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Effects of the nicotinic $\alpha 7$ receptor partial agonist GTS-21 on NMDA-glutamatergic receptor related deficits in sensorimotor gating and recognition memory in rats

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Abstract

Rationale—Disturbances in information processing and cognitive function are key features of schizophrenia. Nicotinic $\alpha 7$ acetylcholine receptors ($\alpha 7$ -nAChR) are involved in sensory gating and cognition, thereby representing a viable therapeutic strategy.

Objectives and methods—We investigated the effects of GTS-21, an $\alpha 7$ -nAChR partial agonist, on prepulse inhibition (PPI) of acoustic startle in two pharmacologic impairment models in Wistar male rats: NMDA-glutamate receptor antagonism by MK-801 and dopamine receptor agonism by apomorphine. The cognitive effects of GTS-21 were assessed using the object recognition task (ORT) at short (3 h) and long (48 h) delays in Sprague-Dawley male rats. Pharmacological specificity was assessed by methyllycaconitine (MLA) coadministration with GTS-21.

Results—In the PPI task, GTS-21 (1–10 mg/kg) alone did not alter the PPI response or startle amplitude. Coadministration of GTS-21 with MK-801 (0.1 mg/kg) or apomorphine (0.5 mg/kg) abolished the pharmacologic-induced PPI impairment as did the antipsychotics clozapine (5.0 mg/kg) and haloperidol (0.3 mg/kg). MK-801 alone increased startle amplitude which was blocked by GTS-21. In the ORT, GTS-21 (0.1–10 mg/kg) reversed the MK-801 (0.08 mg/kg)-induced memory deficit at the 3 h delay and enhanced memory at the 48 h delay, an effect abolished by MLA (0.313–5 mg/kg).

Conclusions—The results extend our preclinical pharmacological understanding of GTS-21 to include the ability of GTS-21 to modulate NMDA-glutamate receptor function, in vivo. Given the role of NMDA-glutamate receptor involvement in schizophrenia, $\alpha 7$ -nAChR agonists may

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represent a novel treatment strategy for the pathophysiological deficits of schizophrenia and other psychiatric disorders.

Keywords

Cognition; Nicotinic $\alpha 7$ receptor; NMDA-glutamate function; Object recognition memory; Sensorimotor gating; Schizophrenia

Introduction

Schizophrenia is a debilitating, lifelong disease affecting approximately 1 % of the world population (Saha et al. 2005; Sullivan 2012). While this complex psychiatric disorder is commonly associated with positive and negative symptoms (e.g., hallucinations, alterations in mood, and social withdrawal), approximately 80 % of this patient population suffers from widespread deficits in information processing, attention, and executive memory function (Green and Braff 2001), thereby severely impacting the individual's ability to perform routine daily activities and maintain social independence.

Although the precise etiology of schizophrenia is unknown, glutamate *N*-methyl-D-aspartate (NMDA) receptor function appears to play an important role especially considering its involvement in cellular plasticity and learning and memory processes (Goff and Wine 1997; Olney and Farber 1995). Accumulating evidence suggests that many of the behavioral abnormalities associated with schizophrenia may be due to a dysfunctional NMDA receptor system (for review, see Synder and Gao 2013). The observation that noncompetitive NMDA antagonists (e.g., phencyclidine (PCP), ketamine, and dizocilpine (MK-801)) produce psychotomimetic effects in humans that resemble "schizophrenic-like" symptoms as well as the exacerbation of the symptoms in schizophrenic patients further implicates NMDA transmission (Lathi et al. 2001; Malhortra et al. 1997). Postmortem studies of schizophrenic patients have also shown decreases in NMDA receptor binding, gene transcription, and protein expression in several key brain regions (Akbarian et al. 1996; Beneyto and Meador-Woodruff 2008; Kornhuber et al. 1989). Preclinically, NMDA receptor antagonists produce many of the observed neurochemical, behavioral, and cognitive impairments associated with schizophrenia (De Lima et al. 2005; Geyer et al. 2001; Rodefer et al. 2005), thereby supporting their use as pharmacological research tools.

Nicotinic $\alpha 7$ acetylcholine receptors ($\alpha 7$ -nAChRs) are located on glutamate and GABA neurons and are involved in the regulation of neurotransmitter release (Fabian-Fine et al. 2001; Huang et al. 2011; Radcliffe et al. 1999). NMDA receptors, like $\alpha 7$ -nAChRs, are found on dendrites and cell bodies of cortical and hippocampal neurons, produce fast excitatory postsynaptic effects, and are highly calcium (Ca^{2+}) permeable. Inhibition of NMDA receptors (via an NMDA receptor antagonist) reduces Ca^{2+} influx and signaling processes (Olney and Farber 1995). Activation of $\alpha 7$ -nAChRs with an exogenous agonist would enhance Ca^{2+} influx and potentially restore the dysfunctional neuronal circuitry affected by NMDA receptor suppression (for review, see Timofeeva and Levin 2011). Several lines of neurobiological and genetic evidence have implicated the $\alpha 7$ -nAChR in the pathophysiology of schizophrenia (Hurst et al. 2013; Wallace and Bertrand 2013) leading to

the development of novel $\alpha 7$ -nAChR agonists as potential therapeutic agents for the treatment of diminished cognitive, sensory gating, and social capabilities associated with schizophrenia. Preclinical studies with $\alpha 7$ -nAChR agonists (e.g., GTS-21, EVP-6124, RG-3487, SSR-180711, and TC-5619) have demonstrated robust procognitive effects (e.g., recognition memory, working memory, and executive function) across multiple cognitive domains (e.g., cortical and hippocampal) in young and aged rodents (Arendash et al. 1995; Hauser et al. 2009; Pichat et al. 2007; Prickaerts et al. 2012; Skidmore et al. 2012; Rezvani et al. 2009; Wallace et al. 2011) and nonhuman primates (Bitner et al. 2010; Briggs et al. 1997; Rodefer et al. 2007; Wallace-Boone et al. 2009; Wallace et al. 2010). In animal models bearing resemblance to schizophrenia, $\alpha 7$ -nAChR agonists are capable of reversing the acute and chronic effects of NMDA receptor antagonists (ketamine, PCP, and MK-801) on learning and memory function in rodents (Hashimoto et al. 2006, 2008; McLean et al. 2011; Pichat et al. 2007; Roncarati et al. 2009; Wallace et al. 2011) and nonhuman primates (Buccafusco and Terry 2009; Cannon et al. 2013). Nicotinic $\alpha 7$ -receptor agonists are also effective in reversing the naturally occurring $\alpha 7$ -nAChR auditory-evoked potential deficit observed in DBA mice as well as the pharmacologic-induced sensory gating disruption (Hajos et al. 2005; Hashimoto et al. 2005; Radek et al. 2012; Stevens et al. 1998). Effects of $\alpha 7$ -nAChR agonists on information gating have been extended to include reversal of pharmacologic-induced disruption of prepulse inhibition (PPI) of acoustic startle. The majority of studies have focused on attenuating the PPI impairment induced by the dopamine agonist apomorphine (Hauser et al. 2009; Kohnomi et al. 2010; Roncarati et al. 2009; Wallace et al. 2011) with fewer studies assessing the role of NMDA receptor function on sensorimotor gating (Dunlop et al. 2009; Ghiron et al. 2010; Kohnomi et al. 2010).

In accordance with the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) and Treatment Unit for Research on Neurocognition and Schizophrenia (TURNS) initiatives to identify cognitive paradigms in humans and laboratory animals that increase predictability and clinical translation (TURN S.ucla.edu; Young et al. 2009), our laboratory has developed several behavioral models that may increase the likelihood of clinical success of novel drug targets to treat schizophrenia and other neuropsychiatric disorders. One such task is the PPI paradigm which assesses information processing (specifically sensorimotor gating) and involves the cortico-striatal-pallidal-pontine circuitry (Geyer and Braff 1987; Swerdlow et al. 2001). Second is the spontaneous object recognition task (ORT) which assesses visual learning and memory and involves cortical and hippocampal substrates (Lyon et al. 2012). Both behavioral models allow for translation between animal and human studies (Braff et al. 2001; Hong et al. 2008; Kitagawa et al. 2003; Kumari et al. 2001; Zhang et al. 2012).

The present study assessed the ability of the $\alpha 7$ -nAChR partial agonist GTS-21 (also known as DMXB-A; Kem 2000) in rats to (1) alter startle amplitude or PPI response when administered alone, (2) ameliorate the pharmacologic-induced deficits in PPI elicited by the NMDA receptor antagonist MK-801 and the dopamine receptor agonist apomorphine, (3) reverse the MK-801-induced object recognition memory deficit following a short 3 h delay interval when memory formation remains intact, and (4) enhance object recognition memory following a long 48 h delay interval when complete forgetting occurs in the control animals.

Materials and methods

Subjects

Experimentally naive, male Sprague-Dawley (3–4 months old; Hilltop Lab Animals, Scottsdale, PA) and male Wistar rats (2–4 months old; Harlan Sprague-Dawley, Inc., Indianapolis, IN) were used. Animals were housed two to three per cage (45×30×18-cm polycarbonate cage with corncob bedding) in a vivarium of constant temperature (21–23 °C) and humidity (40–50 %). Lighting was maintained on a 12-h light/dark cycle (7:00 a.m.–7:00 p.m.), and food and water were available ad libitum. All behavioral testing was performed during the light portion (9 a.m.–5 p.m.) of the light/dark cycle (Monday to Friday). The strains of rats, Sprague-Dawley and Wistar, were selected to allow direct comparisons with our previously published results (Callahan et al. 2013; Terry et al. 2006; Wallace et al. 2011). The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health), and all experimental protocols were approved by the Institutional Animal Care and Use Committees at Memory Pharmaceuticals Corporation and Georgia Regents University.

Prepulse inhibition of acoustic startle

The PPI procedure was adapted from Mansbach and Geyer (1989) and conducted as previously described (Terry et al. 2006). Briefly, male Wistar rats were acclimated to laboratory conditions (i.e., tail marking, daily handling, and weighing) for at least 3 days prior to experimentation. Tests were conducted in four standard startle chambers (San Diego Instruments, San Diego, CA) each consisting of a Plexiglas tube (diameter 8.2 cm, length 25 cm), placed in a sound-attenuated chamber. This tube was mounted on a plastic frame that contained a piezoelectric accelerometer which recorded and transferred the motion of the tube. Our PPI testing procedure consisted for four experimental sessions (chamber habituation, a startle and prepulse level alone presentation session, a PPI group assignment session, and PPI pharmacological testing, see below). Experimentation began 30 min after the animals were transported to the laboratory, and the animals remained in the laboratory for an additional 10 min following study completion. At day 1, the subjects were placed in one of the startle test chambers for a period of 20 min (without any startle stimuli) as an initial period of acclimation to the apparatus. At day 2, the subjects were placed in the test chamber and exposed to a 5-min acclimation period during which a continuous 70-dB background white noise was present. Following acclimation, 15 trials of acoustic startle (120 dB, 40-ms duration) and 3 trials of each prepulse level (5, 10, and 15 dB above background, 20-ms duration) were pseudorandomly presented. This session was conducted in order to reduce the highly variable response to the initial acoustic startle exposures as well as to ensure that the prepulse stimuli (alone) had no significant effect on the startle response. For group assignment (day 3) and pharmacological testing (day 4), the following PPI protocol was used. The subjects were placed in the test chamber and acclimated for 1 min followed by a pseudo-random presentation of 12 startle trials, 12 no-stimulus (null) trials, and 12 trials of each prepulse level/startle trials for a total of 60 trials (see below). The intertrial interval ranged from 10 to 30 s, and the total experimental session lasted for about 25 min. The startle trials consisted of single 120-dB white noise bursts lasting 40 ms; background noise was set at 70 dB and continuous throughout the session. The PPI trials consisted of a

prepulse (20-ms burst of white noise with intensities of 75, 80, and 85 dB) followed by 100 ms later by a startle stimulus (120 dB, 40-ms white noise). During the no-stimulus (null) trial, no startle noise was presented, but the movement of the rat was scored. This represented a control trial for detecting differences in overall activity. The resulting movement of the rat in the startle chamber was measured during 100 ms after startle stimulus onset (sampling frequency 1 kHz), rectified, amplified, and fed into a computer that calculated the maximal response over the 100-ms period. Basal startle amplitude was determined as the mean amplitude of the 12 startle trials. The mean startle amplitude reactivity value obtained for each animal from the group assignment PPI session (day 3) was used to equate the treatment groups. Animals with mean startle reactivity values less than 200 or greater than 2,000 (arbitrary units) were eliminated from further study. This criterion was established due to our previous observations that subjects with very low or very high startle values either failed to exhibit a prepulse-dependent decrease in startle amplitude or exhibited quite variable responses to PPI test compounds. During this day 3 PPI session, the subjects were administered saline (subcutaneously (sc)) to reduce the likelihood of any injection artifact prior to pharmacological test assessment. PPI was calculated according to the following formula: $100 - 100 \times (\text{PPx}/\text{P120})$, in which PPx is the mean of the 12 PPI trials (i.e., for each individual prepulse intensity) and P120 is the basal startle amplitude. The average level of PPI was calculated (mean of the responses to pp75, pp80, or pp85) and analyzed separately. Experimental groups contained the following number of subjects: GTS-21 alone ($N=8$ rats/treatment), GTS-21 plus apomorphine (vehicle $N=13$ rats, apomorphine $N=10$ rats, haloperidol $N=10$ rats, GTS-21 1 and 3 mg/kg $N=10$ rats, and 10 mg/kg $N=8$ rats; 2 rats in this dose group failed to meet the startle reactivity criteria and were eliminated from further study), and GTS-21 plus MK-801 (vehicle $N=10$ rats, MK-801 $N=10$ rats, clozapine $N=10$ rats, GTS-21 1 mg/kg $N=10$ rats, 3 mg/kg $N=12$ rats, and 10 mg/kg $N=9$ rats; 1 rat failed startle reactivity criteria). These experimental groups provided sufficient sample size to obtain statistical significance. The animals were tested only once, and blind testing procedures were followed (i.e., the investigator was unaware of treatment assignment).

Spontaneous object recognition task

The ORT was adapted from Ennaceur and Delacour (1988). Briefly, male Sprague-Dawley rats were acclimated to laboratory conditions (i.e., tail marking, daily handling, and weighing) for at least 3 days prior to experimentation. During experimentation, the animals were transported to the laboratory and acclimated for 30 min prior to initiating the experimental phase; the animals remained in the laboratory for 15 min following study completion.

Habituation—The animals were acclimated, weighed, and individually placed in a dimly lit (10 lux) training/testing environment (an opaque plastic chamber, 78.7 cm×39.4 cm×31.7 cm with bedding on the floor) for 10 min of chamber exploration. The ORT chamber was placed on a table positioned along the short wall of the laboratory. HVAC ventilation provided masking noise to reduce any extraneous background noise, and there were no room orienting cues or wall-mounted visual cues (except for the small B/W camera positioned above the ORT chamber). Bedding material was placed in the chamber prior to habituation

and allowed to become saturated with animal odors. Bedding remained in the chamber for experimental duration; any animal droppings were removed.

Training trial—Twenty-four hours after the habituation session, the animals were acclimated, weighed, and injected with test compound (drug or vehicle) and after the appropriate pretreatment interval, placed in the chamber with their nose facing the center of a long wall and allowed to explore two identical objects for 10 min. The animal's behavior was observed and recorded on videotape via a camera located 69 cm above the chamber; the investigator sat quietly 10–15 ft. away from the ORT chamber.

Test trial—After a retention delay interval of 3 or 48 h (a delay interval that produced recall or forgetting, respectively), the animals were returned to the laboratory, acclimated, and tested for object novelty (i.e., recognition memory). Two objects (one object identical to training (familiar) and a new novel object) were placed in the chamber, and the animal was allowed to explore the objects for 5 min. Experimental objects to be discriminated were a plastic multicolored Duplo-Lego block configured tower (12 cm in height, 6 cm in width) paired with a ceramic conical-shaped green Christmas tree salt/pepper shaker (12 cm in height, 5 cm in diameter); all objects existed in duplicate. The objects were placed 19.3 cm from the sides of the two short walls and 19.3 cm from the sides of the long walls of the chamber; distance between the two objects was approximately 40 cm. The role of familiar and novel object as well as chamber position of object was randomly assigned across subjects and treatments, and objects were cleaned between sessions with a dilute 50 % ETOH solution to eliminate olfactory cues. Object exploration occurred when the animal directed its nose to the object at a distance of 2 cm and/or touching it with its nose, rearing up against the object to investigate if the object was also considered exploration, whereas physically climbing on the object, using the object to support itself while rearing to investigate the chamber arena or digging at the base of the objects, was not considered appropriate object exploratory behavior. The primary behavioral measure was time (s) spent exploring both objects separately and calculated as the difference of exploration time between the novel object and familiar object during the test trial and defined as the recognition index (RI). For data inclusion, the rat had to emit at least 4 s of exploration on each object and obtain a total of at least 12 s of object exploration. Experimental groups contained eight rats per treatment which provided sufficient sample size to observe statistical significance. Animals were tested only once, and object exploration time was scored live under blind testing methods (i.e., the investigator was unaware of treatment assignment).

Drugs

Unless otherwise noted, all compounds were prepared in physiological saline (0.9 % NaCl) and injected in a volume of 1 ml/kg. Doses refer to the weight of the salt, except where noted. Drugs used, suppliers, pretreatment intervals, and route of administration were the following: apomorphine HCl (Sigma-Aldrich, St Louis, MO; 10 min, sc), clozapine free base (Memory Pharmaceuticals, Montvale, NJ; 30 min, intraperitoneally (ip)), GTS-21 2 HCl (Memory Pharmaceuticals; 60 min, orally (po), 2 ml/kg; 30 min, ip), haloperidol free base (Sigma-Aldrich; 30 min, ip), methyllycaconitine citrate (MLA; Tocris Bioscience,

Ellisville MO, 60 min, ip), and (+)-MK-801 maleate (Sigma-Aldrich; 30 min, ip (ORT); 10 min, sc (PPI)). Clozapine and haloperidol were dissolved in 0.1 N acetic acid, diluted with deionized water and the pH adjusted to ~5.0 with 0.1 N NaOH to the desired concentration.

Data analysis

For one and two factor comparisons, analysis of variance (with repeated measures when indicated) was used followed by the Student Newman Keuls or Dunnett's method (for comparisons with vehicle controls only) for post hoc analysis (SigmaPlot 11.2). All results are expressed as the mean ($\pm$ SEM). Differences between means from experimental groups were considered significant at the $p < 0.05$ level.

Results

Prepulse inhibition of acoustic startle

Administration of GTS-21 (1–10 mg/kg, ip) alone did not significantly alter the PPI response across the individual prepulse levels (Fig. 1a) or when PPI was averaged across the three (75, 80, and 85 dB) prepulse intensity levels (Fig. 1b). There was a significant main effect of prepulse intensity [$F(2, 56) = 86.74, p = 0.0001$] but no significant treatment $\times$ prepulse level interaction. GTS-21 startle amplitude responses were not significantly different from control (Fig. 1c).

In the MK-801-induced deficit PPI model (Fig. 2), there was a significant overall main effect of drug treatment [$F(5, 55) = 5.15, p = 0.0006$] and a significant effect across the individual prepulse levels [$F(2, 110) = 57.83, p < 0.0001$]. Statistical analysis also indicated a significant treatment $\times$ prepulse level interaction [$F(10, 110) = 2.03, p < 0.037$]. Post hoc comparisons indicated that MK-801 (0.1 mg/kg, sc) when given alone significantly diminished the PPI response at all three prepulse intensities. Clozapine (5 mg/kg, ip) significantly reversed the MK-801-induced PPI deficit demonstrating model validation. Pretreatment with GTS-21 (1–10 mg/kg, ip) prior to MK-801 significantly and dose-dependently ameliorated the PPI deficits. The improvement by GTS-21 was apparent across the individual prepulse levels (Fig. 2a) and when the data were averaged across the three prepulse levels (Fig. 2b). Examination of the drug treatments on startle amplitude (Fig. 2c) revealed a significant overall main effect of treatment ($F(5, 55) = 3.78, p = 0.005$). Post hoc analysis indicated that MK-801 alone resulted in a significant increase in startle reactivity compared with vehicle control. Combination of GTS-21 with MK-801 counteracted the MK-801-induced startle increase to vehicle levels. Startle reactivity following clozapine plus MK-801 was not significantly different from vehicle.

In the apomorphine-induced deficit PPI model (Fig. 3), there was a significant overall main effect of drug treatment [$F(5, 55) = 7.81, p < 0.0001$] and a significant effect across the individual prepulse levels [$F(2, 110) = 87.69, p < 0.0001$]. There was also a significant treatment $\times$ prepulse level interaction [$F(10, 110) = 2.10, p < 0.03$]. Post hoc comparisons indicated that apomorphine (0.5 mg/kg, sc) when given alone significantly diminished the PPI response. Haloperidol (0.3 mg/kg, ip) significantly reversed the apomorphine-induced PPI deficit demonstrating model validation. Pretreatment with GTS-21 (1–10 mg/kg, ip)

with apomorphine significantly reversed the PPI deficit. This improvement was apparent across the individual prepulse levels (Fig. 3a) as well as when the data were averaged (Fig. 3b). Startle reactivity across the various treatment groups was not significantly different from vehicle (Fig. 3c).

Object recognition memory

Oral administration of GTS-21 (0.1–10 mg/kg) prior to object recognition training produced a significant, dose-dependent increase in recognition memory when tested at the 48 h retention delay interval (an interval that produces complete forgetting) [$F(5, 42)=17.19$, $p<0.0001$]. Test doses of 0.3, 1, 3, and 10 mg/kg of GTS-21 were significantly different from vehicle and produced RI times of 11 to 18 s (Fig. 4a). Similar effects were also observed following intraperitoneal administration of GTS-21 [$F(3, 28)=17.42$, $p<0.0001$]. Test doses of 1 and 10 mg/kg were significantly different from vehicle and resulted in RI times of 10 to 17 s (Fig. 4b). Assessment of the total object exploration time during the 10 min training session after oral or intraperitoneal administration indicated nonsignificant treatment group differences. The average object exploration time after oral and intraperitoneal administration was 80.7 ± 7.2 and 88.8 ± 6.9 s, respectively. Total object exploration time during the 5 min test session was not significantly different across treatments, and the average exploration time for oral and intraperitoneal administration was 47.6 ± 4.2 and 44.1 ± 4.9 s, respectively.

To assess the specificity of $\alpha 7$ -nAChRs in mediating the procognitive effects of GTS-21, the subjects were administered the $\alpha 7$ -nAChR antagonist MLA (0.313–5 mg/kg, ip) in combination with GTS-21 (1 mg/kg, po) prior to object recognition training (Fig. 4c). MLA (1.25 and 5 mg/kg) dose-dependently antagonized the recognition memory enhancement effects of GTS-21 at the 48 h retention delay interval [$F(4, 31)=11.61$, $p<0.0001$]. Total object exploration time during the 10 min training session was not significantly different across treatments, and the average object exploration time was 83.8 ± 5.9 s. Exploration time during the 5 min test session was not significantly different with an average object exploration time of 40 ± 4.6 s. Administration of MLA (1 and 10 mg/kg, ip) alone at a 1 h retention delay interval did not significantly alter intact recognition memory (data not shown). RI times following vehicle and MLA 1 and 10 mg/kg were 19.6 ± 1.7 , 17.8 ± 2.6 , and 21.1 ± 2.7 s, respectively. Total object exploration time during the 10 min training session was not significantly different and resulted in an average object exploration time of 92.5 ± 6.3 s. Exploration time during the 5 min test session was not significantly different with an average object exploration time of 49 ± 5.1 s.

To determine the potential NMDA-glutamate receptor involvement in modulating the procognitive effects of GTS-21, we assessed the ability of GTS-21 to reverse the recognition memory impairment produced by MK-801 using a short (3 h) retention delay interval (where object recognition memory remains intact). MK-801 (0.08 mg/kg, ip) given prior to training resulted in a significant disruption of recognition memory formation (Fig. 4d). Pretreatment with GTS-21 (1–10 mg/kg, ip) dose-dependently reversed the MK-801-induced recognition memory deficit [$F(4, 35)=16.53$, $p<0.0001$]. Total object exploration time during the 10 min training session was not significantly different across treatments, and the average object

exploration time was 79.9 ± 6.8 s. Exploration time during the 5 min test session was not significantly different and resulted in an average object exploration time of 40.8 ± 3.6 s.

Discussion

In the present investigation, six main findings were observed: (1) in the PPI task, the $\alpha 7$ -nAChR partial agonist GTS-21 administered alone did not alter the PPI response or startle reactivity response in comparison with vehicle treated animals; (2) coadministration of GTS-21 with the NMDA-glutamate receptor antagonist MK-801 or the dopamine receptor agonist apomorphine abolished the pharmacologic-induced PPI impairment producing effects similar to the antipsychotics clozapine and haloperidol, respectively; (3) MK-801, but not apomorphine, resulted in a significant increase in startle reactivity when given alone, and GTS-21 in combination with MK-801 counteracted this startle increase; startle amplitude following coadministration of the antipsychotics with MK-801 or apomorphine was not different from control values; (4) in the ORT, GTS-21 completely reversed the MK-801-induced memory deficit at a short 3 h delay interval when object recognition memory is intact in control animals; (5) GTS-21 enhanced long-term recognition memory following the 48 h delay interval when complete forgetting occurs; and (6) the procognitive effects of GTS-21 were completely reversed by the antagonist MLA suggesting $\alpha 7$ -nAChR specificity in the response.

Disturbances in information processing and the inability to attend appropriately to incoming sensory stimuli have been widely confirmed in schizophrenia (Adler et al. 1998; Braff et al. 2001). This sensory overload has been associated with deficits in attention, cognitive processing, and daily living (Braff and Light 2004). PPI is a cross-species gating paradigm that involves both sensory stimuli and motor responses (i.e., sensorimotor gating) and involves a number of neural substrates (e.g., cortical, striatal, and hippocampal) deficient in schizophrenia (Kumari et al. 2003; Swerdlow et al. 2001). Utilization of the PPI pharmacologic-induced model (e.g., dopamine agonists and NMDA receptor antagonists) is based on the premise that dopaminergic tone is often elevated in patients with schizophrenia (Kapur and Mamo 2003) and that glutamatergic (NMDA) receptor function is depressed (Synder and Gao 2013). In the present study, the $\alpha 7$ -nAChR partial agonist GTS-21 (Kem 2000) when given alone was without effect on PPI or startle reactivity; however, GTS-21 administration ameliorated the apomorphine- and MK-801-induced PPI disruption. The GTS-21 effects were dose-dependent across the individual prepulse intensity levels (75, 80, and 85 dB) as well as when the prepulse levels were averaged. Interestingly, startle amplitude following MK-801 alone, but not apomorphine, was increased and GTS-21 counteracted this MK-801 startle response. Increases in startle magnitude following NMDA receptor antagonists have previously been reported (Larrauri and Levin 2012; Levin et al. 2007; Mansbach and Geyer 1989) and may represent a “drug class” effect. Since GTS-21 alone did not affect startle amplitude or PPI responses, it seems unlikely that the GTS-21 effects on reversing the MK-801-induced PPI deficit were simply due to behavioral artifact or re-normalization of MK-801’s startle baseline. In reviewing the GTS-21 plus MK-801 PPI and startle alone effects, test doses of 3 and 10 mg/kg of GTS-21 reversed the MK-801 PPI deficit whereas only the 10-mg/kg dose of GTS-21 reduced the MK-801 increased startle response. Moreover, it has been suggested (Larrauri and Levin 2012; Levin et al.

2007) that a drug's effect on startle amplitude and PPI may be distinct events and that the interpretation of pharmacological specificity on PPI responding might only be in question if the test drug results in reductions in startle amplitude, which was not the case for GTS-21.

Studies involving $\alpha 7$ -nAChR agonists (e.g., RG-3487, SEN-12333, TC-5619, and tropisetron) have routinely demonstrated positive effects in their ability to attenuate apomorphine-induced PPI disruption (Hauser et al. 2009; Kohnomi et al. 2010; Roncarati et al. 2009; Wallace et al. 2011); however, fewer PPI studies have shown consistent effects (Dunlop et al. 2009; Ghiron et al. 2010, but see Kohnomi et al. 2010) with $\alpha 7$ -nAChR agonist-induced modulation of NMDA receptor antagonists. Our results demonstrated a robust effect of GTS-21 on reversing the MK-801 PPI deficit in a manner similar to that of the antipsychotic clozapine (Levin et al. 2007; Mansbach et al. 2001; Terry et al. 2006) thereby strengthening the observations that $\alpha 7$ -nAChR agonists can modulate depressed NMDA receptor function. Additionally, the effects of $\alpha 7$ -nAChR agonists on sensori-motor gating appear to be receptor specific as the improved PPI response is attenuated by the $\alpha 7$ -nAChR antagonist MLA (Kohnomi et al. 2010).

The present effects with GTS-21 (as well as other $\alpha 7$ -nAChR agonists) are interesting in light of reports that nicotine normalizes sensorimotor gating deficits in schizophrenic patients (Hong et al. 2008; Kumari et al. 2001), an effect blocked by the nonselective nAChR antagonist mecamylamine implicating nAChRs and potentially $\alpha 7$ -nAChRs (George et al. 2006). While the sensorimotor effects of $\alpha 7$ -nAChR agonists have not been directly assessed in schizophrenics, GTS-21 and tropisetron have reversed the P50 auditory-evoked sensory gating deficits in this population (Koike et al. 2005; Olincy et al. 2006; Shiina et al. 2010; Zhang et al. 2012). There is no evidence of a causal relationship between sensory gating response and cognitive dysfunction (Swerdlow et al. 2006), however PPI improvements in schizophrenics following nicotine appear to correlate with improved prefrontal executive function (Rabin et al. 2009). Nicotinic $\alpha 7$ agonists (e.g., GTS-21 and tropisetron) administered to schizophrenics have led to improvements in both sensory gating and cognitive function (Koike et al. 2005; Olincy et al. 2006; Shiina et al. 2010; Zhang et al. 2012) and therefore, PPI might serve as a translational research tool to assess potential treatment strategies for cognitive deficits associated with schizophrenia.

Visual learning and memory deficits are also observed in schizophrenic patients (Aleman et al. 1999; Ranganath et al. 2008), and the ORT has been recommended by the TURNs and MATRICS groups as a potential translational preclinical behavioral paradigm to evaluate "episodic-like" memory and screen for putative procognitive therapeutics (Lyon et al. 2012; Young et al. 2009). The ORT is a rodent model of (nonspatial) recognition memory, which by definition consists of two components, a recollective (episodic) component and a familiarity component (Squire et al. 2004). While debated, evidence suggests that the perirhinal cortex and hippocampus are involved in recognition memory of rodents (Ainge et al. 2006; Barker et al. 2007; Clark et al. 2000) and humans (Buffalo et al. 2006; Reed and Squire 1997), including schizophrenic patients (Exner et al. 2008; Turetsky et al. 2003). Our object recognition results demonstrated that GTS-21 was capable of reversing spontaneous long-term (48 h) recognition memory deficits as well as short-term (3 h) deficits following acute MK-801 administration. The long-term memory enhancing effects of GTS-21 were

dose-dependently reversed by MLA suggesting $\alpha 7$ -nAChR specificity in mediating the response. As a drug class, $\alpha 7$ -nAChR agonists have produced robust “stand-alone” improvements on recognition memory to include the enhancement of information encoding during acquisition (drug given prior to training session) as well as memory consolidation/storage (drug given immediately after training session; see Prickaerts et al. 2012; Wallace et al. 2011). In addition, $\alpha 7$ -nAChR agonists have been shown to reverse pharmacologic (scopolamine and/or NMDA receptor antagonists)-induced recognition memory impairments at short delays (e.g., 1–3 h) when recognition memory is intact in control subjects (present results; Ghiron et al. 2010; Hashimoto et al. 2006, 2008; Pichat et al. 2007; Prickaerts et al. 2012; Roncarati et al. 2009). Typically pharmacologic-induced impairment models are used to represent a particular disease state (eg., scopolamine-Alzheimer’s disease and NMDA receptor antagonist-schizophrenia) and/or to ascertain the underlying mechanism(s) of action associated with the test compound. The results from these preclinical studies need to be interpreted with caution, particularly as it relates to clinical translation. For example, while $\alpha 7$ -nAChR agonists are capable of reversing pharmacologic-induced memory deficits (as are antipsychotic medications; Neill et al. 2010), $\alpha 7$ -nAChR agonists also produce “stand-alone” memory enhancing effects (whereas antipsychotic medications do not, Callahan unpublished observations; Terry et al. 2007). This distinction becomes important as we determine the predictive validity of the ORT and its particular relevance for discovering therapeutics for cognitive dysfunction in schizophrenia.

Initial clinical studies with the $\alpha 7$ -nAChR agonists GTS-21 (Kitagawa et al. 2003), RG 3487 (MEM 3454, Callahan et al. 2006), and EVP-6124 (Hilt et al. 2009) in normal human volunteers showed encouraging positive effects on episodic recognition memory as well as executive function from different cognitive test batteries (i.e., CogState and Cognitive Drug Research (CDR) Battery). These procognitive effects were extended with GTS-21 in schizophrenic patients using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS, Olincy et al. 2006) and in mild to moderate Alzheimer’s disease patients with RG 3487 using the CDR battery (Wallace and Porter 2011) and with EVP-6124 using either CogState or ADAS-Cog-13 (www.Envivopharma.com). Unfortunately, $\alpha 7$ -nAChR agonists (e.g., EVP-6124, GTS-21, RG 3487, TC-5619, and tropisetron) have produced mixed results in larger population studies with schizophrenic patients (Freedman et al. 2008; Lieberman et al. 2013; Meltzer et al. 2011; Shiina et al. 2010; Umbricht et al. 2009; Zhang et al. 2012). Overall, there appears to be therapeutic potential for this drug class in modulating cognitive function, but achieving a positive primary outcome depends greatly on the therapeutic doses and cognitive test batteries that are administered (e.g., RBANS, CDR, CogState, or MATRICS Consensus Cognitive Battery). Interestingly, $\alpha 7$ -nAChR agonists have also shown promising effects on reducing the negative symptoms associated with schizophrenia (as measured by the Scale for the Assessment of Negative Symptoms (SANS) and the Positive and Negative Syndrome Scale (PANSS); Freedman et al. 2008; Lieberman et al. 2013; Meltzer et al. 2011; Noroozian et al. 2013; Umbricht et al. 2009), although a recent phase 2b with TC-5619 failed to replicate earlier findings (www.Targacept.com). To date, the $\alpha 7$ -nAChR agonist EVP-6124 is proceeding in phase 3 clinical development for the treatment of cognitive impairment and reduction of the negative symptoms associated with schizophrenia (www.

Envivopharma.com). We will have to wait for these results to determine any beneficial effect of $\alpha 7$ -nAChR agonists on schizophrenia.

In summary, the present results extend our pharmacological understanding of the $\alpha 7$ -nAChR agonist GTS-21 in its ability to improve sensorimotor gating in established pharmacologic models of impaired PPI as well as its ability to produce procognitive effects on short- and long-term object recognition memory. Each of these preclinical behavioral paradigms has scientific merit to be used as translational research tools to develop new pharmacological treatments for schizophrenia. Moreover, considering the substantial positive preclinical evidence with $\alpha 7$ -nAChR agonists along with the encouraging clinical data, activation of the $\alpha 7$ -nAChR via an agonist action warrants further investigation as potential therapeutic options for schizophrenia and other disease states with disrupted cognition.

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Fig. 1.

a Effects of GTS-21 (1–10 mg/kg, ip) administered alone on PPI across the individual prepulse intensities (75, 80, and 85 dB). **b** Effects of GTS-21 on PPI averaged across the prepulse intensity levels. **c** Effects of saline and GTS-21 on startle amplitude. Bars represent the mean ($\pm$ SEM) values for each treatment ($N=8$ rats/treatment). AU arbitrary units, VEH vehicle, GTS GTS-21

Fig. 2.

a Effects of MK-801 (0.1 mg/kg, ip) alone and in combination with GTS-21 (1–10 mg/kg, ip) on MK-801-induced PPI deficits across the individual prepulse intensities (75, 80, and 85 dB). **b** Effects of GTS-21 on MK-801-induced PPI deficits averaged across the prepulse intensity levels. **c** Effects of drug treatments on startle amplitude. Bars represent mean ($\pm$ SEM) values for each treatment ($N=9-12$ rats/treatment). AU arbitrary units, VEH vehicle, MK MK-801, CLOZ clozapine, GTS GTS-21. Asterisk indicates significantly different ($p<0.05$) from vehicle. Plus sign indicates significantly different ($p<0.05$) from MK-801

Fig. 3.

a Effects of apomorphine (0.5 mg/kg, sc) alone and in combination with of GTS-21 (1–10 mg/kg, ip) on apomorphine-induced PPI deficits across the individual prepulse intensities (75, 80, and 85 dB). **b** Effects of GTS-21 on apomorphine-induced PPI deficits averaged across the prepulse intensity levels. **c** Effects of drug treatments on startle amplitude. Bars represent mean ($\pm$ SEM) values for each treatment ($N=8-13$ rats/treatment). AU arbitrary units, VEH vehicle, APO apomorphine, HAL haloperidol, GTS GTS-21. Asterisk indicates significantly different ($p<0.05$) from vehicle. Plus sign indicates significantly different ($p<0.05$) from apomorphine

Fig. 4.

Effects of GTS-21 (0.1–10 mg/kg) administered either orally (**a**) or intraperitoneally (**b**) on object recognition memory assessed at a 48 h retention delay interval. **c** Effects of the $\alpha 7$ -nAChR receptor antagonist MLA (0.313–5 mg/kg, ip) in combination with GTS-21 (1 mg/kg, po) at a 48 h delay interval. **d** Effects of MK-801 (0.08 mg/kg, ip) in combination with GTS-21 (1–10 mg/kg, ip) on object recognition memory at a 3 h delay interval. Bars represent the mean ($\pm$ SEM) values for each treatment ($N=8$ rats/treatment). Asterisk indicates significantly different ($p<0.05$) from vehicle response. Plus sign indicates significantly different ($p<0.05$) from GTS-21 response. Number sign indicates significantly different ($p<0.05$) from MK-801 response