Scopolamine induced deficits in a battery of rat cognitive tests: comparisons of sensitivity and specificity

Donald Bartholomew Hodges Jr[a,c], Mark D. Lindner[b], John B. Hogan[b], Kelly M. Jones[b] and Etan J. Markus[c]

Despite much research, the cognitive effects of scopolamine hydrobromide, a cholinergic antagonist, remain controversial. Scopolamine affects multiple systems each of which can impact behavior. One way to tease apart the effects of the drug is to determine the effects of low scopolamine doses on different abilities. The present experiments compared the effects of low doses of scopolamine on a single group of rats conducting a battery of behavioral tasks: Morris water maze, radial arm maze, delayed non-matching to position tasks, and fixed ratio 5 bar pressing. The behavioral battery ranged from tasks having little cognitive demand to those thought to be based more on attention and spatial-working memory. Control experiments using additional groups of rats assessing peripheral versus central effects were conducted with both liquid and dry reinforcement and with methyl scopolamine. Furthermore, the 5-choice serial reaction time test assessed scopolamine effects on attention. The data show a wide spectrum of central and peripheral cholinergic involvement. The central effects include attention and motor initiation, both of which impact and interact with the mnemonic function of acetylcholine. These results show that a limited disruption of the central cholinergic system can have profound effects on attention and/or psychomotor control before any measurable mnemonic disruption. *Behavioural Pharmacology* 20:237–251 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Behavioural Pharmacology 2009, **20**:237–251

Keywords: attention, behavioral models, delayed non-matching to position, muscarinic, radial maze, rat, water maze, working memory

[a]General Pharmacology-Neuroscience Core, Drug Innovation and Realization, Vertex Pharmaceuticals Inc., Cambridge, Massachusetts, [b]Neuroscience Biology, Research and Development, Bristol-Myers Squibb Company, Wallingford and [c]Deparment of Psychology, University of Connecticut, Storrs, Connecticut, USA

Correspondence to Dr Donald Bartholomew Hodges Jr, PhD, Vertex Pharmaceuticals Inc., 130 Waverly Street, Cambridge, MA 02139, USA
E-mail: donald_hodges@vrtx.com

Received 4 April 2008 **Accepted as revised** 17 February 2009

Introduction

Anticholinergic agents, such as the muscarinic receptor antagonist scopolamine, produce cognitive deficits in humans, rats, and monkeys (Bartus *et al.*, 1978; Murray *et al.*, 1991; Pontecorvo *et al.*, 1991; Rupniak *et al.*, 1991). However, high doses of scopolamine have been shown to cause impairment in many cognitive tasks, and therefore interpreting the results is complex. Acetylcholine is widespread in the brain, and therefore scopolamine could potentially impact many different systems. Similarly 'cognitive tasks' rely, to some degree, on noncognitive processes, such as motivation and motor ability, which could potentially impact the subjects' performance (Hodges, 1996). Given these confounds, it is not surprising that reports on the cognitive effects of scopolamine vary widely across labs and tasks (see Blokland, 1996; Ebert and Kirch, 1998). Although there is a large literature examining the effects of scopolamine, the research is mostly focused on the effect of high doses and/or on a single task.

One strategy to distinguish between the different effects of scopolamine is to examine how the same drug dose impacts on the performance in tasks that differ in their nonmnemonic demands. A second approach is to use very-low doses of the drug, determining at what dose the different behavioral effects are first seen.

This study examined one of scopolamine's well-documented cognitive effects, impairment of 'working memory'. The effects on working memory were assessed using low doses and a range of working memory tasks. These included the eight-arm radial maze (RAM; Olton and Samuelson, 1976), Morris water maze (MWM; Morris *et al.*, 1982), and delayed non-matching to position (DNMTP; Ruotsalainen *et al.*, 1997).

To ensure consistency, the same animals were tested in different tasks. Testing was counterbalanced and/or repeated to control for possible drug tolerance and carryover effects. In addition, factors such as reward type and the central versus peripheral effects of scopolamine were examined. In most of the tasks, there was an effect of scopolamine on performance. However, the degree to which this effect was related to working memory and other cognitive and noncognitive functions varied. Changes in attention and/or psychomotor control (i.e. ability to coordinate timely and appropriate responses to stimuli) occurred at low doses, long before any measurable working memory disruption was found.

0955-8810 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/FBP.0b013e32832c70f5

Methods

Subjects

All rats were singly housed males, maintained on scheduled feedings of 15–20 g per day of standard rat chow (Noyes Formula A/I; Research Diets, New Brunswick, New Jersey, USA). Study 1 used Long–Evans Hooded (LE) rats (Harlan Sprague Dawley Inc., Indianapolis, Indiana, USA), which were 3 months old (335 ± 2.2 g) at the start of testing and 10 months old (392 ± 3.5 g, $n = 42$) at study completion. Study 2 used naive LE rats (Harlan Sprague Dawley Inc.), which were 9 months old (447 ± 4.0 g, $n = 53$). Studies 3 and 4 started training with a group of 75 naive Sprague–Dawley (SD) rats (Harlan Sprague Dawley Inc.), which were 11 months old (423 ± 1.9 g). Study 5 used 32 rats (452 ± 3.0 g) selected from the SD rats completing Study 4. All experimental procedures were reviewed and approved by the Bristol-Myers Squibb Institutional Animal Care and Use Committee and conducted in an Association for Accreditation of Laboratory Animal Care accredited facility, in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2003).

Study 1: behavioral battery

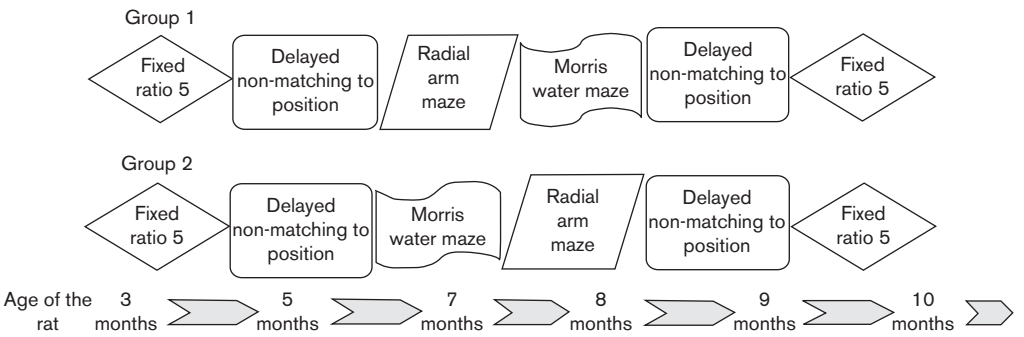
Study 1 was a behavioral battery of fixed ratio 5 (FR5), DNMTP, MWM, and RAM to compare the sensitivity to low doses of scopolamine of these tasks. In each task, after the animals were trained to asymptote and scores were stable for 3 days, 2 weeks of drug testing was continued. During testing, each rat received an intraperitoneal injection (1 ml/kg) of saline or one of five scopolamine doses 20 min before the daily test procedure, using a within-subject, counterbalanced Latin square design (Fig. 1). This was repeated using a different dose order the next week. The 20-min pretest injection interval was chosen because the plasma half-life of scopolamine is approximately 20 min (Lyeth *et al.*, 1992). To control for order effects, rats were divided into two subgroups: one subgroup received the radial maze before the water maze and vice versa for another. Animals also received the DNMTP both before and after the FR5 task. The repetition of the DNMTP and FR5 tasks allowed for examinations of possible drug sensitization/tolerance, and overtraining effects.

Morris water maze

MWM testing was conducted in a 1.5-m diameter × 0.6-m depth black polyvinyl water tank, located off center in a large [length (L) 6.1 × width (W) 6.1 × height (H) 2.6 m] well-lit room (260 lux) containing many stable extra-maze cues. The tank contained a black hidden escape platform (14.5 cm diameter) submerged 1.0 cm below the water surface. The water was 45 cm deep and 22 ± 1°C. Data were collected using an EthoVision video tracking system (version 2.0; Noldus Information Technology Inc., Leesburg, Virginia, USA). Rats were initially trained with four trials per day for 5 days, with the submerged platform fixed in the center of one quadrant. This was followed by a repeated-acquisition task where the platform location was changed daily, but kept at least 15 cm from the wall. Start points were also varied for each trial, all at least 45 cm from the platform. Within a given day, the platform location remained fixed; rats were given three trials with the platform submerged and a fourth with the platform visible. This was repeated for 3 days after which drug testing was conducted. Training scores were stable over these 3 days. The information trial was the rat's first swim trial of the day: the rat was required to find the new placement of the submerged platform. Working memory trials were the second and third swim trials of the day: the rat used the information gained in the first swim trial to find the submerged platform.

In each trial, rats were placed in the tank facing the wall, and allowed to swim until they found the platform. If the rat did not find the platform in 1 min, it was placed on the platform by the experimenter. Rats were given 10 s to stay

Fig. 1



Experimental design for Study 1. The animals were given a battery of tests. To control for order effects, half the animals received the water maze before the radial maze. Animals also received the delayed non-matched to position (DNMTP) both before and after the fixed ratio 5 (FR5) task. The repetition of the DNMTP and FR5 tasks also allowed for examinations of possible drug sensitization/tolerance, and overtraining effects.

on the platform, and a 10 min intertrial interval. Cumulative error (Gallagher *et al.*, 1993), the sum of the mean distance (cm) from the platform for each second (sampled at 6 Hz) was recorded.

Radial arm maze

The eight radial maze arms (L 73.7 × W 8.9 × H 10.8 cm; Coulbourn Instruments, Allentown, Pennsylvania, USA) extended from a central hub, were placed on a circular table 90 cm above the floor in a small (L 2.7 × W 2.7 × H 2.6 m), dimly lit (40 lux) room. A 45 mg food pellet (Noyes Formula A/I; Research Diets) was used to bait a maze arm. Rats were tested on the RAM with four 'once-baited' dark arms (dark drop pans) to test spatial-working memory; alternating with four 'never-baited' white arms (white drop pans) to test cued-reference memory (Oler and Markus, 1998; Ward *et al.*, 1999). In this version of the RAM, aged and hippocampal-lesioned animals only perform well on the cued-reference task (Oler and Markus, 1998; Ward *et al.*, 1999). Each rat was placed in the central hub for 10 s, the hub doors were opened, and it remained in the maze until all four baited arms were visited or 10 min elapsed. Entry into a previously visited baited arm was scored as a spatial-working memory error; and entry into a never-baited arm scored as a cued-reference memory error (Beninger *et al.*, 1986). Training continued to asymptote. After the rats showed 3 days of three or more consecutive correct working memory choices, drug testing was conducted.

Operant conditioning chambers

The FR5, DNMTP, and food pellet consumption studies were conducted in 16 operant conditioning chambers (model # H10-11R-TC; Coulbourn Instruments). Each operant conditioning chamber was enclosed in a sound and light-attenuating box (with vent fan). Two retractable levers were located on the outer bays of one wall with a house light located at the top of the central bay. The food magazine was located on the central bay of the opposite wall. Free access to water was provided during all sessions. Data were collected using Coulbourn Instruments Graphics States I software (version 1.014-00; Coulbourn Instruments).

Fixed ratio 5

During the FR5 training, rats were presented with a single lever and every fifth lever press was reinforced by the delivery of a food pellet (45 mg food pellet = 0.142 kcal; Noyes Formula A/I; Research Diets). After every 50 presses, the side on which the lever was presented alternated. Rats were given one daily session lasting for 20 min or until 15 g of food were delivered, and the number of lever presses was recorded. No rat achieved 15 g of food reward in a 20 min session. Rats were trained until bar pressing reached asymptote and was stable over 3 days.

Delayed non-matching to position

For the DNMTP, either the right or left lever was presented, and retracted as soon as it was pressed. A retention delay followed (0, 4, 8, or 12 s, in pseudo-random order) at the end of which the rat had to poke its nose into a food bin on the other side of the operant conditioning chamber, after which both levers were presented. (For the pseudo-random order, listed delays were randomized into a sequence, which was completed before another randomized sequence began.) The rat was rewarded with a 45 mg pellet for choosing the lever that had not been presented (non-match). After the rat pressed one of the choice levers, both levers were retracted for 5 s and the house light turned off. To prevent side biases, incorrect trials were followed by a trial on the same side until a correct choice was made. Training continued until rats reached 85% correct choices at each delay value and was stable over 3 days.

Study 2: dry food consumption

Rats were placed in an operant conditioning chamber and five food pellets were dropped into the food trough. As soon as the pellets were eaten, another five pellets were dropped into the food trough. This continued until 50 pellets had been eaten or 30 min passed. Each rat received a single injection of scopolamine (0, 0.005, 0.01, 0.03, 0.05, and 0.10 mg/kg; $n = 8$, 9, 8, 9, 9, and 7, respectively) 20 min before testing.

Study 3: FR5 and DNMTP – scopolamine versus methyl-scopolamine and liquid reward versus dry food reward

In Study 3, experiments were conducted to compare performance with a liquid reward versus a dry food reward, 30 min after receiving scopolamine or methyl-scopolamine. For the DNMTP task, doses of 0.0, 0.025, and 0.20 mg/kg were used with dry food reward, and 0.0, 0.1, and 0.2 mg/kg with liquid reward. For FR5, doses of 0.0, 0.1, 0.2, and 0.4 mg/kg were used with both reward types. In these studies, eight of the 16 operant conditioning chambers had the pellet feeders replaced with liquid dippers (model # H14-05R; Coulbourn Instruments). The liquid reward was 0.6 ml of 50 : 50 sweetened condensed milk and water (0.144 kcal). The rats were divided into two subgroups, those receiving a liquid reward ($n = 30$) and those receiving a dry food reward ($n = 25$).

Study 4: 5-choice serial reaction time test

The 5-choice serial reaction time test (CSRTT) was carried out in specially designed operant conditioning chambers (model # H10-11R-TC; Coulbourn Instruments), consisting of five response holes and a food magazine on the opposite wall. Rats were trained (100 trials/day) to nose poke into the illuminated (100 lux) response hole, which was randomly presented among the five locations, to receive food pellets, with progressively shorter light/cue

durations, until the 2-s cue duration was reached. The rationale for this relatively easy attention task was to show a scopolamine-induced deficit. During testing, rats were allowed a 5-s limited hold after the end of the cue to respond. If the animal did not respond within the limited hold, it was recorded as an 'omitted' trial. Incorrect responses were followed by 5 s timeout with house lights on. The rats were divided into five groups (0.0, 0.05, 0.1, 0.2, and 0.4 mg/kg; $n = 6$, 7, 6, 7, and 6, respectively), and injected with scopolamine 30 min before testing.

Drugs

Scopolamine hydrobromide and scopolamine methyl bromide (methyl-scopolamine; both from Sigma, St. Louis, Missouri, USA) were dissolved in isotonic saline in free base equivalents. Drug solutions were prepared fresh each day. Vehicle used was isotonic saline. All injections were intraperitoneal at 1 ml/kg. Given the wide array of reported central effects for scopolamine, rather than injecting drug into a specific brain region a systemic injection was used.

Data analyses

Data are presented in the text and all figures as mean ± SEM. Statistical analyses were performed with SAS-PC (version 8.02). Analyses of variance (ANOVAs) were conducted using the procedures for general linear models, with options for repeated measures where appropriate (SAS Institute Inc., 1999). One-way ANOVAs were used for Studies 2 and 4, and factorial analyses were used for Studies 1 and 3 (for details, see below).

Omega squared (ω^2) is a measure of effect size and is relatively unaffected by sample size (Cohen, 1988); it represents the proportion of total variance accounted for by an effect (Dodd and Schultz, 1973). The ω^2 values of less than 0.06 are considered small, 0.06–0.15 are considered medium sized, and greater than 0.15 large (Cohen, 1988). Effect sizes for comparison across tasks were calculated using the standardized mean difference between a treatment group and a control group in terms of a unit-free outcome variable, '*d*' or Cohen's *d* (Glass *et al.*, 1981; Cohen, 1988). Cohen's *d* estimates were adjusted taking into account sample size (Hedges and Olkin, 1985).

In Study 1, cumulative error swim distances in the MWM, for the first or 'information' trial, the average of the second and third trials, and the fourth trial (visible platform) were analyzed using a $6 \times 2 \times 2$ ANOVA, with the within-group factors scopolamine dose (five doses plus saline) and test period (two dosing regimes) and the between-group factor, subgroup (two groups, tested in the order MWM–RAM or RAM–MWM). The same analysis was used for spatial-working memory, cued-reference memory, total arm entries, and latency per arm in the RAM. For the DMTP, the percentage of correct choices, sample latency, total latency, and number of completed trials were analyzed using a $6 \times 4 \times 4 \times 2$ ANOVA, with the within-group factors dose (0, 0.01, 0.03, 0.05, 0.10 and 0.30 mg/kg), retention delays (0, 4, 8 and 12 s), and test period, and the between-group factor, subgroup (as described above). Bar presses at FR5 were analyzed in a $6 \times 4 \times 2$ ANOVA, with the within-group factors dose (0.005, 0.01, 0.03, 0.05, and 0.10 mg/kg) plus saline and test period, and the between-group factor, subgroup (as described above).

In the first part of Study 3, lever presses were analyzed in a 2×4 ANOVA with the between-group factor reward type (liquid vs. dry food) and the within-group factor, dose of either scopolamine or methyl-scopolamine (saline, 0.1, 0.2, and 0.4 mg/kg). In the second part of Study 3, the percentage of correct choices for liquid reward was analyzed in a 5×5 ANOVA with the within-group factors of treatment (saline, 0.1 and 0.2 mg/kg methyl-scopolamine, and 0.1 and 0.2 mg/kg scopolamine) and retention delays (0, 2, 4, 8, and 12 s). The same analysis was used for the percentage of correct choices for dry food reward, except that the drug doses were saline, 0.025 and 0.2 mg/kg. Sample latency and total latency were analyzed using a 2×3 ANOVA, with the between-group factor reward group (liquid vs. dry chow) and the within-group factor, scopolamine dose (saline, 0.2 mg/kg methyl-scopolamine, and 0.2 mg/kg scopolamine).

Results

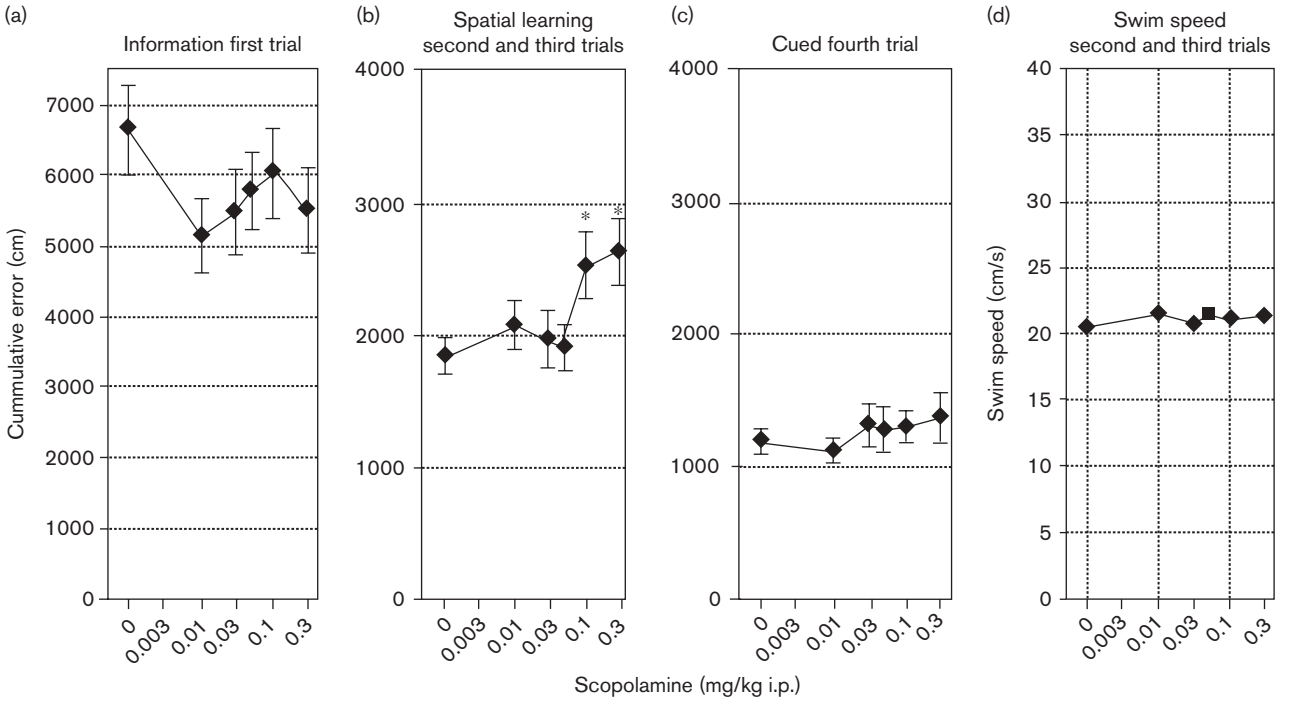
Study 1: behavioral battery

Morris water maze

The ANOVA failed to detect a significant main effect of scopolamine dose on measures of cumulative error during the 'information trial' [$F(5,200) = 1.48$, NS, $\omega^2 = 0.01$; Fig. 2a]. With repeated testing, there was a reduction in cumulative error from the first to second test period, indicating an improved initial search strategy with experience; however, this accounted for a small portion of variance in cumulative error distance measures [$F(1,40) = 13.39$, $P < 0.01$, $\omega^2 = 0.03$]. The subgroup by test period interaction was significant, but accounted for a small proportion of variance [$F(1,40) = 17.3$, $P < 0.01$, $\omega^2 < 0.01$]. Given that the test period and subgroup, although significant, had very little impact on behavior, Fig. 2 shows the overall effects of scopolamine dose on cumulative error distances.

For the two working memory trials, there was a significant main effect for scopolamine dose, but it only accounted for a small proportion of variance in the cumulative error distance measure [$F(5,200) = 2.27$, $P = 0.049$, $\omega^2 = 0.03$; Fig. 2b]. With repeated testing, cumulative error distance improved, which resulted in a significant main effect for test period that accounted for a large portion of variance

Fig. 2



Scopolamine effects on different aspects of the Morris water maze task (Study 1). (a) Information trial – platform placed in a novel location. There was no significant effect of scopolamine on cumulative search error. (b) Spatial working memory trials – after finding the platform in the first trial, rats were better at finding the platform in the two subsequent trials. Planned comparisons with saline control showed a significant disruption of performance only at 0.10 and 0.30 mg/kg doses [$F(1,40) = 4.76$, $P < 0.05$ and $F(1,40) = 6.19$, $P < 0.02$, respectively]. (c) Visible platform trial – in the fourth trial, the platform was visible and placed in a new location. There was no effect of scopolamine on cumulative search errors, or (d) swim speed. Values are expressed as mean ± SEM; *$P < 0.05$ versus control. There were also significant effects of test period and subgroup; however, their effect sizes were small (not shown in the figure, see text). i.p., intraperitoneal.

in cumulative error distance measures [$F(1,40) = 15.28$, $P < 0.01$, $\omega^2 = 0.12$]. However, the effects of different doses of scopolamine did not change with repeated testing (NS). Cumulative error swim distances during the fourth trial, to the visible platform, showed no significant effect of scopolamine dose [$F(5,200) = 0.55$, NS, $\omega^2 < 0.01$; Fig. 2c]. No significant effect of scopolamine dose was detected for the average swim speed of the second and third trials (Fig. 2d). The doses of scopolamine used were well below doses that induce thigmotaxis, and no rats in this study showed thigmotaxis.

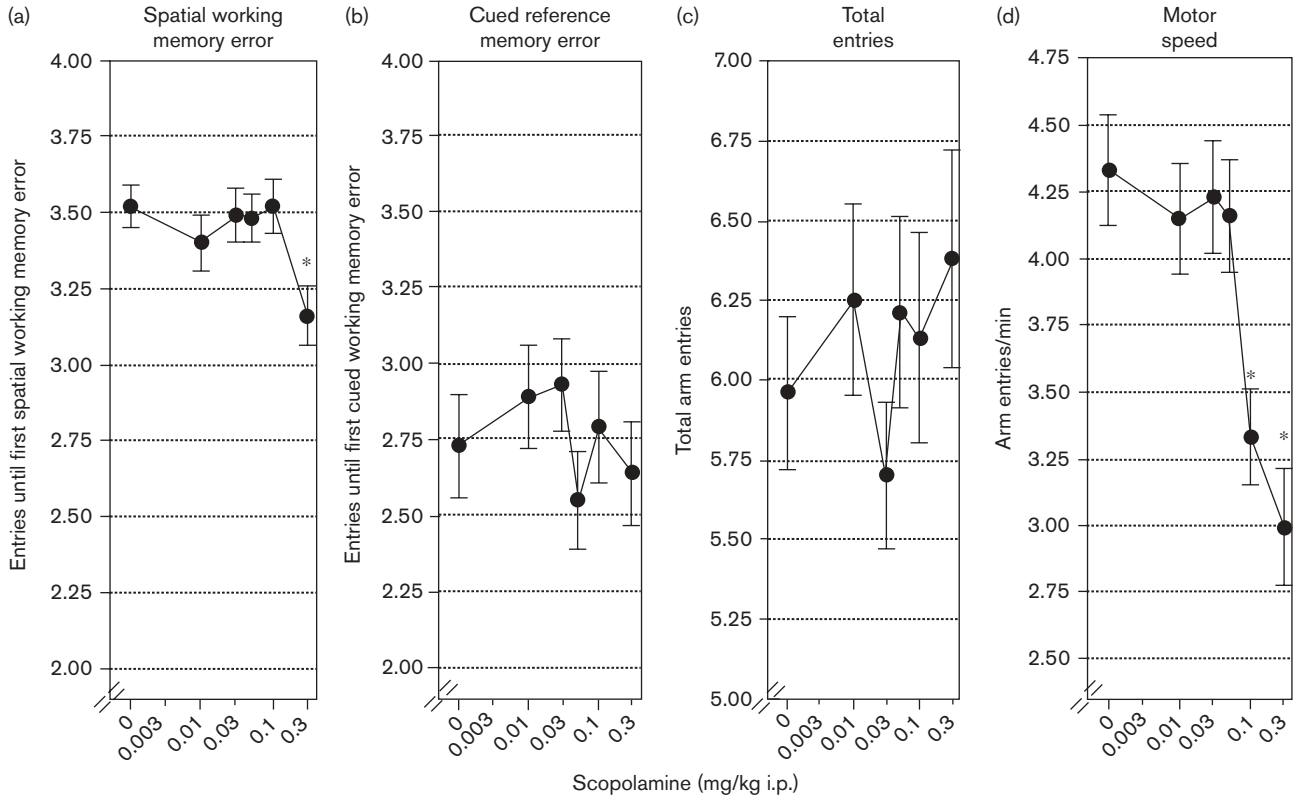
Radial arm maze

Correct entries until the first spatial-working memory error (Fig. 3a) were affected by scopolamine dose, but this accounted for only a small proportion of variance [$F(5,200) = 2.39$, $P = 0.04$, $\omega^2 = 0.03$]. There was also a significant main effect of subgroup, which did not account for a large proportion of variance [$F(5,200) = 4.09$, $P = 0.05$, $\omega^2 = 0.05$]. Correct entries until the first cued-reference memory error (Fig. 3b) were not affected by scopolamine dose (NS, $\omega^2 < 0.06$). There was a significant effect of test period, which accounted for a large proportion of variance in the cued-reference memory measure [$F(5,200) = 28.44$, $P < 0.01$, $\omega^2 = 0.30$]. Thus, with repeated testing, the rats improved their cued-reference memory performance. Total arm entries (Fig. 3c) were not affected by scopolamine dose (NS, $\omega^2 < 0.06$). Arm entries per minute (Fig. 3d) were affected by dose and accounted for a large proportion of variance [$F(5,200) = 16.89$, $P < 0.01$, $\omega^2 = 0.25$]. None of the other factors or interactions in the analysis was significant ($P > 0.10$) or accounted for any substantial proportion of variance ($\omega^2 < 0.06$).

Delayed non-matching to position

The percentage of correct choices was affected by dose, which accounted for a large proportion of the variance [$F(5,170) = 29.48$, $P < 0.01$, $\omega^2 = 0.30$; Fig. 4a]. There was also a significant effect of retention delay, which accounted for a large proportion of variance [$F(3,102) = 32.23$, $P < 0.01$, $\omega^2 = 0.15$]. With repeated testing, the percentage of correct choices increased but this effect accounted for only a small portion of the variance [$F(3,102) = 6.36$, $P < 0.01$, $\omega^2 = 0.04$]. The effects of dose changed with retention delays, resulting in a

Fig. 3



Scopolamine effects on different aspects of the radial arm maze task (Study 1). (a) Correct arm entries until the first working memory error. Planned comparisons with saline control showed that spatial working memory was disrupted at a 0.3 mg/kg scopolamine dose [$F(1,40) = 11.17$, $P < 0.01$]. (b) The correct arm entries until the first cued reference memory error were as unaffected by scopolamine. (c) The total number of arm entries was unaffected by scopolamine. (d) The rate of arm entries (number of entries/min) showed, by planned comparisons with saline control, a scopolamine-induced disruption at 0.1 and 0.3 mg/kg [$F(1,40) = 20.33$, $P < 0.01$ and $F(1,40) = 40.61$, $P < 0.01$, respectively]. Values are expressed as mean ± SEM; *$P < 0.05$ versus control. i.p., intraperitoneal.

significant dose by delay interaction [$F(15,525) = 3.48$, $P < 0.01$, $\omega^2 = 0.02$]. The small ω^2 value implies this interaction, although significant, had only a little impact on behavior.

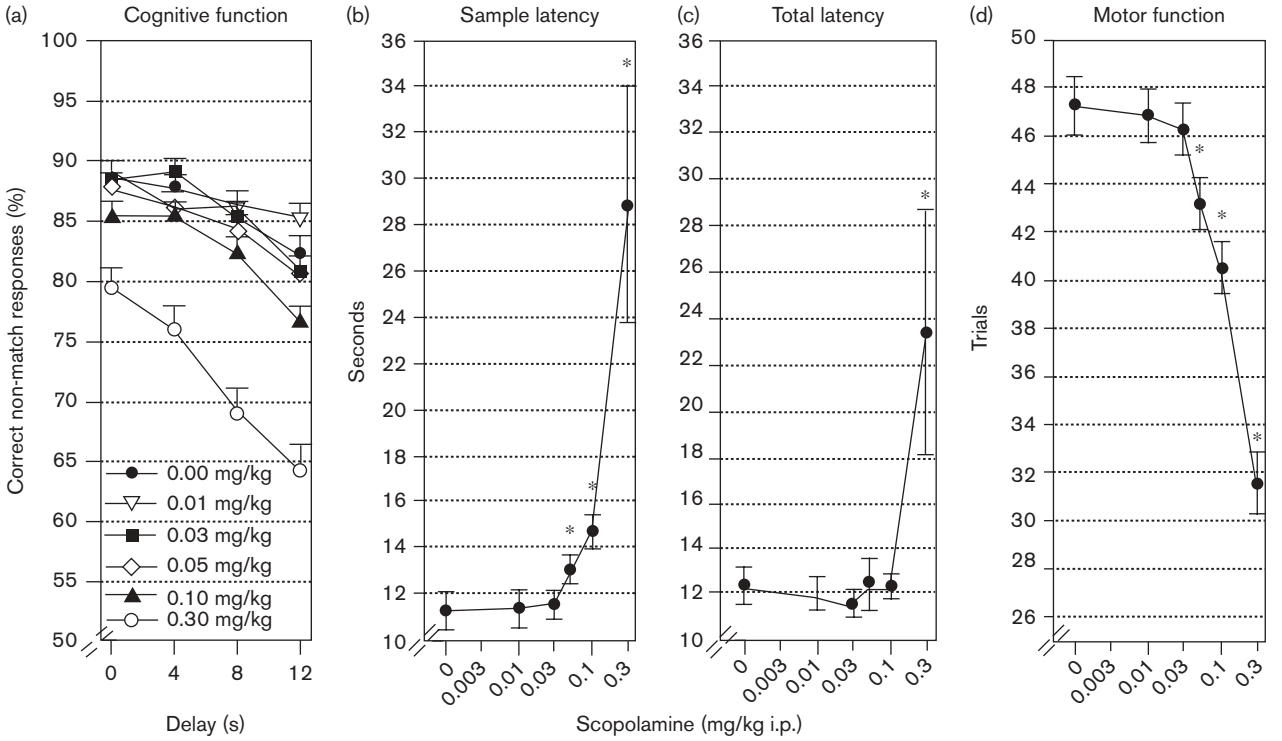
Sample latency (time taken to press the target lever at the beginning of each trial) was affected by dose, which accounted for a large proportion of variance [$F(5,185) = 47.25$, $P < 0.01$, $\omega^2 = 0.40$; Fig. 4b]. Total latency (time from when the rat pressed the sample lever to when it pressed a choice lever) was also affected by dose, which accounted for a moderate proportion of variance [$F(5,190) = 4.21$, $P < 0.01$, $\omega^2 = 0.06$; Fig. 4c]. There was also a significant effect of repeated testing, but this accounted for only a small portion of variance in total latency [$F(3,114) = 2.70$, $P = 0.05$, $\omega^2 = 0.03$]. The number of completed trials was affected by dose, which accounted for a large proportion of the variance [$F(5,190) = 10.87$, $P < 0.01$, $\omega^2 = 0.34$; Fig. 4d]. There was also a significant effect of repeated testing, which accounted for a large portion of the variance in the number of completed trials [$F(3,111) = 14.23$, $P < 0.01$, $\omega^2 = 0.28$].

Fixed ratio 5

The number of bar presses was significantly affected by dose and accounted for a large proportion of the variance [$F(5,200) = 99.98$, $P < 0.01$, $\omega^2 = 0.47$; Fig. 5a]. Repeated testing increased bar pressing and accounted for a large portion of the variance [$F(3,120) = 42.24$, $P < 0.01$, $\omega^2 = 0.42$]. The effects of different doses of scopolamine on bar pressing changed over time, resulting in a significant dose by test period interaction [$F(15,600) = 2.79$, $P < 0.01$], but this interaction was small ($\omega^2 = 0.02$). None of the other factors in the analysis was significant (NS) or accounted for any substantial proportion of variance ($\omega^2 < 0.06$). Given that the dose by test period interaction, although significant, had very little impact on behavior, Fig. 5a shows the overall effects of scopolamine doses on bar pressing.

To determine any change in bar pressing over the 20-min trial, FR5 data were broken down into bar presses per two 10-min bins and analyzed in a 6×2 mixed ANOVA, with the within-group factors scopolamine dose (0.005, 0.01, 0.03, 0.05, and 0.10 mg/kg) plus saline and time

Fig. 4



Scopolamine effects on different aspects of the delayed non-matching to position task (Study 1). (a) The percentage of correct non-match responses showed a significant scopolamine-induced disruption of working memory. At the zero retention delay, only the highest dose (0.3 mg/kg) was significantly different from saline showing a delay independent of disruption. Planned comparisons with saline control showed a significant disruption of performance at 0.10 and 0.30 mg/kg [$F(1,34)=13.82$, $P<0.01$ and $F(1,34)=39.34$, $P<0.01$, respectively] across the retention delays. For clarity, significant differences from control are not shown. (b) Sample latencies: time from sample lever presentation until it was pressed increased with a relatively low dose of scopolamine (0.05 mg/kg). Planned comparisons with saline control showed a significant disruption of performance at 0.05, 0.10, and 0.30 mg/kg [$F(1,38)=6.38$, $P<0.02$, $F(1,38)=23.61$, $P<0.01$, and $F(1,38)=13.24$, $P<0.01$, respectively]. (c) Total latency, time from sample lever pressed to pressing a choice lever, was disrupted only at the highest scopolamine dose (0.30 mg/kg). Planned comparisons with saline control showed a significant disruption of performance at 0.30 mg/kg [$F(1,38)=4.21$, $P<0.05$]. (d) Total trials completed (regardless if correct) showed a scopolamine-induced disruption already with the relatively low dose of 0.05 mg/kg. Planned comparisons with saline control showed a significant disruption of performance at 0.05, 0.10, and 0.30 mg/kg [$F(1,37)=23.33$, $P<0.02$, $F(1,37)=28.65$, $P<0.01$, and $F(1,37)=72.01$, $P<0.01$ respectively]. Values are expressed as mean ± SEM; *$P<0.05$ versus control. i.p., intraperitoneal.

(two 10-min time bins). Both dose [$F(5,1002) = 7.54$, $P < 0.01$] and time bin [$F(1,1002) = 160.32$, $P < 0.01$] significantly affected the performance, but there was no significant dose by time bin interaction ($P > 0.10$). At all six doses, more bar presses were seen in the first 10 min than for the second 10-min period.

Study 2: dry food consumption

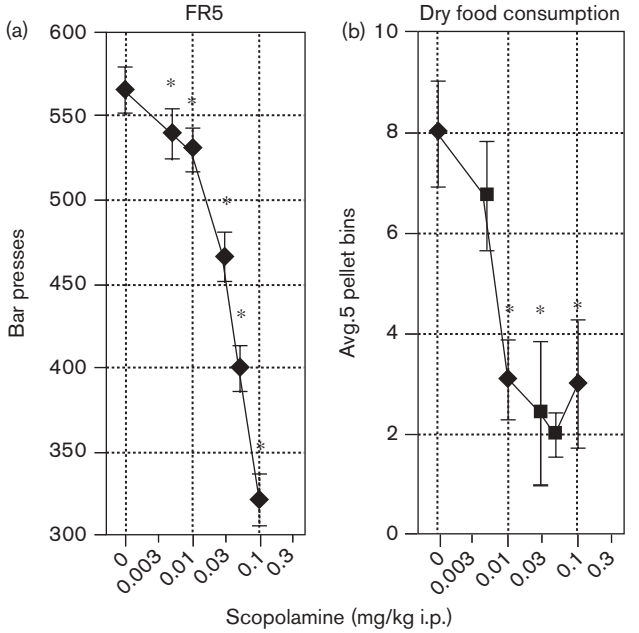
Scopolamine, in humans, is known to cause 'dry mouth' and may reduce salivation in the rat through the M3 receptors prevalent in the salivary glands. The FR5 results might be explained by scopolamine-induced 'dry mouth' reducing the rat's ability to consume dry food pellets. Therefore, an additional group of rats was given the dry food consumption test to determine whether there was any scopolamine-induced impairment in their ability to consume dry food (Fig. 5b). The saline control condition rats consumed approximately 40 pellets in 30 min. At 0.01–0.1 mg/kg scopolamine doses, rats were only consuming approximately 15 pellets in 30 min. A significant treatment effect was observed for total food pellets consumed [$F(5,45) = 6.16$, $P < 0.01$].

The dry food consumption task showed a dose-related impairment on the ability to consume dry food from 0 to 0.01 mg/kg scopolamine, but no additional effect was seen at the higher doses (Fig. 5b). These results show that at higher doses, ability and motivation to consume dry food remain constant. If ability and motivation to consume dry food remain constant, then scopolamine in the FR5 task must be affecting another factor, possibly psychomotor ability. In the FR5 task (Fig. 5a), changes in the ability to consume dry food likely influenced bar pressing, but the dose-related decrease in bar pressing could not be explained.

Study 3: FR5 and DNMTP – scopolamine versus methyl-scopolamine and liquid reward versus dry food reward

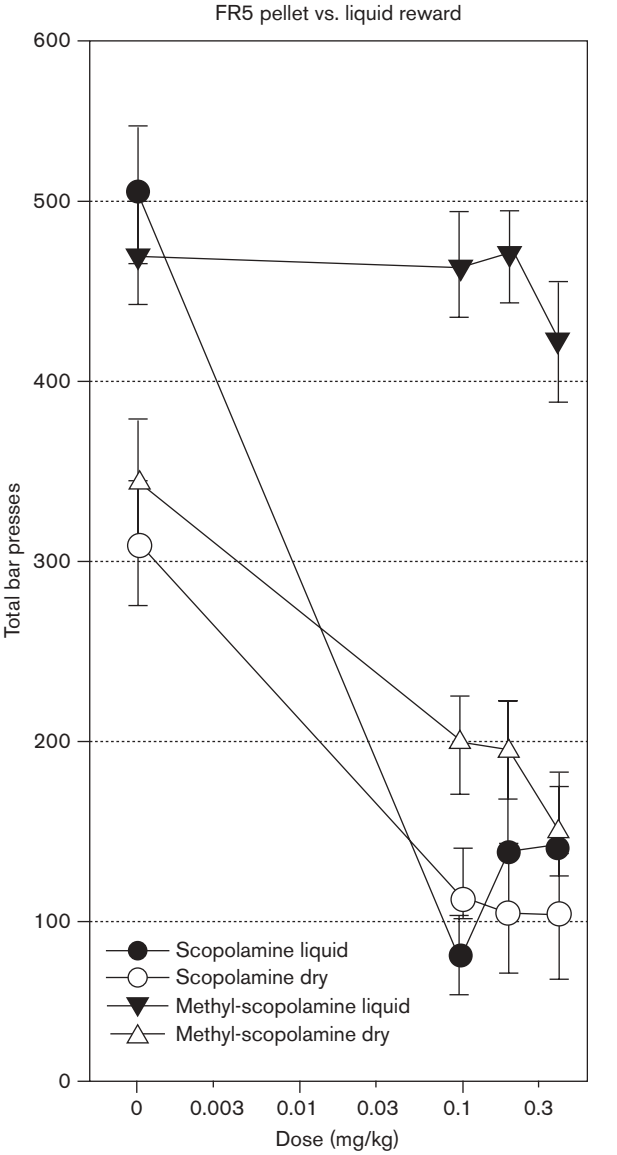
Rats were tested on the FR5 using liquid reward and dry food rewards and two types of scopolamine (Fig. 6). With

Fig. 5



Scopolamine effects on tasks involving the consumption of a large amount of dry food reward. (a) The fixed ratio 5 (FR5) task (Study 1): a 0.1 mg/kg dose of scopolamine reduced total bar presses from a baseline of approximately 550 presses (110 pellets) to approximately 325 (65 pellets). A significant scopolamine-induced disruption was found at a low dose of 0.005 mg/kg. Planned comparisons with saline control showed a significant disruption of performance at the 0.005, 0.010, 0.03, 0.05, and 0.10 mg/kg [$F(1,40)=6.25$, $P<0.02$, $F(1,40)=29.17$, $P<0.01$, $F(1,40)=82.73$, $P<0.01$, $F(1,40)=222.19$, $P<0.01$, and $F(1,40)=256.27$, $P<0.01$, respectively]. There was also a significant dose by test period interaction; however, the effect size was small (not shown in the figure, see text). (b) Dry food consumption (Study 2): the control study measured the effects of scopolamine on the ability to eat multiple reward pellets. The number of pellets consumed during a 30-min session was reduced from approximately 40 pellets (eight bins) to 15 pellets (three bins). Least significant difference post-hoc comparisons showed that a dose of 0.01 mg/kg was sufficient to significantly disrupt the rat's ability to eat multiple pellets. Values are expressed as mean ± SEM; *$P<0.05$ versus control. i.p., intraperitoneal.

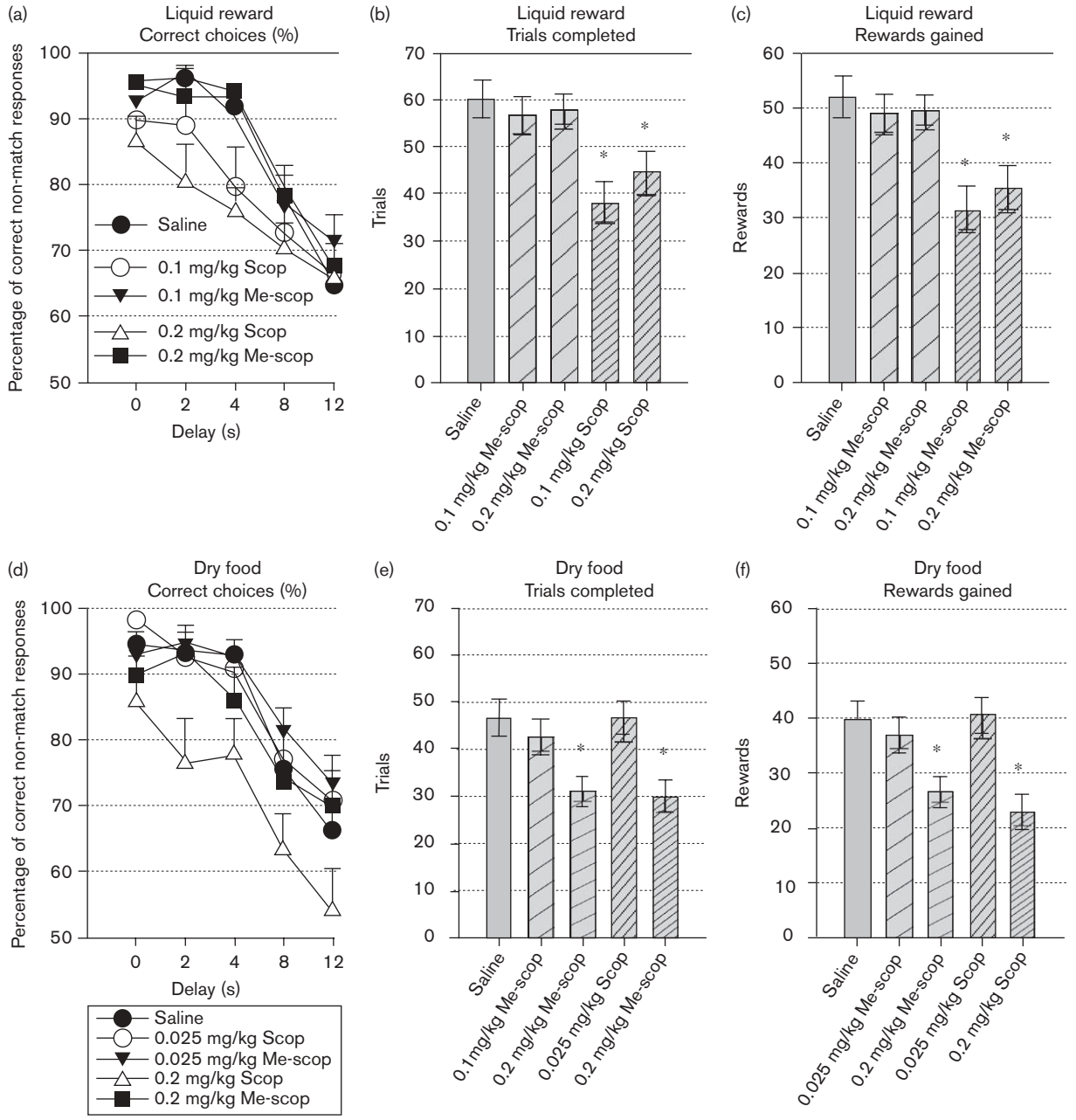
Fig. 6



Central versus peripheral scopolamine effects. In the fixed ratio 5 (FR5) task (Study 3), scopolamine at all doses used significantly ($P<0.05$ vs. control) reduced total bar presses in both liquid and dry reward groups. In the liquid reward group, planned comparisons with saline control showed that 0.1, 0.2, and 0.4 mg/kg scopolamine treatment decreased total lever presses [$F(1,29)=110.46$, $P<0.01$, $F(1,29)=52.46$, $P<0.01$, and $F(1,29)=50.70$, $P<0.01$, respectively]. In the dry chow reward group, planned comparisons with saline control showed that 0.1, 0.2, and 0.4 mg/kg scopolamine treatment decreased total lever presses [$F(1,24)=37.37$, $P<0.01$, $F(1,24)=38.30$, $P<0.01$, and $F(1,24)=29.23$, $P<0.01$, respectively]. Methyl-scopolamine significantly reduced bar pressing at all doses used in the dry food reward group, but had no effect in the liquid reward group. In the liquid reward group, planned comparisons with saline control showed that methyl-scopolamine treatments were not significantly different. In the dry chow reward group, planned comparisons with saline control showed that 0.1, 0.2, and 0.4 mg/kg methyl-scopolamine treatment decreased total lever presses [$F(1,24)=21.38$, $P<0.01$, $F(1,24)=19.73$, $P<0.01$, and $F(1,24)=36.51$, $P<0.01$, respectively]. For clarity, significant differences from control are not shown. Values are expressed as mean ± SEM.

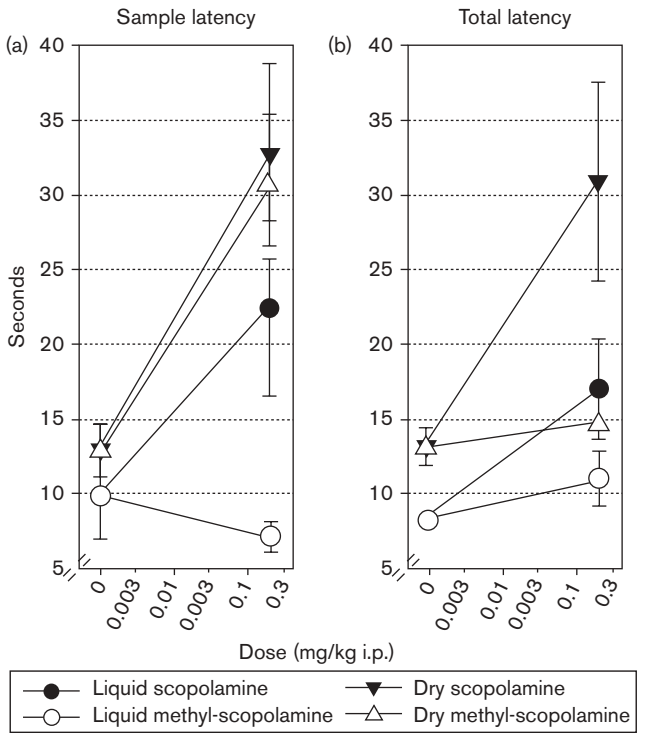
scopolamine, there was only a trend toward significance for reward type, which accounted for only a small proportion of variance [$F(1,53) = 3.72$, $P = 0.059$, $\omega^2 = 0.02$]. There was a significant main effect of scopolamine, which accounted for a large proportion of variance in total lever presses [$F(1,159) = 55.47$, $P < 0.01$, $\omega^2 = 0.46$]. With methyl-scopolamine, there was a significant main effect for reward type, which accounted for a large proportion of variance [$F(1,53) = 49.49$, $P < 0.01$, $\omega^2 = 0.79$], and a significant main effect of methyl-scopolamine, which accounted for a moderate proportion of variance in total lever presses [$F(1,159) = 10.39$, $P < 0.01$, $\omega^2 = 0.10$]. There was also a significant methyl-scopolamine by reward-type interaction, which accounted for a small proportion of variance [$F(1,159) = 5.47$, $P < 0.01$, $\omega^2 = 0.05$]. Although an increase in lever pressing was seen with liquid reward

Fig. 7



Central versus peripheral scopolamine effects – the delayed non-matching to position task (Study 3). (a) The percentage of correct non-match responses for liquid reward showed a significant scopolamine treatment effect ($P<0.05$). Planned comparisons with saline control showed that 0.1 mg/kg scopolamine treatment reduced the percentage of correct choices [$F(1,21)=4.05$, $P=0.06$], as did 0.2 mg/kg scopolamine group [$F(1,21)=9.01$, $P<0.01$], whereas the two methyl-scopolamine doses did not differ from control. The effect was delay-independent. (b) Total completed trials for liquid reward: planned comparisons with saline control again showed that both doses of scopolamine reduced the number of completed trials [$F(1,21)=17.95$, $P<0.01$, and $F(1,21)=13.77$, $P<0.01$, for 0.1 and 0.2 mg/kg, respectively]. The two methyl-scopolamine doses did not differ from control. (c) Total rewards gained for liquid reward: planned comparisons with saline control showed that both doses of scopolamine reduced the number of rewards gained [$F(1,21)=17.54$, $P<0.01$ and $F(1,21)=13.72$, $P<0.01$, respectively]. The two methyl-scopolamine doses did not differ from control. (d) The percentage of correct non-match responses for dry food reward: planned comparisons with saline control showed that only 0.2 mg/kg of scopolamine treatment reduced the percentage of correct choices [$F(1,24)=9.60$, $P<0.01$]; none of the other treatments had significant effects. (e) Total completed trials for dry food reward: planned comparisons with saline control also showed that 0.2 mg/kg scopolamine and 0.2 mg/kg methyl-scopolamine both reduced the number of completed trials [$F(1,24)=17.18$, $P<0.01$ and $F(1,24)=25.84$, $P<0.01$, for scopolamine and methyl scopolamine, respectively]. None of the other treatments had significant effects. (f) Total rewards gained for dry food reward: planned comparisons with saline control showed that 0.2 mg/kg scopolamine and 0.2 mg/kg methyl-scopolamine both reduced total rewards gained [$F(1,24)=19.34$, $P<0.01$ and $F(1,24)=28.93$, $P<0.01$, for scopolamine and methyl-scopolamine, respectively]. None of the other treatments had significant effects. Values are expressed as mean ± SEM; *$P<0.05$ versus control. Me, methyl; Scop, scopolamine.

Fig. 8



Central versus peripheral scopolamine effects – the delayed non-matching to position task latencies (Study 3). (a) Sample latency: in the liquid reward group, planned comparisons with saline control showed that the 0.2 mg/kg scopolamine treatment increased sample latencies [$F(1,21) = 5.05$, $P = 0.04$], but 0.2 mg/kg methyl-scopolamine did not. In the dry chow reward group, planned comparisons with saline control indicated that both scopolamine and methyl-scopolamine increased sample latencies [$F(1,24) = 10.25$, $P < 0.01$ and $F(1,24) = 13.80$, $P < 0.01$, respectively]. Methyl-scopolamine affected latency under dry but not liquid reward conditions. (b) Total latency: in the liquid reward group, planned comparisons with saline control showed that 0.2 mg/kg scopolamine treatment increased total latencies [$F(1,21) = 6.93$, $P = 0.02$], but 0.2 mg/kg methyl-scopolamine did not. In the dry chow reward group, planned comparisons with saline control showed that 0.2 mg/kg scopolamine treatment increased total latencies [$F(1,24) = 6.49$, $P = 0.02$], but 0.2 mg/kg methyl-scopolamine did not. Methyl-scopolamine had no effect in either reward condition. For clarity, significant differences from control are not shown. Values are expressed as mean ± SEM. i.p., intraperitoneal.

versus dry food reward, scopolamine caused disruption with liquid reward, but methyl-scopolamine did not.

To further examine the peripheral effects of scopolamine, rats were tested on DNMTP using liquid reward and dry food reward and two types of scopolamine. The percentage of correct choices for liquid reward was affected by treatment, which accounted for a moderate proportion of the variance [$F(4,84) = 4.82$, $P < 0.01$, $\omega^2 = 0.07$; Fig. 7a]. There was a significant main effect for retention delays, which accounted for a large proportion of variance in the percentage of correct choices [$F(4,84) = 39.02$, $P < 0.01$, $\omega^2 = 0.54$]. The number of completed trials and the number of rewards gained were analyzed using a one-way ANOVA. There was a significant main effect of treatment on number of completed trials, which accounted for a large proportion of variance [$F(4,84) = 8.33$, $P < 0.01$, $\omega^2 = 0.25$; Fig. 7b], and similarly, a significant main effect of treatment on the number of rewards gained , which accounted for a large proportion of variance [$F(4,84) = 8.43$, $P < 0.01$, $\omega^2 = 0.25$; Fig. 7c].

The percentage of correct choices for dry food reward was significantly affected by treatment, which accounted for a large proportion of the variance in the percentage of correct responses [$F(4,96) = 8.33$, $P < 0.01$, $\omega^2 = 0.12$; Fig. 7d]. The main effect for retention delays was statistically significant and also accounted for a large proportion of variance in the percentage of correct choices [$F(4,96) = 33.87$, $P < 0.01$, $\omega^2 = 0.47$]. There was also a significant effect of treatment on number of completed trials, which accounted for a large proportion of the variance [$F(4,96) = 11.76$, $P < 0.01$, $\omega^2 = 0.30$; Fig. 7e]. The effect of treatment on number of rewards gained was also significant and accounted for a large proportion of the variance [$F(4,96) = 13.68$, $P < 0.01$, $\omega^2 = 0.33$; Fig. 7f].

In the liquid reward group, there was a significant main effect of scopolamine dose on sample latency (latency to respond to sample lever) (Fig. 8a), which accounted for a large proportion of variance in latency to respond to the sample lever [$F(1,42) = 5.92$, $P = 0.01$, $\omega^2 = 0.18$]. In the dry chow reward group, the main effect of scopolamine dose was also significant and accounted for a large proportion of variance in the latency to respond to the sample lever [$F(1,48) = 6.35$, $P < 0.01$, $\omega^2 = 0.17$].

In the liquid reward group, there was a significant effect of dose on total latency (from pressing the sample lever to responding to target levers) (Fig. 8b), which accounted for a large proportion of variance in total latency [$F(1,42) = 4.54$, $P = 0.02$, $\omega^2 = 0.14$]. In the dry chow reward group, there was also a significant effect of dose, which accounted for a large proportion of variance in total latency [$F(1,48) = 5.64$, $P = 0.01$, $\omega^2 = 0.15$].

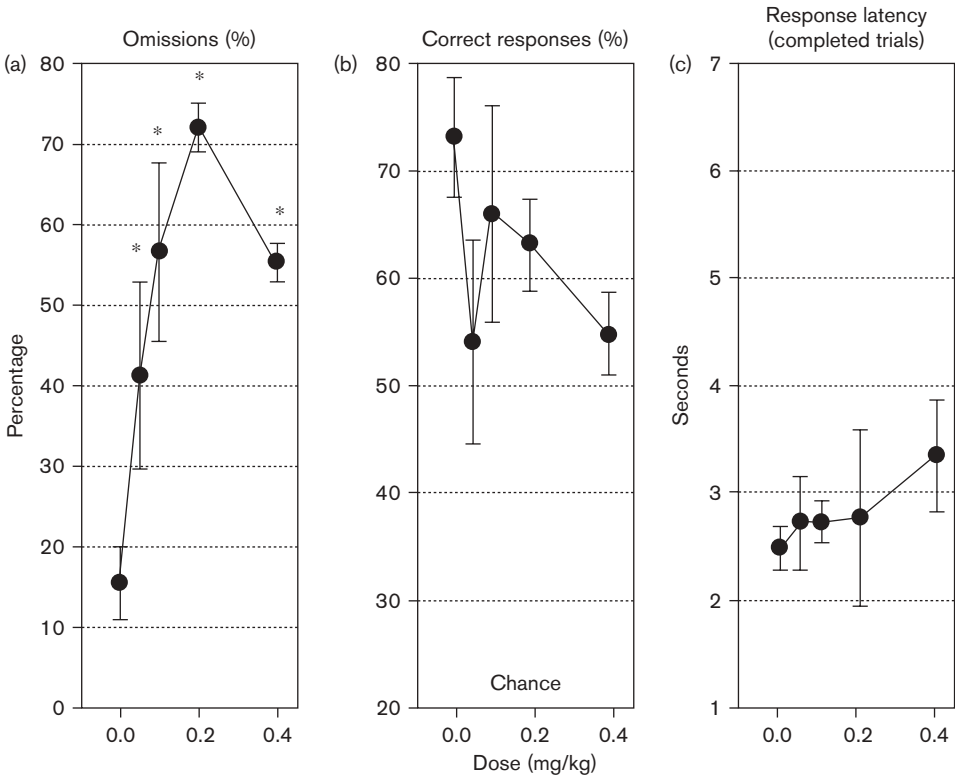
Study 4: 5-choice serial reaction time test

Rats from Study 3 were tested on a 5-CSRTT task to measure scopolamine effects on visuo-spatial attentional performance. All measures were analyzed by ANOVA with treatment as the main factor (Fig. 9). There was a significant effect of treatment on the percentage of omissions [$F(4,31) = 7.41$, $P < 0.01$; Fig. 9a]. There was no significant effect of scopolamine treatment on the percentage of correct responses [$F(4,31) = 1.22$, $P > 0.10$; Fig. 9b], or latencies to respond during completed trials [$F(4,30) = 2.42$, $P = 0.07$; Fig. 9c].

Discussion

In this study, we examined a well-documented cognitive effect of scopolamine, impairment of 'working memory'.

Fig. 9



Scopolamine effects on different aspects of the 5-choice serial reaction time test task (Study 4). (a) Percentage of omissions. Planned comparisons with saline control showed that 0.1, 0.2, 0.3, and 0.4 mg/kg scopolamine increased the percentage of omissions [$F(1,31)=5.6$, $P<0.05$; $F(1,31)=13.19$, $P<0.01$; $F(1,31)=26.74$, $P<0.01$; and $F(1,31)=12.36$, $P<0.01$, respectively]. Scopolamine increased the percentage of trials omitted. For trials in which the animals responded, there was no effect of scopolamine treatment on (b) the percentage of correct responses and (c) latencies to respond. Values are expressed as mean ± SEM; *$P<0.05$ versus control.

Given the ubiquitous nature of acetylcholine, interfering with the cholinergic system is likely, not only influencing mnemonic processes but also many other facets of behavior. To distinguish among the different effects of scopolamine, we examined how similar low drug doses impact performance on tasks that differ in their non-mnemonic demands. A single group of animals was given tasks that ranged from one thought to have little cognitive demand (i.e. FR5) to those based more on attention and spatial working memory (i.e. RAM, MWM). Scopolamine was shown to disrupt all working memory measures but not reference memory measures. In most tasks, there was also an effect of scopolamine on motor function measures (decreased bar pressing rate and radial maze speed). Control experiments, using different groups of animals, examined possible confounding factors, such as reward type and scopolamine's central versus peripheral effects. Testing the same animals on an array of tasks allowed for a better assessment of drug effects than using a single task. However, repeated testing could introduce confounds, such as carryover effects from one task to the next or the development of tolerance/sensitization.

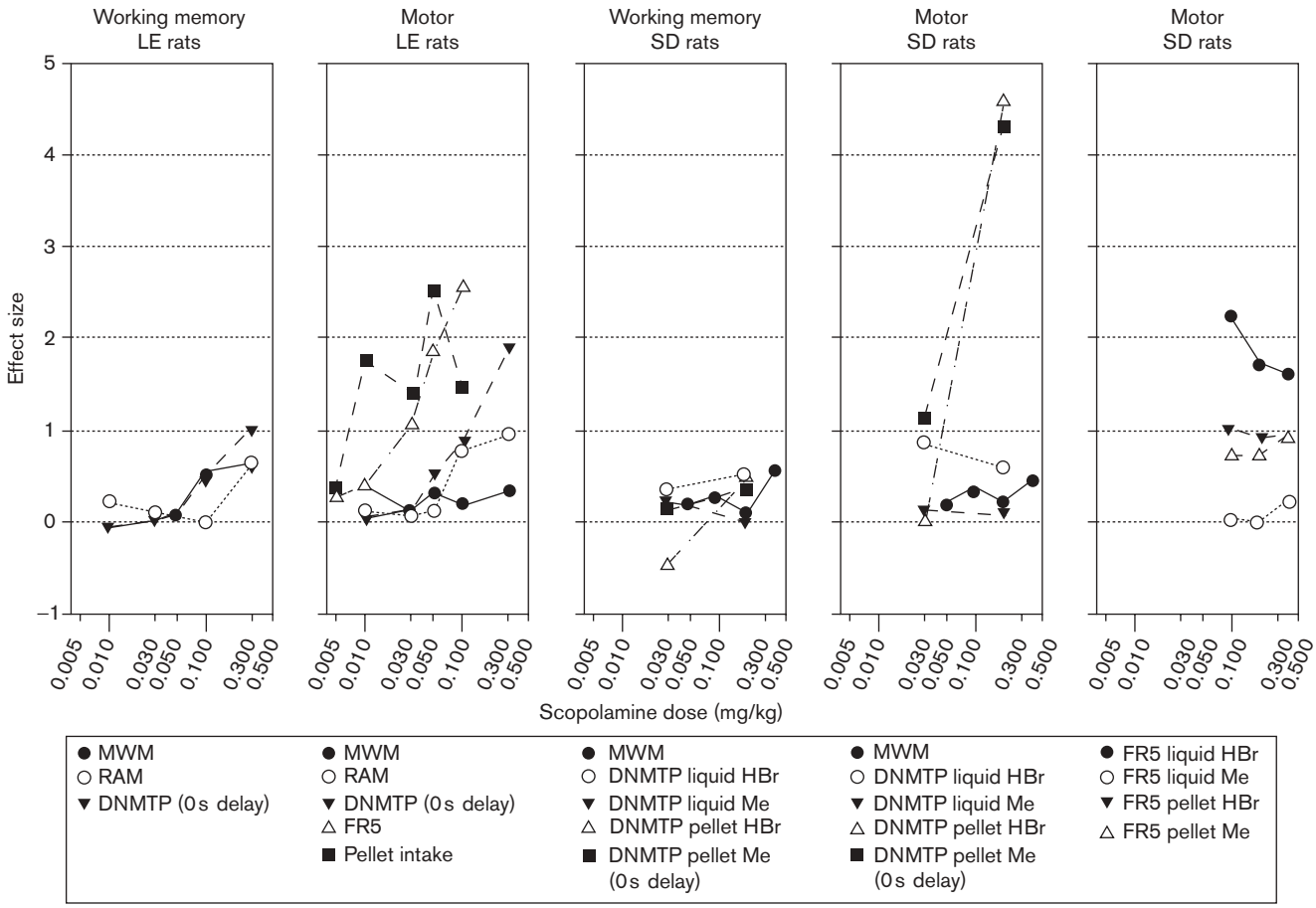
Effect of repeated testing

Rats were well trained before drug testing, and a Latin square design was used for each task to control for treatment and individual differences. Despite the pretraining, there was improved performance with repeated testing on some aspects of the tasks. Specifically, some MWM and DNMTP cognitive measures, as well as the FR5 total presses and MWM swim speed, showed improved performance with repeated testing. Importantly, besides a small interaction with FR5 bar presses ($\omega^2 < 0.06$) the improved performance did not interact with scopolamine treatment.

Tolerance and test order

Animals can, over multiple injections, develop tolerance or sensitivity to the test drug (Timmons and Hamilton, 1990b). Comparing the FR5 and DNMTP task results at the beginning and end of the test battery, the treatment by test period interactions were small ($\omega^2 < 0.06$), indicating no development of tolerance or sensitization. The use of very-low scopolamine doses and the fact that injections were spread out over a 6-month period were likely factors in avoiding drug

Fig. 10



Effect size comparisons across tasks. Effect size calculations used summary statistics (*n*, means, and standard deviations). The effect size is the standardized mean difference between a treatment group and a control group in terms of a unit-free outcome variable, '*d*' (Glass *et al.*, 1981; Cohen, 1988). Effect size estimates were adjusted taking into account sample size (Hedges and Olkin, 1985). The working memory (WM) measures in the Long–Evans Hooded (LE) rats showed the ability of scopolamine to disrupt WM. In the Sprague–Dawley (SD) rats, the WM measure for the Morris water maze (MWM) was similar to that seen in the LE rats. Furthermore, the delayed non-matching to position (DNMTP) WM measures with scopolamine and methyl-scopolamine (which does not cross blood–brain barrier) showed that the WM disruption is of central origin. In both LE and SD rats, the WM disruption was not as robust as that seen in the motor measures. MWM swim speed in both LE and SD was unaffected by scopolamine, but the other motor measures showed robust scopolamine effects. The effects of scopolamine and methyl-scopolamine showed that though there were peripheral scopolamine effects when dry reward pellets were used, there was a strong central effect on the motor measures. Methyl-scopolamine did not affect motor measures when a liquid reward was used but scopolamine did. Overall, the effect sizes show a stronger scopolamine effect on motor measures than on WM measures. HBr, scopolamine hydrobromide; Me, methyl-scopolamine, both dosed in free base equivalents.

tolerance and sensitivity issues (Park *et al.*, 2000; Ortega-Alvaro *et al.*, 2005). Another potential effect of repeated testing is that experience with one task may affect the performance in the subsequent task (Burghardt *et al.*, 2004). The behavioral battery design allowed for the examination of two different carryover effects. First, the sequence of FR5 and DNMTP was reversed, and second, half of the animals were given the MWM before the RAM, and this sequence was reversed for the second group. Besides a weak ($\omega^2 < 0.06$) effect on correct entries until first spatial working memory error in the RAM, there were no other order effects found, and no interactions of task order with scopolamine dose.

Central versus peripheral effects

Systemic injections of scopolamine can have both central and peripheral effects. The control studies showed that there was a central effect of scopolamine on psychomotor control and scopolamine disruption of working memory was not related to peripheral effects of scopolamine. The only peripheral effect shown in the control studies was scopolamine-induced 'dry mouth'. Scopolamine is known to induce drying of the mouth or 'dry mouth' in humans (Spinks *et al.*, 2004; Renner *et al.*, 2005). In the dry food consumption control study, 'dry mouth' could explain how a low dose of scopolamine (0.01 mg/kg) can disrupt a rat's ability to eat multiple food pellets. No peripheral scopolamine effect was shown in the FR5 and DNMTP

control studies when a liquid reward was used. Methyl-scopolamine, which does not readily cross the blood–brain barrier (Timmons and Hamilton, 1990a), decreased FR5 lever-pressing performance when using dry food reward but not liquid reward. Similarly, methyl-scopolamine affected the DNMTP task only when dry food reward was used. Nevertheless, scopolamine decreased FR5 and DNMTP performance even when using a liquid reward. These data show that a peripheral effect was manifested when using dry food rewards. In addition, there was also a central effect, because with a liquid reward, scopolamine caused similar disruptions as those seen with dry food pellets in the FR5 and DNMTP control studies.

In the behavioral battery, there was likely a scopolamine-induced 'dry mouth' effect and the central effect on motor function measures as well. The decrease in motor function could potentially be interpreted as peripheral effect: this cannot be ruled out, as methyl-scopolamine was not tested in the behavioral battery. Although a peripheral effect on motor ability in Study 1 cannot be refuted, this is unlikely, as the peripheral effects of scopolamine are related to its anticholinergic effect on parasympathetic postsynaptic receptors and hyperactivity would be expected as a peripheral effect. In these studies, hyperactivity was not seen at the low doses of scopolamine used. In Study 3, methyl-scopolamine was used in the SD rats. The two rat strains used in these studies may differ in ability to perform a particular task (Whishaw *et al.*, 2003; Rosen *et al.*, 2006) and/or susceptibility to a pharmacological manipulation (Gleason *et al.*, 1999; Rosen *et al.*, 2006). In these studies, both LE and SD rats showed similar scopolamine response profiles, with no differences in their abilities to perform the behavioral tasks. It is unlikely that the response to scopolamine in the two strains was by differing physiological mechanisms. Furthermore, if the effects on motor ability were purely peripheral, then motor disruption should be seen in the MWM, and in the DNMTP similar effects would be expected for both sample latency and total latency. This was not the case, as seen in Figs 4 and 8.

Motor function versus attention versus memory

A scopolamine-induced central disruption limited to the motor system is unlikely, because in some motor tasks there was no effect of scopolamine. The MWM swim speed and 5-CSRTT response latencies on correct trials were also unaffected by high scopolamine doses. In addition, at 0.05 and 0.10 mg/kg doses there was a delay in response to the sample lever in DNMTP, but there was no effect of scopolamine on total latency (pressing lever, turning to food hopper, and back to choose a bar). Presumably, the ability to produce this sequence of motor behaviors was unaffected. Taken together, it would seem that factors related to movement initiation rather than motor ability *per se*, played a role in the increased latencies that were observed.

Attention

In the 5-CSRTT control study, scopolamine increased omissions with a trend for increased response latency. However, in those trials in which the animals responded, there was no drug effect on correct choice. If scopolamine was primarily affecting attention, it should have reduced the percentage of correct choices as well as causing the animals to miss trials (omissions). Although these results do not argue against a scopolamine effect on attention, they suggest a disruption in the ability to initiate a motor response (psychomotor function). This was also seen in the DNMTP, where scopolamine dose-dependently increased the latency to respond to the sample lever. However, once started, the ability to perform a well-rehearsed sequence of movements (total latency) was unaffected by scopolamine. Although scopolamine increased omissions in the 5-CSRTT, it did not affect the rat's ability to finish trials once initiated (Higgs *et al.*, 2000). An increased number of omissions reflects possible failures in detection (Chudasama *et al.*, 2004) or disruption of sustained attention (Jones and Higgins, 1995; Nelson *et al.*, 2002). If a rat was unable to sustain its attention to task, it would not immediately detect and react to task stimuli. This would result in disrupted motor performance (e.g. DNMTP sample latency), and in certain timed trials increased trial omissions (e.g. 5-CSRTT). The FR5 disruption in bar pressing and the motor delay in the RAM could be related to the inability to sustain attention but could also be because of impaired psychomotor function. Disruption of movement initiation or psychomotor function could also explain the inability to react to task stimuli. These performance changes induced by scopolamine were likely independent of changes in basic sensory and motor ability, or in motivation (Jones *et al.*, 1995; Higgs *et al.*, 2000). Taken together, these data suggest that in addition to attention, scopolamine affects the psychomotor function.

Working memory

A problem in accessing working memory distinguishes a mnemonic deficit from a disruption in attention (Park *et al.*, 2000). Significant deficit in attention and/or psychomotor function could disrupt working memory measures. Working memory in the RAM and DNMTP was disrupted by scopolamine, but motor performance measures were affected at doses not disrupting the working memory. In the DNMTP, scopolamine caused a delay-independent decrease in choice accuracy. Furthermore, an increase in retention delay interval should increase working memory errors. Although the interaction between retention interval and drug was significant, the magnitude of the interaction was relatively minor.

These data suggest that in the DNMTP, the drug effect was related to the attentional aspects of the task (Dunnett, 1985). In the MWM learning set task, scopolamine caused disruption of working memory (second and third trials) but not the animals' search strategy (initial trial of the day) or motor performance. The scopolamine-induced deficits in working memory seen in the RAM, DNMTP, and MWM may be caused by disruption of the cortical cholinergic system modulating both attentional and mnemonic processing (Gu, 2002).

A comparison of tasks

Each task in the battery and the other four control studies depended on the integrity of many different systems only some of which were cognitive. Thus, factors such as perceptual ability, motivational state, and motor ability all played a role in these 'cognitive' tasks (Hodges, 1996). In models designed to detect a memory deficit it is unlikely that other subtle behavioral changes would be detected, especially in a single behavioral task. Assessing the relative involvement of scopolamine in mnemonic and nonmnemonic function requires a comparison of the effect of scopolamine in different types of tasks. In the five tasks of the battery, a direct comparison of minimal dose needed to induce a disruption showed scopolamine to be most potent in the FR5 and DNMTP (with large, $\omega^2 > 0.3$, effects already apparent at 0.05 mg/kg). This was followed by the MWM (with small, ω^2 approximately 0.03, effects from 0.1 mg/kg, in LE rats) and by the RAM (with small, ω^2 approximately 0.05, effects from 0.3 mg/kg).

To compare the effect size across all five studies (Fig. 10), a unit-free outcome variable was used (Glass *et al.*, 1981; Cohen, 1988). In both the LE and SD rats, the most robust and least robust responses to scopolamine were seen in the motor function domain. The FR5 total bar pressing and DNMTP trials completed had the most robust response to scopolamine, yet the MWM swim speed effect was very small. This difference was not explained by the use of dry food, as a robust response to scopolamine was seen in both FR5 and DNMTP when using a liquid reward. Thus, the motor disruption is not likely related to a disruption of physical ability, but to attention and/or psychomotor function. The working memory measures were relatively similar in size across tasks, but were relatively small when compared with the motor measures. This suggests that scopolamine perturbs attention and psychomotor function before affecting working memory. Disruption of working memory by scopolamine may be based on the interference with attentional control and cognitive processes involved with movement initiation (LaBar *et al.*, 1999; Simon *et al.*, 2002).

As a whole, the results of these studies show that low doses of scopolamine had limited peripheral effects (e.g. salivation), but had a wide spectrum of central effects, including disruption of attention and movement initiation, both of which impact and interact with the mnemonic function. These results show that a limited disruption of the central cholinergic system can have profound effects on attention and/or psychomotor control before any measurable mnemonic disruption.

Acknowledgements

This study was supported by the Bristol-Myers Squibb Co., Pharmaceutical Research Institute; UConn FRS445142; NIH #R29-A613941-01A1.

References

Bartus RT, Fleming D, Johnson HR (1978). Aging in the rhesus monkey: debilitating effects on short-term memory. *J Gerontol* **33**:858–871.

Beninger RJ, Wirsching BA, Jhamandas K, Boegman RJ, el-Defrawy SR (1986). Effects of altered cholinergic function on working and reference memory in the rat. *Can J Physiol Pharmacol* **64**:376–382.

Blokland A (1996). Acetylcholine: a neurotransmitter for learning and memory? *Brain Res Brain Res Rev* **21**:285–300.

Burghardt PR, Fulk LJ, Hand GA, Wilson MA (2004). The effects of chronic treadmill and wheel running on behavior in rats. *Brain Res* **1019**:84–96.

Chudasama Y, Dalley JW, Nathwani F, Bouger P, Robbins TW (2004). Cholinergic modulation of visual attention and working memory: dissociable effects of basal forebrain 192-IgG-saporin lesions and intraprefrontal infusions of scopolamine. *Learn Mem* **11**:78–86.

Cohen J (1988). *Statistical power analysis for behavioral sciences*. 2nd ed. Hillsdale, New Jersey: Lawrence Erlbaum Associates.

Dodd DH, Schultz RF Jr (1973). Computational procedures for estimating magnitude of effect for some analysis of variance design. *Psychol Bull* **79**:391–395.

Dunnett SB (1985). Comparative effects of cholinergic drugs and lesions of nucleus basalis or fimbria-fornix on delayed matching in rats. *Psychopharmacology (Berl)* **87**:357–363.

Ebert U, Kirch W (1998). Scopolamine model of dementia: electroencephalogram findings and cognitive performance. *Eur J Clin Invest* **28**:944–949.

Gallagher M, Burwell R, Burchinal M (1993). Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci* **107**:618–626.

Glass GV, McGaw B, Smith ML (1981). *Meta-analysis in social research*. Newbury Park: Sage Publications.

Gleason TC, Dreiling JL, Crawley JN (1999). Rat strain differences in response to galanin on the Morris water task. *Neuropeptides* **33**:265–270.

Gu Q (2002). Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity. *Neuroscience* **111**:815–835.

Hedges LV, Olkin I (1985). Estimation of a single effect size: parametric and nonparametric methods. In: *Statistical methods for meta-analysis*. New York: Academic Press. pp. 76–104.

Higgs S, Deacon RM, Rawlins JN (2000). Effects of scopolamine on a novel choice serial reaction time task. *Eur J Neurosci* **12**:1781–1788.

Hodges H (1996). Maze procedures: the radial-arm and water maze compared. *Brain Res Cogn Brain Res* **3**:167–181.

Jones DN, Higgins GA (1995). Effect of scopolamine on visual attention in rats. *Psychopharmacology (Berl)* **120**:142–149.

Jones DN, Barnes JC, Kirkby DL, Higgins GA (1995). Age-associated impairments in a test of attention: evidence for involvement of cholinergic systems. *J Neurosci* **15**:7282–7292.

LaBar KS, Gitelman DR, Parrish TB, Mesulam M (1999). Neuroanatomic overlap of working memory and spatial attention networks: a functional MRI comparison within subjects. *Neuroimage* **10**:695–704.

Lyeth BG, Ray M, Hamm RJ, Schnabel J, Saady JJ, Poklis A, *et al.* (1992). Postinjury scopolamine administration in experimental traumatic brain injury. *Brain Res* **569**:281–286.

Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982). Place navigation impaired in rats with hippocampal lesions. *Nature* **297**:681–683.

Murray TK, Cross AJ, Green AR (1991). Reversal by tetrahydroaminoacridine of scopolamine-induced memory and performance deficits in rats. *Psychopharmacology (Berl)* **105**:134–136.

National Research Council (2003). *Guide for the care and use of laboratory animals*. Washington, DC: National Academy Press.

Nelson CL, Burk JA, Bruno JP, Sarter M (2002). Effects of acute and repeated systemic administration of ketamine on prefrontal acetylcholine release

and sustained attention performance in rats. *Psychopharmacology (Berl)* **161**:168–179.

Oler JA, Markus EJ (1998). Age-related deficits on the radial maze and in fear conditioning: hippocampal processing and consolidation. *Hippocampus* **8**:402–415.

Olton DS, Samuelson RJ (1976). Remembrance of places passed: spatial memory in rats. *J Exp Psychol Anim Behav Process* **2**:97–116.

Ortega-Alvaro A, Gibert-Rahola J, Mico JA (2005). Influence of chronic treatment with olanzapine, clozapine and scopolamine on performance of a learned 8-arm radial maze task in rats. *Prog Neuropsychopharmacol Biol Psychiatry* **30**:104–111.

Park S, Knopick C, McGurk S, Meltzer HY (2000). Nicotine impairs spatial working memory while leaving spatial attention intact. *Neuropsychopharmacology* **22**:200–209.

Pontecorvo MJ, Clissold DB, White MF, Ferkany JW (1991). N-methyl-D-aspartate antagonists and working memory performance: comparison with the effects of scopolamine, propranolol, diazepam, and phenylisopropyladenosine. *Behav Neurosci* **105**:521–535.

Renner UD, Oertel R, Kirch W (2005). Pharmacokinetics and pharmacodynamics in clinical use of scopolamine. *Ther Drug Monit* **27**:655–665.

Rosen JB, West EA, Donley MP (2006). Not all rat strains are equal: differential unconditioned fear responses to the synthetic fox odor 2,4,5-trimethylthiazoline in three outbred rat strains. *Behav Neurosci* **120**:290–297.

Ruotsalainen S, MacDonald E, Miettinen R, Puumala T, Riekkinen P Sr, Sirvio J (1997). Additive deficits in the choice accuracy of rats in the delayed non-matching to position task after cholinolytics and serotonergic lesions are non-mnemonic in nature. *Psychopharmacology (Berl)* **130**:303–312.

Rupniak NM, Samson NA, Tye SJ, Field MJ, Iversen SD (1991). Evidence against a specific effect of cholinergic drugs on spatial memory in primates. *Behav Brain Res* **43**:1–6.

SAS Institute Inc. (1999). *SAS/STAT User's Guide, Version 8*. Cary, North Carolina: SAS Institute Inc.

Simon SR, Meunier M, Piettre L, Berardi AM, Segebarth CM, Boussaoud D (2002). Spatial attention and memory versus motor preparation: premotor cortex involvement as revealed by fMRI. *J Neurophysiol* **88**:2047–2057.

Spinks AB, Wasiak J, Villanueva EV, Bernath V (2004). Scopolamine for preventing and treating motion sickness. *Cochrane Database Syst Rev*: CD002851.

Timmons CR, Hamilton LW (1990a). Psychopharmacological concepts. In: *Drugs, brains and behavior*. New Brunswick, New Jersy: Rutgers University.

Timmons CR, Hamilton LW (1990b). Tolerance, drug abuse and habitual behaviors. In: *Drugs, brains and behavior*. New Brunswick, New Jersey: Rutgers University.

Ward MT, Stoelzel CR, Markus EJ (1999). Hippocampal dysfunction during aging II: deficits on the radial-arm maze. *Neurobiol Aging* **20**:373–380.

Whishaw IQ, Gorny B, Foroud A, Kleim JA (2003). Long–Evans and Sprague–Dawley rats have similar skilled reaching success and limb representations in motor cortex but different movements: some cautionary insights into the selection of rat strains for neurobiological motor research. *Behav Brain Res* **145**:221–232.