Pharmacology, Biochemistry and Behavior 95 (2010) 146–157

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

ABT-594 improves performance in the 5-choice serial reaction time task under conditions of increased difficulty, sub-chronic dosing, and in poorly-performing subjects

Eric G. Mohler [a,*], Stanley R. Franklin [a], Lynne E. Rueter [a], Gerard B. Fox [b], Michael W. Decker [a], Kaitlin E. Browman [a]

[a] Neuroscience Research, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6115, United States
[b] Experimental Imaging, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6115, United States

ARTICLE INFO

Article history:
Received 10 July 2009
Received in revised form 8 December 2009
Accepted 23 December 2009
Available online 11 January 2010

Keywords:
Acetylcholine
Nicotine
Attention
α4β2
5-Choice serial reaction time task

ABSTRACT

Several studies have tested nicotinic receptor ligands in the 5-Choice Serial Reaction Time Task (5-CSRTT) with varying results. Some investigators have increased attentional demands by modifying task parameters or using aged or poor performing rats to observe treatment effects. This study examined the α4β2 nicotinic agonist ABT-594 in the 5-CSRTT using a variety of manipulations to determine optimal conditions for observing enhancement. ABT-594 had no effect in drug-naïve adult rats that self-initiated trials. Constant trial presentation decreased accuracy and omissions, with the latter significantly attenuated by acute administration of ABT-594 (0.019–0.062 μmol/kg). Sub-chronic treatment (0.019 μmol/kg) initially impaired drug-naïve subjects, but significant improvements in accuracy and decreased omissions were observed after 5 days of dosing. In 18–22 month-old rats, attentional demands were altered by interspersing blocks of trials with different stimulus durations. Acute ABT-594 (0.062 μmol/kg) enhanced accuracy performance in poor performing rats (<70% accuracy) but not in those that performed well (>80% accuracy), while omissions were decreased in both groups. Sub-chronic treatment with (0.019 μmol/kg) decreased omissions in all rats, but enhanced accuracy primarily in poor performing rats. These experiments demonstrate that an α4β2 nicotinic agonist can enhance attention, but accuracy effects may only be observed under specific conditions. Moreover, a reduction in omissions was more reliably observed than improvements in accuracy, resulting in a net increase in signals successfully detected.

1. Introduction

Deficits in attention are observed in a variety of disorders of the central nervous system, such as Attention Deficit Hyperactivity Disorder (ADHD), schizophrenia, and Alzheimer's disease (Allain et al., 2007). Epidemiological studies indicate greater instances of tobacco smoking in schizophrenic and ADHD patients, possibly as a form of self-medication (Newhouse et al., 2004). In controlled studies, therapeutic effects of nicotine on attention have been reported in Alzheimer's disease (Jones et al., 1992; White and Levin, 1999), schizophrenia (Rezvani and Levin, 2001), and ADHD (Levin et al., 1996). Accordingly, several nicotinic receptor ligands are in development for the treatment of Alzheimer's disease, schizophrenia, and ADHD (Taly et al., 2009). In order to investigate improvements in attention observed with nicotine and nicotinic receptor ligands, several studies have used rodent attention tasks, such as the 5-Choice Serial Reaction Time Task (5-CSRTT) (Carli et al., 1983) or the Sustained Attention Task (McGaughy and Sarter, 1995; Bushnell et al., 1997). These studies have demonstrated varying degrees of improvement in performance, often depending upon specific task parameters, treatment schedules, and even the strain of rats. For example, improvements in attention with nicotine have been noted by increasing task demands with weak signals or altered inter-trial intervals (Mirza and Stolerman, 1998; Hahn et al., 2002), constant rather than subject-paced trials (Bizarro et al., 2004; Day et al., 2007), various rat strains (Mirza and Bright, 2001) and/or chronic treatment (Semenova et al., 2007). These effects of nicotine do not appear to be merely modulating motivation for performing the 5-CSRTT, as the use of various feeding schedules prior to testing yields a different pattern of results (Bizarro and Stolerman, 2003). In the Sustained Attention Task, nicotine improved performance on non-signal trials at low doses (Rezvani et al., 2002), but not high doses (Turchi et al., 1995; Rezvani et al., 2002).

Further complicating the understanding of the effects of nicotine on attention is the possibility that effects may be the result of activity at multiple receptor subtypes. To more specifically determine the receptor subtypes that mediate the effects of nicotine, other recent studies have used agonists and antagonists selective for the α4β2 and α7 nicotinic receptors. In general, these studies have found a greater role for α4β2 receptors relative to α7 receptors for nicotine-induced

* Corresponding author. Neuroscience Research, Dept 4N5, Bldg AP9A-LL, 100 Abbott Park Rd, Abbott Park, IL 60064-6115, United States. Tel.: +1 847 936 5836; fax: +1 847 938 0072.
E-mail address: Eric.Mohler@abbott.com (E.G. Mohler).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.12.019

enhancements in the 5-CSRTT. For example, treatment with the α4β2 agonist SIB 1765F, but not the α7 agonist AR-R 17779, significantly improved attention (Grottick and Higgins, 2000). In a separate study, the α4β2 agonist epibatidine, but not the α7 agonist AR-R 17779 improved 5-CSRTT performance (Hahn et al., 2003). Dihydro-β-erythroidine (DHβE) selectivity inhibits β2 sub-units compared to α7 receptors (Jensen et al., 2005) and accordingly the enhancing effects of nicotine were blocked with DHβE but not the α7 antagonist methyllycaconitine (Blondel et al., 2000; Grottick et al., 2003). However, a role for α7 receptors in attention has been suggested by experiments testing α7 knock-out mice in the 5-CSRTT (Young et al., 2004). Furthermore, the α7 agonist/5-HT_3 antagonist MEM3454 improved performance on signal trials, but not non-signal trials in the Sustained Attention Task, although a role for 5-HT_3 cannot be completely excluded (Rezvani et al., 2009). Additional studies with other compounds may clarify the extent to which α7 receptors may contribute to attention.

While α4β2 receptor agonists have been shown to improve attention performance in multiple measures, such as accuracy, omissions, anticipatory responses, and latency to correct responses, these effects have not been consistent. For example, beneficial effects of SIB 1765F were only noted in poor performing rats receiving sub-chronic treatment (Grottick and Higgins, 2000) or in aged rats greater than 24 months old (Grottick et al., 2001). Epibatidine resulted in enhanced accuracy throughout a session but the α4β2 agonist ABT-418 only improved 5-CSRTT performance during the first 10 min (Hahn et al., 2003), or improved SAT accuracy in intact but not basal forebrain lesioned rats (McGaughy et al., 1999). In order to understand the nature of enhancement via the α4β2 nicotinic receptor, we investigated the effects of ABT-594, a potent α4β2 agonist with some α3β4 activity (see Donnelly-Roberts et al., 1998) in the 5-CSRTT under a variety of different parameters. ABT-594 has previously demonstrated significant cognition enhancing effects in a monkey delayed-match-to-sample paradigm under normal and distractor conditions (Buccafusco et al., 2007). In contrast to previous studies investigating α4β2 agonists that used different compounds and different testing conditions which varied from investigator to investigator, we used the same α4β2 agonist while systematically manipulating different parameters in order to determine the optimal conditions in which enhancement of attention could be observed. As many of the studies characterizing potential cognitive enhancers that act at the α4β2 receptor have used a variety of different 5-CSRTT paradigms to varying degrees of success, we were interested in determining optimum conditions for identifying cognition enhancing molecules with the 5-CSRTT in a drug discovery environment.

Performance was initially assessed with the standard version of the 5-CSRTT (e.g., Carli et al., 1983) in adult rats (9–11 months of age), and then task difficulty was increased by switching rats from self-initiated to constantly presented trials (e.g., Bizarro et al., 2004). Due to reported differential effects of acute and chronic nicotine (e.g., Semenova et al., 2007), the effects of five days of treatment were examined with constant trial presentation. Rats trained with the self-initiated trials paradigm and tested daily in the constant trial presentation version eventually adapt to the latter procedure, making it difficult to observe treatment effects (unpublished observations). Therefore, a group of rats was trained and tested entirely with constant trial presentation. However, in a previous study with rats trained from the start using constant trial presentation, significant effects of nicotine were observed initially, but this effect was not as robust with subsequent testing (Day et al., 2007). Because some experiments with nicotine have observed effects more readily in aged subjects or those that perform the task poorly (Grottick and Higgins, 2002; Semenova et al., 2007), the effects of ABT-594 were next investigated in 18–22 month-old rats. As previous studies have observed differential effects under variable stimulus durations (e.g., Stolerman et al., 2000), poor and good performing rats were compared to investigate susceptibility to changes in stimulus properties. Finally, older rats received sub-chronic dosing with ABT-594 (two days of pre-treatment and five days of testing) to reduce the potential for performance decrements observed in the previous sub-chronic study.

2. Methods

All studies were carried out in accordance with the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals adopted by the U.S. National Institutes of Health in a laboratory accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.1. Subjects

Male hooded Lister rats were obtained from Charles River Labs (UK) and housed individually in a temperature and humidity-controlled environment on a 12-h light–dark cycle. The age of rats at testing was 9–11 months in Experiments 1–3, and 18–22 months in Experiments 4–5. Body weights at test ranged from 440 to 515 g and daily food intake was restricted to maintain these 85% free feeding levels. Water was available in home cages without restriction.

2.2. Apparatus

Eight 5-choice serial reaction time chambers, controller hardware, and software were obtained from Cambridge Cognition (through Lafayette Instruments, Lafayette IN, USA). Each chamber (interior dimensions of 26×26×26 cm) was housed in a wooden sound-attenuating enclosure equipped with a ventilation fan and micro-camera to allow observation of subject without disruption. The rear wall of each chamber was concavely curved and contained 9 apertures, each 2.5 cm square, 3 cm deep, and 2 cm above the wire mesh floor. Only 5 of the apertures were used in these experiments, with intervening holes covered with metal inserts. An illuminated green LED located at the rear of each aperture served as the discriminative stimulus. 45-mg rodent food pellets (TestDiet, Richmond IN, USA) were delivered to the food magazine in the wall opposite the light stimuli. This delivery magazine was equipped with a clear Plexiglas panel that the rat pushed to obtain food, activating a switch that registered the food retrieval.

2.3. Training procedures

Five experiments were conducted. The first three experiments used the subject-initiated protocol originally described by Carli et al. (1983). Rats in Experiments 4 and 5 were trained and tested under a procedure similar to that described by Hahn et al. (2003) in which trials were constantly presented.

2.3.1. Subject-initiated trial protocol

Rats were trained to respond to a 0.5-s visual stimulus presented randomly in one of the five stimulus apertures. Each session began with house light illumination and delivery of a single food pellet to the food magazine. Each trial was initiated by the rat pushing the Plexiglas panel to either collect a pellet or restart the trial sequence after a timeout period, resulting in illumination of one of the 5 stimulus lights for 0.5 s following a 5-s inter-trial interval (ITI). Timeout periods (house light off, no trial initiation) lasted 5 s and occurred (1) following failure to respond within 5 s of stimulus offset, (2) after incorrect hole choice, and (3) following premature responses (aperture nose pokes during ITIs). Inappropriate responses of the first type above were identified as "omissions", the second type "incorrect choice responses", and the third type "premature responses". Rats were trained to nose poke into the lighted, or recently lit, aperture beginning with a 60-s lighting of single aperture followed by a non-cued, 60-s period (limited hold period) in

which any nose poke into that aperture during either period produced a food pellet. Rats were advanced through a series of reductions of the trial lights until they reached the 0.5-s stimulus duration (SD) and met performance requirements of greater than 80% correct response and fewer than 20% omitted trials. Failure to respond during either the SD period or the subsequent limited hold period was registered as an omission error and punished by a 5-s timeout (house light off, no response effects). A nose poke in one of the 4 unlit holes (incorrect response) also resulted in a 5-s timeout. During each session the light stimulus was presented an equal number of times in each of the five holes in a random order. Daily sessions lasted either 30 min or until 100 trials were completed. Drug test sessions were extended to 160 trials where noted.

2.3.2. Constant trial presentation protocol

In general, training is similar to the procedure described above, with the exception that trials were initiated automatically 5 s following food presentations or timeouts. In addition, 5-s timeout periods were only initiated by an incorrect response during the SD or limited hold period. Neither omission errors nor ITI responses were punished with timeouts. The SD was 0.5 s except in Experiment 4 where the stimulus duration was varied within a session as indicated below. Sessions lasted 30 min or ended with completion of 100 or 160 trials, as indicated below.

2.4. Drug preparation and administration

ABT-594 (Holladay et al., 1998) with p-Toluenesulfonic Acid salt was synthesized at Abbott Laboratories. Doses of 0.0062, 0.019, or 0.062 μmol/kg (2.3 μg/kg, 7 μg/kg, or 23 μg/kg as the weight of salt) were mixed in sterile saline. Rats received a 1 ml/kg injection 30 min before testing. In all acute experiments (Experiments 1, 2, and 4), rats received 3 doses of ABT-594 and vehicle according to a counter-balanced schedule. In the two sub-chronic experiments, rats received vehicle or ABT-594 (0.019 μmol/kg) for 5 days (Experiment 3) or 7 days (Experiment 5). In Experiment 5, after a two-week washout, rats received the opposite treatment for 7 days.

2.5. Testing procedures

2.5.1. Experiment 1: standard 5-CSRTT

Rats (9 months old) were trained and tested with the standard version of the 5-CSRTT with self-initiation of trials. Rats were administered ABT-594 (0.0062, 0.019, or 0.062 μmol/kg) or vehicle acutely 30 min before a testing session according to a counter-balanced schedule ($n=18$). Test sessions lasted for 160 trials or 30 min, whichever occurred first. Rats were tested with drug or vehicle two times per week and received daily maintenance training for 3 other days per week (rats were not trained on weekends). Rats received approximately 70 days of training before drug testing was initiated. Rats met baseline criteria of >80% accuracy and less than 20% omissions prior to each drug session.

2.5.2. Experiment 2: constant trial presentation

This experiment used the same rats as Experiment 1 after a two-week washout period. Rats received daily training sessions with the standard version of the 5-CSRTT except during drug sessions, when the non-subject-initiated protocol was used. Rats were acutely administered ABT-594 (0.0062, 0.019, or 0.062 μmol/kg) or vehicle 30 min before a testing session according to a counter-balanced schedule ($n=19$). Test sessions lasted for 160 trials or 30 min, whichever occurred first, and were conducted two days per week. Rats received daily maintenance training with the standard version of the 5-CSRTT for 3 other days per week (rats were not trained on weekends). Rats met baseline criteria of >70% accuracy and less than 25% omissions prior to each drug session.

2.5.3. Experiment 3: constant trial presentation and sub-chronic dosing

Rats (11 months old) that had not previously been administered ABT-594 received sub-chronic treatment with a 0.019 μmol/kg dose ($n=12$) or vehicle ($n=11$) 30 min before a testing session for 5 consecutive days. These rats were initially trained on the standard version of the 5-CSRTT with self-initiation of trials, but were tested with ABT-594 on the non-subject-initiated protocol. Rats received approximately 75 days of training before drug testing was initiated. Rats met baseline criteria of >70% accuracy and less than 25% omissions prior to sub-chronic treatment.

2.5.4. Experiment 4: good and poor performing rats with multiple stimulus durations

As rats trained with the self-initiated trials paradigm and tested daily in the constant trial presentation version eventually adapt to the latter procedure, rats (18–22 months of age) were trained exclusively on the non-subject-initiated protocol periodically over 14 months. These rats had received ABT-594 in other studies and were tested in this experiment after a minimum washout period of 2 weeks. Due to a bimodal distribution of accuracy performance, rats were separated into "Poor" ($n=12$) and "Good" ($n=12$) performing groups with a median split. The sensitivity of "Poor" and "Good" rats to alterations in attention demands during test sessions was investigated in a paradigm in which the SD was varied per block of 20 trials as follows: Block 1 SD was 500 ms; Block 2 SD was 400 ms; Block 3 SD was 750 ms; Block 4 SD was 1000 ms; Block 5 SD was 500 ms. Rats were administered ABT-594 (0.0062, 0.019, or 0.062 μmol/kg) or vehicle acutely 30 min before a testing session according to a counter-balanced schedule two days per week. Rats received daily maintenance training with the standard non-subject-initiated version of the 5-CSRTT (with SD = 500 ms) for 3 other days per week (rats were not trained on weekends). Rats met baseline criteria of less than 25% omissions (no criteria for accuracy due to the inclusion of poor performing rats).

2.5.5. Experiment 5: sub-chronic dosing in good and poor performing rats

Rats previously tested in Experiment 4 (plus 1 additional "Poor" rat and 1 additional "Good" rat) received sub-chronic treatment with ABT-594 (0.019 μmol/kg) or vehicle for 7 days ("Poor" $n=13$ and "Good" $n=13$). No training occurred during the first two days of treatment, and then rats received ABT-594 or vehicle 30 min before a test session using the non-subject-initiated protocol for 5 consecutive days. After a two-week washout, rats received the opposite treatment for 7 days. At the time of testing, rats had received periodic training over the course of 16 months. Rats met baseline criteria of less than 25% omissions (no criteria for accuracy due to the inclusion of poor performing rats).

2.6. Data analysis

The major response measures of interest were: accuracy (percentage of correct responses), computed as $100\times[\text{correct responses}/(\text{correct}+\text{incorrect responses})]$; omission errors (the percentage of trials in which no response is made during or immediately following the SD); premature responses (the number of nose pokes into any stimulus aperture during inter-trial intervals); perseverative responses (the number of nose pokes into any stimulus aperture after a correct response and before collecting a food reward); the latency to correct response (the mean time between SD onset and a nose poke into the correct hole); the latency to incorrect response (the mean time between SD onset and a nose poke into an incorrect hole); the latency to collect reward (the mean time between a correct response and a nose poke into food hopper); number of correct responses and number of incorrect responses. Dose–response studies were analyzed with repeated-measures analysis of variance (ANOVA) followed by post-hoc testing using Dunnett's multiple comparison test. Sub-chronic studies were analyzed

Table 1
Acute effects of ABT-594 on task performance in naïve rats with the self-initiated trials version of the 5-CSRTT (Experiment 1, $n=18$). Results are expressed as means ± SEM (**$P<0.01$ vs. vehicle with Dunnett's multiple comparison tests).

	Vehicle	0.0062 μmol/kg	0.019 μmol/kg	0.062 μmol/kg
Accuracy (%)	81.34 ± 1.71	81.11 ± 2.21	78.19 ± 2.31	77.0 ± 2.68
Omissions (%)	7.0 ± 1.49	7.0 ± 1.21	7.70 ± 1.03	9.13 ± 1.87
# Premature responses	11.89 ± 2.12	7.72 ± 1.27	10.0 ± 1.85	12.89 ± 3.52
# Perseverative responses	14.0 ± 2.14	12.39 ± 2.70	14.33 ± 2.94	13.72 ± 3.70
Correct response latency (s)	0.53 ± 0.07	0.54 ± 0.11	0.54 ± 0.13	0.54 ± 0.03
Incorrect response latency (s)	1.62 ± 0.15	1.61 ± 0.08	1.62 ± 0.09	1.23 ± 0.09**
Reward collection latency (s)	1.2 ± 0.04	1.26 ± 0.05	1.27 ± 0.05	1.39 ± 0.06**
# Correct responses	115.6 ± 4.99	118.7 ± 4.80	109.9 ± 5.02	88.11 ± 7.79**
# Incorrect responses	26.11 ± 2.45	26.89 ± 2.880	29.83 ± 2.86	25.44 ± 2.83

with repeated-measures ANOVA with day and drug as separate repeated factors. In this case Bonferroni comparisons were used for post-hoc analysis.

3. Results

3.1. Experiment 1: standard 5-CSRTT

Naïve rats tested in the standard 5-CSRTT did not demonstrate any significant effects of treatment with ABT-594 on measures of accuracy, omissions, premature responses, perseverative responses, or latency to correct response, P's >0.05 (see Table 1). Significant effects of treatment were observed for latency to incorrect response, $F(3, 71) = 3.804$, $P<0.05$, and latency to collect rewards, $F(3, 71) = 12.83$, $P<0.0001$. Post-hoc comparisons found that after receiving the 0.062 μmol/kg dose of ABT-594, rats had significantly shorter latencies to respond on incorrect trials, $P<0.05$, and significantly longer latencies to collect reward, $P<0.001$, compared to vehicle. When the number of correct and incorrect responses was examined, there was a significant effect of treatment, $F(3, 71) = 9.565$, $P<0.0001$, on correct responses and no effect on incorrect responses, $P>0.05$. A post-hoc comparison found that rats completed significantly fewer correct trials after receiving the 0.062 μmol/kg dose compared to vehicle treatment, $P<0.01$. One rat was not included in the analysis because it failed to respond after receiving the 0.062 μmol/kg dose of ABT-594.

3.2. Experiment 2: constant trial presentation

After a 2-week washout, the same rats were tested using the constant trial presentation paradigm. In general, constant trial presentation decreased accuracy and the number of correct responses, and increased omissions, premature responses, and the latency to correct response (see Table 2). No treatment effects were noted on accuracy, premature responses, perseverative responses, or latencies for correct or incorrect responses, P's >0.05. Treatment with ABT-594 significantly decreased the number of omissions, $F(3, 75) = 2.850$, $P<0.005$, and post-hoc tests found that rats omitted significantly fewer trials after receiving the 0.019 and 0.062 μmol/kg doses of ABT-594 compared to vehicle. A significant increase in the latency to collect food reward was also observed, $F(3, 75) = 8.023$, $P<0.0001$, primarily a result of the 0.062 μmol/kg doses of ABT-594 ($P<0.001$ vs. controls). When the number of correct responses was examined, there was a significant effect of treatment, $F(3, 75) = 3.633$, $P<0.05$. A post-hoc test found that the 0.019 and 0.062 μmol/kg doses of ABT-594 resulted in significantly more correct responses compared to vehicle treatment, $P<0.01$ and $P<0.05$, respectively. In contrast, no effects were observed on the number of incorrect responses, $P>0.05$.

3.3. Experiment 3: constant trial presentation and sub-chronic dosing

A separate set of rats originally trained on the standard paradigm and completely naïve to ABT-594 treatment was administered the 0.019 μmol/kg dose for 5 consecutive days while being tested on the constant trial presentation version of the 5-CSRTT (Fig. 1). A significant main effect of treatment was only observed for latency to collect reward, $F(1, 21) = 4.801$, $P<0.05$, all other measures $P>0.05$. Significant effects of day were observed for accuracy, $F(4, 84) = 5.937$, $P<0.001$; omissions, $F(4, 84) = 8.016$, $P<0.001$; perseverative responses, $F(4, 84) = 2.610$, $P<0.05$; latency to collect reward, $F(4, 84) = 2.572$, $P<0.05$; number of correct responses, $F(4, 84) = 9.713$, $P<0.001$; and number of incorrect responses, $F(4, 84) = 2.504$, $P<0.05$, all other measures $P>0.05$. In addition, significant treatment by day interactions were found for accuracy, $F(4, 84) = 11.09$, $P<0.001$; omissions, $F(4, 84) = 13.48$, $P<0.001$; perseverative responses, $F(4, 84) = 2.847$, $P<0.05$; latency to correct choice, $F(4, 84) = 4.35$, $P<0.005$; latency to collect reward, $F(4, 84) = 2.525$, $P<0.05$; number of correct responses, $F(4, 84) = 18.64$, $P<0.001$; and number of incorrect responses, $F(4, 84) = 5.184$, $P<0.001$, all other measures $P>0.05$.

Post-hoc tests generally demonstrated performance disruption on the first day of ABT-594 treatment, with significantly poorer accuracy, $P<0.001$, significantly increased omissions, $P<0.05$, significantly longer latency to collect rewards, $P<0.05$, significantly fewer correct choices, $P<0.001$, and significantly more incorrect choices, $P<0.05$.

Table 2
Acute effects of ABT-594 on task performance in drug-experienced rats with the constant trial presentation version of the 5-CSRTT (Experiment 2, $n=19$). Results are expressed as means ± SEM (*$P<0.05$, **$P<0.01$ vs. vehicle with Dunnett's multiple comparison test).

	Vehicle	0.0062 μmol/kg	0.019 μmol/kg	0.062 μmol/kg
Accuracy (%)	77.79 ± 1.86	80.54 ± 2.41	81.36 ± 1.47	80.24 ± 1.89
Omissions (%)	17.47 ± 2.15	14.87 ± 1.87	8.35 ± 1.30**	11.48 ± 1.60**
# Premature responses	127.7 ± 14.31	117.4 ± 14.09	115.7 ± 11.43	99.21 ± 9.79
# Perseverative responses	26.00 ± 8.27	21.79 ± 5.58	25.11 ± 7.40	25.79 ± 5.12
Correct response latency (s)	0.65 ± 0.10	0.61 ± 0.04	0.59 ± 0.02	0.58 ± 0.03
Incorrect response latency (s)	1.94 ± 0.10	1.86 ± 0.09	1.75 ± 0.11	1.72 ± 0.11
Reward collection latency (s)	1.28 ± 0.04	1.29 ± 0.04	1.32 ± 0.05	1.53 ± 0.06**
# Correct responses	103.6 ± 4.66	110.5 ± 5.0	119.6 ± 3.34**	114.11 ± 3.98*
# Incorrect responses	28.47 ± 1.94	25.68 ± 2.88	27.00 ± 1.88	27.63 ± 2.47

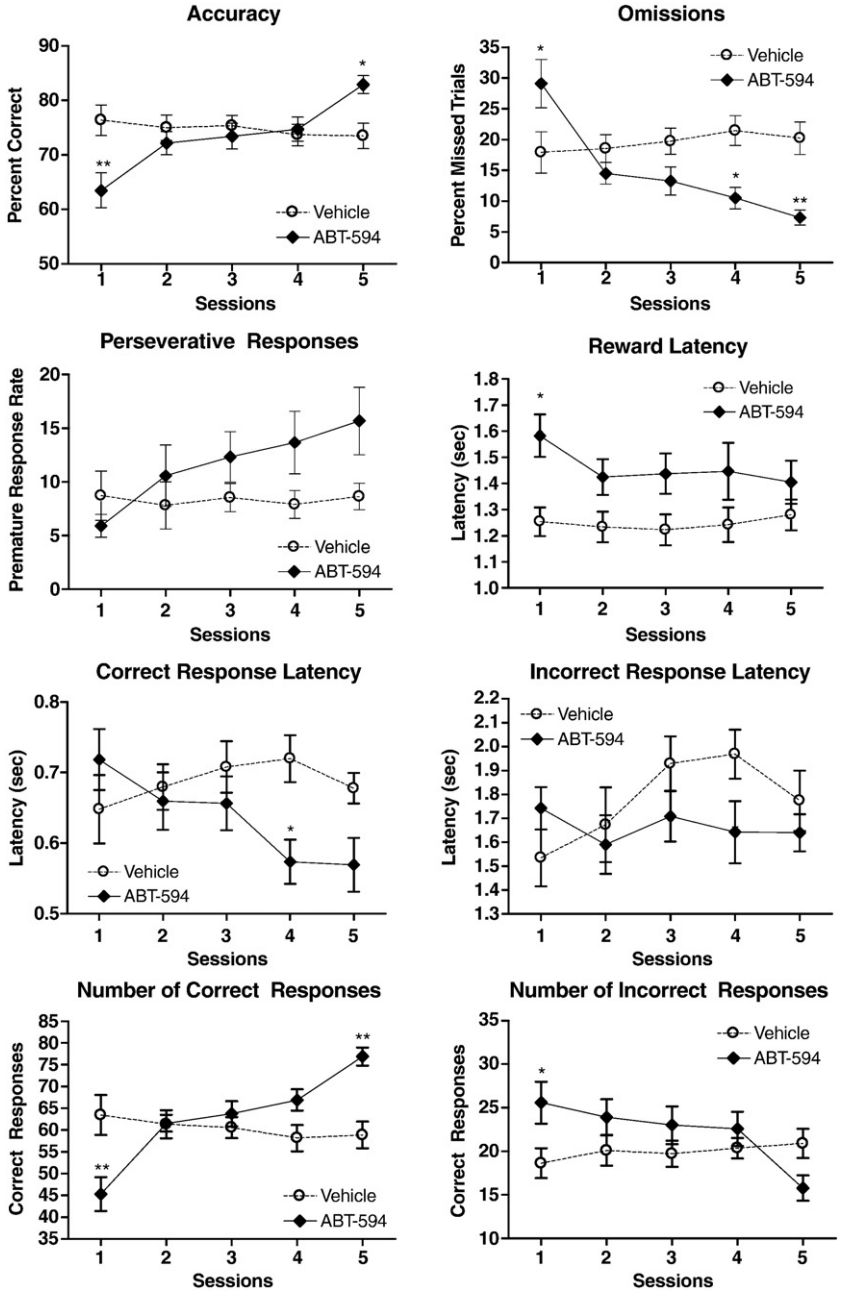

Fig. 1. Sub-chronic effects of ABT-594 on task performance in naïve rats with the constant trial presentation version of the 5-CSRTT (Experiment 3, $n = 11$ for vehicle group, $n = 12$ for ABT-594 group). Results are expressed as means ± SEM (*$P<0.05$, **$P<0.01$ vs. vehicle with Bonferroni post-hoc tests).

On days 4 and 5 of treatment, however, significant improvements in performance began to emerge. Accuracy was significantly better than vehicle on day 5, $P<0.05$. Omissions were significantly lower in ABT-594 treated rats on day 4, $P<0.05$, and day 5, $P<0.01$. Correct response latency was significantly faster in ABT-594 mice on day 4, $P<0.05$. An increase in the number of correct responses was observed on day 5, $P<0.05$ without any change in the number of incorrect responses.

3.4. Experiment 4: good and poor performing rats with multiple stimulus durations

A separate set of rats was trained exclusively on the constant trial presentation version of the 5-CSRTT and tested at 18 months of age. Due to a bimodal distribution of performance on accuracy, rats were separated into "poor discriminator" and "good discriminator" groups using a median split. Baseline accuracies for the "Poor" and "Good" groups were 67% and 86%, respectively. "Poor" rats had a baseline omission rate of 10% while "Good" rats had an omission rate of 8%. In this experiment, rats were tested in a paradigm with stimulus durations varying from one block of 20 trials to the next (1st block = 500 ms, 2nd block = 400 ms, 3rd block = 750 ms, 4th block = 1000 ms, and 5th block = 500 ms).

3.4.1. Poor discriminators

For the measure of accuracy, significant effects of treatment, $F(3, 33) = 3.616$, $P<0.05$ and stimulus duration, $F(4, 44) = 7.743$, $P<0.0001$ were observed in "Poor" rats (see Table 3). A post-hoc test found that "Poor" rats receiving 0.062 μmol/kg of ABT-594 had significantly improved accuracy compared to vehicle treatment, $P<0.05$. Not surprisingly, rats were more accurate with longer stimulus durations as evidenced by significantly better performance with the 750- or 1000-ms stimuli compared to the 400 and 500-ms stimuli (P's<0.05). On the

Table 3
Acute effects of ABT-594 on task performance in "Poor" performing aged rats with varying stimulus durations in the constant trial presentation version of the 5-CSRTT (Experiment 3, $n=13$). Results are expressed as means ± SEM (*$P<0.05$, **$P<0.01$ vs. vehicle with Bonferroni multiple comparison test).

	Treatment	500 ms (B1)	400 ms (B2)	750 ms (B3)	1000 ms (B4)	500 ms (B5)
Accuracy (%)	Vehicle	65.91 ± 4.14	66.24 ± 4.05	74.51 ± 3.98	73.46 ± 3.95	67.42 ± 4.05
	0.0062 μmol/kg	67.31 ± 2.71	66.17 ± 4.06	73.87 ± 2.85	73.04 ± 3.58	72.95 ± 3.74
	0.019 μmol/kg	74.34 ± 4.72	71.56 ± 3.60	75.28 ± 3.25	74.45 ± 3.62	76.44 ± 3.09
	0.062 μmol/kg	66.68 ± 4.48	71.50 ± 4.12	83.22 ± 3.88	79.29 ± 4.41	79.29 ± 4.41
Omissions (%)	Vehicle	9.21 ± 3.25	4.58 ± 1.99	3.75 ± 1.25	5.83 ± 1.93	8.75 ± 4.13
	0.0062 μmol/kg	3.95 ± 2.16	3.75 ± 1.64	4.17 ± 1.21	2.50 ± 1.31	4.17 ± 1.04
	0.019 μmol/kg	1.32 ± 0.69	0.42 ± 0.42	0.42 ± 0.42	2.92 ± 1.30	2.92 ± 0.97
	0.062 μmol/kg	7.02 ± 2.77	2.92 ± 1.68	0.83 ± 0.56	1.25 ± 0.65	2.92 ± 0.97
# Premature responses	Vehicle	27.00 ± 4.08	30.58 ± 4.44	31.50 ± 6.63	28.25 ± 5.82	30.00 ± 5.89
	0.0062 μmol/kg	31.42 ± 4.51	34.83 ± 4.91	28.50 ± 5.07	28.17 ± 6.20	29.42 ± 5.20
	0.019 μmol/kg	32.25 ± 5.77	34.67 ± 5.05	30.25 ± 6.51	32.83 ± 5.38	29.75 ± 5.72
	0.062 μmol/kg	27.67 ± 5.85	30.17 ± 5.68	28.75 ± 5.64	28.50 ± 7.03	24.67 ± 6.98
# Perseverative responses	Vehicle	0.08 ± 0.08	0.00 ± 0.00	0.25 ± 0.18	0.17 ± 0.17	0.50 ± 0.36
	0.0062 μmol/kg	0.17 ± 0.17	0.00 ± 0.00	0.25 ± 0.25	0.00 ± 0.00	0.00 ± 0.00
	0.019 μmol/kg	0.08 ± 0.08	0.50 ± 0.29	0.67 ± 0.35	0.58 ± 0.37	0.33 ± 0.19
	0.062 μmol/kg	0.17 ± 0.11	0.17 ± 0.11	0.25 ± 0.25	0.25 ± 0.18	0.08 ± 0.08
Correct response latency (s)	Vehicle	0.53 ± 0.06	0.49 ± 0.06	0.59 ± 0.07	0.67 ± 0.15	0.52 ± 0.06
	0.0062 μmol/kg	0.52 ± 0.04	0.47 ± 0.04	0.52 ± 0.03	0.61 ± 0.05	0.48 ± 0.02
	0.019 μmol/kg	0.46 ± 0.03	0.44 ± 0.05	0.47 ± 0.03	0.55 ± 0.06	0.50 ± 0.05
	0.062 μmol/kg	0.56 ± 0.02	0.50 ± 0.05	0.51 ± 0.03	0.50 ± 0.03	0.48 ± 0.03
Incorrect response latency (s)	Vehicle	1.49 ± 0.17	1.37 ± 0.22	1.24 ± 0.26	0.85 ± 0.19	1.24 ± 0.16
	0.0062 μmol/kg	1.10 ± 0.17	1.03 ± 0.14	1.15 ± 0.20	1.45 ± 0.24	1.45 ± 0.24
	0.019 μmol/kg	0.91 ± 0.14	0.84 ± 0.13	0.84 ± 0.16	0.93 ± 0.19	1.22 ± 0.17
	0.062 μmol/kg	0.88 ± 0.17	1.09 ± 0.25	0.60 ± 0.13	0.61 ± 0.18	1.04 ± 0.15
Reward collection latency (s)	Vehicle	1.29 ± 0.10	1.22 ± 0.07	1.26 ± 0.07	1.28 ± 0.07	1.34 ± 0.14
	0.0062 μmol/kg	1.27 ± 0.09	1.24 ± 0.07	1.33 ± 0.12	1.24 ± 0.07	1.28 ± 0.07
	0.019 μmol/kg	1.38 ± 0.10	1.24 ± 0.06	1.31 ± 0.06	1.31 ± 0.06	1.31 ± 0.09
	0.062 μmol/kg	1.77 ± 0.15*	1.57 ± 0.11*	1.47 ± 0.07	1.48 ± 0.09*	1.50 ± 0.11
# Correct responses	Vehicle	11.33 ± 0.80	12.67 ± 0.82	14.42 ± 0.89	13.92 ± 0.85	12.33 ± 0.99
	0.0062 μmol/kg	12.33 ± 0.64	12.83 ± 0.90	14.17 ± 0.59	14.25 ± 0.75	14.00 ± 0.77
	0.019 μmol/kg	13.92 ± 0.87*	14.25 ± 0.72	15.00 ± 0.66	14.42 ± 0.67	14.83 ± 0.59*
	0.062 μmol/kg	11.92 ± 1.01	13.92 ± 0.87	16.50 ± 0.76	16.67 ± 0.85*	15.32 ± 0.90**
# Incorrect responses	Vehicle	8.67 ± 0.80	7.33 ± 0.82	5.58 ± 0.89	6.08 ± 0.85	7.67 ± 0.99
	0.0062 μmol/kg	7.67 ± 0.64	7.17 ± 0.90	5.83 ± 0.59	6.00 ± 0.75	6.00 ± 0.77
	0.019 μmol/kg	6.08 ± 0.87	5.75 ± 0.72	5.00 ± 0.66	5.17 ± 0.67	5.17 ± 0.59
	0.062 μmol/kg	8.08 ± 1.01	6.08 ± 0.87	3.50 ± 0.76	3.33 ± 0.85	4.58 ± 0.90

measure of omissions, "Poor" rats demonstrated significant effects of treatment, $F(3, 33)=5.646$, $P<0.005$ and stimulus duration, $F(4, 44)=2.768$, $P<0.05$. Post-hoc testing showed a significant overall decrease in omissions with the 0.019 and 0.062 μmol/kg doses of ABT-594, P's<0.05. In addition, rats tended to make fewer omissions with longer stimulus durations, although none of the post-hoc comparisons was significant. When premature and perseverative responses were examined, no significant effect of treatment or treatment by stimulus duration interaction was observed in "Poor" rats, P's>0.05 On the measure of latency to correct response, a significant effect of stimulus duration was found in "Poor" rats, $F(4, 44)=2.836$, $P<0.05$, which appears primarily to be a result of longer latencies with increasing stimulus durations. No significant effects were observed for latency to an incorrect response, $P>0.05$. For latency to collect reward, significant effects of treatment, $F(3, 33)=17.723$, $P<0.001$, stimulus duration, $F(4, 44)=3.118$, $P<0.05$, and the interaction, $F(12, 132)=3.118$, $P<0.05$, were observed in "Poor" rats. Post-hoc tests showed that the 0.062 μmol/kg dose of ABT-594 significantly increased overall reward latency, $P<0.01$, particularly during the 1st 500-ms block, the 400-ms block and the 1000-ms block (P's<0.05).

When the number of correct responses was examined, significant effects of treatment, $F(3, 33)=5.541$, $P<0.005$, stimulus duration, $F(4, 44)=14.179$, $P<0.0001$, and the interaction, $F(12, 132)=1.881$, $P<0.05$ were observed in "Poor" rats. Post-hoc tests showed that the 0.019 and 0.062 μmol/kg doses were significantly increasing the number of correct responses, P's<0.05. When individual treatment by stimulus duration effects were examined, an increase in correct responses was observed with the 0.019 μmol/kg dose of ABT-594 during the 1st and 2nd 500-ms blocks, P's<0.05, and with the 0.062 μmol/kg dose during the 1000-ms block ($P<0.05$) and 2nd 500-ms block ($P<0.01$). For incorrect responses, significant effects of treatment, $F(3, 33)=2.967$, $P<0.05$ and stimulus duration, $F(4, 44)=11.882$, $P<0.001$ were observed in "Poor" rats. While the 0.062 μmol/kg dose of ABT-594 tended to decrease incorrect responses, this was not significant for any individual stimulus duration. Stimulus duration effects were noted such that fewer errors were made with longer stimulus durations compared to shorter durations and more errors were made early in a session compared to late in a session (1st 500-ms block vs. 750-ms; 1st 500-ms block vs. 1000-ms; 1st 400-ms block vs. 750-ms; 1st 400-ms block vs. 1000-ms; 1st 500-ms block vs. 2nd 500-ms block; all P's<0.05).

3.4.2. Good discriminators

In "Good" performing rats there was no overall significant effect of treatment on accuracy, $P>0.05$, but there was an effect of stimulus duration, $F(4, 44)=7.428$, $P<0.0001$, mostly due to improved performance on longer stimulus durations (see Table 4). Specifically, rats had significantly better performance with the 1000-ms stimulus compared to the 400- and 500-ms stimuli (P's<0.05), as well as the 750-ms stimulus compared to the 400-ms stimulus ($P<0.05$). "Good" rats also showed a significant effect of treatment on omissions, $F(3, 33)=9.046$, $P<0.0005$, as all doses of ABT-594 significantly reduced overall omissions P's<0.05. When the premature and perseverative responses were examined, no significant effect of treatment was observed in "Good" rats, P's>0.05. A significant effect of stimulus duration was observed for premature responses in "Good" rats, $P(4, 44)=4.876$, $P<0.005$, as premature responses tended to decrease with longer lengths. For "Good" rats, significant effects of treatment, $F(3, 33)=3.241$, $P<0.05$, stimulus duration, $F(4, 44)=2.710$, $P<0.05$, and the interaction, $F(12, 132)=1.952$, $P<0.05$ were observed on latencies to correct responses. Post-hoc tests indicated a significant decrease

Table 4
Acute effects of ABT-594 on task performance in "Good" performers aged rats with varying stimulus durations in the constant trial presentation version of the 5-CSRTT (Experiment 4, $n=13$). Results are expressed as means ± SEM (*$P<0.05$, **$P<0.01$ vs. vehicle with Bonferroni multiple comparison test).

	Treatment	500 ms (B1)	400 ms (B2)	750 ms (B3)	1000 ms (B4)	500 ms (B5)
Accuracy (%)	Vehicle	87.99 ± 1.53	85.89 ± 2.84	87.28 ± 2.55	91.94 ± 2.05	88.29 ± 1.41
	0.0062 μmol/kg	85.40 ± 2.05	87.75 ± 2.32	89.75 ± 2.03	91.31 ± 1.56	88.97 ± 2.12
	0.019 μmol/kg	87.50 ± 2.15	84.90 ± 1.49	94.45 ± 1.49	95.81 ± 1.21	86.59 ± 1.64
	0.062 μmol/kg	81.58 ± 2.99	80.38 ± 4.29	89.96 ± 3.19	92.71 ± 1.94	87.14 ± 1.96
Omissions (%)	Vehicle	8.33 ± 2.29	7.50 ± 2.72	12.92 ± 3.82	7.92 ± 2.42	10.00 ± 2.38
	0.0062 μmol/kg	5.26 ± 2.42	5.00 ± 1.38	4.17 ± 2.12	4.17 ± 1.83	9.17 ± 2.03
	0.019 μmol/kg	1.32 ± 0.69	2.92 ± 1.67	1.67 ± 0.71	1.25 ± 0.90	3.33 ± 1.12
	0.062 μmol/kg	6.14 ± 3.60	2.50 ± 1.44	2.08 ± 0.97	2.08 ± 1.15	3.75 ± 2.90
# Premature responses	Vehicle	10.08 ± 1.32	10.17 ± 2.92	8.92 ± 1.96	8.08 ± 1.72	7.67 ± 2.25
	0.0062 μmol/kg	9.58 ± 1.40	12.25 ± 1.92	9.25 ± 2.20	6.83 ± 1.63	6.83 ± 1.63
	0.019 μmol/kg	12.08 ± 1.64	13.50 ± 3.02	11.08 ± 2.07	6.92 ± 1.68	10.00 ± 2.19
	0.062 μmol/kg	11.58 ± 2.14	14.83 ± 2.91	10.58 ± 2.66	11.42 ± 3.04	10.67 ± 2.18
# Perseverative responses	Vehicle	0.17 ± 0.17	0.08 ± 0.08	0.25 ± 0.25	0.67 ± 0.51	0.25 ± 0.18
	0.0062 μmol/kg	0.08 ± 0.08	0.25 ± 0.25	0.25 ± 0.18	0.25 ± 0.18	0.08 ± 0.08
	0.019 μmol/kg	1.00 ± 0.83	1.42 ± 1.08	1.67 ± 1.26	1.50 ± 1.17	1.42 ± 0.10
	0.062 μmol/kg	0.00 ± 0.00	0.42 ± 0.42	0.25 ± 0.25	0.08 ± 0.08	0.08 ± 0.08
Correct response latency (s)	Vehicle	0.50 ± 0.02	0.51 ± 0.03	0.50 ± 0.02	0.54 ± 0.03	0.52 ± 0.05
	0.0062 μmol/kg	0.51 ± 0.04	0.49 ± 0.03	0.49 ± 0.04	0.54 ± 0.04	0.51 ± 0.03
	0.019 μmol/kg	0.45 ± 0.03	0.40 ± 0.01*	0.45 ± 0.02	0.47 ± 0.03	0.48 ± 0.03
	0.062 μmol/kg	0.62 ± 0.07**	0.46 ± 0.03	0.47 ± 0.02	0.52 ± 0.04	0.48 ± 0.03
Incorrect response latency (s)	Vehicle	2.05 ± 0.30	1.58 ± 0.31	1.31 ± 0.45	1.71 ± 0.50	1.84 ± 0.35
	0.0062 μmol/kg	1.43 ± 0.24	1.07 ± 0.19	1.76 ± 0.43	1.50 ± 0.33	1.36 ± 0.26
	0.019 μmol/kg	1.23 ± 0.21	1.12 ± 0.20	0.90 ± 0.26	1.14 ± 0.33	1.30 ± 0.21
	0.062 μmol/kg	1.45 ± 0.28	1.27 ± 0.21	1.02 ± 0.49	1.46 ± 0.51	1.48 ± 0.44
Reward collection latency (s)	Vehicle	1.22 ± 0.06	1.24 ± 0.06	1.76 ± 0.48	1.30 ± 0.08	1.31 ± 0.06
	0.0062 μmol/kg	1.21 ± 0.06	1.26 ± 0.06	1.25 ± 0.05	1.27 ± 0.05	1.29 ± 0.05
	0.019 μmol/kg	1.24 ± 0.07	1.27 ± 0.08	1.32 ± 0.07	1.35 ± 0.08	1.34 ± 0.07
	0.062 μmol/kg	1.67 ± 0.14*	1.57 ± 0.10*	1.49 ± 0.10	1.50 ± 0.10	1.47 ± 0.09
# Correct responses	Vehicle	15.33 ± 0.50	16.00 ± 0.85	15.25 ± 0.82	16.92 ± 0.57	15.92 ± 0.57
	0.0062 μmol/kg	15.33 ± 0.45	16.67 ± 0.50	17.25 ± 0.64*	17.50 ± 0.45	16.17 ± 0.55
	0.019 μmol/kg	16.42 ± 0.45	16.50 ± 0.57	18.58 ± 0.36**	18.92 ± 0.26*	16.75 ± 0.41
	0.062 μmol/kg	14.67 ± 0.88	15.75 ± 0.95	17.67 ± 0.73 **	18.17 ± 0.47	16.75 ± 0.59
# Incorrect responses	Vehicle	4.67 ± 0.50	4.00 ± 0.85	4.75 ± 0.82	3.08 ± 0.54	4.08 ± 0.57
	0.0062 μmol/kg	4.57 ± 0.45	3.33 ± 0.50	2.75 ± 0.45	2.50 ± 0.45	3.83 ± 0.55
	0.019 μmol/kg	3.58 ± 0.45	3.50 ± 0.57	1.42 ± 0.36	1.08 ± 0.26	3.25 ± 0.41
	0.062 μmol/kg	5.33 ± 0.88	4.25 ± 0.95	2.33 ± 0.73	1.83 ± 0.47	3.25 ± 0.59

in latencies with the 0.019 μmol/kg doses of ABT-594, although the 0.062 μmol/kg dose significantly increased latencies during the 1st block with the 500-ms stimulus duration. No significant effects were found for the latency to make an incorrect response, $P>0.05$. For latency to collect reward, only a significant effect of treatment, $F(3, 33)=5.417$, $P<0.005$, was found. Post-hoc tests found that the 0.062 μmol/kg dose of ABT-594 significantly increased reward latency during the 1st 500-ms block and the 400-ms block (P's<0.05).

In "Good" rats, significant effects of treatment, $F(3, 33)=3.814$, $P<0.05$, stimulus duration, $F(4, 44)=12.074$, $P<0.0001$, and the interaction, $F(12, 132)=2.451$, $P<0.01$ were observed upon the number of correct responses. Post-hoc tests indicated significant improvement on the number of correct responses overall with the 0.019 μmol/kg dose of ABT-594, particularly during the 750-ms ($P<0.01$) and 1000-ms ($P<0.05$) stimulus durations. Furthermore, during the 750-ms block the 0.0062 and 0.062 μmol/kg doses also increased the number of correct responses ($P<0.05$ and $P<0.01$, respectively). For incorrect responses, a significant effect of stimulus duration, $F(4, 44)=14.591$, $P<0.001$ was observed in "Good" rats, as less fewer errors were made with longer stimulus durations compared to shorter durations and more errors were made early in a session compared to late in a session (1st 500-ms block vs. 750-ms; 1st 500-ms block vs. 1000-ms; 1st 400-ms block vs. 750-ms; 1st 400-ms block vs. 1000-ms; 1st 500-ms block vs. 2nd 500-ms block; all P's<0.05).

3.5. Experiment 5: sub-chronic dosing in good and poor performing rats

Approximately 2 months after Experiment 4, rats received vehicle or a 0.019 μmol/kg dose of ABT-594 for 7 consecutive days. No testing occurred during the first two days of drug administration to minimize the chance of performance decrements on the first day of testing. Testing with the constant trial presentation version of the 5-CSRTT began on the third day of treatment. As with Experiment 4, rats were separated into "poor discriminator" and "good discriminator" groups using a median split. Baseline accuracies for the "Poor" and "Good" groups were 66% and 85%, respectively. "Poor" rats had a baseline omission rate of 12% while "Good" rats had an omission rate of 10%.

3.5.1. Poor discriminators

In "Poor" rats, treatment with ABT-594 significantly improved accuracy, $F(1, 12)=8.242$, decreased omissions, $F(1, 12)=8.763$, $P<0.05$, increased premature responses, $F(1, 12)=8.700$, $P<0.05$, decreased correct response latency, $F(1, 12)=12.665$, $P<0.01$, decreased incorrect response latency, $F(1, 12)=16.581$, $P<0.005$, and increased reward collection latency, $F(1, 12)=6.119$, $P<0.05$. No significant effects of day or treatment by day interactions were found, $P>0.05$. Post-hoc tests demonstrated that in rats receiving ABT-594, there was significant enhancement of accuracy on days 2–3, $P<0.05$ (see Fig. 2). Omissions were decreased on day 4 only, $P<0.05$, although an effect on day 1 narrowly missed significance ($P=0.057$). For premature responses, post-hoc tests did not indicate a significant increase on any individual day. Significant decreases in correct response latency were found on days 2 and 3, $P<0.05$ and a significant increase in reward collection latency was observed on day 1, $P<0.05$ (see Fig. 3). On the measure of perseverative responses, there was a significant effect of day, $F(4, 48)=3.039$, $P<0.05$, but no effect of treatment or interaction, $P>0.05$. Post-hoc testing found that "Poor" rats receiving ABT-594 made significantly more perseverative

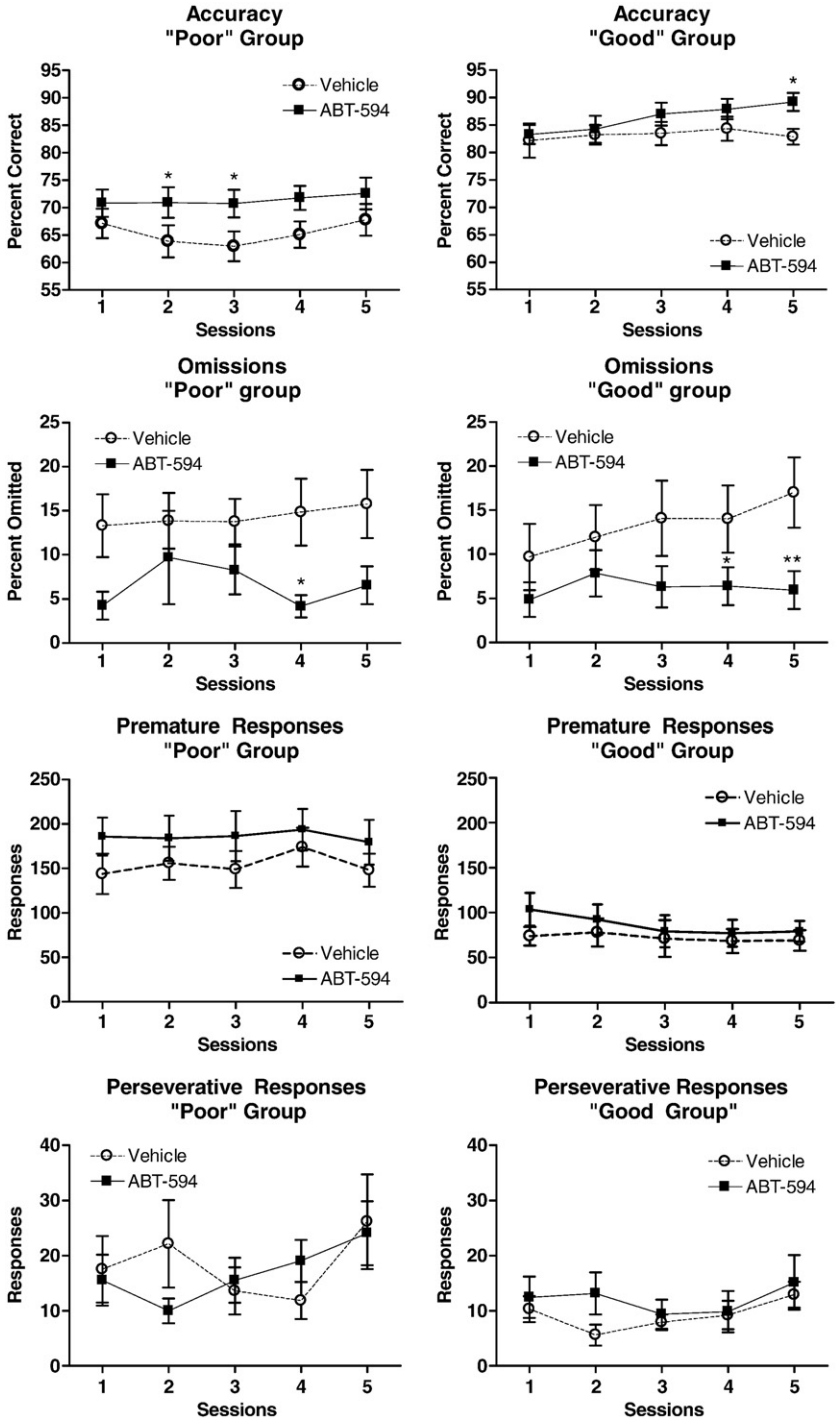

Fig. 2. Sub-chronic effects of ABT-594 on accuracy, omissions, premature and perseverative responses in "Poor" and "Good" groups of drug-experienced rats (constant trial version of the 5-CSRTT). Results are expressed as means ± SEM (*P<0.05, **P<0.01 vs. vehicle with Bonferroni post-hoc tests. #P<0.05 vs. vehicle with paired t-test).

responses on day 4 only, P<0.05. When the number of correct responses was examined, a significant effect of treatment, $F(1, 12) = 18.831$, P<0.001 was observed in "Poor" rats, but no effect of day and no treatment by day interaction, P's>0.05. Post-hoc tests showed that ABT-594 treatment significantly increased the number of correct responses on days 1 and 3–5, P's<0.05. In contrast, no significant effects were observed on the number of incorrect responses, P>0.05.

3.5.2. Good discriminators

In contrast to the "Poor" discriminating rats, there was no overall significant effects of treatment on accuracy in "Good" rats, P>0.05, but there was a significant effect of day, $F(4, 48) = 3.011$ P<0.05 (see Fig. 2). A Post-hoc test showed improved performance in the ABT-594 treated rats compared to vehicle by the 5th day of testing, P<0.05. On the measure of omissions, "Good" rats demonstrated a significant effect of treatment, $F(1, 12) = 11.322$, P<0.01, day, $F(4, 48) = 3.279$, P<0.05, and a treatment by day interaction, $F(4, 48) = 2.967$, P<0.05. Post-hoc tests demonstrated that in "Good" rats receiving ABT-594, there was a significant decrease in omissions on days 4–5, P<0.05. For premature and perseverative responses, "Good" rats did not show an effect of treatment, P>0.05, but an effect of day was observed, $F(4, 48) = 3.977$, P<0.01 and $F(4, 48) = 3.025$, P<0.05, respectively as premature

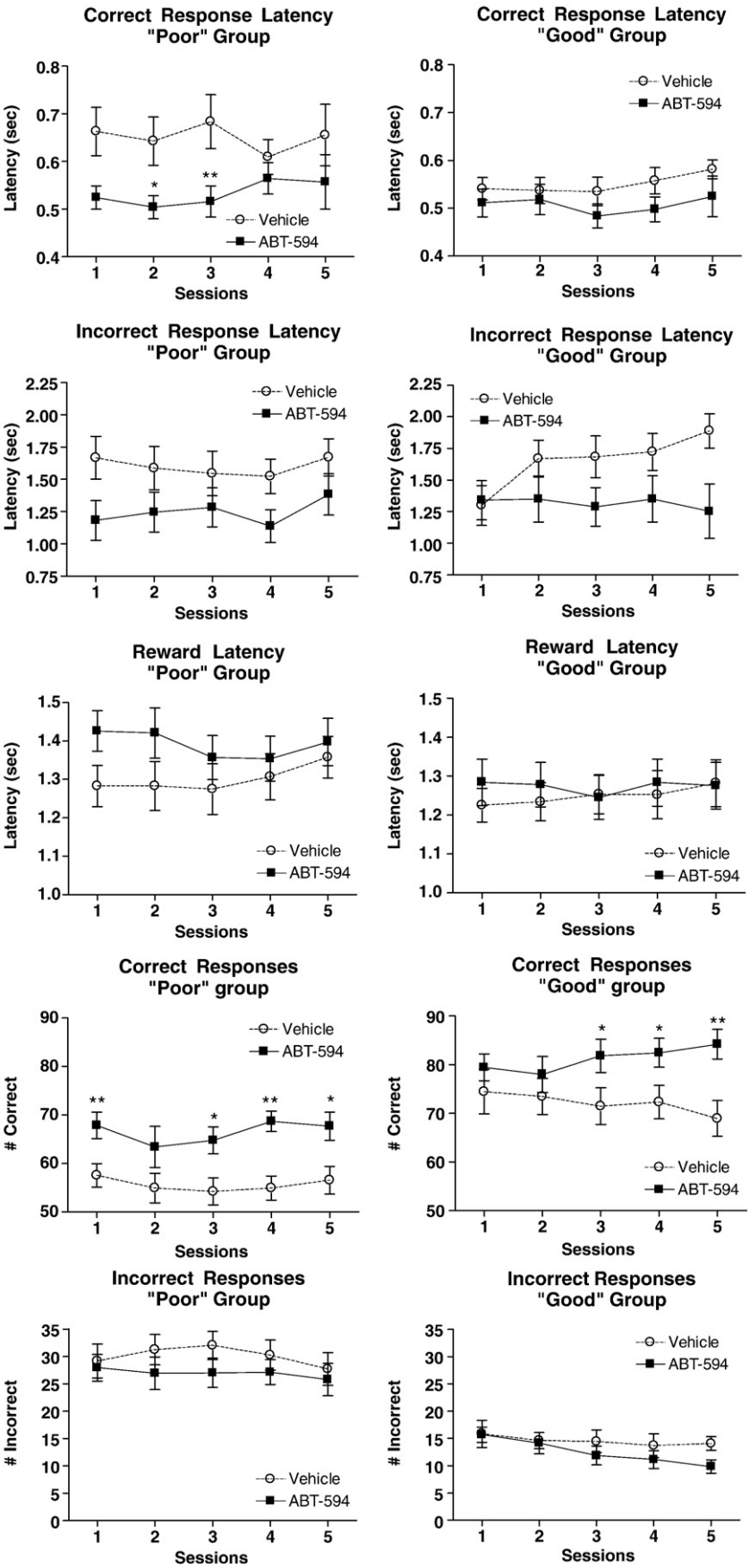

Fig. 3. Sub-chronic effects of ABT-594 on latency measures and the number of correct and incorrect responses in "Poor" and "Good" groups of drug-experienced rats (constant trial version of the 5-CSRTT). Results are expressed as means ± SEM (*$P<0.05$, **$P<0.01$ vs. vehicle with Bonferroni post-hoc tests).

responses tended to decline over five days of testing while perseverative responses tended to increase. No significant effects were observed on correct response latency or reward collection latency in "Good" rats, $P>0.05$. For latency to incorrect responses, significant effects of treatment, $F(1, 12)=6.369$, $P<0.05$, day, $F(4, 48)=3.382$, $P<0.05$, and a treatment by day interaction, $F(4, 48)=3.824$, $P<0.01$ were observed (see Fig. 3). While ABT-594 treatment reduced latencies, the effect was not significant on any individual day, $P>0.05$. When the number of correct responses was examined in "Good" performing rats, significant effects of treatment, $F(1, 12)=15.347$, $P<0.01$ and a treatment by day interaction, $F(4, 48)=4.494$, $P<0.005$, were observed. Post-hoc tests indicated significant improvement on the number of correct trials on days 3–5 with the 0.019 μmol/kg dose of ABT-594, $P<0.05$. No significant effect treatment was observed on the number of incorrect responses, $P>0.05$, although there was a significant effect of day, $F(4, 48)=3.627$, $P<0.05$, as performance improved over 5 days of testing.

4. Discussion

The present studies indicate that ABT-594, a potent agonist of the α4β2 receptor improves 5-CSRTT performance in multiple measures, such as accuracy, omissions, and latency to correct responses. However, ABT-594 and other α4β2 receptor agonists may only enhance accuracy under very specific conditions. In contrast, omissions are robustly decreased across multiple conditions, and are often accompanied by an increase in correct responses without a corresponding increase in incorrect responses, suggesting that the increased responding is not at the expense of decreased accuracy. Furthermore, the increase in correct responses is not accompanied by an increase in premature responses, with the exception of the "Poor" rats in Experiment 5. Interestingly, ABT-594 significantly increased the latency to collect reward in all of the experiments. As this increase was accompanied by increases in correct responses and decreases in omissions in most cases, it does not appear to reflect reduced motivation or satiation. Moreover, perseverative responses were only increased in Experiment 3, likely a result of daily testing with constant trial presentation, suggesting that perseverative behavior is not an explanation for the increased latency to collect reward. Latencies to correct responses tended to be faster in ABT-594 treated rats, suggesting there is no overall slowing of reaction time. The "Good" performers in Experiment 5 did not show increased reward latency, and the "Poor" group latencies were similar to control rats by the end of the experiment, suggesting the effect may disappear over time. Together, these data suggest that ABT-594, and possibly other α4β2 agonists, consistently improve attention in the 5-CSRTT, but effects on accuracy are difficult to obtain and are highly dependent on subject variables, such as age or baseline performance, task variables, such as stimulus duration, and dosing variables, such as sub-chronic treatment.

Rats tested in Experiment 1 with the self-initiated trials version of the 5-CSRTT showed no enhancement of performance on any measure with ABT-594. The lack of an enhancement in this paradigm is not surprising, as conditions of low event rate or weak signals have been necessary to observe enhancement with nicotine (Mirza and Stolerman, 1998). Therefore, in Experiment 2 task demands were increased by testing rats on the constant trial presentation version of the 5-CSRTT two times per week during drug treatment sessions. With cue presentation no longer self-paced, the presentation of cues was less predictable. Nicotine has been demonstrated to have more evident effects on subjects that have greater cingulate cortex activation with less predictive cues in human functional imaging studies (Giessing et al., 2007). The change in predictability from self-paced to constant trials was hypothesized to allow a similar therapeutic window in the present study. Rats tended to make more omissions and premature responses and had a lower accuracy rate with constant trial presentation. Treatment with ABT-594 did not significantly improve accuracy, but did significantly decrease omissions and increase the number of correct responses. These results are consistent with findings that nicotine (Bizarro et al., 2004) and epibatidine (Hahn et al., 2003) increase the number of trials to which rats respond, resulting in a net increase in the number of correct responses. In contrast to some studies with nicotine using the constant trial presentation task (Day et al., 2007; Bizarro et al., 2004), ABT-594 did not significantly increase premature responses. This is consistent with studies examining epibatidine and the α4β2 agonist ABT-418 in this paradigm (Hahn et al., 2003).

More robust treatment effects have been observed with sub-chronic dosing of nicotine (Hahn and Stolerman, 2002; Semenova et al., 2007) or the α4β2 agonist SIB 1765F (Grottick and Higgins, 2000). Experiment 3 examined the effect of sub-chronic administration of ABT-594 in rats maintained on the self-initiation version of the 5-CSRTT, but tested daily during drug administration with the constant trial presentation paradigm. Consistent with previous studies examining chronic nicotine treatment (Hahn and Stolerman, 2002; Semenova et al., 2007), a decrement in accuracy and an increase in omissions were observed with initial treatment in drug naïve rats. Rats rapidly became tolerant to the effects of ABT-594 as normal performance was observed by the second day of dosing. By the fifth day of treatment significant improvements in accuracy as well as the number of correct responses and a decrease in omissions were observed. In addition, a significant reduction in latency to correct responses was observed on the fourth day of treatment and this pattern, although not significant, continued on the fifth day. These data are generally consistent with a study using the α4β2 agonist SIB 1765F (Grottick and Higgins, 2000), with the impairment observed on day 1 and lag of the accuracy effect until day 5 likely due to the lack of pre-treatment of the drug and use of well-performing subjects in the present study. To minimize the potential for performance decrements, rats in Experiment 5 received ABT-594 for two days prior to testing. ABT-594 significantly improved accuracy in "Poor" rats by the second day of testing compared to vehicles. In contrast, "Good" performing rats that were well accustomed to the more demanding paradigm only showed modest enhancement of accuracy. However, both good and poor groups of rats showed significant reductions in omitted trials. Consistent with young rats, ABT-594 significantly increased the number of trials to which rats responded without impairing accuracy, resulting in a net increase in the number of correct responses without increasing errors. This suggests that rats receiving ABT-594 are not indiscriminately responding to more trials, but rather are maintaining the same accuracy rate. While sub-chronic treatment may allow the demonstration of treatment effects that are not obvious with acute dosing, drug effects may be less obvious in rats that perform the task well. These data may explain why other α4β2 agonists, such as ABT-418 failed to enhance accuracy in an acute study (Hahn et al., 2003).

The ability of nicotine to improve attention in humans (Hahn et al., 2007) and rats (Stolerman et al., 2000; Hahn et al., 2002) is also affected by the strength of cues, such that an effect is not observed with weaker targets. Alterations of signal strength have previously been shown to result in differential effects of nicotine treatment (Mirza and Stolerman, 1998) as very short stimulus durations tended to reduce effects of nicotine (Stolerman et al., 2000; Hahn et al., 2002). In the present study this is most apparent in Experiment 4, where multiple signal durations were used. As young rats perform very well with stimulus durations of 500 ms or longer, Experiment 4 tested "Poor" and "Good" 18–22 month-old rats with signal strength varying within a session. Differences between vehicle and ABT-594 performance on measures of accuracy and number of correct responses tended to be larger with longer signal durations, particularly in "Poor" rats. It has been hypothesized that nicotine may enhance the alerting response to external stimuli, and this activity is greater with stimuli that are more prominent (Hahn et al., 2007). In this case, there was a significant interaction with stimulus duration, such that longer stimulus durations tended to result in larger effects. Since multiple

stimulus durations were presented within a single session, the effects of vigilance over time could contribute to the findings. This would be more likely in the poor group, where performance generally declines over the session, compared to the good group where vehicle performance was fairly consistent.

Overall, these studies suggest that ABT-594 primarily improves attention when performance is sub-optimal. Sub-optimal performance may be induced with changes in event rate, or may be a result of natural changes that occur with aging. As the aged subjects in these experiments were only tested with constant trial presentation, it is not clear if the effects of ABT-594 are limited to this paradigm. The finding that drug-induced improvements in performance are more readily apparent in rats that perform poorly is consistent with studies showing that improvements in attention with nicotine may be more readily observed in rats with an MK-801 induced deficit (Rezvani and Levin, 2003; Rezvani et al., 2008), than in rats that perform well. Moreover, while several studies have demonstrated that nicotine enhances attention in normal human subjects (e.g., Foulds et al., 1996; Levin et al., 1998), other investigations have not observed any differences (see Newhouse et al., 2004 for a review). The effects of nicotinic agonists may be more readily observed in patients with attentional disorders, such as ADHD, Alzheimer's disease or schizophrenia, than in normal volunteers, as attention performance may be near peak performance (Newhouse et al., 2004). Similarly, the most robust effects of ABT-594 on attention in the present studies were observed in rats that have poor baseline performance on the 5-CSRTT. As such, these experiments suggest that enhanced attention may be difficult to observe in normal rats and humans except under specific conditions, but in subjects with poor performance, such as those with reduced attention associated with a disease state, nicotinic agonists may be of therapeutic benefit.

In conclusion, modulation of attention in the 5-CSRTT with $\alpha4\beta2$ agonists, as with nicotine, is highly dependent on dosing schedules, task parameters, and the dependent measures analyzed. ABT-594 tended to improve attention when task parameters allowed for enhancements to be observed. In the normal rats, drug effects were typically observed only on omissions unless performance is degraded. In poor rats, the effect of treatment may be more apparent in both accuracy and omissions. The number of correct responses more consistently allowed the observation of treatment effects than the accuracy measure, reflecting the ability of ABT-594 to create a net increase in stimuli that were successfully detected. Sub-chronic dosing may also allow treatment effects to emerge that may not otherwise be apparent. Consistent with the findings of numerous previous studies, these experiments show that the 5-CSRTT is a useful tool for investigating the pharmacology of attention. However, if conditions are not optimal, the ability to observe treatment effects may be decreased, thereby limiting the utility of the 5-CSRTT. Several factors need to be considered when designing experiments for novel cognitive enhancers, particularly those with novel mechanisms, as any one set of experimental variables and/or treatment schedules may or may not allow the identification of a molecule of interest.

References

Allain H, Akwa Y, Lacomblez L, Lieury A, Bentué-Ferrer D. Impaired cognition and attention in adults: pharmacological management strategies. Neuropsychiatr Dis Treat 2007;3(1):103–16.

Bizarro L, Patel S, Murtagh C, Stolerman IP. Differential effects of psychomotor stimulants on attentional performance in rats: nicotine, amphetamine, caffeine and methylphenidate. Behav Pharmacol 2004;15(3):195–206.

Bizarro L, Stolerman IP. Attentional effects of nicotine and amphetamine in rats at different levels of motivation. Psychopharm (Berl) 2003;170(3):271–7.

Blondel A, Sanger DJ, Moser PC. Characterisation of the effects of nicotine in the five-choice serial reaction time task in rats: antagonist studies. Psychopharm (Berl) 2000;149(3):293–305.

Buccafusco J, Terry Jr AV, Decker MW, Gopalakrishnan M. Profile of nicotinic acetylcholine receptor agonists ABT-594 and A-582941, with differential subtype selectivity, on delayed matching accuracy by young monkeys. Biochem Pharmacol 2007;74(8):1202–11.

Bushnell PJ, Oshiro WM, Padnos BK. Detection of visual signals by rats: effects of chlordiazepoxide and cholinergic and adrenergic drugs on sustained attention. Psychopharmacology (Berl) 1997;134(3):230–41.

Carli M, Robbins TW, Evenden JL, Everitt BJ. Effects of lesions to ascending noradrenergic neurons on performance on a 5-choice serial reaction time task in rats: implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. Behav Brain Res 1983;9:361–80.

Day M, Pan JB, Buckley MJ, Cronin E, Hollingsworth PR, Hirst WD, et al. Differential effects of ciproxifan and nicotine on impulsivity and attention measures in the 5-choice serial reaction time test. Biochem Pharmacol 2007;73(8):1123–34.

Donnelly-Roberts DL, Puttfarcken PS, Kuntzweiler TA, Briggs CA, Anderson DJ, Campbell JE, et al. ABT-594 [(R)-5-(2-azetidinylmethoxy)-2-chloropyridine]: a novel, orally effective analgesic acting via neuronal nicotinic acetylcholine receptors: I. In vitro characterization. J Pharmacol Exp Ther 1998;285(2):777–86.

Foulds J, Stapleton J, Swettenham J, Bell N, McSorley K, Russell MA. Cognitive performance effects of subcutaneous nicotine in smokers and never-smokers. Psychopharm (Berl) 1996;127(1):31–8.

Giessing C, Fink GR, Rösler F, Thiel CM. fMRI data predict individual differences of behavioral effects of nicotine: a partial least square analysis. J Cogn Neurosci 2007;19(4):658–70.

Grottick AJ, Haman M, Wyler R, Higgins GA. Reversal of a vigilance decrement in the aged rat by subtype-selective nicotinic ligands. Neuropsychopharm 2003;28(5):880–7.

Grottick AJ, Higgins GA. Effect of subtype selective nicotinic compounds on attention as assessed by the five-choice serial reaction time task. Behav Brain Res 2000;117(1–2):197–208.

Grottick AJ, Higgins GA. Assessing a vigilance decrement in aged rats: effects of pre-feeding, task manipulation, and psychostimulants. Psychopharm (Berl) 2002;164(1):33–41.

Grottick AJ, Wyler R, Higgins GA. A study of the nicotinic agonist SIB-1553A on locomotion and attention as measured by the five-choice serial reaction time task. Pharmacol Biochem Behav 2001;70(4):505–13.

Hahn B, Ross TJ, Yang Y, Kim I, Huestis MA, Stein EA. Nicotine enhances visuospatial attention by deactivating areas of the resting brain default network. J Neurosci 2007;27(13):3477–89.

Hahn B, Sharples CG, Wonnacott S, Shoaib M, Stolerman IP. Attentional effects of nicotinic agonists in rats. Neuropharm 2003;44(8):1054–67.

Hahn B, Shoaib M, Stolerman IP. Nicotine-induced enhancement of attention in the five-choice serial reaction time task: the influence of task demands. Psychopharm (Berl) 2002;162(2):129–37.

Hahn B, Stolerman IP. Nicotine-induced attentional enhancement in rats: effects of chronic exposure to nicotine. Neuropsychopharm 2002;27(5):712–22.

Holladay MW, Wasicak JT, Lin NH, He Y, Ryther KB, Bannon AW, et al. Identification and initial structure–activity relationships of (R)-5-(2-azetidinylmethoxy)-2-chloropyridine (ABT-594), a potent, orally active, non-opiate analgesic agent acting via neuronal nicotinic acetylcholine receptors. J Med Chem 1998;41(4):407–12.

Jensen AA, Frølund B, Liljefors T, Krogsgaard-Larsen P. Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations. J Med Chem 2005;48(15):4705–55.

Jones GMM, Sahakian BJ, Levy R, Warburton DM, Gray JA. Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. Psychopharmacology 1992;108:485–94.

Levin ED, Conners CK, Silva D, Hinton SC, Meck WH, March J, et al. Transdermal nicotine effects on attention. Psychopharm (Berl) 1998;140(2):135–41 80.

Levin ED, Conners CK, Sparrow E, Hinton SC, Erhardt D, Meck WH, et al. Nicotine effects on adults with attention-deficit/hyperactivity disorder. Psychopharmacology 1996;123(1):55–63.

McGaughy J, Decker MW, Sarter M. Enhancement of sustained attention performance by the nicotinic acetylcholine receptor agonist ABT-418 in intact but not basal forebrain-lesioned rats. Psychopharmacology 1999;144(2):175–82.

McGaughy J, Sarter M. Behavioral vigilance in rats: task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. Psychopharmacology 1995;117(3):340–57.

Mirza NR, Bright JL. Nicotine-induced enhancements in the five-choice serial reaction time task in rats are strain-dependent. Psychopharm (Berl) 2001;154:8-12.

Mirza NR, Stolerman IP. Nicotine enhances sustained attention in the rat under specific task conditions. Psychopharm (Berl) 1998;138(3–4):266–74.

Newhouse PA, Potter A, Singh A. Effects of nicotinic stimulation on cognitive performance. Curr Opin Pharmacol 2004;4:36–46.

Rezvani AH, Bushnell PJ, Levin ED. Effects of nicotine and mecamylamine on choice accuracy in an operant visual signal detection task in female rats. Psychopharmacology 2002;164(4):369–75.

Rezvani AH, Kholdebarin E, Brucato FH, Callahan PM, Lowe DA, Levin ED. Effect of R3487/MEM3454, a novel nicotinic alpha7 receptor partial agonist and 5-HT3 antagonist on sustained attention in rats. Prog Neuropsychopharmacol Biol Psychiatry 2009;33(2):269–75.

Rezvani AH, Levin ED. Cognitive effects of nicotine. Biol Psychiatry 2001;49(3):258–67.

Rezvani AH, Levin ED. Nicotinic–glutamatergic interactions and attentional performance on an operant visual signal detection task in female rats. Eur J Pharmacol 2003;465(1–2):83–90.

Rezvani AH, Tizabi Y, Getachew B, Hauser SR, Caldwell DP, Hunter C, et al. Chronic nicotine and dizocilpine effects on nicotinic and NMDA glutamatergic receptor regulation: interactions with clozapine actions and attentional performance in rats. Prog Neuropsychopharmacol Biol Psychiatry 2008;32(4):1030–40.

Semenova S, Stolerman IP, Markou A. Chronic nicotine administration improves attention while nicotine withdrawal induces performance deficits in the 5-choice serial reaction time task in rats. Pharmacol Biochem Behav 2007;87(3):360–8.
Stolerman IP, Mirza NR, Hahn B, Shoaib M. Nicotine in an animal model of attention. Eur J Pharmacol 2000;393(1–3):147–54.
Taly A, Corringer PJ, Guedin D, Lestage P, Changeux JP. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. Nat Rev Drug Discov 2009;8(9):733–50.
Turchi J, Holley LA, Sarter M. Effects of nicotinic acetylcholine receptor ligands on behavioral vigilance in rats. Psychopharmacology 1995;118(2):195–205.
White HK, Levin ED. Four-week nicotine skin patch treatment effects on cognitive performance in Alzheimer's disease. Psychopharmacology 1999;143(2):158–65.
Young JW, Finlayson K, Spratt C, Marston HM, Crawford N, Kelly JS, et al. Nicotine improves sustained attention in mice: evidence for involvement of the alpha7 nicotinic acetylcholine receptor. Neuropsychopharmacology 2004;29(5):891–900.