. Involvement of rodent prefrontal cortex subregions in strategy switching. *Behavioral Neuroscience*. 1999; 113:32–41. [PubMed: 10197904]
- Rassoulpour A, Wu H-Q, Ferré S, Schwarcz R. Nanomolar concentrations of kynurenic acid reduce extracellular dopamine levels in the striatum. *Journal of Neurochemistry*. 2005; 93:762–765. [PubMed: 15836634]
- Robbins TW. Shifting and stopping: fronto-striatal substrates, neurochemical modulation and clinical implications. *Philosophical Transactions of the Royal Society B-Biological Sciences*. 2007; 362:917–932.
- Robbins TW, Arnsten AFT. The neuropsychopharmacology of fronto-executive function: Monoaminergic modulation. *Annual Review of Neuroscience*. 2009; 32:267–287.
- Robbins TW, Roberts AC. Differential regulation of fronto-executive function by the monoamines and acetylcholine. *Cerebral Cortex*. 2007; 17:151–160.
- Rollnik JD, Borsutzky M, Huber TJ, Mogk H, Seifert J, Emrich HM, Schneider U. Short-term cognitive improvement in schizophrenics treated with typical and atypical neuroleptics. *Neuropsychobiology*. 2002; 45:74–80. [PubMed: 11893863]
- Rygula R, Walker SC, Clarke HF, Robbins TW, Roberts AC. Differential contributions of the primate ventrolateral prefrontal and orbitofrontal cortex to serial reversal learning. *Journal of Neuroscience*. 2010; 30:14552–14559. [PubMed: 20980613]
- Scarr E, Cowie TF, Kanellakis S, Sundram S, Pantelis C, Dean B. Decreased cortical muscarinic receptors define a subgroup of subjects with schizophrenia. *Molecular Psychiatry*. 2009; 14:1017–1023. [PubMed: 18317461]
- Schubert MH, Young KA, Hicks PB. Galantamine improves cognition in schizophrenic patients stabilized on risperidone. *Biological Psychiatry*. 2006; 60:530–533. [PubMed: 16806095]
- Schwarcz R, Pelliccari R. Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities. *Journal of Pharmacology and Experimental Therapeutics*. 2002; 303:1–10. [PubMed: 12235226]
- Schwarcz R, Rassoulpour A, Wu H-Q, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenate content in schizophrenia. *Biological Psychiatry*. 2001; 50:521–530. [PubMed: 11600105]
- Shank RP, Leo GC, Zielke HR. Cerebral metabolic compartmentation as revealed by nuclear magnetic resonance analysis of D-[1–¹³C]glucose metabolism. *Journal of Neurochemistry*. 1993; 61:315–323. [PubMed: 8515279]
- Speciale C, Hares K, Schwarcz R, Brookes N. High-affinity uptake of L-kynurenine by a Na⁺-independent transporter of neutral amino acids in astrocytes. *Journal of Neuroscience*. 1989; 9:2066–2072. [PubMed: 2723766]
- Shepard PD, Joy B, Clerkin L, Schwarcz R. Micromolar brain levels of kynurenic acid are associated with a disruption of auditory sensory gating in the rat. *Neuropsychopharmacology*. 2003; 28:1454–1462. [PubMed: 12700696]
- Stefani MR, Groth K, Moghaddam B. Glutamate receptors in the rat medial prefrontal cortex regulate set-shifting ability. *Behavioral Neuroscience*. 2003; 117:728–737. [PubMed: 12931958]
- Stefani MR, Moghaddam B. Systemic and prefrontal cortical NMDA receptor blockade differentially affect discrimination learning and set-shift ability in rats. *Behavioral Neuroscience*. 2005; 119:420–528. [PubMed: 15839788]
- Stone TW, Forrest CM, Mackay GM, Stoy N, Darlington LG. Tryptophan, adenosine, neurodegeneration and neuroprotection. *Metabolic Brain Disease*. 2007; 22:337–352. [PubMed: 17712616]
- Swartz KJ, During MJ, Freese A, Beal MF. Cerebral synthesis and release of kynurenic acid: an endogenous antagonist of excitatory amino acid receptors. *Journal of Neuroscience*. 1990; 10:2965–2973. [PubMed: 2168940]

- Tait DS, Brown VJ. Lesions of the basal forebrain impair reversal learning but not shifting of attentional set in rats *Behavioural Brain Research*. 2008; 187:100–8. [PubMed: 17920704]
- Vasey MW, Thayer JF. The continuing problem of false positives in repeated measures ANOVA in psychophysiology – a multivariate solution. *Psychophysiology*. 1987; 24:479–489. [PubMed: 3615759]
- Vidal C, Changeux JP. Nicotinic and muscarinic modulations of excitatory synaptic transmission in the rat prefrontal cortex in vitro. *Neuroscience*. 1993; 56:23–32. [PubMed: 7901807]
- Volk DW, Eggan SM, Lewis DA. Alterations in metabotropic glutamate receptor 1 alpha and regulator of G protein signaling 4 in the prefrontal cortex in schizophrenia. *American Journal of Psychiatry*. 2010; 167:1489–1498. [PubMed: 20889653]
- Volk DW, Lewis DA. Prefrontal cortical circuits in schizophrenia. *Current Topics in Behavioral Neuroscience*. 2010; 4:485–508.
- Winterer G, Weinberger DR. Genes, dopamine, and cortical signal-to-noise ratio in schizophrenia. *Trends in Neurosciences*. 2004; 27:683–690. [PubMed: 15474169]
- Wonodi I, Schwarcz R. Cortical kynurenine pathway metabolism: a novel target for cognitive enhancement in schizophrenia. *Schizophrenia Bulletin*. 2010; 36:211–218. [PubMed: 20147364]
- Wonodi I, Stine OC, Sathyaikumar KV, Roberts RC, Mitchell BD, Hong LE, Kajii Y, Thaker GK, Schwarcz R. Downregulated kynurenine 3-monooxygenase gene expression and enzyme activity in schizophrenia and genetic association with schizophrenia endophenotypes. *Archives of General Psychiatry*. 2011 in press.
- Woo TUW, Shrestha K, Lamb D, Minns MM, Benes FM. N-methyl-D-aspartate receptor and calbindin-containing neurons in the anterior cingulate cortex in schizophrenia and bipolar disorder. *Biological Psychiatry*. 2008; 64:803–809. [PubMed: 18585682]
- Wu HQ, Pellicciari R, Schwarcz R. Bidirectional regulation of extracellular dopamine by endogenous kynurenic acid in the rat medial prefrontal cortex. *Society for Neuroscience Abstracts*. 2006; 32:624.3.
- Wu HQ, Pereira EFR, Bruno JP, Pellicciari R, Albuquerque EX, Schwarcz R. The astrocyte-derived alpha7 nicotonic receptor antagonist kynurenic acid controls extracellular glutamate levels in prefrontal cortex. *Journal of Molecular Neuroscience*. 2010; 40:204–210. [PubMed: 19690987]
- Zmarowski A, Wu H-Q, Brooks JM, Potter MC, Pellicciari R, Schwarcz R, Bruno JP. Astrocyte-derived kynurenic acid modulates basal and evoked cortical acetylcholine release. *European Journal of Neuroscience*. 2009; 29:529–538. [PubMed: 19187269]

Figure 1.

(Top Panel) Effects of systemic kynurenine administration (100 mg/kg, i.p.) on extracellular levels of kynurenic acid (KYNA) and glutamate in the PFC. The two analytes were determined in the same microdialysate samples ($n = 5$ rats). Kynurenine led to a marked increase in KYNA levels in the first post-injection collection, reaching a maximum 2 h later, and approaching baseline levels by the end of the microdialysis session. Glutamate levels exhibited a mirror image profile, decreasing in the first collection interval, reaching a nadir 90 min later, and returning to baseline levels 2 ½ h after injection. **(Bottom Panel)** Effects of a combined systemic administration of kynurenine (100 mg/kg, i.p.) and galantamine (3 mg/kg, i.p.) on extracellular levels of KYNA or glutamate in the PFC. The two analytes were again determined in the same microdialysate samples ($n = 5$ rats). KYNA levels exhibited the same pattern of elevation seen following kynurenine alone (top panel). 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 's < 0.05). See text for absolute baseline concentrations.

Figure 2.

Mean trials to criterion ($\pm$ S.E.M.) 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. Kynurenine-treated rats exhibited marked deficits in the ability to make an extra-dimensional shift (ED), and this deficit was normalized following pretreatment with galantamine. ($n = 5-6$ 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 's < 0.05).

Figure 3.

Representative photomicrograph showing bilateral placements of guide cannula 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). The left and right guide cannulae terminated approximately 200 μm posterior and anterior, respectively, to this section.

Figure 4.

Mean trials to criterion ($\pm$ S.E.M.) for drug treatment groups in various stages of the attentional set-shifting task. The four drug conditions received an intra-PFC infusion of either aCSF or galantamine ($5 \mu\text{M}$) 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 (100 mg/kg). The results were strikingly similar to those seen in Figure 2. Neither local perfusion of aCSF nor the delivery of galantamine to saline-treated controls affected the results seen in Experiment 2 (Figure 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 's < 0.05).

Table 1

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

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
				Corn-cob bedding
				Gravel
Texture	Paper	Velour fabric	Felt fabric	Yarn
	Parafilm	Cotton fabric	Cotton fabric	Cotton fabric