Improving unbiased left/right training of rats and use of physostigmine to counteract scopolamine-induced short-term memory impairment.

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Summary
Rationale: The DNMTP task measures short-term / working memory uncontaminated by learning capacity, spatial abilities, motor performance or general motivational and arousal factors. However, DNMTP training of rats can take two months, and we aimed to reduce this. *Methods:* Two experiments were conducted on rats in an operant DNMTP task. Improvements were made on the training procedure. The method was validated by replicating the effect of scopolamine on working memory. The experiments also explored the influence of physostigmine in reversing impairment induced by scopolamine. Thus in experiment 1, ten Lewis rats were trained in an operant DNMTP task (1, 2, 4 and 8 s delay intervals) before 9 of them received vehicle, scopolamine, saline or combinations of scopolamine and physostigmine. In experiment 2, ten Lewis rats (5 old and 5 young) were trained in the same task (1, 2, 4, 8 and 16 s delay intervals). There were six treatments: 0.05 mg/kg scopolamine, 0.1 mg/kg physostigmine or 0.15 mg/kg physostigmine, control involving saline or involving no injection and no handling, and finally a combined treatment of 0.05mg/kg scopolamine and 0.15 mg/kg physostigmine. In both experiments scopolamine significantly reduced correct responses, nose-pokes and lever presses compaired to control conditions. Furthermore, in experiment 2, there was insignificant difference between saline and combined scopolamine/physostigmine for correct responses and for delay prior to pressing the sample lever. As expected, there was significant difference between scopolamine and combined scopolamine/physostigmine for correct responses, for delay prior to pressing the sample lever and for delay prior to pressing the non-matching lever. As a result, the animals were ready for drug injection after 17 days from habituation and the method ensured that there were no drop-outs due to left or right lever preference. This is a shorter training period than previously thought necessary. The brief training method was validated by replicating the effect of scopolamine on working memory.

Introduction
The importance of the neurotransmitter acetylcholine (AChE) in various aspects of memory is demonstrated in the psychopathology of Alzheimer's disease (AD). The disruption of working or short term memory by scopolamine through its anticholinergic effects has been considered to model the working memory deficit in AD patients and used as such in research. This applies both at preclinical (*Zhang & O'Donnell, 2000*) and clinical (*Robbins et al., 1997*) levels of research. Performance of healthy volunteers on various cognitive memory tasks has been significantly reduced by scopolamine when concurrent secondary tasks have been run, suggesting selective impairment by scopolamine of the central executive mechanism (*Rusted, 1988*) or attention (*Mirza & Stolerman, 2000*). Scopolamine has also produced a dose-dependent disruption of performance in rats that is similar to lesions to central cholinergic systems (*Chudasama & Muir, 1997; Dunnett, 1985*).

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Enhancing the cholinergic function has been a therapeutic strategy for treating the cognitive decline associated with AD (*Iversen, 1998*). The acetylcholinesterase inhibitor, rivastigmine (Exelon) appears to attenuate the working memory impairment with humans (*Wesnes et al., 2002*) as well as impairment induced by scopolamine in an operant delayed non-matching to position (DNMTP) task in rats (*Ballard & McAllister 1999*). The DNMTP task measures short-term / working memory uncontaminated by learning capacity, spatial abilities, motor performance or general motivational and arousal factors. Reduction in motor coordination or motivation is indicated by a reduction in the number of trials completed and effect on cognitive processing is indicated by a reduction in the number of correct choices. The DNMTP task has been widely used as a model to investigate the cognitive effects of cholinergic manipulation and the method is sensitive to working memory deficits induced by scopolamine (*Aggleton et al., 1991; Ballard & McAllister, 2000; Dunnett, 1985; McAllister, 2001*). Yet the present training procedure is time-consuming. It can take more than eight weeks to train rats on DNMTP tasks following habituation and the drop-out rate can be high during training, e.g. due to left or right lever preference.

The National Institutes of Health stated in 1997 that when administered to people the short-acting antiglaucoma agent, physostigmine, aided and improved performance of working memory by enhancing levels of AChE between neurons in the brain. The drug may even have a modulatory influence on central emotional processes such as anxiety apparent with AD patients (*Sienkiewicz-Jarosz, et al. 2000*). In a recent review, however, Coelho Filho & Birks (2002) conclude that the evidence of beneficial effectiveness of physostigmine for the symptomatic treatment of AD is limited (in part by its short half-life), and adverse effects remain common leading to a high rate of withdrawal. Behavioral studies show conflicting results. Physostigmine alone has induced mild but significant enhancement of memory performance in rats in some studies (*Dunnett, 1985*), but not in others (*Murray et al., 1991*). Studies appear to agree that physostigmine can reverse memory deficits induced by scopolamine in DMTP and DNMTP tasks (*Dawson et al. 1991; Murray et al. 1991*).

Two successive experiments were conducted on rats in an operant DNMTP task. Improvements were made on the DNMTP training procedure, the animals showed no left or right lever preference and were ready for drug injection after a shorter training period than previously thought necessary. The method was validated by replicating the effect of scopolamine on working memory. The experiments also explored the influence of physostigmine on working memory by attempting to reverse impairment induced by scopolamine.

Materials and Methods

Subjects

Fifteen untrained male Lewis rats were used, aged approximately 12 weeks and weighing 230–280 g at the start of testing. Each animal was housed alone in a cage, under a 12-h light/dark cycle, and with free access to water throughout. Food intake was restricted to maintain about 85% of normal body weight.

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985).

Apparatus

A single rat modular test chamber was used (MED-Associates Inc., Lafayette IN, USA) located in a sound attenuating cubicle. The operant chamber was attached to an Intel Celeron 800 MHz computer via a PCI Interface Connection Package. Software in-volved a MED-PC Version IV with Delphi 4 Compiler. The test chamber was equipped with two retractable levers, positioned on each side of a central food magazine. A head-entry detector registered nose-pokes. A house light was positioned on

the opposite panel in the center by the ceiling, two stimulus lights were above each lever, and another light illuminated the food tray. Enticement was provided by 45 mg food pellets (Bio-serv), which were dispensed one at the time into the food magazine.

Drugs

Scopolamine hydrobromide, SCOP, and physostigmine salicylate, PHYS, were dissolved in 0.9% saline and administered in a dosage volume of 1.0 ml/kg.

Procedures for experiment 1

Habituation to chamber

House light was illuminated throughout all sessions. Step one. (1 day) Five grams of 45 mg food pellets were placed in the food magazine at the start of the session which lasted 30 minutes per rat. Step two. (2 days) The session started with both levers exposed. A press on either lever resulted in withdrawal of both and a food pellet was dispensed into the food magazine. Both levers were exposed automatically 1 s after retraction. A single session was scheduled per animal each day (45 min. and 50 min. respectively). Step three. (5 days) Each rat got a 50 min. session per day which consisted of single lever being released into the chamber. It was retracted on pressing and a food pellet was dispensed into the magazine. A head entry into the food magazine resulted in exposure of either the left or the right lever (random) and the next trial started.

Non-matching to position (NMTP) training

Step four. (2 days) House light was illuminated during a 50 min. session which started with a 5 s rest period, then a single lever released into the chamber. It was retracted on pressing it and the light inside the food magazine was illuminated. A head entry into the food magazine turned off the light and released the opposite lever (non-matching to position). A press on the opposite lever resulted in its retraction and a food pellet was dispensed into the magazine. The next trial started with a 5 s rest period followed by either lever (random) being released into the chamber. Step five. (4 days) A trial started with a 5 s rest period followed by a single lever being released. It was retracted upon pressing it and the light inside the food magazine was illuminated. A head entry into the food magazine turned off the light and released both levers. Correct response consisted of a press on the non-matching to position lever which resulted in its retraction and a food pellet into the magazine. The next trial began with a 5 s rest followed by either lever being released. Incorrect response consisted of a press on the same (sample) lever which resulted in its retraction and a "time-out" period for 10 s with the house light off. The house light turned on after time-out, and the next trial could begin. In six days the rats reached criteria of >95% correct responses and no bias for a lever.

Delayed non-matching to position (DNMTP) training

Step six. (2 days) Same as step four, except for the introduction of a delay. The delay began when the sample lever was pressed and ended with the first nose-poke after the scheduled delay, of 1, 2, 4 or 8 s. All four delay options occurred randomly within four trials. The nose-poke following the delay turned off the light and released the opposite lever (non-matching to position). A press on the opposite lever resulted in its retraction and a food pellet into the magazine. Then the next trial started with a 5 s rest period followed by either lever (random) being released into the chamber. Step seven. (10 days) A trial started with a 5 s rest period followed by release of the sample lever. It was retracted on pressing it and the light inside the food magazine was illuminated. The first nose-poke following the scheduled delay turned off the magazine light and released both levers. Correct and incorrect responses were defined as in step five. The DNMTP training took 12 days for rats to reach criteria of >90% correct responses for all delays and no pre-ference for left or right lever.

Treatment protocol

There were three treatments: (i) 0.05 mg/kg SCOP administered 20 min. prior to testing, (ii) control (saline) administered 20 min prior to testing, and (iii) a combined treatment of 0.05 mg/kg SCOP administered 20 min prior to testing followed 5 min later by injection of 0.1 mg/kg PHYS.

Experimental schedule

Ten subjects started and completed the training, one of which failed to demonstrate 95% correct responses for all delay conditions. The remaining nine rats were divided into three equally performing groups of three rats each, based on correct responses in the last training session. Difference between correct responses of the three groups was insignificant according to Kruskal-Wallis Chi Square at 1, 2, 4 and 8 s delay. Experimental procedure followed the schedule shown in Table 1 and continued for 15 days.

Each rat was tested at the same time throughout the experiment. Following drug and saline administration, rats were placed back into their houses. Three training days were included as a second control condition. During training, rats were taken out of their houses 20 min. prior to the experiment and handled but not injected with anything.

Procedures for experiment 2

Habituation to chamber

Five untrained rats went through the three habituation steps and house light was illuminated throughout all sessions. Step one. (first half of day one) Three grams of 45 mg food pellets were placed in the food magazine at the start of the session which lasted 30 minutes per rat. Step two. (second half of day one) The session started with both levers exposed. A press on either lever resulted in withdrawal of both and a food pellet was dispensed into the food magazine. Both levers were exposed automatically 1 s after retraction. A single 30 min. session was scheduled per animal. Step three. (3 days) Each rat got a 60 min. session per day which consisted of a single lever being released into the chamber. It was retracted on pressing it and a food pellet was dispensed into the magazine. A head entry into the food magazine resulted in exposure of either the left or the right lever (random) and the next trial started.

Non-matching to position (NMTP) training

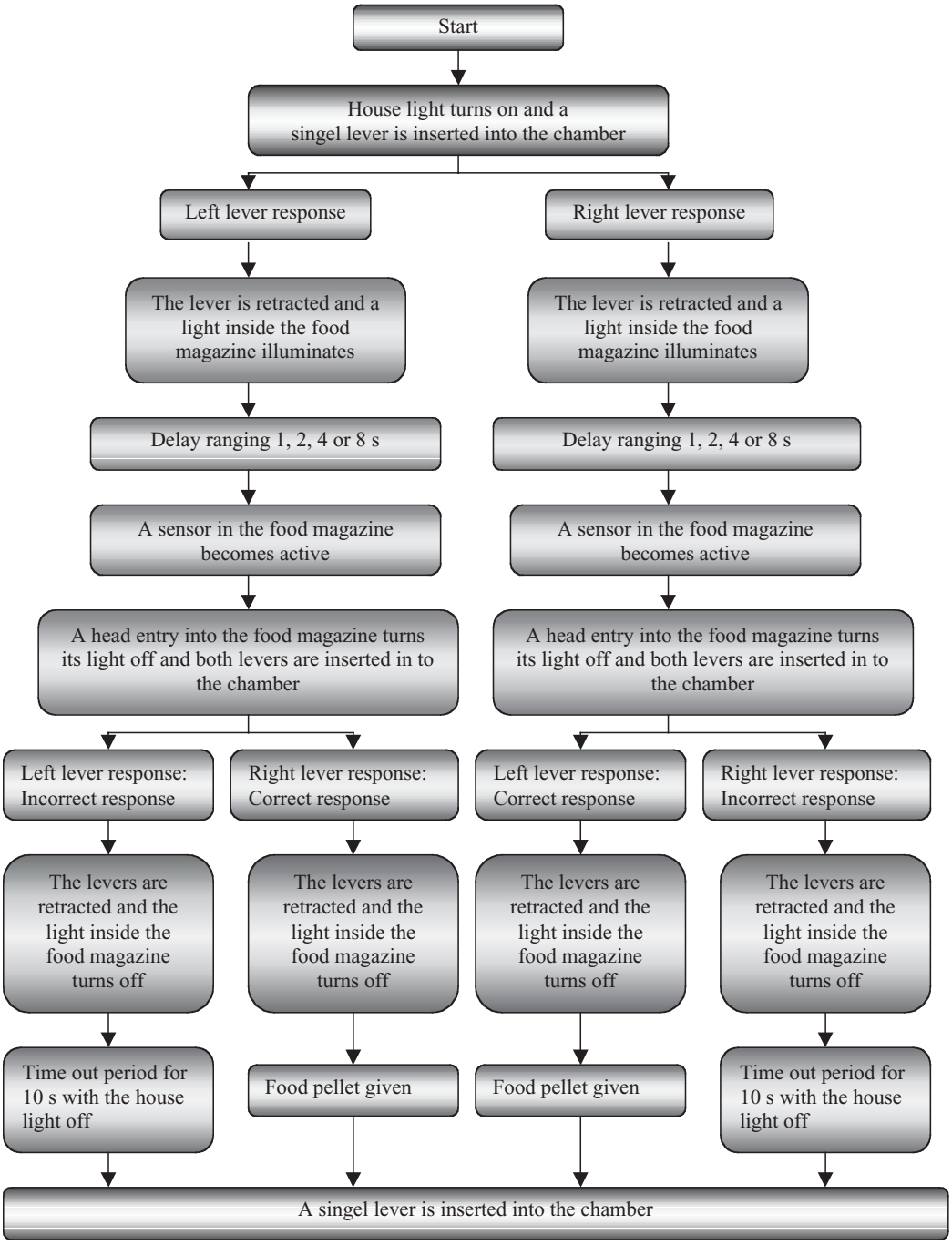
Five previously trained rats were randomly selected (by the throw of a dice) from experiment 1 and added at this stage to the group of five untrained rats that had completed steps 1 to 3. Step four. (2 days) House light was illuminated during a 50 min. session that started with a 5 s rest period followed by a single lever being released into the chamber. It was retracted on pressing it and the light inside the food magazine was illuminated. A head entry into the food magazine turned off the light and released the opposite lever (non-matching to position). A press on the opposite lever resulted in its retraction and a food pellet was dispensed into the magazine. The next trial started with a 5 s rest period followed by either lever (random) being released into the chamber.

Delayed non-matching to position (DNMTP) training

Step five. (1 days) Same as step four, except for the introduction of a delay. The delay began when the sample lever was pressed and ended with the first nose-poke after the scheduled delay, of 1, 2, 4 or 8 s. All four delay options occurred randomly within four trials. The nose-poke following the delay turned off the light and released the opposite lever

Table 1. The first seven days of the 15 day experimental schedule.

Condition	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
SCOP	Group A	Group C	Group B	Group A	Training	Group C	Group B
Saline	Group B	Group A	Group C	Group B	Training	Group A	Group C
SCOP+PHYS	Group C	Group B	Group A	Group C	Training	Group B	Group A


Start
House light turns on and a singel lever is inserted into the chamber
Left lever response
Right lever response
The lever is retracted and a light inside the food magazine illuminates
The lever is retracted and a light inside the food magazine illuminates
Delay ranging 1, 2, 4 or 8 s
Delay ranging 1, 2, 4 or 8 s
A sensor in the food magazine becomes active
A sensor in the food magazine becomes active
A head entry into the food magazine turns its light off and both levers are inserted in to the chamber
A head entry into the food magazine turns its light off and both levers are inserted in to the chamber
Left lever response: Incorrect response
Right lever response: Correct response
Left lever response: Correct response
Right lever response: Incorrect response
The levers are retracted and the light inside the food magazine turns off
The levers are retracted and the light inside the food magazine turns off
The levers are retracted and the light inside the food magazine turns off
The levers are retracted and the light inside the food magazine turns off
Time out period for 10 s with the house light off
Food pellet given
Food pellet given
Time out period for 10 s with the house light off
A singel lever is inserted into the chamber

(non-matching to position). A press on the opposite lever resulted in its retraction and a food pellet into the magazine. Then the next trial started with a 5 s. rest period followed by either lever (random) being released into the chamber. Step six. (3 days) Procedure same as in step five. The scheduled delay was expanded to 1, 2, 4, 8 or 16 s. All five delay options occurred within five trials, not randomly but starting with 1 s, then 2 s and so on. Furthermore, failing to respond to a lever was now defined as a missed trial or omission. If the rat did not press the sample lever within 30 s, the house light turned off for 10 s and a new trial started. Step seven (7 days) A trial started with a 5 s rest period followed by the release of the sample lever. It was retracted on pressing and the light inside the food magazine was illuminated. The fist nose-poke following the scheduled delay turned off the magazine light and released both levers.

Problems occurred with the 12-h light/dark cycle for a couple of nights during housing early in step seven resulting in a temporary drop in correct responses and possibly prolonging the training period.

Treatment protocol

There were six treatments: (i) 0.05 mg/kg SCOP administered 20 min. prior to testing, (ii) 0.1 mg/kg PHYS or (iii) 0.15 mg/kg PHYS administered 15 min. prior to testing, (iv) control involving saline administered 20 min prior to testing or (v) control involving no injection and no handling, and (vi) a combined treatment of 0.05mg/kg SCOP administered 20 min prior to testing followed 5 min later by injection of 0.15 mg/kg PHYS.

Experimental schedule

Five untrained and five previously trained subjects started and completed the training, one of which failed to demonstrate >90% correct at 1 s and 2 s delay, and >50% correct responses at 16 s delay. No left or right-hand side preference was observed. The remaining nine rats were divided into three equally performing groups of three rats each, based on correct responses in the last four DNMTP training sessions. Each group had a combination of "old" and "young" rats. Difference between correct responses of the three groups was insignificant according to Kruskal-Wallis Chi Square at 1, 2, 4, 8 and 16 s delay. Experimental procedure followed the schedule shown in Table 4 and continued for 16 days.

Each rat was tested at the same time throughout the experiment. The control involved four "training" days. Prior to the first training day the rats were divided into two control groups, 1 and 2 respectively, each balanced for old and new rats and performance in the last four DNMTP training sessions. Control 1 got saline injection and control 2 got no injection during the first training day. During the second training day, control 1 got no injection and control 2 got saline injection, and so on.

Statistical analysis

Nonparametric tests were selected for analysis of data from both experiments. Nonparametric tests make no assumption about the shape of the population distribution, nor do they assume homogeneity of variance, i.e. that two or more samples studied have equal amount of spread. This is especially relevant in a study with few cases, where scores do not necessarily follow a normal curve and measures

Table 2. The first eight days of the 16 day experimental schedule.

Condition	Day 1		Day 2	Day 3	Day 4	Day 5	Day 6		Day 7	Day 8
SCOP+PHYS	Control 1 (sal)	Control 2	Group A	Group C	Group B	Group A	Control 1	Control 2 (sal)	Group C	Group B
PHYS			Group B (0,15)	Group A (0,15)	Group C (0.15)	Group B (0,10)			Group A (0,10)	Group C (0,10)
SCOP			Group C	Group B	Group A	Group C			Group B	Group A

(lever pressing and nose-pokes) reflect an ordinal scale variable. Furthermore, each animal is compared to itself under different conditions (although means are reported for sake of clarity).
In the preliminary experiment tests were two tailed (2-t). In the confirmatory follow-up study one tailed (1-t) tests were used for the main relationships.

Results

Experiment 1

Differences between treatment conditions
Significant difference was obtained between four conditions (SCOP, SCOP+PHYS, Saline, Training) for correct responses: Friedman Chi-Square=10.20 (n=9, df=3, p<.05, 2-t). There was also significant difference between conditions for number of nose-pokes and lever presses, see Table 3.
As expected, there was insignificant difference between saline and training for correct responses, for nose-pokes, for delay prior to pressing sample lever and for delay prior to pressing non-matching lever.
As expected, significant difference was observed between SCOP and Saline conditions for correct responses Wilcoxon Signed Ranks Test z=-2.67 (p=.008, 2-t), for nose-pokes Wilcoxon z=-2.67 (p=.008, 2-t), for delay prior to pressing sample lever Wilcoxon z=-2.67 (p=.008, 2-t), and for delay prior to pressing non-matching lever Wilcoxon z=-2.67 (p=.008, 2-t).
Counter to expectation, significant difference was observed between SCOP+PHYS and Saline conditions for correct responses Wilcoxon Signed Ranks Test z=-2.19 (p=.03, 2-t), for nose-pokes Wilcoxon z=-2.67 (p=.008, 2-t), for delay prior to pressing sample lever Wilcoxon z=-2.67 (p=.008, 2-t), and for delay prior to pressing non-matching lever Wilcoxon z=-2.43 (p=.02, 2-t).
Significant difference was not found between SCOP and SCOP+PHYS for correct responses, for nose-pokes, for delay prior to pressing sample lever and for delay prior to pressing non-matching lever.

Differences in delays
There was significant difference between conditions (1, 2, 4 and 8 s) for correct responses and the performance declined as the delay got longer, see

Table 3. Main results of the three treatment conditions and training day for: correct responses in %, number of nose-pokes, delay prior to sample lever in seconds and delay prior to non-matching to position lever in seconds.

	SCOP	SCOP+PHYS	Saline	Training	Difference
Correct responses %					Friedman Chi-Square =10.20 p<.05
Mean	95.12	95.98	97.65	97.15	
Sd	4.95	3.05	1.64	2.41	
Nose-pokes					Friedman Chi-Square =19.80, p<.001
Mean	1311.75	1316.14	1748.69	1759.86	
Sd	326.52	374.86	437.19	451.53	
Delay sample lever					Friedman Chi-Square =19.40, p<.001
Mean	10.57	4.60	2.75	2.70	
Sd	36.52	4.07	1.87	1.87	
Delay non-matching					Friedman Chi-Square =19.93, p<.001
Mean	1.33	1.51	0.79	0.75	
Sd	1.31	2.59	0.22	0.13	

Table 4. Greatest deterioration occurred for the SCOP treatment condition: Friedman Chi-Square=16.87 (n=9, df=3, p<.01, 2-t). The difference between correct responses for SCOP and Saline at 8 s delay was significant, Wilcoxon Signed Ranks Test z=-2.55 (p=.01, 2-t). The difference between correct responses for SCOP and SCOP+PHYS at 8 s was not significant, Wilcoxon z=-1.01 (p=.31, 2-t).

Experiment 2

Differences between treatment conditions

Significant difference was obtained between the six conditions; PHYS (0.10), PHYS (0.15), SCOP, SCOP+PHYS and the two control conditions (saline and no injection) - for correct responses: Friedman Chi-Square=13.44 (n=9, df=5, p=.01, 1-t). There was also significant difference between conditions for number of nose-pokes and lever presses, see Table 5.

As expected, there was insignificant difference between the two control conditions, saline and no handling/no injection for correct responses, for nose-pokes, for delay prior to pressing sample lever and for delay prior to pressing non-matching lever.

As expected, significant difference was observed between SCOP and Saline condition for correct responses Wilcoxon Signed Ranks Test z=-2.43 (p=.008, 1-t), for nose-pokes Wilcoxon z=-1.72 (p=.05, 1-t), and for delay prior to pressing non-matching lever Wilcoxon z=-2.67 (p=.004, 1-t), but not for delay prior to pressing sample lever Wilcoxon z=-0.42 (n.s.).

As expected, there was insignificant difference between SCOP+PHYS and Saline for correct responses Wilcoxon Signed Ranks Test z=-1.01 (n.s.) and for delay prior to pressing sample lever Wilcoxon z=-1.36 (n.s.). The difference became significant however for nose-pokes Wilcoxon z=-1.96 (p=.03, 1-t), and for delay prior to pressing non-matching lever Wilcoxon z=-2.67 (p=.004, 1-t).

As expected, there was significant difference between SCOP and SCOP+PHYS for correct responses Wilcoxon Signed Ranks Test z=-2.67 (p=.004, 1-t), for delay prior to pressing sample lever Wilcoxon z=-1.72 (p=.04, 1-t) and for delay prior to pressing non-matching lever Wilcoxon z=-1.72 (p=.04, 1-t), but not for nose-pokes Wilcoxon =-0.06 (n.s.).

Table 4. Comparison of the the three treatment conditions and training day. Correct responses in % for 1, 2, 4 and 8 s delay.

Delay	1 s	2 s	4 s	8 s	Difference
SCOP					Friedman Chi-Square =16.87 p<.01
Mean	97.67	95.18	95.15	91.48	
Sd	2.34	10.99	6.19	6.40	
SCOP+PHYS					Friedman Chi-Square =8.73, p<.05
Mean	97.00	96.83	96.23	93.21	
Sd	3.53	3.23	3.95	5.89	
Saline					Friedman Chi-Square =21.93, p<.01
Mean	98.41	99.16	97.70	94.66	
Sd	1.76	1.26	2.04	4.67	
Training					Friedman Chi-Square =13.67, p<.01
Mean	98.31	97.83	97.46	94.09	
Sd	1.94	2.40	2.51	6.51	

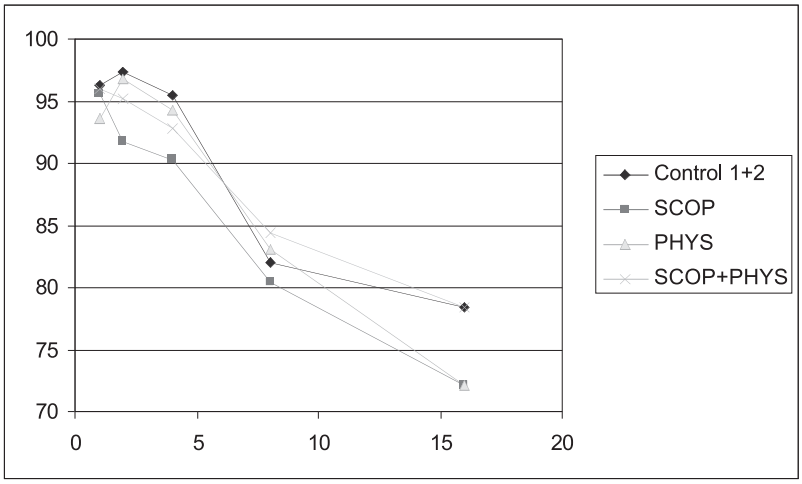
Table 5. Main results of the six treatment conditions for:correct responses in %, number of nose-pokes, delay prior to sample lever in seconds and delay prior to non-matching to position lever in seconds.

	PHYS 0,10	PHYS 0.15	SCOP	SCOP + PHYS 0.15	Control saline	Control no inject.	Difference
Correct responses %							Friedman Chi-Square =13.44, p<.05
Mean	88.48	87.72	86.14	89.42	90.12	89.84	
Sd	9.0	7.38	8.89	8.64	7.61	10.90	
Nose-pokes							Friedman Chi-Square =24.17, p<.001
Mean	1759.00	1345.44	1720.11	1719.50	2090.83	2318.22	
Sd	849.67	590.59	717.35	680.54	1107.13	873.49	
Delay sample Lever							Friedman Chi-Square =30.70, p<.001
Mean	12.69	15.71	5.56	7.01	6.38	4.53	
Sd	16.59	12.81	5.12	7.69	6.66	4.22	
Delay non-Matching							Friedman Chi-Square =36.91, p<.001
Mean	1.15	6.88	1.05	1.17	0.83	0.82	
Sd	0.57	5.19	0.46	0.51	0.24	0.18	

Table 6. Comparison of the six treatment conditions, correct responses in % for 1, 2, 4, 8 and 16 s delay.

Delay	1 s	2 s	4 s	8 s	16 s	Difference
PHYS 0.10						Friedman Chi-Square =28.86, p<.001
Mean	93.77	97.13	94.59	83.36	73.18	
Sd	5.81	3.10	9.11	14.94	17.67	
PHYS 0.15						Friedman Chi-Square =25.20, p<.001
Mean	93.34	96.54	94.02	82.78	71.12	
Sd	5.34	3.14	4.51	15.78	16.89	
SCOP						Friedman Chi-Square =29.24, p<.001
Mean	95.61	91.70	90.22	80.44	72.12	
Sd	3.55	8.22	10.99	13.85	12.75	
SCOP+PHYS						Friedman Chi-Square =26.04, p<.001
Mean	95.98	95.16	92.84	84.36	78.38	
Sd	4.20	8.31	8.66	12.58	15.29	
Saline						Friedman Chi-Square =28.74, p<.001
Mean	97.14	97.97	96.51	81.21	77.17	
Sd	3.19	2.65	4.25	17.78	15.31	
No injection						Friedman Chi-Square =29.30, p<.001
Mean	95.44	96.67	94.47	82.68	79.51	
sd	7.81	5.90	7.53	20.32	18.53	

Figure 1. Comparison of four treatment conditions, correct responses in % for 1, 2, 4, 8 and 16 s delay.



Differences in delays

There was significant difference between conditions (1, 2, 4, 8 and 16 s) for correct responses and the performance declined as the delay got longer, see Table 6. The difference between correct responses for SCOP and saline at 16 s delay was marginally significant, Wilcoxon Signed Ranks Test z=-1.60 (p=.06, 1-t), see Figure 1. The difference between correct responses for SCOP and SCOP+PHYS at 16 s was also significant, Wilcoxon z=-2.07 (p=.02, 1-t). The difference between correct responses for saline and SCOP+PHYS was not significant, Wilcoxon Signed Ranks Test z=-0.53 (n.s.).

The effect of physostigmine

The PHYS (0,15 mg/kg) condition differed significantly from the saline control condition for correct responses Wilcoxon Signed Ranks Test z=-2.31 (p=.02, 2-t), for nose-pokes Wilcoxon z=-2.43 (p=.015, 2-t), and for delay prior to pressing sample lever Wilcoxon z=-2.67 (p=.008, 2-t) and for delay prior to pressing non-matching lever Wilcoxon z=-2.52 (p=.01, 2-t). The PHYS (0,10 mg/kg) also differed signficantly from saline for nose-pokes, and both delay measures, but not for correct responses. The difference was more profound for the larger dose condition of PHYS (0,15 mg/kg). During the study we observed some adverse symptoms related to the 0.15 mg/kg dose, that were substantiated by the different number of omissions for the two PHYS conditions. Greater number of omissions was observed for the larger dose (19 on average during a session) compared to the smaller dose (14 on average), and rats on the larger dose obtained most of their omissions at the beginning of a session.

Pretrained rats versus new arrivals

The "old" pretrained rats did significantly better overall than "young" newly trained rats. There was a greater number of responses and nose pokes, and correct responses were higher for all delays, see Table 7.

Discussion

Two successive experiments were conducted on rats in an operant DNMTP task. Improvements were made on the DNMTP training procedure such that the animals were ready for drug injection after 17 days from habituation. This is a shorter training period than previously thought necessary. It

Table 7. Overall performance of "old" pretrained rats compared to performance of newly trained "young" rats.

Sessions (n)	Old rats (n=64)		Young rats (n=80)		Mann-Whitney U Test
	Mean	SD	Mean	SD	
Number of responses	176.9	34.4	110.7	49.1	U=754.5 (z=-7.26, p<.001)
Correct responses (%)	94.8	3.8	80.5	8.5	U=356.0 (z=-8.86, p<.001)
Nose pokes	2345.6	702.6	1116.0	407.3	U=313.5 (z=-9.03, p<.001)
Correct responses (1 s)	98.1	2.5	91.9	7.3	U=1158.0 (z=-5.84, p<.001)
Correct responses (2 s)	98.6	3.3	91.1	10.7	U=1184.0 (z=-5.93, p<.001)
Correct responses (4 s)	98.4	2.5	86.8	11.6	U=845.0 (z=-7.16, p<.001)
Correct responses (8 s)	92.7	6.6	69.6	17.4	U=604.0 (z=-7.87, p<.001)
Correct responses (16 s)	85.8	9.9	62.0	17.3	U=673.5 (z=-7.59, p<.001)
Omissions	4.9	8.2	17.5	15.9	U=1218.5 (z=-5.43, p<.001)

involves teaching rats to go directly from pressing the left lever, poking nose into magazine, to pressing right lever, and vice versa --- to obtain food. Furthermore, this ensures no dropouts due to lever preference. Previous training models have taught the animals through trial and error to press the "correct" lever through discovery. The brief training method was validated by replicating the effect of scopolamine on working memory.

By using the "old" pretrained rats (following diet), the training period can even be cut down to a few days. The pretrained rats required only a few sessions to reach former response rate and accuracy. Laboratories could keep pretrained animals in stock for testing working memory (and other faculties) with different drugs. This could result in longer working life for the rats (housed together between studies) and simulates closer the real life situation, where people of all ages are in need of particular drugs, not only the young ones.

Herremans et al. (1996) have argued that scopolamine does not affect working memory, but impairs discriminability in a delay-dependent manner only in animals that used mediating behavior. We argue that an essential part of keeping items in working memory while allocating them to long term storage is rehearsal and the use of various external and internal cues, hence the term working memory. Animals and humans use visual cues to remember things. Thus, it should not be surprising that scopolamine infusions disrupt mediating behavior during tasks. Accordingly, the use of barriers can only influence the outcome by increasing the number of omissions in treatment condition, as was the case with Ballard and McAllister (1999).

The experiments also explored the influence of physostigmine on working memory. Impairment by scopolamine was reversed by a large dose of physostigmine (0.15 mg/kg). However, such dose appears to have an adverse effect when provided on its own without scopolamine injection. In general, the studies show physostigmine to have an impairing effect on short term working memory when used without scopolamine. The effects of the cholinesterase inhibitors physostigmine and heptyl physostigmine on memory and performance deficits induced by scopoloamine were studied by Murray et al. (1991) and Dawson et al. (1991) using DNMTP and DMTP tasks respectively. The performance deficits induced in these tasks by scopolamine were reversed by doses of the cholinesterase inhibitors. Our findings are in line with these results.

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