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Profile of nicotinic acetylcholine receptor agonists ABT-594 and A-582941, with differential subtype selectivity, on delayed matching accuracy by young monkeys

Jerry J. Buccafusco [a,b,1,*], *Alvin V. Terry Jr.* [a,b,1], *Michael W. Decker* [c,2], *Murali Gopalakrishnan* [c,2]

[a] *Department of Pharmacology and Toxicology, Alzheimer's Research Center, Medical College of Georgia, Augusta, GA 30912, USA*
[b] *Veterans Administration Medical Center, Augusta, GA 30904, USA*
[c] *Abbott Labs, Neuroscience Research, 100 Abbott Park Rd, Abbott Park, IL 60064-6125, USA*

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ABSTRACT

ABT-594 and A-582941 are high affinity neuronal nicotinic acetylcholine receptor agonists with differential selectivity for the α4β2 and the α7 subtypes, respectively. This study was designed to determine whether either compound, like nicotine also possesses cognitive-enhancing ability. The compounds were administered by intramuscular injection to young adult Rhesus monkeys trained to perform two versions of a computer-assisted delayed matching-to-sample (DMTS) task. ABT-594 (0.115–3.7 μg/kg) significantly improved DMTS accuracies, shifting the retention curve (accuracy–delay relationship) to the right in a parallel fashion. DMTS accuracy also was maintained during the sessions initiated 24 h after compound administration. Because task accuracy was improved during short delay trials, a separate study was performed in which non-predictable distractors were inserted within the DMTS format to impair accuracy. The 0.115 μg/kg dose of ABT-594 almost completely reversed distractor-impaired performance associated with short delay trials. The α7 nAChR agonist, A-582941 (1.14–38 μg/kg) also significantly improved DMTS accuracies. The compound produced a significant improvement during long delay trials. The effect was twice as robust for long delay as compared with short delay trials and A-582941 was not as effective as ABT-594 in improving short delay trial accuracy. A-582941 also failed to sustain task improvement during sessions run 24 h after dosing. These data are consistent with the ability of subtype-preferring nicotinic receptor agonists to enhance specific components of working memory and cognitive function, and they suggest that differential subtype selectivity could result in varied pharmacological response profiles.

* *Corresponding author at*: Alzheimer's Research Center, Medical College of Georgia, 1120-15th Street, Augusta, GA 30912-2300, USA. Tel.: +1 706 721 6355; fax: +1 706 721 9861.
E-mail address: jbuccafu@mcg.edu (J.J. Buccafusco).

[1] The author was a collaborator who studied these compounds in the monkey DMTS task.

[2] The author is an employee of Abbott Labs.

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1. Introduction

Central nicotinic acetylcholine receptors (nAChRs) serve as potential therapeutic targets for a wide number of human diseases [1–4]. For example, nicotine and a number of nAChR agonists have been evaluated as potential cognition-enhancing agents, particularly for the treatment of Alzheimer's disease [for review, 1,4,5–10]. Our laboratory has had the opportunity to study a wide variety of compounds targeting nicotinic receptors for cognition enhancement in non-human primates. These compounds produce varying degrees of improvement in delayed matching-to-sample (DMTS) task accuracies in both young adult and aged (>19-year-old) macaques [4,6,11]. Nicotine and the synthetic nicotinic agonists ABT-418, ABT-089, and SIB-1553A were shown to improve accuracies by monkeys performing the standard DMTS task [12–14]; but they were also effective in a version of the DMTS task that measures attention, i.e., in which non-predictable distractors are inserted within the DMTS format [15,16].

Some of these early nAChR ligands were not sufficiently subtype-selective to avoid some of the more common nicotinic side effects. The synthesis and characterization of novel synthetic nAChR agonists binding selectively to either the hetero-oligomeric α4β2 or to the homo-oligomeric α7 nAChR subtype has uncovered the specific pharmacological responses mediated by the two subtypes. One of the most studied compounds, both preclinically and clinically, largely for its potential as an analgesic agent [17], is ((*R*)-5-(2-azetidinylmethoxy)-2-chloropyridine) (ABT-594) [18]. ABT-594 displays high affinity in displacing [^{3}H](−)-cytisine from α4β2 neuronal nAChRs (K_i = 37–55 pM), and has greater than 180,000-fold selectivity for the α4β2 subtype relative to the α1β1δγ neuromuscular nicotinic receptor labeled by [^{125}I]α-bungarotoxin [19]. ABT-594 is approximately 25 times more potent than nicotine in displacing [^{3}H](−)-cytisine binding to α4β2 receptors; but exhibits three-fold less affinity relative to nicotine binding α7 homo-oligomeric nicotinic receptors (estimated by displacement of [^{125}I]α-bungarotoxin binding). Though epibatidine is 60-fold more potent than ABT-594 in α7 binding assays, the compounds are equipotent with respect to α4β2 receptors. Thus, ABT-594 is significantly more selective for α4β2 receptors in binding studies than either nicotine or epibatidine. With respect to functional studies, ABT-594 is about 17 times more potent than nicotine at human α4β2 (and more efficacious than nicotine) in the ability to enhance calcium flux in a stable cell line, but the analog is only about 1.5 times more potent than nicotine at human α7 receptors (with the same efficacy) as measured electrophysiologically in transfected oocytes. Thus, for ABT-594 the available evidence indicates a net improvement over nicotine selectivity with respect to the α4β2 and α7 subtypes by a factor of 75 in binding studies and slightly more than a factor of 10 in functional studies [18]. In a related *in vivo* study ABT-594 treatment in rats was shown to increase the brain expression of fibroblast growth factor-2 mRNA [20]. Consistent with its *in vitro* binding profile, these effects of ABT-594 were antagonized by the partly α4β2-selective nicotinic receptor antagonist dihydro-β-erythroidine, but not by the selective α7 antagonist methyllycaconitine.

The α7 homo-oligomeric subtype of nAChRs is not as highly expressed as the α4β2 subtype, but compounds targeting α7 receptors also have been developed for therapeutic potential [4,21–23]. Agonists for α7 receptors have been reported to produce neuroprotective activity [24–27] and to enhance cognition in a variety of experimental models [28–30]. A preliminary report regarding the pharmacological characterization of A-582491 (2-methyl-5-(6-phenyl-pyridazin-3-yl)-octahydro-pyrrolo[3,4-*c*]pyrrole) indicated significant α7 subtype selectivity. The compound also stimulated MAPK/ERK1/2/CREB phosphorylation in PC12 cells, a CNS signaling pathway involved in learning and memory consolidation, and increased ERK1/2 and CREB phosphorylation in mouse cingulate cortex and hippocampus. Unlike some of the initially disclosed quinuclidine-derived α7 agonists, A-582941 exhibited excellent CNS penetration at least in rodents [31].

The purpose of this study was to compare the abilities of ABT-594 and A-582941 to improve accuracies by monkeys in their performance of the DMTS task. The expectation was that differences between the pharmacological responses evoked by the two compounds might reflect their relative selectivities for the two major nAChR subtypes.

2. Methods

2.1. Study subjects

All procedures were reviewed and approved by the Medical College of Georgia Institutional Animal Care and Use Committee and they are consistent with AAALAC guidelines. The subjects used in this study (Table 1), seven adult male Rhesus monkeys (*Macaca mulatta*) were individually housed at the Animal Behavior Center of the Medical College of Georgia in stainless steel cages composed of multiple 127 cm × 71 cm × 66 cm units. To promote psychological well-being toys and foraging tubes were provided routinely and monkeys were allowed to observe television programs each afternoon after testing and on weekends. The subjects were well trained (>100 individual sessions) in the delayed matching-to-sample (DMTS) task. The animals were maintained on tap water (unlimited) and standard laboratory monkey chow (Harlan Teklad Laboratory monkey diet, Madison, WI) supplemented with fruits and vegetables. The animals were maintained on a feeding schedule in which all food was removed from cages at about 06:30 h, and replaced after completion of testing of all subjects for the day (at about 16:30 h). During testing additional food intake was derived from 300 mg reinforcement food pellets (commercial composition of standard monkey chow and banana flakes, Noyes Precision food pellets, P.J. Noyes Co., Lancaster, NH) obtained during experimental sessions. On weekends animals were fed without time restrictions. The monkeys were maintained on a 12 h light–dark cycle and were tested each week day between 09:00 and 14:00 h. Room temperature and humidity was maintained at 72 ± 1 °C and 52 ± 2%, respectively. In addition to the demographic information provided in Table 1, each of the subjects had participated in one or more previous studies in which potential cognitive enhancing agents were evaluated. Compounds were administered during acute studies no more than twice per week. The behavioral characteristics

Table 1 – Subject Information

Subject I.D.	Sex	Age (years)	Weight (kg)	Delay interval (sec)			
				Species	Short	Medium	Long
23	M	18	10	Rhesus	50	110	220
24	M	11	7.2	Rhesus	50	120	220
308	M	9	9.6	Rhesus	80	140	220
18	M	10	9.8	Rhesus	55	100	130
573	M	10	11.8	Rhesus	5	10	20
281[a]	M	16	11.6	Rhesus	4	6	9
618	M	10	11.8	Rhesus	5	10	15
Mean		12	10.3		36	71	119
S.E.M.		1.3	0.6		11.6	22.5	38.9

[a] Not available for the A-582941 series.

and general physiognomy of each animal is well known to our technical staff. At the end of each test period, animals are examined for signs of untoward drug effects. Food intake and the quantity and quality of stools are noted for any deviation from normal. Finally, animals will generally decrease responding and/or task latencies will dramatically increase in response to GI drug-related side effects. In this study no side effects or long-lasting effects were observed, and all animals were provided at least a 4 week washout period prior to the studies.

2.2. *Delayed matching-to-sample (DMTS)*

2.2.1. *Standard task*

Test panels attached to each animal's home cage present the delayed matching-to-sample (DMTS) task by using a computer-automated system [32]. A touch-sensitive screen (15 in. AccuTouch LCD Panelmount TouchMonitor)/pellet dispenser unit (Med Associates) mounted in a light-weight aluminum chassis was attached to the home cage. The stimuli included red, blue, and yellow rectangles. A trial was initiated by presentation of a sample rectangle (with a 3D look) composed of one of the three colors. The sample rectangle remained in view until the monkey touched within its borders to initiate a pre-programmed delay (retention) interval. Following the delay interval, the two choice rectangles located below where the sample had been were presented. One of the two choice rectangles was presented with its color matching the stimulus color, whereas the other (incorrect) choice rectangle was presented as one of the two remaining colors. A correct (matching) choice was reinforced. Non-matching choices were neither reinforced nor punished. The inter-trial interval was 5 s and each session consisted of 96 trials. The presentation of stimulus color, choice colors, and choice position (left or right on the screen) were fully counterbalanced so as to relegate non-matching (mediating) strategies to chance levels of accuracy. Five different presentation sequences were rotated through each daily session to prevent the subjects from memorizing the first several trials.

Monkeys exhibit individual capabilities to maintain matching-to-sample accuracy, particularly as delay intervals are extended. Delay intervals were established during several non-drug sessions or vehicle sessions run prior to initiating the study. The duration for each delay interval was adjusted until three levels of performance difficulty were obtained: zero delay (85–100% of trials answered correctly); short delay interval (75–84% correct); medium delay interval (65–74% correct); and long delay interval (55–64% correct) representing each animal's limit in terms of DMTS performance. Zero delay (nearly simultaneous matching) was included as a control to monitor the animals' ability to maintain reference memory, and the ability to match-to-sample. The assignment of retention intervals based upon an individual's baseline levels of task accuracy is necessary to avoid ceiling effects in the most proficient animals during studies, while also serving to insure that each animal begins testing at relatively the same level of task difficulty [33]. Accuracy values obtained for each difficulty level (retention interval) were averaged and recorded as the mean percent correct. Effects were calculated as the absolute change from vehicle-associated accuracy. A 3 min interval was allowed for the animal to respond after a sample or choice presentation. Failure to respond initiated the next trial in the sequence. Each trial that does not receive a response is deemed not applicable and the % trials correct is determined only from the total number of trials actually completed. At least 64 trials must be completed to constitute a valid session. In addition to session accuracy and the number of completed trials, two (median) response latencies also were measured: the ''sample latency'', which is the time between presentation of the sample color and the animal touching the sample rectangle; and the ''choice latency'', which is the time between presentation of the choice rectangles and the animal touching one of the choice rectangles. Latencies were recorded to the nearest 100th of a second [34].

2.2.2. *Distractor DMTS*

Distractor stimuli were presented in a non-predictable manner to the test subject on 18 of the 96 trials completed during distractor DMTS sessions. The stimuli were presented simultaneously with the sample and choice rectangles for 3 s and they consisted of a random pattern of the three colored rectangles flashing in an alternating manner. The distractor images were comprised of the same three colors used for sample and choice stimuli presentation. The total duration of onset for a given colored rectangle was 0.33 s. Immediately as one colored rectangle was extinguished, a different colored rectangle was presented. Thus, during presentation of the distractor, each color was presented in random order for each rectangle three separate times. Distractor stimuli were

present an equal number of times on trials with short, medium, and long delay intervals. The remaining trials were completed with no delay interval or distractor and they are randomly placed throughout the test session.

2.3. Drug administration protocol

ABT-594 [((*R*)-5-(2-azetidinylmethoxy)-2-chloropyridine) tosylate] and A-582941 [2-methyl-5-(6-phenyl-pyridazin-3-yl)-octahydro-pyrrolo[3,4-*c*]pyrrole dihydrochloride] were synthesized at Abbott (Abbott Park, IL). Stock solutions in sterile normal saline were freshly prepared at the start of each test day. Doses were administered as a single injection in a thigh muscle in a volume of 0.035 ml/kg. During standard DMTS testing vehicle was usually administered on Mondays, and compound was administered on Tuesdays and Thursdays. On Wednesdays and Fridays animals were tested with no compound or vehicle preceding the test. Distractor sessions were administered no more than three times every 2 weeks so as to preclude the animals becoming tolerant to the distractor. Doses were generally administered in ascending order to avoid potential side effects associated with the higher doses. All references to doses indicate the salt form of the compound. The dose ranges to be studied in monkeys were selected to match plasma concentrations where efficacy was observed in earlier rodent models of cognition. The time interval between drug administration and the initiation of DMTS testing for each compound was based on studies in satellite monkeys. With ABT-594 the *T*max was achieved in about 6 min ($t1/2 = 1.5$ h). Accordingly, a 10 min pretreatment time was chosen for testing. With A-582941, the *T*max was achieved in approximately 20 min ($t1/2 = 2.4$ h); accordingly, a 30 min pretreatment time was chosen for testing.

2.4. Statistical analyses

The following parameters were recorded for all trials during all test sessions (i.e., 96-trials per session): % trials correct on trials with zero, short, medium, and long delay intervals and task latencies. Data were subdivided according to delay interval for each 24-trial delay component of the session. Two task latencies were recorded and collapsed across delay intervals: the sample latency (time interval between presentation of the sample stimulus and the subject touching the sample rectangle), and the choice latency (time interval between presentation of the choice stimuli and the subject pressing a choice response rectangle). Data were analyzed by use of a multi-factorial analysis of variance (ANOVA) with repeated measures (SAS, JMP statistical software package). For post hoc analyses, a least squares means orthogonal multi-comparison *t*-test was used to compare individual means. For each table/figure (below) error values denoted by ± indicates the standard error of the mean. Differences between means from experimental groups were considered significant at the $P < 0.05$ level (two-sided test). Trends toward significance were considered when $P < 0.10$. For each analysis in which the duration of the four delay intervals appeared as an independent variable, these were always statistically significant in the ANOVA tables ($P < 0.01$).

3. Results

3.1. ABT-594

3.1.1. Standard DMTS testing

For this experimental series, part of the dose–response relationship was obtained on two separate occasions in the same subjects (the two highest doses were examined only one time). The data for duplicate doses were combined in the analysis. During the course of this study standard DMTS vehicle sessions were performed on eight occasions (four for each dose–response relationship). These values were averaged to provide the vehicle baseline means against which all subsequent drug sessions were compared. The mean vehicle accuracies for zero, short, medium, and long delay intervals were, respectively, 93.2, 76.9, 70.4, and 66.8% trials correct. Treatment with ABT-594 produced a significant improvement in task accuracies ($F_{6,442} = 5.17$, $P < 0.0001$). The effect of the two most effective doses (0.23 and 0.46 μg/kg) of ABT-594 on standard DMTS accuracies are presented in Fig. 1 for those

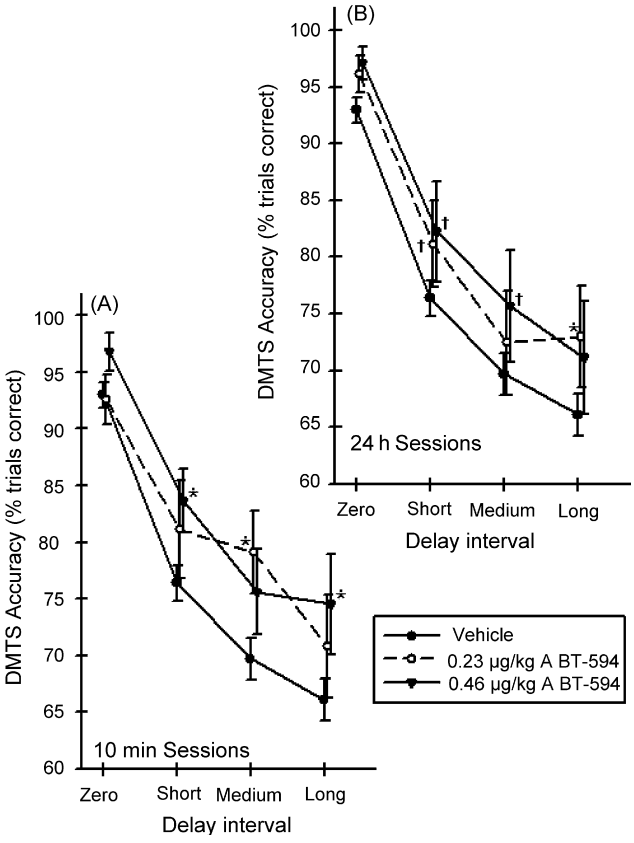

Fig. 1 – The effect of ABT-594 on accuracy in the standard DMTS task by adult Rhesus monkeys. Test sessions were initiated 10 min (panel A) or 24 h (panel B) after compound administration. Data points and error bars were slightly displaced for clarity of presentation. Each data point represents the mean ± S.E.M. derived from six subjects. *$P < 0.05$; †$P < 0.10$ as compared with respective vehicle means. Only vehicle means, and those obtained from sessions initiated after administration of either the 0.23 or 0.46 μg/kg doses of ABT-594 are plotted as a function of delay interval for clarity (see Fig. 2).

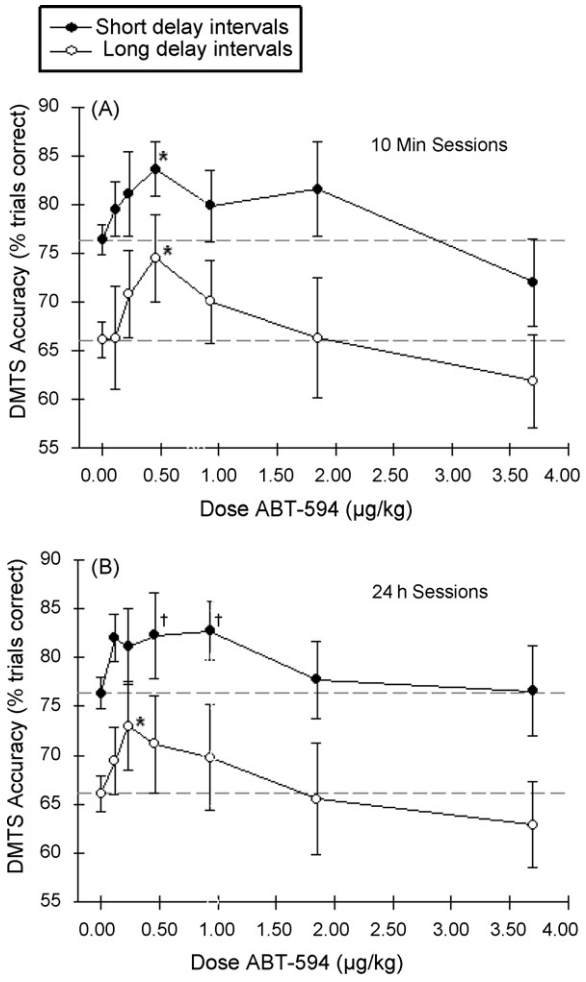

Fig. 2 – The effect of ABT-594 on accuracy in the standard DMTS task by adult Rhesus monkeys. Data associated with short and long delay trials are plotted as a function of dose. Test sessions were initiated 10 min (panel A) or 24 h (panel B) after compound administration. The 0 mg/kg dose represents the data derived from vehicle administration in which testing was initiated 10 min later. Each data point represents the mean ± S.E.M. derived from six subjects. *P < 0.05; †P < 0.10 as compared with respective vehicle means.

sessions initiated 10 min after compound administration. Compound treatment resulted in a significant increase in task accuracies associated with the short delay trial after the 0.23 μg/kg dose ($t = 2.0$, $P = 0.048$), and with long delay trials after the 0.23 ($t = 3.02$, $P = 0.0027$) and 0.46 ($t = 2.38$, $P = 0.018$) μg/kg doses. Overall the effect was to shift the memory retention curve after compound administration to the right of the vehicle curve in an approximately parallel fashion. In fact, the increase in accuracy during long delay trials (11.6% above control) was not that different from the increase during short delay trials (8.8% above control). The complete dose–response relationships obtained during short and long delay trials are presented in Fig. 2. The dose–response profiles suggest an inverted U-type relationship with the effect peaking at about the 0.46 μg/kg dose for each delay interval. Inspection of

Table 2 – Combined (all vehicle and dose values) median choice and sample task latencies obtained during the initial (10 min) sessions after ABT-594 or A-582941 administration

	Mean (sec)	Standard error
ABT-594		
Choice	3.624	0.541
Sample	1.534	0.541
ABT-594 non-distractor		
Choice	3.466	0.528
Sample	1.629	0.528
ABT-594 distractor		
Choice	4.541	0.648
Sample	1.704	0.648
A-582941		
Choice	4.204	0.442
Sample	2.121	0.442

No drug treatment produced a statistically significant change from vehicle-latency means.

Table 1 indicates that subjects 573, 281 and 618 were assigned much shorter delay intervals as compared to the other subjects. This is because subjects 573, 281 and 618 were less experienced. However, the lack of task experience did not contribute to their responses to ABT-594. In analyzing the changes from baseline (across all four delay intervals) for each subject after receiving each dose of ABT-594, there were no significant differences among the individual subjects' increase in task improvement to the compound ($F_{6,58} = 0.705$, $P = 0.65$).

ABT-594 produced no significant effect on either sample or choice task latencies ($F_{6,219} = 0.186$, $P = 0.98$). Averaged latency values for this series are presented in Table 2.

During the sessions run 24 h after compound administration, the increase in task accuracy obtained during the previous day's sessions was generally maintained ($F_{6,442} = 2.85$, $P = 0.001$). There was a significant increase in task accuracy associated with the long delay trial after the 0.23 μg/kg dose ($t = 2.03$, $P = 0.043$). There also were trends towards significant increases in accuracies during short delay trials after the 0.46 ($t = 1.7$, $P = 0.09$) and 0.93 ($t = 1.8$, $P = 0.07$) μg/kg doses; and during medium delay trials after the 0.46 μg/kg dose ($t = 1.7$, $P = 0.09$). Again, the memory retention curve was shifted to the right of the vehicle curve. The complete dose–response relationships obtained during short and long delay trials during 24 h sessions are presented in Fig. 2. The dose–response profiles appeared to be similar to those obtained during the previous day's (10 min) sessions.

3.1.2. *Distractor DMTS testing*

Because the ABT-594 improved short delay accuracies in the standard DMTS task, we elected to attempt to confirm possible effects of the compound to enhance attentional components of memory (reverse distractibility). For this experimental series, part of the dose–response relationship was obtained on two separate occasions in the same subjects (the two highest doses were examined only one time). The data for duplicate doses were combined in the analysis. During the course of this study standard DMTS vehicle sessions were performed on two occasions (one for each dose–response relationship).

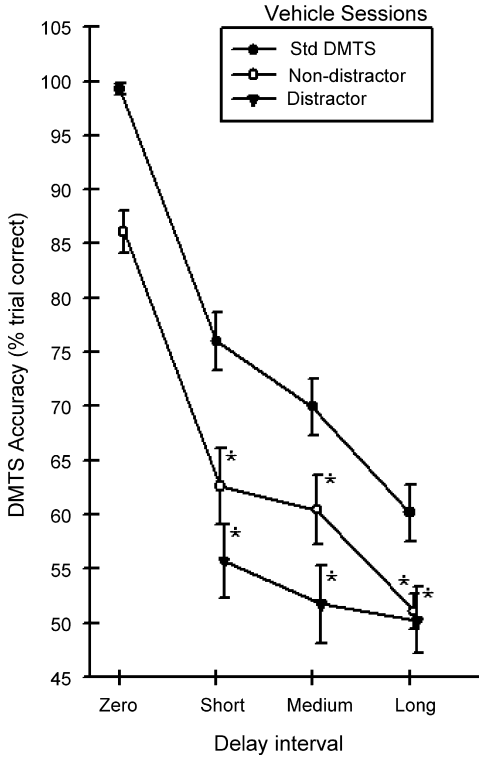

Fig. 3 – Comparison of standard DMTS and distractor DMTS vehicle (sterile normal saline) sessions with respect to task accuracies by adult Rhesus monkeys. Test sessions were initiated 10 min after compound administration. Standard DMTS indicates the values derived from standard vehicle DMTS sessions run prior to the distractor-DMTS series. "Non-distractor" and "Distractor" respectively indicate the 72 non-distractor trials and the 24 distractor trials included in the distractor-DMTS task. $^{*}P < 0.05$ as compared with respective standard DMTS means.

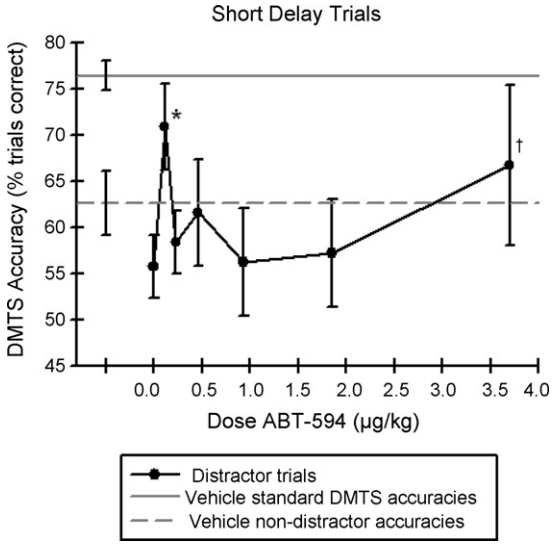

Fig. 4 – Effect of ABT-594 on short delay trial accuracies of the distractor DMTS task by adult Rhesus monkeys plotted as a function of dose. Test sessions were initiated 10 min after compound administration. The solid horizontal line and error bar indicate the mean ± S.E.M. for short delay as derived from standard vehicle DMTS sessions run prior to the distractor series. The dashed horizontal line and error bar indicates the mean ± S.E.M. for vehicle short delay trials obtained from non-distractor trials that were a component of the distractor-DMTS sessions. $^{*}P < 0.05$; $^{\dagger}P < 0.10$ as compared with vehicle distractor mean (0.0 μg/kg).

The number of vehicle sessions was kept to a minimum to avoid the possibility of subjects becoming tolerant to the distractor. For each session there were 24 randomly placed distractor trials and 72 non-distractor trials. These two sets of data were analyzed separately. Mean vehicle accuracies were compared between the standard DMTS trials, and the non-distractor trials and distractor trials (Fig. 3). In general, the accuracies obtained both during non-distractor trials and distractor trials were lower than those obtained in the standard DTMS task ($F_{1,187} = 27.9$, $P < 0.0001$). The decrease in non-distractor trial accuracies represents a carryover effect from the presence of the distractor trials; and provides an additional challenge to the attentional component of the paradigm [16,35]. Treatment with ABT-594 produced a significant improvement in distractor task accuracies ($F_{8,438} = 15.3$, $P < 0.0001$). Post hoc analysis indicated that the effect was mainly restricted to short delay trials (Fig. 4) after the 0.115 μg/kg dose ($t = 2.88$, $P = 0.0042$), and possibly after the 3.7 μg/kg dose ($t = 1.73$, $P = 0.087$). There also were trends towards significant increases in medium delay distractor accuracies after the 0.93 ($t = 1.73$, $P = 0.084$) and 1.85 ($t = 1.70$, $P = 0.089$) μg/kg doses (Fig. 5). There was no significant effect of ABT-594 on non-distractor trial accuracies ($F_{6,302} = 1.59$, $P = 0.149$). ABT-594 produced no significant effect on either sample or choice task latencies associated with non-distractor trials ($F_{6,148} = 1.07$, $P = 0.38$) or distractor trials ($F_{6,150} = 0.53$, $P = 0.78$). Averaged latency values for this series are presented in Table 2. In the course of the study ABT-594 produced no untoward effects in the animal subjects.

3.2. A-582941

3.2.1. *Standard DMTS testing*

For this experimental series, the dose–response relationship was obtained during a single dose series. The mean vehicle accuracies for zero, short, medium, and long delay intervals were, respectively, 99.3, 76.0, 70.0, and 60.2% trials correct. Treatment with ABT-582941 produced a significant improvement in task accuracies ($F_{12,119} = 1.95$, $P = 0.036$). Three of the four doses tested significantly improved either short or long delay accuracies. These data are presented in Fig. 6. Compound treatment resulted in significant increases in task accuracies associated with the short delay trial after the 11.4 μg/kg dose ($t = 2.04$, $P = 0.043$), and with long delay trials after the 1.4 ($t = 2.8$, $P = 0.006$), the 3.8 ($t = 3.19$, $P = 0.002$), and the 11.4 ($t = 3.19$, $P = 0.002$) μg/kg doses. For the highest (38.0 μg/kg) dose of A-582941, a modest trend toward improvement was noted ($t = 1.62$, $P = 0.10$). Overall the effect was to increase accuracy predominantly during long delay trials. In

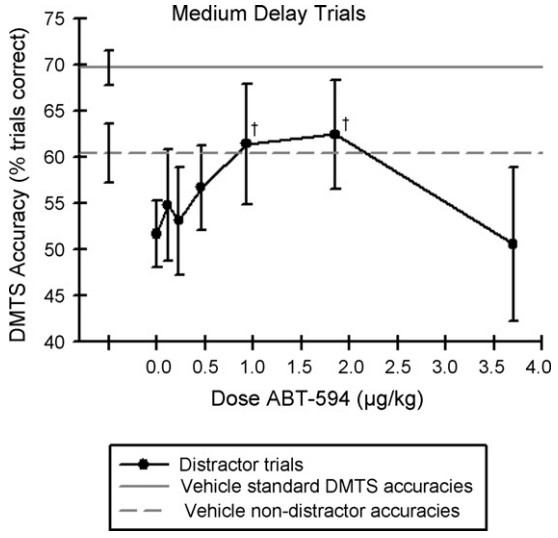

Fig. 5 – Effect ABT-594 on medium delay trial accuracies of the distractor DMTS task by adult Rhesus monkeys plotted as a function of dose. Test sessions were initiated 10 min after compound administration. The solid horizontal line and error bar indicate the mean ± S.E.M. for medium delay as derived from standard vehicle DMTS sessions run prior to the distractor series. The dashed horizontal line and error bar indicates the mean ± S.E.M. for vehicle medium delay trials obtained from non-distractor trials that were a component of the distractor-DMTS sessions. †P < 0.10 as compared with vehicle distractor mean (0.0 μg/kg).

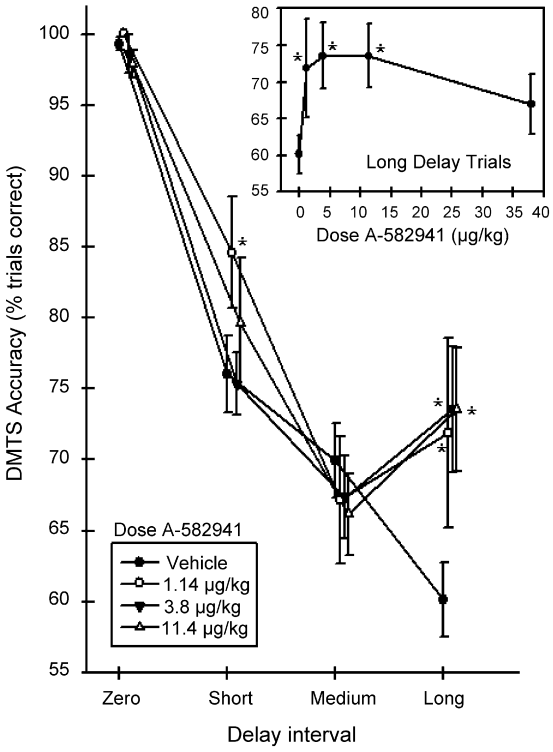

Fig. 6 – The effect of A-582941 on accuracy in the standard DMTS task by adult Rhesus monkeys. Test sessions were initiated 30 min after compound administration. Data points and error bars were slightly displaced for clarity of presentation. Each data point represents the mean ± S.E.M. derived from six subjects. Inset: mean values obtained during long delay trials plotted as a function of dose. *P < 0.05 as compared with respective vehicle (or 0 μg/kg) means. The 38.0 μg/kg dose of A-582941 was not plotted as a function of delay interval for clarity (see inset).

this case, the increase in accuracy during long delay trials (22.2% above control) was about twice the increase during short delay trials (11.3% above control). The complete dose–response relationship obtained during long delay trials are presented in the inset to Fig. 6. The data show a sustained increase in mean task accuracies over the entire dose range. Because the data failed to suggest a robust effect of A-582941 on short delay trial accuracies, the compound was not tested in the distractor version of the task. One other interesting difference between ABT-594 and A-582941 is the inability of the latter compound to significantly improve task accuracies during sessions run 24 h after dosing. Examination of Fig. 7 shows that, though mean long delay accuracies after A-582941 administration were maintained above the vehicle mean for each dose, the effect was not statistically significant ($F_{4,119} = 1.06$, $P = 0.38$). A-582941 produced no significant effect on either sample or choice task latencies ($F_{4,57} = 0.50$, $P = 0.74$). Averaged latency values for this series are presented in Table 2. In the course of the study A-582941 produced no untoward effects in the animal subjects.

4. Discussion

In the present study ABT-594 was shown to produce a significant improvement in standard DMTS task accuracy. A discussion of the mnemonic properties of ABT-594 might be developed from a comparison with the known effects of nicotine and other nicotinic receptor agonists studied under similar circumstances. For example, in young macaques, nicotine and the analogs ABT-418 and ABT-089 each improved DMTS task accuracy with similar potency and efficacy [13,34]. ABT-594 proved to be the most potent of the three, being about 16-fold more potent than nicotine and 13-fold more potent than ABT-418. The difference in (molar) potency between ABT-594 and nicotine as cognition-enhancing agents fit well with the 18-fold difference between ABT-594 and nicotine in binding affinity for human ([^{3}H]-cytisine) α4β2 receptors; but not with the differences between the two compounds' affinities for either human ([^{3}H]-epibatidine) α3βx receptors (136-fold) or with human ([^{125}I]α-bungarotoxin) α7 receptors (four-fold) [18]. It is likely, therefore, that the positive mnemonic effects of ABT-594 are mediated through activation of α4β2 receptors. Though ABT-594 possesses analgesic properties in rodents [17,18], these effects are observed at higher doses than those that produce cognitive enhancement in rodents (unpublished data, Abbott). Consistent with these observations, the effective doses in the current study were sub-analgesic doses, i.e., >200-fold lower than the antinociceptive dose in rats [18].

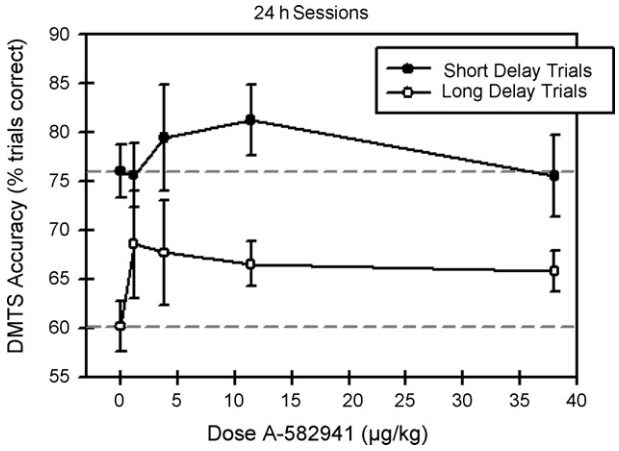

Fig. 7 – The effect of A-582941 on accuracy during short and long delay trials in the standard DMTS task by adult Rhesus monkeys. Test sessions were initiated 24 h after compound administration and data are plotted as a function of dose. Horizontal dashed lines indicate the vehicle means for each delay interval. There were no statistically significant differences between A-582941 means and the respective vehicle means (0 μg/kg).

One aspect of nicotinic pharmacology that we and others have helped to characterize is the protracted profile of cognitive enhancement. Nicotine and certain other agonists have the ability to sustain improvements in memory-related task efficiency long after the compound levels have become insignificant in brain or plasma [11,36,37]. The magnitude of the protracted effect is not always related to the magnitude of the initial effect, and in fact, for some compounds (e.g., GTS-21) the effect measured 24 h after administration is greater than the initial response [38]. In the present study ABT-594 sustained the initial increase in task accuracy to 24 h after administration. Earlier we had speculated that the ability to sustain a protracted response in this model was related to likely interactions at the α7 subtype of nicotinic receptors [37]. This supposition needs to be re-examined, as ABT-594 has reduced affinity for α7 nicotinic receptors relative to nicotine, and A-582941, which is α7-selective failed to produce a significant increase in accuracy during the 24 h sessions. The original concept that α7 receptor activation was responsible for protracted mnemonic improvement was based on the marked effects of GTS-21 as indicated above. However, this compound, while active at the α7 site, is equally effective in binding to α4β2 receptors [38]. In the absence of a significant impact of pharmacokinetic or bioavailability differences between GTS-21 and ABT-594, it is more likely that the hetero-oligomeric subtype is more relevant for nicotine's (and ABT-594's) protracted mnemonic actions. It should be pointed out, however, that other non-nicotinic cognition-enhancing agents share this property [39–42].

In young macaques, nicotine, ABT-418, and ABT-089 each effectively reversed distractor-induced impairment in task performance, particularly during short delay trials [15]. Similar results were obtained with the α4β2-preferring nicotinic agonist SIB-1553A [16]. In this respect each of these compounds were as, or more, effective than methylphenidate [43], a standard treatment for attention deficit disorders. During the standard DMTS series with ABT-594, the plots of accuracy versus delay interval appeared to be shifted to the right of the vehicle curve by the effective doses. This feature of the relationship is suggestive of a role for attention or acquisition to the overall mnemonic action [33]. This was confirmed during the distractor DMTS series. Treatment with ABT-594 was almost completely effective in reversing distractor-impaired performance during Short delay intervals. This was particularly evident in that average Short delay accuracy after treatment was increased above that for non-distractor trials after vehicle treatment. However, unlike the responses to nicotine, ABT-418, ABT-089, and SIB-1553A cited above, the reversal of distractor-impaired accuracy by ABT-594 was restricted to a single dose—the lowest of the series. This limited effectiveness in the distractor series mirrored the relatively modest efficacy of ABT-594 in the standard DMTS series in improving task accuracies (averaging 12% over vehicle). Also in the distractor series, ABT-594 failed to significantly improve non-distractor-impaired accuracies. Thus the participation of more than one nicotinic receptor subtype might be necessary for the full expression of cognition enhancement.

A-582941, which is selective for the α7 subtype, produced a robust increase in standard DMTS accuracy (22% above vehicle), but the effect was mainly restricted to long delay trials—an effect more likely related to enhancing short term retention or recall components of memory than attentional components [33]. These findings support those from an earlier study in rats pointing to a more dominant role for α4β2 receptors as compared with α7 receptors in enhancing attention and vigilance [44]. In that study the α7 receptor-selective nicotinic agonist AR-R17779 failed to improve five-choice serial reaction time accuracy as had nicotine and epibatidine. The authors proposed that the relative selectivity of AR-R17779 for the α7 receptor subtype limited the ability of the compound to improve attention. For compounds without marked subtype selectivity other factors such as the ability to trigger functional effects, as for example, dopamine release, was suggested to be more useful than binding affinities in predicting efficacy for improving attention.

Although it seems that the results of this study may have raised many new questions regarding nicotinic receptor agonists and memory function, the continued study of novel nicotinic compounds with varying degrees of receptor subtype selectivity and other pharmacological properties may help shed light on important aspects of memory processes. Still to be resolved are questions relating to the nature of the protracted positive mnemonic response to nicotinic drugs and other compounds [11]. In addition to the importance of the question related to processes underlying the basic aspects of working memory, there is the practical question of what type of dosing regimen to use in clinical trials. Perhaps the failure or lackluster effects of certain compounds like early nicotinic drugs, and muscarinic cholinergic or adrenergic receptor agonists in clinical trials is related in part to overmedication. This could be the case when the pharmacokinetic aspects of the compound and the pharmacodynamic actions attributed to the compound are not considered together in determining

dosing schedules. The other important issue relates to the obvious specificity of compounds, even within the same pharmacological class, to affect different components of memory. The categorization of compounds by their specific actions on components of memory could engender the rationale for the use of combinations of therapeutic agents in the treatment of disorders of human cognition. The specific targeting of components of memory, such as attention and recall, could result in additive or synergistic effects possibly surpassing the effects obtained to date [6,45,46]. Lastly, a complication in the translation of many cognition-enhancing agents to the clinic is the narrow dose window characterized by inverted-U or -J dose–response relationships. The cause for the loss of efficacy at the higher end of the dose–response could be related to side effect complications. This is particularly relevant for cholinergic agonists which can activate peripheral cholinergic receptors involved in autonomic and somatic motor function. However, the common profile of the inverted-U dose–response among potential cognition-enhancing drugs from many different pharmacological classes suggests that peripheral side effects issues do not completely explain the phenomenon. We have obtained a rather wide therapeutic window for increased DMTS accuracy for the compound MHP-133 which has low-potency activity at multiple drug targets (including nicotinic receptors). MHP-133 was effective in improving DMTS accuracy by macaques over a dose range of 5–100 μg/kg [47]. Future studies will be required to confirm the general applicability of the pharmacological profile exemplified by MHP-133 to widening effective dose windows in cognition enhancement.

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