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A reversible model of the cognitive impairment associated with schizophrenia in monkeys: Potential therapeutic effects of two nicotinic acetylcholine receptor agonists

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ABSTRACT

In monkeys proficient in the performance of a computer-assisted delayed response task, administration of sub-sedative doses of ketamine significantly impaired task performance after the 2 mg/kg dose, producing a decrease in accuracies across all four delay intervals. Ketamine elicited occasional and inconsistent increases in task latencies. But in general processing speed was not dramatically affected by the test dose. Pretreatment with the α7 nicotinic receptor agonist GTS-21 (DMXB-A) [3-[(3E)-3-[(2,4-dimethoxyphenyl) methylidene]-5,6-dihydro-4H-pyridin-2-yl]pyridine] produced a dose-dependent attenuation of ketamine-induced decreases in task accuracies. In fact, the best dose of GTS-21 completely reversed the effects of ketamine. The nicotine metabolite cotinine is a cognitive-enhancer, and active in models predictive of antipsychotic activity. Pretreatment with cotinine did not reverse the task deficits produced by ketamine, and selection of a best dose was necessary to show the activity of cotinine. However, the best dose of cotinine, like GTS-21, completely reversed the ketamine-induced task deficits. Task accuracies were increased relative to their non-ketamine baselines during sessions run 24 h later. The cotinine–ketamine order of administration was reversed to provide a more clinically relevant model, and cotinine post-treatment regimen produced a clear reversal of the ketamine-induced task deficits. The protracted task improvement also was still evident. The DMTS task impairment induced by ketamine was capable of being completely reversed by two compounds that are known to improve working memory and cognition. The model could provide a means of late stage preclinical evaluation of new compounds that address the cognitive impairment associated with major psychotic disease.

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1. Introduction

Schizophrenia is a chronic neuropsychiatric illness with a debilitating array of clinical symptoms that commonly require life-long therapeutic intervention. These symptoms include positive symptoms (e.g., hallucinations, delusions), negative symptoms (e.g., anhedonia, alogia, depression) and cognitive dysfunction (e.g., impaired working memory, attention, etc.) [1]. The primary therapeutic agents used for schizophrenia, known as "antipsychotics", have been shown in most clinical trials to improve the positive behavioral symptoms, however, the negative symptoms of the illness are often not pharmacologically addressed [2]. The older conventional agents (also referred to as typical or first generation antipsychotics) are limited by adverse motor effects (e.g., Parkinsonian symptoms and tardive dyskinesia) whereas the newer agents (referred to as atypical or second generation antipsychotics) are limited by adverse metabolic effects that include abnormal weight gain, development of diabetes mellitus and hyperlipidemias [3,4]. The marked and often florid behavioral symptoms associated with schizophrenia are generally controlled by existing antipsychotic medications, but the cognitive impairment associated with the disease poses a challenge to treatment [5–8]. In schizophrenia, cognitive dysfunction is now believed to have the greatest impact on the measures of overall illness outcome, i.e., the ability to acquire new skills, function in community settings, retain active employment, etc. [9–11]. Essentially two factors have limited progress toward the development of therapeutic agents to treat the cognitive deficits. There is no animal model or behavioral paradigm in animals that reproduces the human cognitive impairment in schizophrenia, though there are several rodent models that are used in the preclinical evaluation of novel antipsychotic agents. These include

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paradigms that estimate the capacity for sensory gating, and the measurement of sustained attention, some of which require the use of psychogenic compounds such as phencyclidine to impair task performance (see Ref. [12]). Secondly, there are few prototypical drugs that are available to validate an animal model that specifically replicates the cognitive impairment associated with schizophrenia. One possibility is the class of drugs that act on nicotinic acetylcholine receptors—particularly the α7 homomeric subtype. Nicotinic α7 receptor agonists have shown promise in studies in animals and in humans in which the goal was to enhance attention, memory, and cognition [13–17]. GTS-21 (DMXB-A) [3-[(3E)-3-[(2,4-dimethoxyphenyl) methylidene]-5,6-dihydro-4H-pyridin-2-yl]pyridine], with its partial selectivity for the α7 subtype has been shown to capitulate these pharmacological properties [18–20] and GTS-21 [21], like nicotine [22], has shown utility in initial clinical trials in schizophrenia.

Cotinine is a primary metabolite of nicotine that can exert measurable effects on certain behaviors, working memory and cognition. Most relevant to this study, cotinine was shown to attenuate the impairment in sensory gating in rats treated with a dopamine receptor agonist [23]. In the rat, the motor response to acoustic startle can be inhibited by the presentation of a low-level acoustic prepulse presented just in advance of the high-level acoustic pulse, thereby providing a measure of sensory gating. Disruption of sensory gating can be produced by dopamine receptor agonists like apomorphine that can induce a schizophrenic-like action in humans. Under the conditions established at baseline, apomorphine treatment suppresses the ability of the prepulse to inhibit acoustic startle. Many drugs with potential antipsychotic actions reverse the effects of apomorphine. Treatment with cotinine significantly reversed the ability of apomorphine to impair the inhibitory effect of the low amplitude prepulse on the motor response to acoustic startle in rats, supporting the possibility that the metabolite shares antipsychotic potential with nicotine. In the same study, cotinine dose-dependently attenuated the impairing actions on the prepulse by the NMDA receptor antagonist dizocilpine (MK-801) [23]. Also very relevant to this study, cotinine increased working memory in Rhesus monkeys, and the drug reduced the effectiveness of task-relevant distractors to impair accuracy in an attentional version of the DMTS task [23]. Attention deficits are also an important feature of the cognitive impairment associated with schizophrenia [12]. Therefore, GTS-21 and cotinine each combine the ability to reverse behavioral processes related to schizophrenia and to improve cognition.

Ketamine at the higher end of its useful dose range is a dissociative anesthetic often used in veterinary medicine and animal research. At low, pre-anesthetic doses ketamine is a non-competitive antagonist of glutamate NMDA receptors, and the drug has been classified a hallucinogen similar to phencyclidine [24]. Ketamine's interaction with NMDA receptors is particularly relevant in view of the known alterations to central glutamatergic neurons in schizophrenia [25,26], and glutamate receptor agonists have demonstrated effectiveness in the disease [27,28]. Interactions with other neural substrates likely contribute to the compound's behavioral profile in humans [29,30]. At sub-sedative doses, ketamine also can impair several aspects of working memory in humans and animals [31–33]. The drug therefore possesses two pharmacological components necessary for an animal model of schizophrenia—hallucinogenic potential and impaired working memory. If ketamine is used at relatively low memory-impairing doses, and only intermittently to avoid tolerance, administration of the drug could constitute a reversible pharmacological model for testing novel compounds that might address the cognitive disturbances in schizophrenia.

It has been our experience that evaluation of compounds for cognition enhancement in non-human primates allows for a greater level of clinical predictability as compared with lower species. Various operant tasks, usually food-motivated, allow for the measurement of abilities which are relevant to human cognition such as attention, strategy formation, reaction time in complex situations and memory for recent events. Although rodent models have proven invaluable during initial drug screening procedures, in late stage preclinical studies, primate models have demonstrated greater levels of clinical predictability than rodent models. It has been our experience over the past 20 years that compounds that are effective in improving cognitive performance in monkeys as assessed in the DMTS task are often also effective in humans [34]. Therefore it seems reasonable to combine DMTS testing in monkeys with low-dose ketamine administration as a model for the cognitive impairment in schizophrenia. In this study we first established a dose of ketamine that impaired working memory without sedation, and which elicited reproducible responses in macaques well trained in the performance of a computer-assisted delayed matching-to-sample (DMTS) task. Both GTS-21 and cotinine were compared for their ability to reverse working memory deficits induced by ketamine in these animals.

2. Methods

2.1. Subjects

Eight Pigtail (*Macaca nemestrina*) monkeys 6–23 years old served as experimental subjects (Table 1). Monkeys 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 [35]. Delayed matching-to-sample (DMTS) testing was conducted once each weekday. During the test-week monkeys were maintained on a modified feeding schedule such that food (standard monkey chow and other supplements) was withheld beginning at 08:00 h and ending at 17:00 h (when all testing at the facility was completed). During testing, animals obtained approximately 75 flavored reinforcement pellets (300 mg) awarded for correct responses. Standard laboratory monkey chow, fresh fruits and vegetables were provided after 17:00 h during the test-week and without modification on weekends. Water was available on an unlimited basis, including during testing. All procedures were reviewed and approved by the Medical College of Georgia Institutional Animal Care and Use Committee and are consistent with AAALAC guidelines. Each subject had previously participated in one or more short-term studies assessing the effects of reversible drugs on DMTS performance and all were well trained in this task. Prior drug experience had produced no observable untoward effects in the

Table 1
Subject information for the study cohort (*Macaca nemestrina*).

ID #	Sex	Years old	Weight (kg)	Delay intervals (s)		
				Short	Medium	Long
119	F	18	9.8	20	60	110
146	M	23	11.7	30	60	200
797	M	17	15.4	45	95	160
c8r	M	9	11.4	5	15	45
pa1	M	15	15.9	15	25	40
p18	F	11	7.8	15	30	75
tp8	F	17	6.6	10	15	35
v6t	M	9	15.6	15	45	90
Mean		14.88	11.78	19.38	43.13	94.38
S.E.M.		1.74	1.28	4.48	9.77	21.14

animals. A minimal washout period of 4 weeks occurred before the initiation of the current study. Although there is a wide range of ages in the study cohort (Table 1), these animals have been in our program for several years and they are proficient in the task. In fact, there was no statistically significant correlation between age and delay interval ($P > 0.09$ for each delay).

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

Test panels attached to each animal's home cage presented the task by using a computer-automated system. A 15-in. touch-sensitive screen (AccuTouch LCD Panelmount TouchMonitor, Elo TouchSystems, Menlo Park, CA) and pellet dispenser unit (Med Associates, St. Albans, VT) 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 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 were presented below and to the right and left of the sample. 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 strategies to chance levels of accuracy. The lack of a response (screen touch) after 3 min of illumination of the sample stimulus was counted as an incorrect response. Five different presentation sequences were rotated through each daily session to prevent the subjects from memorizing the first several trials. Delay intervals were established during numerous non-drug or vehicle sessions prior to initiating the study. The duration for each delay interval was adjusted for each subject until three levels of group performance accuracy were approximated: zero delay interval (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). The assignment of these memory retention intervals based upon an individual's baseline task accuracy is necessary to avoid ceiling effects in the most proficient animals during drug studies, while also serving to insure that each animal begins testing at relatively the same level of task difficulty. The delay intervals were not adjusted during the study. Three response latencies also were measured: the "sample latency", which is the time between presentation of the sample color and the animal pressing in sample rectangle, and the "choice latency" which is the time between presentation of the choice colors and the animal pressing one of the choice rectangles. Choice latencies were divided into those associated with correct and incorrect responses.

2.3. Drug regimens

The study was divided into four experimental series spaced over a 10-month period. The same cohort of 8 monkeys was used in each, except that during the third series (cotinine pretreatment, see below) two subjects were not available for study. The first series evaluated the effects of ketamine alone on DMTS accuracies in the study population. Five doses of ketamine hydrochloride (Butler Animal Health Supply, Dublin, OH) were used (0.1–4 mg/kg). Though DMTS sessions were run each weekday during the study, ketamine was never administered in a regimen more frequently than once per week. Preliminary studies had established that the weekly dosing would obviate the development of tolerance to ketamine's ability to impair DMTS accuracy. In the second series, one of five doses of GTS-21 (a gift from Memory Pharmaceuticals, Montvale, NJ) (2.5–40 μg/kg) was administered 30 min prior to ketamine, and DMTS testing was initiated 30 min after ketamine (2 mg/kg) administration. In the third series one of six doses of (−)-cotinine (Sigma–Aldrich, St. Louis, MO) (0.05–3 mg/kg) was administered 15 min prior to ketamine, and DMTS testing was initiated 30 min later. In the fourth series, ketamine was administered first, followed 30 min later by one of five doses (0.05–1.2 mg/kg) of cotinine. DMTS testing was initiated 15 min after cotinine. During control sessions, drug vehicle (sterile, normal saline) was administered in place of GTS-21 or cotinine, and vehicle was administered twice in series to control for pre- and post-treatment injection of test drugs and ketamine. Compound solutions were prepared just before use. They were weighed to the nearest 0.1 mg and dissolved in vehicle for an injection volume of 0.035 ml/kg. Injections were given in the thigh muscle. The doses chosen for GTS-21 and cotinine were based on prior experience with the compounds in the standard DMTS task [19,23]. The timing for the ketamine injection relative to initiating testing was determined from earlier pilot experiments.

2.4. Statistics

Data for correct percent were subdivided according to delay interval for each 24-trial delay component of the session. All statistical analyses were performed on raw data (% trials correct) except that control performances were obtained by calculating for each delay, the averaged accuracy from multiple vehicle sessions obtained from each monkey. Data were analyzed by use of a multi-factorial analysis of variance (ANOVA) with repeated measures (SAS, JMP statistical software package). An 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 (2-sided test). Trends toward significance were considered at the $P < 0.10$ but >0.05.

3. Results

3.1. Ketamine dose-response

During control DMTS sessions mean task accuracies conformed to the delay interval categories described above: zero delay, 97.9; short delay, 82.8, medium delay, 70.3, and long delay, 56.3, % trials correct (see Fig. 1C). Administration of ketamine 45 min prior to DMTS testing produced a significant decrease in task accuracies ($F_{5,7} = 25.5, P < 0.0001$). The effect was statistically significant after animal received the 2 mg/kg ($t = 6.75$, $P < 0.0001$) and 4 mg/kg ($t = 6.93$, $P < 0.0001$) doses (Fig. 1A). The effects of ketamine on median task latencies are presented in Fig. 1B. There was a statistically significant increase in mean latencies ($F_{5,7} = 5.1$, $P < 0.0003$) that was specific to mean sample latencies associated with the 2 mg/kg ($t = 3.14$, $P < 0.002$) and 4 mg/kg ($t = 4.38$, $P < 0.0001$) doses. Choice latencies were not significantly affected by ketamine. The data for each dose of ketamine are plotted vs. delay interval in Fig. 1C. The decrements in accuracy noted in Fig. 1A were not specific to a particular delay interval. After the 2 mg/kg dose, accuracies during zero, short, and medium delay intervals were significantly decreased ($P < 0.001$); long delay trial accuracy was nearly significantly decreased ($P = 0.097$). After the 4 mg/kg dose, all accuracies during zero, short, medium, and long delay intervals were significantly decreased relative to vehicle ($P < 0.006$). On the day after ketamine administration (24-h sessions) with no additional pre-test administration, task

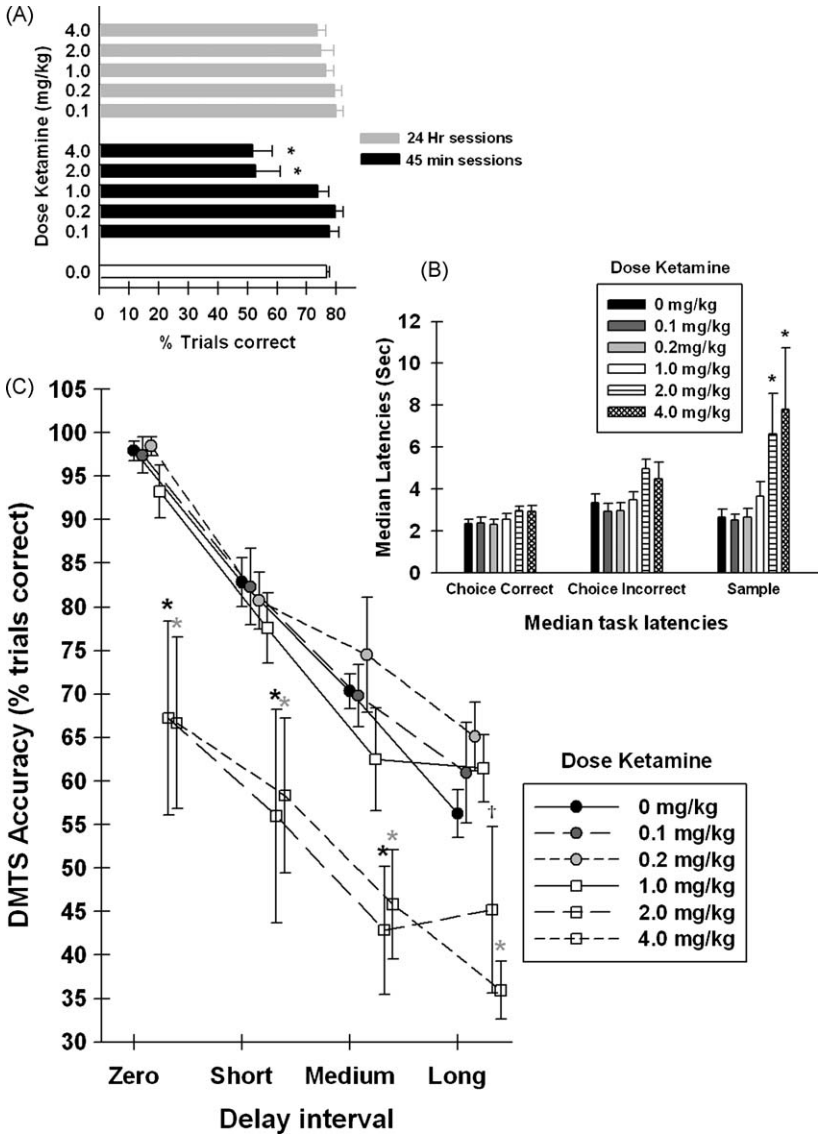

Fig. 1. The effect of ketamine on DMTS task performance by 8 pigtail monkeys. (A) Data represent the averaged accuracies for each of four delay intervals. Subjects received vehicle (saline) 45 min before testing (45 min sessions), and a subsequent session was run 24 h later without further pre-test treatment (24 h sessions). (B) The effect of ketamine on median task latencies. (C) Accuracy data plotted vs. delay interval. The "0 mg/kg" dose refers to sessions in which testing was preceded by vehicle administration. Each value represents the mean ± S.E.M. Data points are slightly offset to better illustrate the error bars. *Significantly different from respective vehicle value ($P < 0.05$); †($P < 0.10$).

accuracies were again at vehicle levels (Fig. 1A). The 2 mg/kg dose of ketamine was used for all subsequent series because it was the lowest dose that produced a highly significant decrease in task accuracies, without dramatically affecting task latencies.

3.2. GTS-21–ketamine series

In this series pretreatment with vehicle before ketamine again resulted in a significant decrease in task accuracies relative to vehicle–vehicle treatment, and GTS-21 pretreatment significantly attenuated the ketamine deficits ($F_{6,7} = 10.2$, $P < 0.0001$). Ketamine-induced decrements were most apparent for zero, short, and medium delay intervals ($P < 0.0002$), and nearly significant for long delay intervals ($P = 0.072$). Pretreatment with GTS-21 significantly attenuated the ketamine-induced decreases in overall (all 4 delay accuracies averaged) task performance (Fig. 2A). Significant task improvement was specific to the 20 μg/kg ($t = 2.79$, $P = 0.006$) and 40 μg/kg ($t = 2.32$, $P = 0.021$) doses. In this series ketamine (pretreated only with vehicle) produced no statistically significant affects on task latencies, and the same was true for sessions in which GTS-21 preceded the ketamine injection (Fig. 2B). An individual best dose of GTS-21 was selected for each animal that represented the highest overall accuracy value among the 5 doses tested. For the group, the average best dose was 18.1 μg/kg. The data are plotted as a function of delay interval in Fig. 2C (the data for the 20 μg/kg dose from the GTS-21 dose-response series are also presented; also see Table 2). The best dose of GTS-21 was associated with a highly significant, and virtually complete reversal, of the ketamine-induced decrease in task accuracies ($F_{3,7} = 26.7$, $P < 0.0001$). The effect of GTS-21 pretreatment was statistically significant from respective vehicle–ketamine means for zero, short, and medium delay trials ($P < 0.008$). There were no residual effects on task accuracies during sessions run 24 h after ketamine.

3.3. Cotinine–ketamine series

In this series pretreatment with vehicle before ketamine resulted in a significant decrease in task accuracies relative to vehicle–vehicle treatment ($F_{7,5} = 11.7$, $P < 0.0001$). The

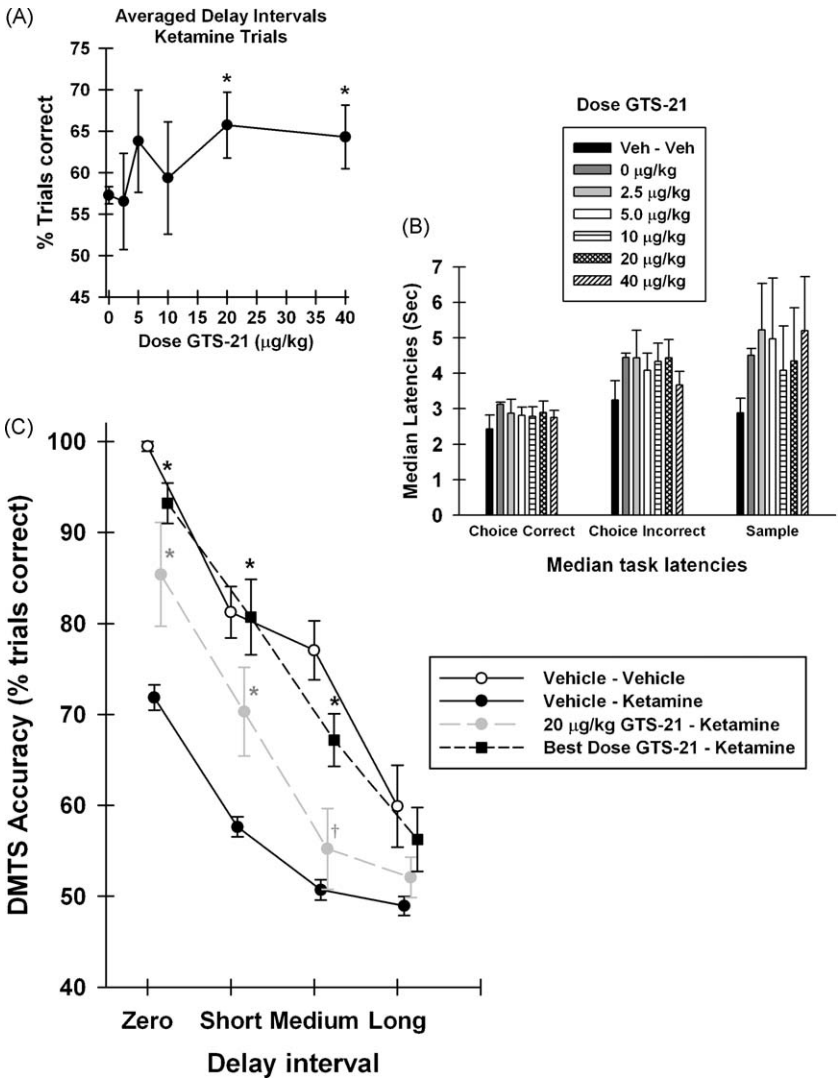

Fig. 2. The effect of pretreatment with GTS-21 on ketamine-induced decrements in DMTS performance by 8 pigtail monkeys. (A) GTS-21 was administered 30 min prior to ketamine, and DMTS testing was initiated 30 min after ketamine (2 mg/kg) administration. Data represent the averaged accuracies for each of four delay intervals. The "0 mg/kg" dose refers to sessions in which ketamine was preceded by vehicle. (B) The effect of GTS-21-vehicle and GTS-21-ketamine regimens on median task latencies. "Veh–Veh" indicated sessions in which two consecutive vehicle injections were made before testing. (C) The data associated with the 20 μg/kg dose of GTS-21, and with the averaged best dose (highest overall accuracy value among the doses tested) are plotted vs. delay interval. "Vehicle–Vehicle" indicates control data in which there was no administration of ketamine. "Vehicle–Ketamine" indicated control data in which vehicle preceded 2 mg/kg ketamine. Each value represents the mean ± S.E.M. Data points are slightly offset to better illustrate the error bars. *Significantly different from respective Vehicle–Ketamine value ($P < 0.05$); †($P < 0.10$).

decrements (Fig. 3C) were most apparent for zero, short, medium, and long delay intervals ($P < 0.003$). However, cotinine treatment failed to attenuate the ketamine-induced decreases in overall task accuracies (Fig. 3A). As in the first series ketamine treatment was associated with a significant increase in task latencies ($F_{7,5} = 3.44$, $P < 0.002$). But in this instance significant increases were obtained during sample latencies ($t = 4.20$, $P < 0.0001$), and during incorrect choice latencies ($t = 2.55$, $P = 0.012$). Cotinine pretreatment did not significantly reduce the duration of these latencies, but neither were their any increased latencies (relative to vehicle–vehicle means) in cotinine pretreated animals (Fig. 3B). An individual best dose of cotinine was selected for each animal that represented the highest overall accuracy value among the 6 doses tested. For the group, the average best dose was 1.13 mg/kg. The data are plotted as a function of delay interval in Fig. 3C (the data for the 0.05 mg/kg dose from the cotinine dose-response series are also presented; also see Table 2). The best dose of cotinine was associated with a highly significant, and virtually complete reversal, of the ketamine-induced decrease in task accuracies ($F_{2,5} = 30.1$, $P < 0.0001$). The effect of cotinine pretreatment was statistically significant from respective vehicle–ketamine means for zero and medium delay trials ($P < 0.007$) and nearly statistically significant for short and long delay trials ($P < 0.070$). During sessions run 24 h after ketamine (with no other pre-test administration) task accuracies were significantly increased relative to vehicle–vehicle means run the day prior ($F_{6,5} = 2.55$, $P = 0.022$). All of the improvements in task accuracies (Fig. 4 inset) were associated with medium delay trials after animals received the 0.1, 1.2, and 3 mg/kg doses ($P < 0.04$), and nearly significantly after the 0.6 mg/kg dose ($P = 0.056$). Task accuracies during sessions run 24 h after vehicle or after the 0.1 and 1.2 mg/kg cotinine–ketamine combinations are plotted vs. delay interval presented in Fig. 4.

3.4. Ketamine–cotinine series

To evaluate cotinine in a more clinically relevant treatment strategy, an alternate regimen was adopted in which ketamine preceded cotinine prior to DMTS testing. In this series pretreatment with ketamine before vehicle resulted in a significant decrease in task accuracies relative to vehicle–vehicle treatment

Table 2
DMTS accuracies (means ± S.E.M.) for the three studies presented by delay interval.

Pretreatment	Post-treatment	Delay interval			
		Zero	Short	Medium	Long
GTS-21 0 μg/kg	Ketamine 0 mg/kg	99.48 0.53	81.26 2.84	77.08 3.24	59.91 4.52
GTS-21 0 μg/kg	Ketamine 2 mg/kg	71.87 1.40	57.64 1.09	50.70 1.12	48.94 1.04
GTS-21 2.5 μg/kg[a]	Ketamine 2 mg/kg	75.00 6.93	60.73 6.35	47.03 6.08	43.44 6.92
GTS-21 5 μg/kg	Ketamine 2 mg/kg	80.21 6.38	69.78 7.03	55.23 7.21	50.01 5.22
GTS-21 10 μg/kg	Ketamine 2 mg/kg	79.68 7.36	63.03 7.93	48.95 7.03	45.83 5.95
GTS-21 20 μg/kg	Ketamine 2 mg/kg	85.40 5.68	70.31 4.88	55.21 4.44	52.08 2.23
GTS-21 40 μg/kg	Ketamine 2 mg/kg	95.83 1.51	79.86 2.29	66.19 2.40	64.13 2.11
Cotinine 0 mg/kg	Ketamine 0 mg/kg	71.52 7.01	59.73 7.33	49.99 5.23	45.45 5.75
Cotinine 0 mg/kg	Ketamine 2 mg/kg	79.18 6.80	62.50 7.29	57.65 7.87	50.72 4.23
Cotinine 0.05 mg/kg[b]	Ketamine 2 mg/kg	77.78 8.23	58.32 8.41	53.47 6.13	43.07 5.23
Cotinine 0.1 mg/kg	Ketamine 2 mg/kg	76.38 4.52	63.19 3.26	55.91 2.71	51.04 2.98
Cotinine 0.3 mg/kg	Ketamine 2 mg/kg	70.83 8.47	52.07 7.04	54.12 6.58	47.20 8.98
Cotinine 0.6 mg/kg	Ketamine 2 mg/kg	81.66 6.79	63.32 6.38	53.32 5.00	47.50 1.66
Cotinine 1.2 mg/kg	Ketamine 2 mg/kg	70.83 7.76	63.20 7.34	51.40 8.85	49.98 5.60
Ketamine 0 mg/kg	Cotinine 0 mg/kg	92.70 2.92	77.09 4.46	62.49 3.70	58.86 3.38
Ketamine 2 mg/kg	Cotinine 0 mg/kg	62.78 7.96	57.29 8.95	44.08 5.06	38.25 6.25
Ketamine 2 mg/kg[c]	Cotinine 0.05 mg/kg	63.69 9.21	49.40 8.74	42.27 6.71	46.37 7.26
Ketamine 2 mg/kg	Cotinine 0.1 mg/kg	69.66 9.91	56.56 8.81	39.89 6.10	41.06 8.54
Ketamine 2 mg/kg	Cotinine 0.3 mg/kg	70.24 9.02	61.30 8.61	52.39 5.21	45.24 5.99
Ketamine 2 mg/kg	Cotinine 0.6 mg/kg	77.97 10.44	67.84 11.34	64.81 6.84	58.87 6.36
Ketamine 2 mg/kg	Cotinine 1.2 mg/kg	88.01 4.49	74.48 3.56	57.80 3.64	49.45 4.75

[a] GTS-21 was administered 30 min before ketamine, and testing was initiated 30 min after ketamine.
[b] Cotinine was administered 15 min before ketamine, and testing was initiated 30 min after ketamine.
[c] Ketamine was administered 30 min before cotinine, and testing was initiated 15 min after cotinine.

and cotinine post-treatment significantly reversed the ketamine deficits ($F_{6,5} = 11.5$, $P < 0.0001$). The ketamine-induced decrements (Fig. 5C) were most apparent for zero, medium, and long delay intervals ($P < 0.015$) and nearly significant for short delay trials ($P = 0.069$). In contrast to the previous series, cotinine post-treatment significantly attenuated the ketamine-induced decreases in overall task accuracies (Fig. 5A). The effects were statistically significant after subjects received the 0.6 and 1.2 mg/kg doses ($P < 0.0001$), and nearly significant after the 0.3 mg/kg dose ($P = 0.086$). In this series, ketamine produced no statistically significant effect on task latencies when the post-treatment was vehicle or when it was one of the doses of ketamine (Fig. 5B). An individual best dose of cotinine was selected for each animal that represented the highest overall accuracy value among the 5 doses tested. For the group, the average best dose was 0.63 mg/kg. The data are plotted as a function of delay interval in Fig. 5C (the data for the 0.6 mg/kg dose from the cotinine dose-response series are also presented; also see Table 2). The best dose of cotinine was associated with a highly significant, and virtually complete reversal, of the ketamine-induced decrease in task accuracies ($F_{2,7} = 31.9$, $P < 0.0001$). The effect of cotinine pretreatment was statistically significant from respective vehicle–ketamine means

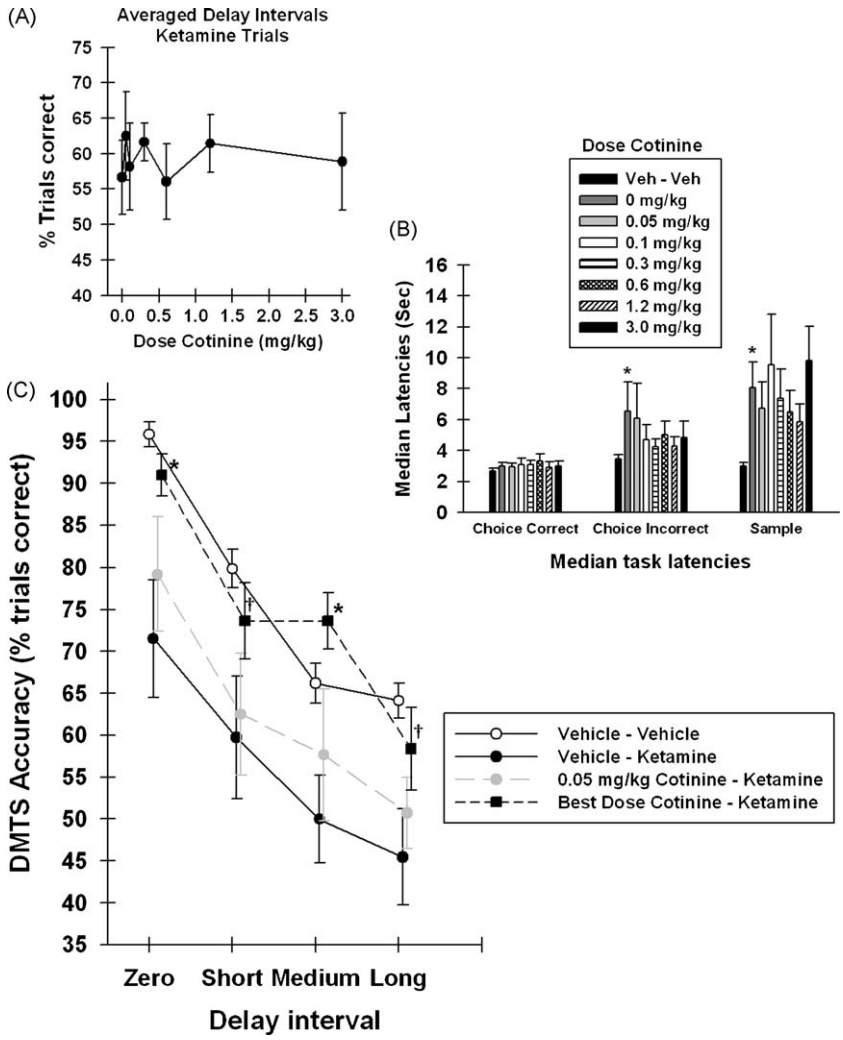

Fig. 3. The effect of pretreatment with cotinine on ketamine-induced decrements in DMTS performance by 6 pigtail monkeys. (A) Cotinine was administered 15 min prior to ketamine, and DMTS testing was initiated 30 min after ketamine (2 mg/kg) administration. Data represent the averaged accuracies for each of four delay intervals. The "0 mg/kg" dose refers to sessions in which ketamine was preceded by vehicle. (B) The effect of cotinine-vehicle and cotinine–ketamine regimens on median task latencies. "Veh–Veh" indicates sessions in which two consecutive vehicle injections were made before testing. (C) The data associated with the 0.05 mg/kg dose of cotinine, and with the averaged best dose (highest overall accuracy value among the doses tested) are plotted vs. delay interval. "Vehicle–Vehicle" indicates control data in which there was no administration of ketamine. "Vehicle–Ketamine" indicated control data in which vehicle preceded 2 mg/kg ketamine. Each value represents the mean ± S.E.M. Data points are slightly offset to better illustrate the error bars. *Significantly different from respective Vehicle–Ketamine value ($P < 0.05$); †($P < 0.10$).

for all four delay intervals ($P < 0.009$). During sessions run 24 h after ketamine (with no other pre-test administration) task accuracies were significantly increased relative to vehicle–vehicle means run the day prior ($F_{5,7} = 2.76$, $P = < 0.020$). All of the statistically significant improvement in task accuracies was associated with the 0.3 mg/kg dose of cotinine during medium ($t = 2.40$, $P = 0.017$) and long ($t = 2.82$, $P = 0.006$) delay trials (Fig. 6).

Administration of the 2 mg/kg dose of ketamine was associated with a significant decrease in the number of trials completed per session (timed-out sessions). The effect depicted in Fig. 7 was statistically significant from vehicle ($F_{4,4} = 18.4$, $P < 0.0001$). The average best dose of GTS-21 in the GTS-21 series, and the best doses of cotinine obtained in the cotinine–ketamine, and ketamine-cotinine series each significantly reversed the ketamine-induced decrease in the number of trials completed ($P < 0.005$).

4. Discussion

Ketamine is an antagonist of NMDA glutamate receptors, a property which explains the drug's ability to impair working memory. However, the ability of ketamine to induce a hypnotic state, its antidepressant activity, and its psychotomimetic activity are not fully shared in the clinical dose range by more selective NMDA antagonists such as MK-801. This discrepancy could be related to ketamine's multimodal action at neural targets, including sigma receptors, phencyclidine receptors [36], various subtypes of opiate, cholinergic and GABA receptor subtypes (see Ref. [30]), and HCN1 pacemaker channels [29]. Though this diversity of action likely occurs optimally at different brain concentrations, the ability of ketamine to mimic many of the symptoms of schizophrenia fits with the complex etiology of the disease and with the efficacy of second generation antipsychotic agents like clozapine which also interacts with several neural targets [37]. For these reasons ketamine was chosen for use as potential pharmacological model for schizophrenia-related cognitive deficits in monkeys. To support the proof of concept for the model, two nicotinic compounds GTS-21 and cotinine were evaluated for their ability to prevent the deficits in DMTS task accuracies produced by the test dose of ketamine. As indicated in Section 1, there also exists preclinical and clinical data supporting the utility of GTS-21 in improving aspects of cognition and other

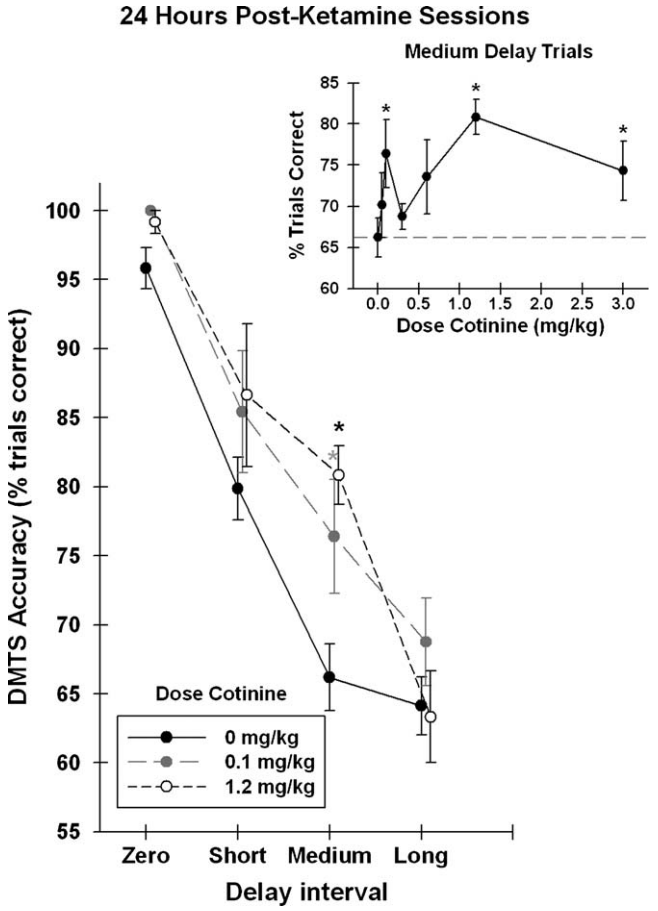

Fig. 4. The residual effect on task accuracies 24 h after pretreatment with cotinine in vehicle- and ketamine (2 mg/kg)-treated monkeys. These data are derived from the sessions run 24 h after those described in Fig. 3, without additional pre-test administration. "0 mg/kg" indicates data derived from subjects when vehicle (no ketamine) was administered the previous day. These data are compared with those in which 0.1 or 1.2 mg/kg cotinine followed by ketamine (2 mg/kg) was administered the previous day. Note: even though only two cotinine doses are presented in the figure, all control and cotinine sessions were included in the statistical analysis. Data points are slightly offset to better illustrate the error bars. Inset: the residual effect of cotinine pretreatment on medium delay accuracy as a function of dose. *Significantly different from respective 0 mg/kg value ($P < 0.05$).

symptoms of schizophrenia. In addition to the evidence provided to support the pharmacological activity of cotinine [23,38–40], this long-lived metabolite of nicotine could play a role in the self-medication smoking provides for an inordinately large proportion of schizophrenic patients [16].

Initially we needed to determine a standard test dose of ketamine. Ketamine administration did not generate a smooth dose-response relationship. Instead the task accuracy was rather constant from 0.1–1 mg/kg, with the 2 mg/kg dose providing the first significant degree of task impairment. The abrupt change in performance from 1 to 2 mg/kg could represent the recruitment of one or more of the neural targets indicated above. In fact, the decreases in zero delay accuracies after ketamine suggest direct effects of the drug on attentional components of memory [41]. Low doses of the amnestic drug scopolamine exert similar decreases in zero and short delay accuracies in the monkey DMTS task, however, scopolamine's actions are not as robust as ketamine's for medium and long delay intervals [42]. In fact, the ability of ketamine to dramatically decrease medium and long delay accuracies implies a concomitant effect of the drug on working memory. Higher doses of ketamine than 4 mg/kg were not attempted in order to avoid frank sedation. Notwithstanding the nature of the dose-response relationship, the test dose (2 mg/kg) of ketamine produced a very reproducible impairment in DMTS accuracies under control (vehicle) conditions in each of the four experimental series. Throughout the study ketamine produced occasional and inconsistent increases in task latencies. But in general, processing speed was not dramatically affected by the test dose, suggesting that sedation was not a major component of the ketamine-induced decrement in task accuracies. Ketamine did produce a decrease in accuracies across all four delay intervals such that the accuracy–delay relationship was shifted below and in a roughly parallel manner to the control curve (Fig. 1C). Thus the drug has the potential to interfere with all aspects of working memory–discrimination/attention, encoding and retention [41,42]. Some effect of ketamine on discrimination is suggested by the decrease in zero delay accuracy, though it is not possible to directly determine whether alterations in perception significantly contributed to the deficits. However, during the choice phase of the paradigm, there was little change in task latencies, and none for correct choices, suggesting that the subjects discriminated enough so as not to delay their responses.

Since the cognition-enhancing agent GTS-21 already was reported to have clinical utility in schizophrenia [20] we chose this compound to study among other nicotinic receptor agonists. GTS-21 has displayed efficacy in a variety of rodent models of cognitive impairment and in models assessing behavioral processes related to schizophrenia [43]. However, the compound has not been specifically evaluated in a rodent model of cognitive impairment in schizophrenia. In the present study pretreatment with GTS-21 produced a dose-dependent attenuation of ketamine-induced decreases in task accuracies. In fact, the best dose of GTS-21 completely reversed the effects of ketamine, both on task accuracy and in terms of the number of trials completed per session. It would be difficult to understand the marked efficacy of GTS-21, if the compound were only affecting ketamine-impaired perception, e.g., as a classical antipsychotic agent. Somewhat surprising was the lack of effect of GTS-21 to improve DMTS accuracies on the day after administration. We had reported earlier that GTS-21, like nicotine, exhibits a pharmacodynamic action that results in protracted improvements in cognitive performance [19,42,44]. The lack of a protracted response to GTS-21 was contrasted by cotinine which was quite effective in this regard (see below).

Unlike GTS-21, cotinine pretreatment was not associated with a typical dose-response relationship for reversing the task deficits produced by ketamine. In fact, selection of a best dose was necessary to show the activity of cotinine. However, the best dose of cotinine, like GTS-21, completely reversed the ketamine-induced task deficits. That the doses used for cotinine were active pharmacologically was supported by the observation that DMTS accuracies were increased relative to their non-ketamine baselines during sessions run 24 h later (Fig. 4). We had previously reported this protracted positive mnemonic action of cotinine in monkeys during standard DMTS testing [23]. In the present study, the protracted effects of cotinine could be related to the compound's rather long (15–19 h) plasma half-life [45].

In the final series, the cotinine–ketamine order of administration was reversed to provide a more clinically relevant model (the diagnosis of schizophrenia would normally precede treatment). Surprisingly, the cotinine post-treatment regimen produced a classical dose-response relationship which, as indicated above, was not the case for cotinine pretreatment (Fig. 5A vs. Fig. 3A). In this series the most effective of the doses in the sequence (0.6 mg/kg; Fig. 5A) was similar to the average best dose (0.63 mg/kg) even though 0.6 mg/kg was the best dose for only 3 of the 8 subjects. Also cotinine appeared to be about twice as potent as a post-treatment than as a pretreatment (best dose = 1.13 mg/kg). As with

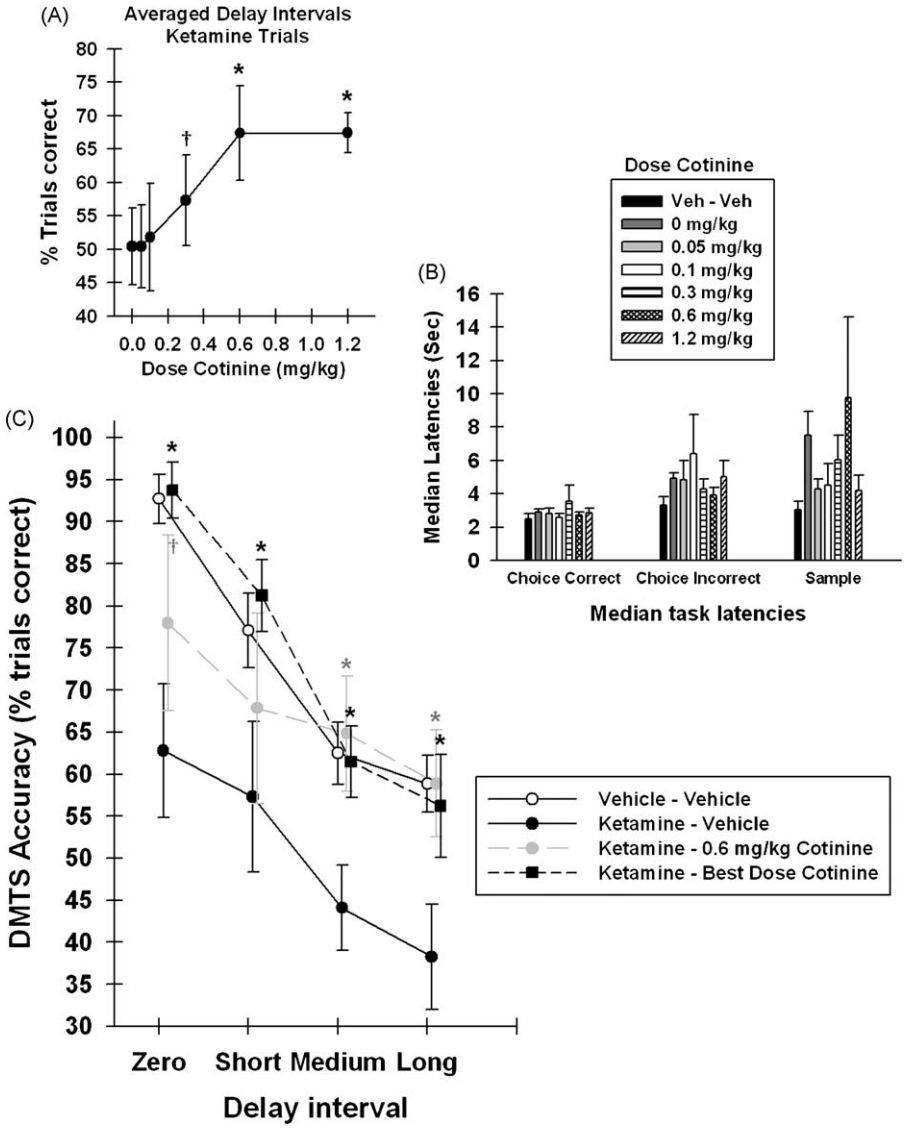

Fig. 5. The effect of post-treatment with cotinine on ketamine-induced decrements in DMTS performance by 8 pigtail monkeys. (A) Ketamine (2 mg/kg) was administered first; followed 30 min later by cotinine, and DMTS testing was initiated 15 min later. Data represent the averaged accuracies for each of four delay intervals. The "0 mg/kg" dose refers to sessions in which vehicle was administered 30 min after ketamine. (B) The effect of vehicle-cotinine and ketamine-cotinine regimens on median task latencies. "Veh–Veh" indicates sessions in which two consecutive vehicle injections were made before testing. (C) The data associated with the 0.6 mg/kg dose of cotinine, and with the averaged best dose (highest overall accuracy value among the doses tested) are plotted vs. delay interval. "Vehicle–Vehicle" indicates control data in which there was no administration of ketamine. "Ketamine-Vehicle" indicated control data in which 2 mg/kg ketamine preceded vehicle. Each value represents the mean ± S.E.M. Data points are slightly offset to better illustrate the error bars. *Significantly different from respective Vehicle–Ketamine value ($P < 0.05$); †($P < 0.10$).

the pretreatment regimen, post-ketamine cotinine resulted in a significant improvement in task accuracies during the sessions run 24 h later (Fig. 6).

Based on the average best doses, GTS-21 was between 35- and 62-fold more potent in reversing ketamine-induced task decrements than was cotinine. GTS-21 [18] was similarly more potent than cotinine [23] in the standard DMTS task. The inability of ketamine to disturb this dose ratio suggests a consistency of the pharmacological basis for the task improvements in both paradigms. Thus ketamine could be used to simulate the cognitive impairment associated with schizophrenia when it is administered to monkeys trained to perform a delayed response task. We show that the DMTS task impairment induced by ketamine in monkeys is reproducible and capable of being completely reversed by two compounds that are known to improve working memory and cognition, and which have relevance to schizophrenia. For future studies either agent could be used as a prototype for comparison with novel compounds. It has been suggested that a modern approach to the treatment of schizophrenia should make use of polypharmacy, i.e., the use of pharmacological agents that have multiple interactions with relevant drug targets, or with the use of several compounds that address the varied symptoms of schizophrenia [37]. The model described in this report could provide a means of late stage preclinical evaluation of new compounds that address the cognitive impairment associated with major psychotic disease. It was particularly exciting to find a protracted improvement in task accuracies in both cotinine series, considering that in the previous day's testing, cotinine was mostly paired with ketamine. Some consideration should be given to evaluating cotinine at least as an adjunct to therapy in schizophrenia. The compound has undergone clinical testing and found to be associated with few, if any side effects [46–48]. Future drug discovery also should be considered taking advantage of cotinine's chemical structure.

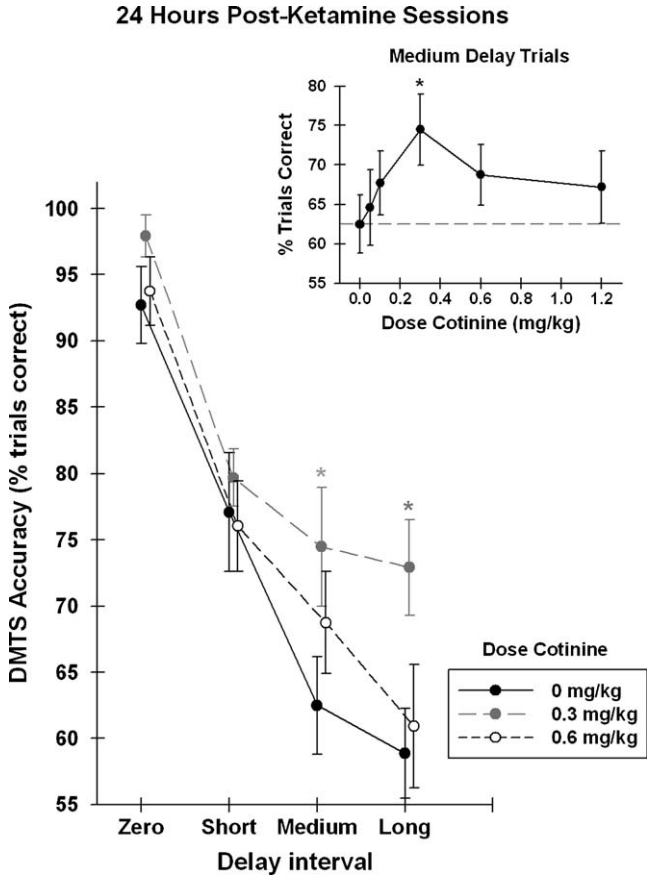

Fig. 6. The residual effect on task accuracies 24 h after post-treatment with cotinine in vehicle- and ketamine (2 mg/kg)-treated monkeys. These data are derived from the sessions run 24 h after those described in Fig. 5, without additional pre-test administration. "0 mg/kg" indicates data derived from subjects when vehicle (no ketamine) was administered the previous day. These data are compared with those in which ketamine (2 mg/kg) followed by 0.3 or 0.6 mg/kg cotinine was administered the previous day. Note: even though only two cotinine doses are presented in the figure, all control and cotinine sessions were included in the statistical analysis. Data points are slightly offset to better illustrate the error bars. **Inset:** The residual effect of cotinine pretreatment on medium delay accuracy as a function of dose. *Significantly different from respective 0 mg/kg value ($P < 0.05$).

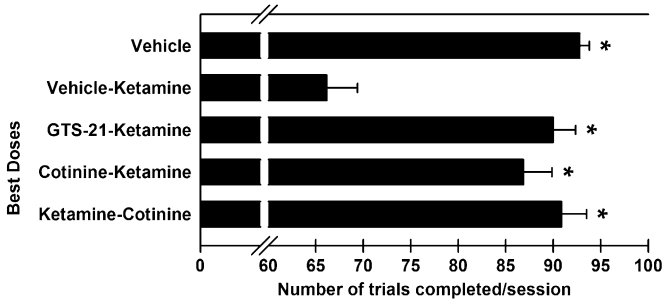

Fig. 7. The ability of GTS-21 and cotinine pretreatment, and cotinine post-treatment to attenuate the ketamine-induced decrease in the number of trials completed per session (timed-out sessions). The average best dose of GTS-21 in the GTS-21 series, and the best doses of cotinine obtained in the cotinine–ketamine, and ketamine–cotinine series each significantly reversed the ketamine-induced decrease in the number of trials completed. The "Vehicle–Ketamine" value represents the mean (±S.E.M.) of all sessions in the study in which ketamine (2 mg/kg) was paired with vehicle. The "Vehicle" value represents all sessions in which only vehicle administrations preceded DMTS testing. *Significantly different from respective Vehicle–Ketamine value ($P < 0.005$).

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