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Reversal of a Vigilance Decrement in the Aged Rat by Subtype-Selective Nicotinic Ligands

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In humans, nicotine has been demonstrated to improve both normal and disordered attention, suggesting potential clinical utility for nicotinic ligands. However, attempts to replicate these findings in the rodent have met with some difficulty, thus hampering the search for specific receptor mechanisms underlying these effects. In the present studies, we sought to characterize the effects of nicotine and subtype-selective ligands in a group of aged rats, which show consistent deficits in sustained attention over prolonged sessions of responding in the five-choice serial reaction time task (5-CSRTT). Following the establishment of a replicable performance improvement with nicotine (0.4 mg/kg), we assessed the effects of both SIB 1765F (1–5 mg/kg) and AR-R17779 (20 mg/kg), agonist ligands with selective affinities for the $\alpha_4\beta_2$ and α_7 receptor sites, respectively. We then attempted to block this effect of nicotine using the high affinity, competitive nicotinic antagonist DHβE (3 mg/kg). Finally, in an attempt to determine whether the psychostimulant profile of nicotinic agonists could be dissociated from their effects on attention, we compared the (R)- and (S)-enantiomers of SIB 1765F in the 5-CSRTT, and in their ability to increase locomotor activity. Reversal of a within-session decline in performance speed and accuracy by nicotine was mimicked by SIB 1765F, but not by AR-R17779, whereas DHβE antagonized all of the performance changes induced by nicotine. Finally, the (S)- but not the (R)-enantiomer increased locomotor activity and improved performance in the 5-CSRTT. These results support a critical involvement for the $\alpha_4\beta_2$ nicotinic receptor in mediating the attention-enhancing properties of nicotine.
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INTRODUCTION

In humans, a number of studies have demonstrated that nicotine can enhance attention, both in normal subjects (Wesnes and Warburton, 1984; Koelega, 1993) and in patients with certain psychiatric disorders (Jones *et al*, 1992; Sahakian *et al*, 1989; White and Levin, 1999; Conners *et al*, 1996; Levin *et al*, 1996; Sanberg *et al*, 1997). This has led to the suggestion that ligands selective for nicotinic receptors would be viable targets for the treatment of disordered attention.

A fundamental step in the development of drugs for CNS dysfunction is the use of appropriate behavioral models in nonhuman species. Research into the attention-enhancing properties of nicotine in rodents has been hampered by the inability to demonstrate attentional enhancement consistently with drugs, which are active in human tests of attention. This most likely results from the high baseline levels of performance typically seen in these tasks, rendering them insensitive to further improvement with pharmacological intervention. In order to bypass this obstacle, the five-choice serial reaction time task (5-CSRTT; Carli *et al*, 1983) has recently been utilized to assess the effects of nicotine, using various manipulations to generate low baseline performance. Thus, under conditions of low event-rate (intertrial interval (ITI) increased to 20 s at test), Mirza and Stolerman (1998) detected significant effects of nicotine on accuracy but not performance speed at a single dose (0.15 mg/kg). In nicotine-sensitized rats, nicotine administered at 0.4 mg/kg was found to improve accuracy and at a lower dose (0.2 mg/kg) to increase performance speed (Grottick and Higgins, 2000), and in subjects performing below criterion, subchronic dosing with single doses of nicotine (0.2 mg/kg) over a five-day period was found to increase response speed and accuracy (Grottick and Higgins, 2000).

Aged rats have been used also as a potential model of disordered attention in the 5-CSRTT. It has been demonstrated recently that when session length is increased, two (but not one)-year-old rats demonstrate a progressive decline in performance accuracy and speed as the session progresses, and that this decline in attention is sensitive to reversal by nicotine (Grottick and Higgins, 2002).

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Studies investigating the role of nicotinic receptor (nAchR) subtypes utilizing agonists with differential activity at the various subtypes have to date provided some direction as to those which may underlie the attention-enhancing properties of nicotine. In poorly performing rats, subchronic dosing with the $\alpha_4\beta_2$ nAchR preferring agonist SIB-1765F (5 mg/kg) (Sacaan *et al*, 1997) increased both speed and accuracy of performance (Grottick and Higgins, 2000). Acute and subchronic administration of the selective α_7 nAchR ligand, AR-R17779 (Gordon *et al*, 1998, Gurley *et al*, 1998), failed to improve performance under standard test conditions (Grottick and Higgins, 2000; Hahn *et al*, 2001), and similarly, the β_4 subunit preferring agonist SIB-1553A (Reid *et al*, 1997; Vernier *et al*, 1999) was found to be ineffective both under normal test conditions and in an aged-rat model identical to that described above (Grottick *et al*, 2001).

The purpose of the present study was to evaluate the pharmacology of the vigilance improvement produced by nicotine treatment and described previously (Grottick and Higgins, 2002; Grottick *et al*, 2001). Initial studies compared nicotine, AR-R17779, and SIB-1765F in the aged-rat vigilance task in which session length was increased from 100 to 250 trials. The performance improvement with nicotine was then characterized further by examining the interaction between nicotine and the high-affinity subtype antagonist DHβE (Chavez-Noriega *et al*, 1997; Harvey and Luetje, 1996).

To date, all compounds demonstrated to enhance attention in rodents also appear to stimulate locomotor activity. In order to assess whether this is consistent for nicotinic ligands, we demonstrated performance improvement in the five-choice task with SIB-1765F, and subsequently isolated its enantiomers ((R)- and (S)-3-ethynyl-5-(1-methyl-pyrrolidin-2-yl)-pyridine but-2-enedioic acid), comparing them directly with SIB-1765F on both locomotor activity and vigilance performance. (S)-3-Ethynyl-5-(1-methyl-pyrrolidin-2-yl)-pyridine but-2-enedioic acid corresponds to SIB-1508Y (Cosford *et al*, 1996), a drug currently in Phase II clinical investigation for the treatment of Parkinson's Disease.

METHODS

All studies were conducted at F Hoffmann-La Roche (Basel, Switzerland) and complied with local Cantonal and Swiss federal law regulating animal experimentation.

5-Choice Serial Reaction Time Task

Subjects. Male Lister Hooded rats (Harlan, Netherlands) weighing 400–500 g were used throughout, and were housed in groups of four in holding rooms at controlled temperature (20–22°C) with a 12 h light/dark cycle (lights on at 06:00 h). In order to motivate subjects to perform the task, access to food was restricted so as to maintain 85% of free feeding body weight. Except during testing water was available *ad libitum* at all times. At the beginning of the studies described, subjects were approximately two years of age.

Apparatus. Five-choice operant chambers (Med Associates Inc., St Albans, VT) housed in sound-insulated and ventilated enclosures were used for all experiments. Each chamber consisted of an aluminum enclosure ($25 \times 30\,cm^2$), containing on one wall a food hopper and house light, and on the opposite wall an array of five square niches ($2.5 \times 2.5 \times 2.5$ cm) arranged on a curved panel and raised 2.5 cm from the grid floor. An LED (standard conditions: 150 lux) was positioned at the rear of each niche. All apertures in the chamber including the food hopper were controlled by a photocell placed across the entrance. Operant chambers were controlled by the Kestrel Control System (Conclusive Solutions, Harlow, UK).

Training procedure. Rats were initially given access to a handful of pellets (45 mg Noyes Formula P Food Pellets) in their home cage. Training commenced with sessions in which the food hopper and five light niches were filled with approximately five pellets each. The five-choice task began with the illumination of the house light and delivery of a food pellet. A nose poke into the magazine tray initiated the first trial, which consisted of an ITI (5 s) followed by the random illumination of one of the five lights for a fixed interval (stimulus duration, SD). If a nose poke was registered in the illuminated niche before the end of either the SD or a fixed interval after this period (limited hold, LH), a further pellet was dispensed and a correct trial registered. An incorrect nose poke (incorrect trial) or failure to respond within the allotted time (missed trial) resulted in a time out (TO) period in which the houselight was extinguished for 5 s. Responding into one of the five niches during the ITI (premature response), or after a correct trial was registered (perseverative response), resulted in a further TO. Finally, if a rat responded into a niche during a TO, the TO was restarted.

Each training session ran for either 100 trials or 60 min, whichever was shorter. Initially, stimulus parameters were such that SD was set at 60 s, and ITI, TO, and LH were 5 s. For all subjects, the SD was progressively reduced until a criterion duration of 0.5 s was achieved. All other parameters remained at their initial levels throughout training and test. Training continued under the target stimulus parameters until subjects had achieved consistent performance above a threshold of 75% correct ((correct/(correct+incorrect))100) and $<20\%$ omissions for at least a two-week period.

After attaining criterion performance, all subjects were run in the five-choice task 2–3 times per week until they had reached approximately two years of age. Rats were then returned to the previous training regimen, and were run five days per week until performance was again stable. Prior to these studies subjects in experiments 1 and 2 had previously been drug treated although at the time that the present studies began, all subjects had been drug-free for at least 8 weeks. Subjects contributing data to experiment 3 were previously described as 1-year old in the study of Grottick and Higgins (2002).

Locomotor Activity Studies

Male Sprague–Dawley rats (RCC Ltd, Fullinsdorf, Switzerland), aged 3–4 months at test were used throughout. The animals were housed four per cage in a light and temperature-controlled environment (lights on: 06:00–18:00 h) with food available *ad libitum*. All testing was

conducted during the animals light phase. A repeated measures design was used for the studies, with rats habituated to the test apparatus (36 × 24 × 19 cm, Benwick Electronics, UK) for 3 × daily 2 h sessions before formal activity testing commenced. A 30-min acclimation period to the test apparatus preceded testing, which was of 90 min duration. A washout period of 2–3 days intervened between each treatment cycle.

Experiment 1: Effect of nicotine, SIB-1765F, and AR-R17779 on a vigilance decrement in aged rats

A dose–response to nicotine in the same cohort of rats was previously reported (Grottick and Higgins, 2002). In the present studies, the effects of nicotine (0.4 mg/kg) (exp 1A), SIB-1765F (1.5 mg/kg) (exp 1B), and AR-R17779 (20 mg/kg) (exp 1C) were examined over prolonged five-choice sessions, running for either 250 trials or 60 min. Each study included its own vehicle control, and doses selected were based on previous studies run in this laboratory, $n = 12$ rats per study.

Experiment 2: Blockade of nicotine-induced changes in performance by DHβE

In an attempt to assess the contribution of nicotinic receptor subtypes to nicotine-induced enhancement of five-choice performance, the high-affinity competitive antagonist DHβE (3 mg/kg) was administered either in the presence or absence of nicotine (0.4 mg/kg, $n = 12$). This dose of DHβE was based on previous studies in this laboratory, which demonstrated a complete blockade of nicotine-induced increase of performance speed (Grottick and Higgins, 2000).

Experiment 3: Effect of enantiomers of SIB-1765F on locomotor activity and vigilance

In this study (exp 3A), rats received either SIB-1765F (1 mg/kg), the (R)- (1 mg/kg) or the (S) (1 mg/kg)-enantiomer prior to extended sessions of five-choice performance ($n = 12$).

For the locomotor activity tests (exp 3B), separate groups of rats ($n = 12$) were used to investigate locomotor effects of the (R)- (0.1–3.0 mg/kg) and (S) (0.1–3.0 mg/kg)-enantiomers. A single dose of SIB-1765F (3 mg/kg) was included in each group as a positive control.

All studies described utilized a fully repeated measures design with treatment pseudorandomly assigned to subjects. Between each treatment day 2–3 days intervened, during which subjects that were trained to perform the five-choice task were run under standard stimulus parameters (100 trials, SD = 500 ms).

Drugs and injections

(−)-Nicotine hydrogen tartrate (Sigma), SIB-1765FIND AR-R17779 (synthesized within the Roche CNS Chemistry department), and DHβE (RBI) were dissolved in 0.9% NaCl solution (saline) and the pH of nicotine and SIB-1765F were adjusted to 7.0 by the addition of sodium hydroxide. Doses are expressed as that of the base, and drugs were administered at a dose volume of 1 ml/kg. The route of administration was subcutaneous, except AR-R17779, which was given by the intraperitoneal route. Pretreatment times were: nicotine and SIB-1765F, 5 min; DHβE, 10 min; and AR-R17779 30 min.

Statistical analysis

For five-choice studies, data were collected in 50-trial response bins and initially analyzed using a repeated measures ANOVA with either two within-subjects factors (exp 1 and 3A: treatment × 50-trial bins) or three within-subjects factors (exp 2: DHβE × nicotine × 50-trial bins). Where main effects of treatment were observed, a further ANOVA on session totals was performed in order to determine significant differences between treatments. For locomotor activity studies (exp 3B), activity counts were initially analyzed by ANOVA with two within-subjects factors (enantiomer dose and time bin). This was followed by a one-way ANOVA of data collapsed over the 90 min test session. Where appropriate, all significant main effects were followed by *post hoc* comparisons using the Newman–Keuls test. Finally, for clarity, omission data from experiment 2 were analyzed and presented as percentages.

RESULTS

Experiment 1: Effect of Nicotine, SIB-1765F, and AR-R17779 on Vigilance Performance in Aged Rats

Nicotine and SIB-1765F produced similar changes in performance. Both increased percent correct responses (nicotine, $(F(1,11) = 9.3,\ p < 0.01)$; SIB-1765F, $(F(2,22) = 13.5,\ p < 0.01)$) (Figure 1), reduced correct latency (nicotine, $(F(1,11) = 17.3,\ p < 0.01)$; SIB-1765F, $(F(2,22) = 20.9,\ p < 0.01)$) (Figure 1), decreased omissions (nicotine, $(F(1,11) = 15.1,\ p < 0.01)$; SIB-1765F, $(F(2,22) = 13.6,\ p < 0.01)$ (Table 1), and increased premature responses (nicotine, $(F(1,11) = 9.9,\ p < 0.01)$; SIB-1765F, $(F(2,22) = 27.4,\ p < 0.01)$). Neither compound altered the latency to collect reward (nicotine, $(F(1,11) = 0.1$, NS); SIB-1765F, $(F(2,22) = 0.1$, NS). For SIB-1765F these changes occurred as a function of trials as revealed by significant response-bin × drug interaction terms (percent correct, $(F(8,88) = 5.6,\ p < 0.01)$; correct latency, $(F(8,88) = 4.8,\ p < 0.01)$; omissions, $(F(8,88) = 5.6,\ p < 0.01)$, premature responses, $(F(8,88) = 8.6,\ p < 0.01)$. For nicotine, a significant interaction term was only observed for correct latency $(F(4,44) = 3.6,\ p < 0.01)$. For percent correct $(F(4,44) = 2.5,\ p = 0.06)$ and omissions $(F(4,44) = 2.5,\ p = 0.06)$ there were clear trends towards an interaction that narrowly failed to reach significance (Figure 1a). AR-R17779 did not affect any measure of performance when analyzed as session totals. Over time, the only change induced by AR-R17779 was an increase in omissions (drug × time bin interaction, $(F(4,44) = 5.3,\ p < 0.01)$).

Experiment 2: Blockade of Nicotine-Induced Changes in Performance by DHβE

In this study, the effects of nicotine were essentially replicated, as demonstrated by significant main effects on accuracy $(F(1,11) = 32.0,\ p < 0.01)$ (Figure 2), latency to make a correct response $(F(1,11) = 32.4,\ p < 0.01)$, and omissions $(F(1,11) = 9.5,\ p < 0.01)$ (Figure 2). In this study, the number of premature responses were not significantly

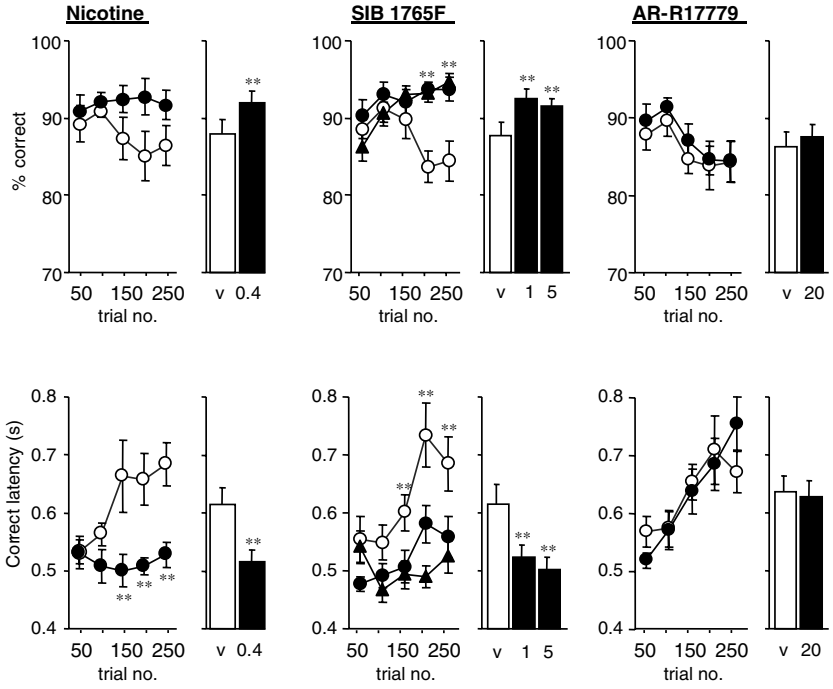

Figure 1 Effect of subtype-selective nicotinic agonists SIB 1765F (1 mg/kg (●), 5 mg/kg (▲)), AR-R17779 (20 mg/kg), and nicotine (0.4 mg/kg) on accuracy and speed of responding in 2-year-old rats performing over sessions of five-choice responding extended to 250 trials ($n = 12$). Data are presented both in 50-trial bins (left panels), and as session totals (right panels). Data points represent means ± SE, *$p < 0.05$, **$p < 0.01$ *vs* vehicle.

Table 1 Performance Measures Following Administration of Subtype Selective Nicotinic Agonists over Extended Sessions of Performance in the 5-CSRTT

Dose (mg/kg)	Omissions	Incorrect latency (s)	Magazine latency (s)	Premature responses	Perseverative responses
Nicotine					
Vehicle	20 ± 2	1.7 ± 0.1	1.6 ± 0.1	15 ± 3	24 ± 4
0.4	11 ± 2**	1.2 ± 0.1**	1.6 ± 0.1	36 ± 5**	27 ± 4
SIB 1765F					
Vehicle	24 ± 5	1.6 ± 0.1	1.7 ± 0.1	9 ± 2	29 ± 5
1	11 ± 2**	1.3 ± 0.1**	1.7 ± 0.1	26 ± 5*	27 ± 2
5	7 ± 1**	1.2 ± 0.1**	1.8 ± 0.1	59 ± 9**	34 ± 4
AR-R17779					
Vehicle	23 ± 5	1.7 ± 0.1	1.6 ± 0.1	7 ± 1	27 ± 4
20	25 ± 3	1.8 ± 0.1	1.6 ± 0.1	8 ± 1	25 ± 3

Results are expressed as means ± SEM. *$p < 0.05$, **$p < 0.01$ in comparison with the respective vehicle control.

changed ($F(1,11) = 3.1$, NS) (Table 2). Additional administration of DHβE to nicotine-pretreated rats altered nicotine's effect on accuracy (nicotine × DHβE interaction, ($F(1,11) = 12.1$, $p < 0.01$)) and correct latency (nicotine × DHβE interaction, ($F(1,11) = 5.3$, $p < 0.05$). *Post hoc* analyses revealed a partial reversal of all nicotine-induced changes: following administration of the nicotine/DHβE combination, both percent correct and correct latency scores differed significantly both from vehicle- and nicotine-treated rats. In this study, nicotine also reduced omissions (Figure 2), although the combination of nicotine and DHβE differed neither from nicotine- nor from vehicle-pretreated rats.

Experiment 3: Effect of SIB-1765F and its (R)- and (S)-Enantiomers on Locomotor Activity and Vigilance Performance

In the vigilance task, a main effect of treatment on accuracy ($F(3,30) = 6.0$, $p < 0.01$), correct latency ($F(3,30) = 11.7$, $p < 0.01$) (Figure 3), and premature responses ($F(3,30) = 5.3$, $p < 0.01$) was recorded (Table 3). *Post hoc* analyses revealed this effect to be because SIB-1765F and its (S)-enantiomer (both 1 mg/kg) significantly improved accuracy and reduced reaction time compared to vehicle-treated controls. The (R)-enantiomer had no effect on any performance measure.

In locomotor activity tests, ANOVA revealed an interaction between enantiomer dose and time bin for both

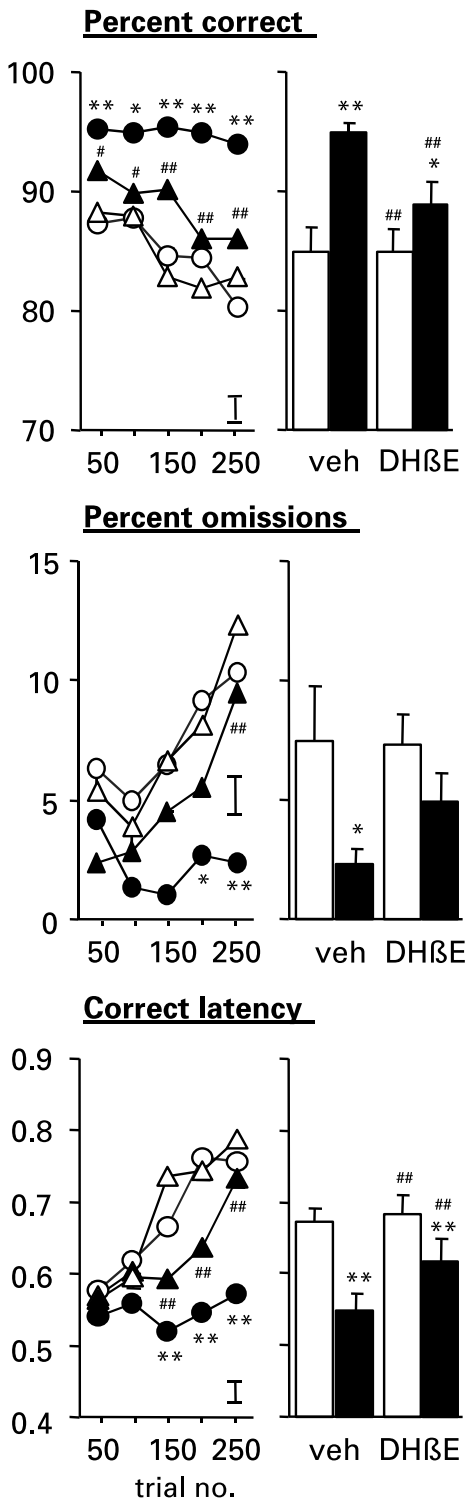

Figure 2 Performance of 2-year-old rats in the 5-CSRTT task following nicotine (0.4 mg/kg), administered either alone, or in combination with DHβE (3 mg/kg): Veh/Veh (○), Nic/Veh (●), Veh/DHβE (△), Nic/DHβE (▲), ($n = 12$). Data are presented both as session totals (right-hand panels; open bars, vehicle; closed bars, nicotine) and in 50-trial time bins (left-hand panels). Data points represent means; mean standard errors are illustrated within the line graphs. *$p < 0.05$ *vs* vehicle, **$p < 0.01$ *vs* vehicle, $^{\#}p < 0.05$ Veh/DHβE *vs* Nic/DHβE, $^{\#\#} < 0.01$ Veh/DHβE *vs* Nic/DHβE.

the (S)-($F(4,440) = 2.7$, $p < 0.01$) and the (R)-enantiomers ($F(40,440) = 1.7$, $p < 0.01$). Further analysis of data collapsed over the 90 min session revealed a main effect of drug treatment in both the (S)- ($F(5,55) = 5.3$, $p < 0.01$) and the (R)-enantiomer studies ($F(5,55) = 6.6$, $p < 0.01$). However, in the (R)-enantiomer study this was accounted for solely by the increase in activity induced by SIB-1765F, as all other doses did not differ from vehicle. The (S)-enantiomer induced a dose-dependent increase in activity. Thus, the (S)- but not (R)-enantiomer of SIB-1765F increased locomotor activity. The effect of SIB-1765F itself was similar across treatment groups (Figure 4).

DISCUSSION

The present studies were designed to investigate further the effects of subtype-selective nicotinic ligands in the 5-CSRTT, utilizing a protocol which has been demonstrated previously to be sensitive to performance enhancement with nicotine. In this protocol, aged rats are subjected to extended sessions of five-choice responding, resulting in performance disruption over time that is not accounted for by satiety, and further is sensitive to reversal by amphetamine, caffeine, and nicotine (Grottick and Higgins, 2002). Here, we replicated the performance improvement with nicotine, and in the same cohort of rats demonstrated similar performance improvement with the $\alpha_4\beta_2$ agonist SIB-1765F, but not the α_7 agonist AR-R17779. Pretreatment with the high-affinity antagonist DHβE partially reversed the improvement seen with nicotine. Finally, the (S)- but not the (R)-enantiomer of SIB-1765F, which has higher functional activity at the $\alpha_4\beta_2$ nAchR (SIB1508Y; Cosford *et al*, 1996), increased locomotor activity and improved attentional performance.

Nicotine and SIB-1765F significantly increased accuracy and response speed and decreased omissions. Each of these performance measures reflect aspects of attention, and are generally consistent with the pattern of effects observed in humans performing sustained attention tasks (Parasuraman and Davies, 1976; Smith and Nutt, 1996; Koelega, 1993). Importantly, each of these changes occurred as a factor of responding, such that beneficial effects only became apparent as control performance declined. Explanations for this effect include a specific reversal of attentional decline, a negation of the onset of fatigue, or the near-asymptotic nature of performance at the beginning of sessions making them impervious to further improvement. Fatigue appears an unlikely explanation, given that we have reported previously a similar reversal of the decrement by increasing stimulus detectability (Grottick and Higgins, 2002). Under these conditions an identical number of responses are made throughout the session, which if a fatigue explanation is invoked would be expected to produce similar within-session declines. However, whether the late-onset effect of nicotine and SIB-1765F reflect a reversal of declining attention late in the session, or an overall attentional enhancement masked by high levels of performance at session onset cannot be confirmed by the present studies.

Although the present study assessed a single dose of AR-R17779 only, this confirms previous studies using the 5-CSRTT, which demonstrate the absence of any performance

Table 2 Effect of Nicotine alone and in Combination with the High-Affinity Nicotine Antagonist DHβE on Five-Choice Performance

Treatment (mg/kg)	Omissions	Incorrect latency (s)	Magazine latency (s)	Premature responses	Perseverative responses
Vehicle/vehicle	19 ± 6	1.8 ± 0.1	1.7 ± 0.1	10 ± 3	21 ± 2
Nicotine (0.4)/vehicle	6 ± 2*	1.6 ± 0.2	2.0 ± 0.2*	19 ± 6	25 ± 3
Vehicle/DHβE (3)	18 ± 3	1.8 ± 0.1	1.7 ± 0.1	8 ± 1	24 ± 3
Nicotine (0.4)/DHβE (3)	12 ± 3	1.6 ± 0.1	1.7 ± 0.1	15 ± 5	23 ± 3

Results are expressed as means ± SEM. *$p<0.05$ compared to vehicle.

Table 3 Effect of SIB 1765F and its Enantiomers on Measures of Five-Choice Performance

Treatment (mg/kg)	Omissions	Incorrect latency (s)	Magazine latency (s)	Premature responses	Perseverative responses
Vehicle	19 ± 4	1.7 ± 0.1	1.8 ± 0.2	9 ± 2	27 ± 4
SIB 1765F (1)	18 ± 5	1.3 ± 0.1**	1.9 ± 0.2	23 ± 5*	34 ± 5
(R)-enantiomer (1)	19 ± 8	1.6 ± 0.1	1.8 ± 0.2	12 ± 3	28 ± 4
(S)-enantiomer (1)	12 ± 3	1.3 ± 0.1**	1.9 ± 0.2	23 ± 4*	31 ± 3

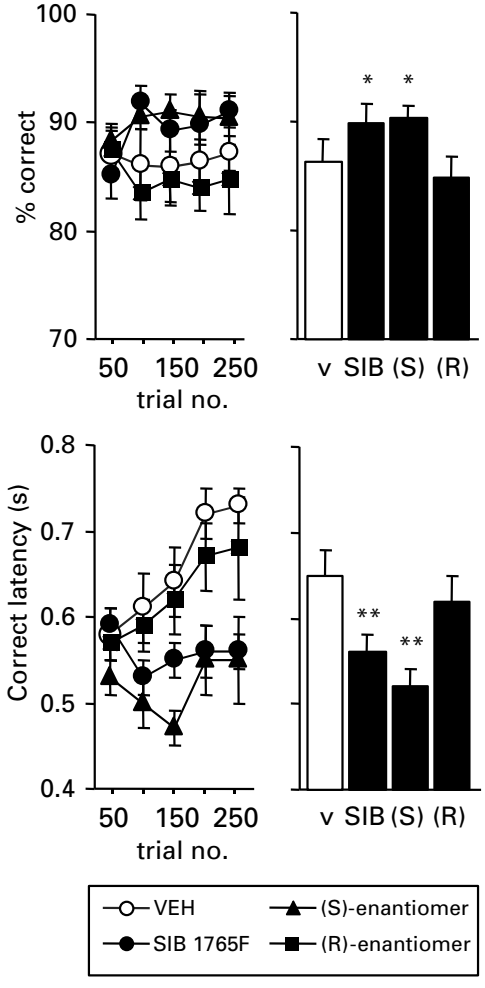

Figure 3 Effect of SIB 1765F (1 mg/kg) and its enantiomers ((S) and (R), both 1 mg/kg) on accuracy (top panel) and correct latency over extended sessions of five-choice responding ($n=12$). Data are presented both in 50-trial bins (left panels), and as session totals (right panels). Data points represent means ± SE, *$p<0.05$, **$p<0.01$ *vs* vehicle.

effect with selective α_7 agonists including AR-R 17779 (Grottick and Higgins, 2000; Hahn *et al*, 2001), or GTS-21 (Blondel *et al*, 1999), and no antagonism of nicotine-induced effects with the α_7-selective blocker methylycaconitine (Blondel *et al*, 2000; Grottick *et al*, 2000a). In the study of Hahn *et al* (2001), AR-R 17779 was tested across a wide dose range (3–24 mg/kg) with no effect at any dose. While improvements in reference and working memory function have been demonstrated with AR-R 17779 (Levin *et al*, 1999), the apparent lack of effect of α_7 ligands in the five-choice task might suggest that these result from selective effects on mnemonic processes, rather than from secondary enhancement of attentional processes.

Increases in response speed and premature responding effected by nicotine in both nontolerant and nicotine-sensitized rats are fully antagonized by coadministration of DHβE (Blondel *et al*, 2000; Grottick and Higgins, 2000). The performance improvement induced by nicotine in the present studies enabled further assessment of the interaction between nicotine and DHβE. Thus, nicotine increased accuracy and magazine latency, and decreased omissions, all of which were partially reversed by pretreatment with DHβE. This partial antagonism could reflect either the dose of antagonist employed, or alternatively an additional, DHβE-insensitive action of nicotine, although given previous studies with nicotinic ligands it is unclear which particular subunit combination this may be.

Experimental manipulations, including both pharmacological and surgical intervention, have demonstrated that the various parameters of five-choice performance reflect distinct and dissociable processes. Despite this, the array of changes exerted by nicotine in the 5-CSRTT have now been demonstrated to be sensitive to antagonism by DHβE.

The (R)- and (S)-enantiomers of SIB-1765F possess differential functional activity at the $\alpha_4\beta_2$ nAchR subtype. While the (S)-enantiomer (SIB-1508Y) increased intracellular Ca^{2+} influx into HEK293 cells expressing $\alpha_4\beta_2$ nAchR with an equivalent potency to nicotine and racemic

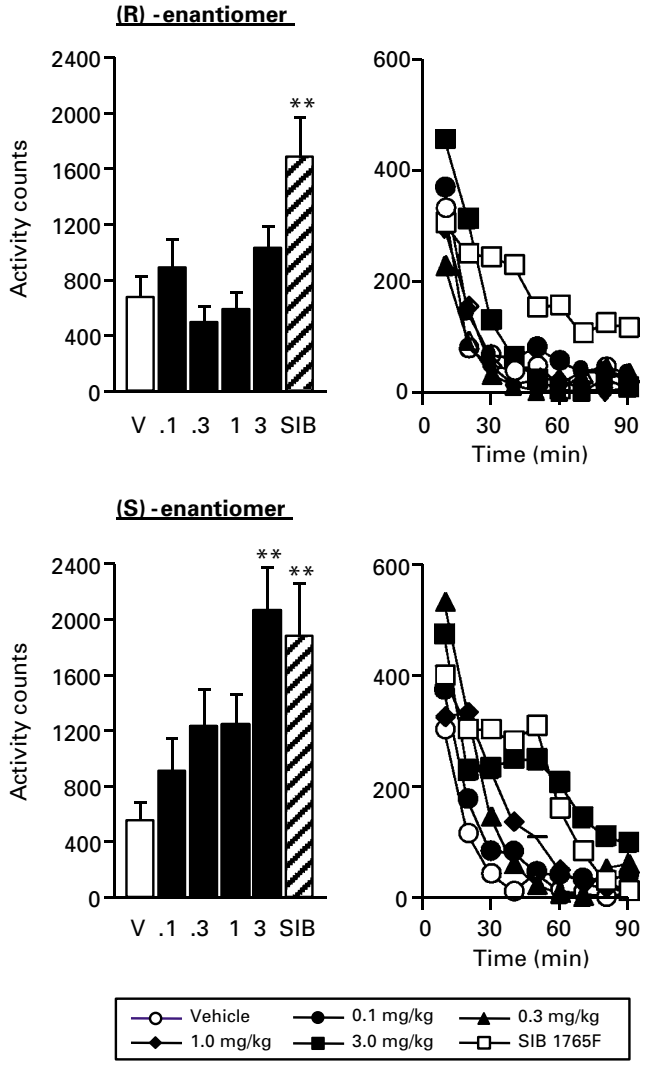

Figure 4 Locomotor activity following administration of the (R)- and (S)-enantiomers (0.1–3.0 mg/kg) of SIB 1765F (3 mg/kg) ($n = 12$). Data represent total activity counts for a 90-min period following injection. Both studies include SIB 1765F (3 mg/kg) for comparison. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* respective vehicle controls.

SIB-1765F, the (R)-enantiomer was inactive in this assay (Cosford *et al*, 1996). Both SIB-1765F and nicotine increase locomotor activity (Menzaghi *et al*, 1997; Clarke and Kumar, 1983a, b; Reavill and Stolerman, 1990; Louis and Clarke, 1998) in a DHβE-sensitive manner (Menzaghi *et al*, 1997; Stolerman *et al*, 1997; Grottick *et al*, 2000a). The present studies demonstrate the (S)-enantiomer to be responsible for both the stimulant effects of SIB-1765F and its effects on attention, as the (S)-, but not (R)-enantiomer increased locomotor activity and produced a similar profile to SIB-1765F in the five-choice task, increasing accuracy and response speed. Although different rat strains were utilized for the locomotor and five-choice studies, previous studies suggest that qualitatively similar results would be expected if the same strain had been used in each test. For example, nicotine-induced increases in locomotor activity (Menzaghi *et al*, 1997; Benwell and Balfour, 1992; Clarke and Kumar, 1983) and changes in five-choice performance (Blondel *et al*, 2000; Mirza and Stolerman, 1998) have been reported in both the Sprague–Dawley and Lister-Hooded rat strain.

Taken together, these data coupled with other studies using subtype-selective agonists and antagonists provide considerable support for the suggestion that the $\alpha_4\beta_2$ site is essential for the attentional-enhancing and locomotor stimulant properties of nicotine, and that these effects are not dissociable.

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