Pharmacology Biochemistry & Behavior, Vol. 32, pp. 971–975. Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Atropine Effects on Delayed Discrimination Performance of Rats[1]

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Received 16 May 1988

ELSMORE, T. F., J. K. PARKINSON, J. R. LEU AND J. M. WITKIN. *Atropine effects on delayed discrimination performance of rats*. PHARMACOL BIOCHEM BEHAV **32**(4) 971–975, 1989.— The effects of atropine sulfate (ATS) and atropine methyl nitrate (ATM) on the conditional discrimination behavior of rats were investigated in eight-hour experimental sessions. Responding of rats was reinforced on either a lighted or a darkened lever depending on whether lights over both levers had been on during the preceding sample portion of the trial. Zero-delay and four-second-delay trials were randomly interspersed. Quality of performance was analyzed using the A′ sensitivity measure of signal detection theory. Both drugs reduced both sensitivity and the percentage of trials on which responding occurred (percent response) below saline treatment levels. The two drugs did not reliably differ from each other in their effects on sensitivity during the zero-delay condition, but reliable differences between the two drugs emerged during the four-second-delay condition at doses above 0.8 mg/kg. Percent response recovered more rapidly for animals treated with ATS than ATM, such that the ATS group did not differ from saline performance by the end of the eight-hour session, whereas the ATM group was still impaired. The data support the conclusion that atropine effects on rate of responding, as measured under complex discriminations, is primarily due to peripheral factors, while effects on qualitative features of performance are central in origin.

Atropine sulfate Atropine methyl nitrate Time course Discrimination Memory Rat

CHOLINERGIC mechanisms have been extensively implicated in the control of memory processes in both humans and animals (3–7, 15, 19, 26), and are implicated in the pathophysiology of senile dementia (12). A typical finding is the production of dose-related decreases in both running rate and accuracy of radial maze performance of rodents by anticholinergic drugs (7,15). In both of these studies, the quaternary salts of the cholinergic drugs (scopolamine methyl bromide, atropine methyl nitrate) did not affect accuracy of performance, but did affect the rate at which the animals ran through the maze. These differential effects have generally been ascribed to the impermeability of the blood-brain barrier to quaternary compounds (9, 11, 29). Thus, effects on rate are assumed to be due to peripheral mechanisms, and effects on accuracy are assumed to represent central antimuscarinic action.

Evidence is accumulating that short-term memory may depend upon the specific nature of the task, and in particular, upon the nature of the stimuli to be remembered. For example, it has been shown that monkeys may solve spatial memory problems more rapidly than nonspatial or symbolic memory problems (16), and that in rats, memory for auditory stimuli may be superior to memory for visual stimuli (28). In rodents, the majority of memory research has been conducted in mazes, in particular the radial maze which assesses primarily spatial memory (13, 15, 17, 18). For a complete description of drug effects on memory, nonspatial tasks must be assessed as well. In primates, there are two commonly used nonspatial memory tests, delayed-matching-to-sample (DMTS) and delayed-nonmatching-to-sample (3,21). In rodents, however, there are no standard tests, although various analogs of primate match- and nonmatch-to-sample have been recently described (1, 2, 20, 22, 24, 28), and one of these has been used in the investigation of cholinergic drug effects in rat (27).

The present experiment was designed to assess the effects of atropine sulfate (ATS), presumed to have both central and peripheral antimuscarinic actions, and atropine methyl nitrate (ATM), presumed to act only peripherally, on performance by rats of a nonspatial delayed conditional discrimination task in which responding was reinforced on either a lighted or a dark lever, depending on whether lights had been present during the earlier (sample) phase of the trial. Both dose-response and time-course data were collected for both drugs.

METHOD

Subjects

Twelve male albino rats (Walter Reed Sprague-Dawley derived) weighing approximately 230 grams were used. The animals were previously trained on the task used for the current experiment

[1]Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NIH publication 86-23, 1985 edition. The views of the authors do not purport to reflect The position of the Department of the Army or the Department of Defense (para 4-3, AR 360-5).

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TABLE 1

PERCENT TRIALS COMPLETED

Drug	Dose	Hours Postinjection 1–2	3–4	5–6	7–8
Saline		95.84 (0.82)	89.22 (1.92)	87.18 (1.47)	89.15 (1.61)
Atropine Sulfate	0.8	83.67 (3.18)	72.31 (5.29)	85.76 (3.52)	91.09 (1.66)
	1.6	80.47 (3.11)	65.19 (3.93)	69.73 (6.65)	84.46 (3.19)
	3.2	77.24 (3.84)	56.04 (4.59)	66.04 (6.26)	74.31 (6.97)
Atropine Methyl Nitrate	0.8	67.85 (7.13)	61.56 (6.16)	71.34 (6.19)	70.52 (6.37)
	1.6	72.42 (5.53)	55.62 (7.06)	58.85 (7.01)	66.77 (7.50)
	3.2	74.93 (4.59)	52.01 (7.74)	47.34 (7.42)	48.84 (6.64)

and had additional extensive training on long delay trials (20). The animals lived in the experimental apparatus for the duration of the experiment, and all food was earned under the experimental contingencies, i.e., a "closed" economy (10). Water was continuously available from a bottle mounted on the side of the cage.

Apparatus

The experiment was conducted with twelve, two-lever operant chambers (Coulbourn Instruments). The response levers were positioned 6.5 cm from the floor of the chamber and separated on the front panel by a food delivery hopper. The chambers were housed in sound- and light-attenuating enclosures. General illumination in the chambers (houselights) was on for 12 hours each day, with light onset at 2200 hours. A Sonalert tone, houselight, stimulus lights and food solenoid were operated by a PDP/8e computer running the SUPERSKED software system (25).

Procedure

The twelve rats were assigned to three groups. During each drug session, one group received saline, one received atropine sulfate (ATS, Sigma Chemical Co.), and the third received atropine methyl nitrate (ATM, Sigma). Drugs were given every 2 or 3 days. Drug doses were given in an ascending order, and each group received all doses of both drugs. Injections were made subcutaneously in a volume of 1 ml/kg. All doses were calculated using the salt form of each drug. A single replication of the dosing regimen was performed 10 days after the first series was completed. Training was continued between determinations. Each 8-hour session began at 1030 hours, 30 min after lights out. On treatment sessions, injections were given between 1015 and 1025 hours.

The sample portion of each trial consisted of the presentation of a Sonalert tone (2900 Hz) for 10 sec either alone or in conjunction with the onset of lights over both response levers. To prevent responding during sample presentation, a response on either lever during the last 5 sec of the 10-sec sample period reset the sample timer to 5 sec. This procedure effectively eliminated responding during the sample. Trial type (i.e., tone + lights or tone alone) was randomly selected so each type would occur approximately 50

TABLE 2

SENSITIVITY (A')

Drug	Dose	Hours Postinjection 1–2	3–4	5–6	7–8
Zero-Sec-Delay Trials					
Saline		0.936 (0.006)	0.947 (0.004)	0.948 (0.006)	0.953 (0.004)
Atropine Sulfate	0.8	0.909 (0.015)	0.898 (0.016)	0.921 (0.012)	0.928 (0.012)
	1.6	0.895 (0.016)	0.919 (0.008)	0.931 (0.008)	0.916 (0.019)
	3.2	0.888 (0.014)	0.833 (0.022)	0.864 (0.019)	0.901 (0.023)
Atropine Methyl Nitrate	0.8	0.881 (0.020)	0.861 (0.029)	0.906 (0.018)	0.922 (0.020)
	1.6	0.900 (0.014)	0.867 (0.024)	0.868 (0.035)	0.879 (0.039)
	3.2	0.910 (0.014)	0.861 (0.025)	0.849 (0.039)	0.889 (0.027)
Four-Sec-Delay Trials					
Saline		0.885 (0.011)	0.898 (0.010)	0.905 (0.009)	0.907 (0.013)
Atropine Sulfate	0.8	0.830 (0.020)	0.824 (0.019)	0.850 (0.022)	0.872 (0.019)
	1.6	0.806 (0.019)	0.781 (0.026)	0.811 (0.033)	0.842 (0.022)
	3.2	0.773 (0.025)	0.707 (0.023)	0.795 (0.023)	0.850 (0.010)
Atropine Methyl Nitrate	0.8	0.810 (0.024)	0.816 (0.026)	0.836 (0.022)	0.856 (0.024)
	1.6	0.800 (0.029)	0.827 (0.029)	0.797 (0.035)	0.827 (0.037)
	3.2	0.832 (0.021)	0.790 (0.039)	0.806 (0.037)	0.838 (0.027)

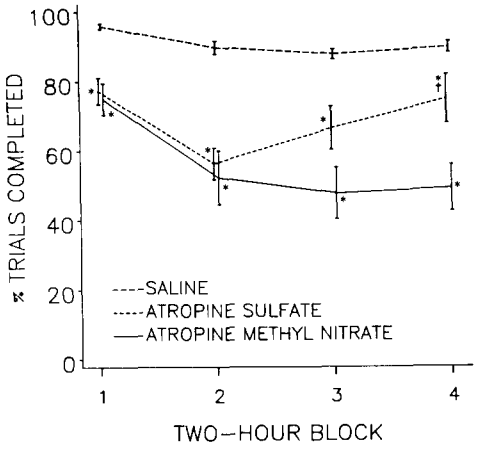

FIG. 1. Effects of 3.2 mg/kg atropine sulfate and atropine methyl nitrate on the percentage of trials completed as a function of time since injection in two-hour blocks. Data were averaged across zero- and 4-sec-delay trials. Each point represents the average of twelve animals. Vertical bars are ±standard errors of the means. Statistically significant differences ($p<0.05$) between a drug and saline are denoted by asterisks, and significant differences between the two drugs are denoted by daggers.

percent of the time.

After sample presentation, a random ($p=0.5$) selection of either a short (0.01-sec, hereafter called "zero") or long (4-sec) delay occurred, after which, the light over one of the response levers was illuminated, with the side selected randomly. A single press within 10 sec on the lighted lever, if the lights had been on during sample presentation, or on the dark lever, if there had been no lights on during the sample presentation resulted in the presentation of a single 45 mg food pellet. A correct response or no response initiated a 30-sec intertrial interval (ITI) during which the chamber was completely dark. An incorrect response initiated a 60-sec ITI. This set of parameters resulted in approximately 60 trials per hour, depending on the accuracy of performance. A correction procedure was employed on all trials to reduce lever bias. If the number of pellets accrued on either lever became more than 5 greater than on the other lever, the probability of that lever being correct on the subsequent trial was reduced to 25 percent until the imbalance was corrected. This procedure is described in more detail elsewhere (20).

Data Analysis

Data from the initial testing and the replication were pooled for analysis. Means were computed for each subject for each treatment. Overall repeated measures analyses of variance were conducted prior to performing pairwise *t*-tests between treatments. All statistical analyses were conducted with the SAS software package on a Digital Equipment Corporation VAX computer. Statistical significance is reported for effects exceeding the 0.05 level of significance. Due to a consistent response bias on zero-delay trials (20), accuracy data were analyzed using a signal detection theory procedure following standard techniques (8,23). In this procedure, β'' is a measure of response bias. The A′ measure, a nonparametric index of "sensitivity" or degree of control by the stimuli in a discrimination procedure, was used to test for drug effects on the quality of discriminative performance. This statistic varies from 0.5 (no stimulus control) to 1.0 (perfect discrimination). A′ and β'' were calculated only for sessions in which responding occurred on greater than 10% of the trials.

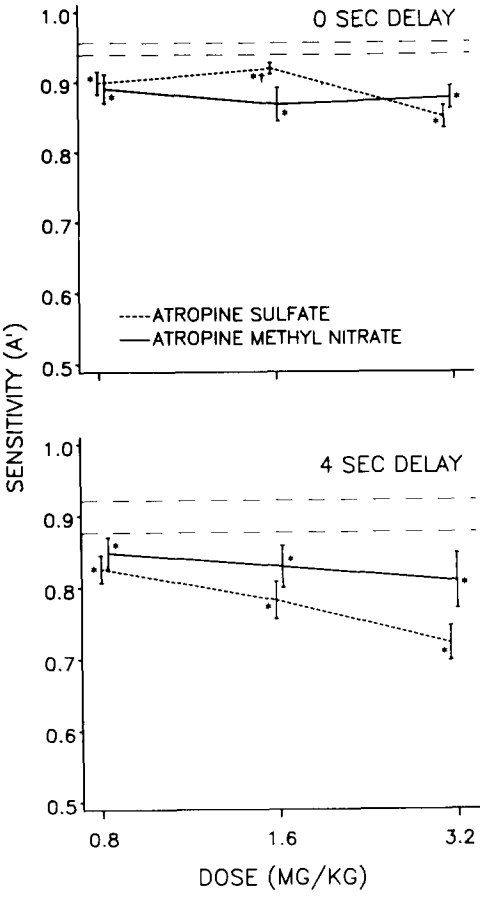

FIG. 2. Dose-effect curves for atropine sulfate and atropine methyl nitrate on sensitivity of responding to the duration of a visual stimulus as indexed by A′. The top frame is from trials with zero delay between the offset of the stimulus and the opportunity to respond, and the bottom frame is from trials with a four-sec delay. The horizontal dashed lines indicate 95% confidence intervals for saline control injections. These data are from the third and fourth hours of an eight-hour experimental session. Each point represents the average of twelve animals. Vertical bars are ±standard errors of the means. Statistically significant differences ($p<0.05$) between a drug and saline are denoted by asterisks, and significant differences between the two drugs are denoted by daggers.

RESULTS

Since sessions were eight hours long, it was possible to derive time course as well as dose-effect functions for both atropine and methyl atropine. Since atropine is known to produce decreases in response rates (14, 15, 21, 31), the time course of drug effects on percentage of trials completed was analyzed. There were no differences in this measure as a function of delay, so zero- and four-sec-delay trials were pooled. Table 1 shows mean percent trials completed in each two-hour block of the session for saline and all doses of both drugs. Numbers in parentheses are standard errors of the means. Separate one-way analyses of variance for repeated measures were conducted for saline, and all drug doses by time since injection. These analyses were all significant [$df(3,33)$, $p<0.05$] with the exception of the 0.8 mg/kg dose of atropine methyl nitrate which depressed responding at all time points. Some of these data are presented graphically in Fig. 1 which shows the effects of saline as well as 3.2 mg/kg of both drugs as a function of time in the session. Both ATS and ATM

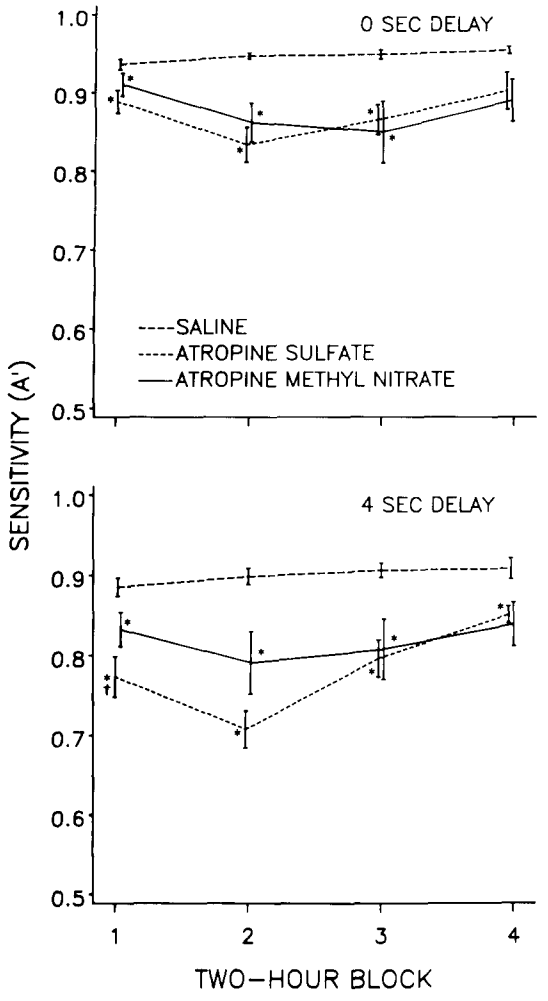

FIG. 3. Time course of 3.2 mg/kg atropine sulfate and atropine methyl nitrate on zero- and four-sec-delay trials. Each point represents the average of twelve animals. Vertical bars are ±standard errors of the means. Statistically significant differences ($p<0.05$) between a drug and saline are denoted by asterisks, and significant differences between the two drugs are denoted by daggers.

produced decreases in this measure which were maximal during the second two-hour block of the session. Statistical significance of the differences between drugs at each time point was assessed by pairwise repeated-measures contrasts between the drugs and saline and between the two drugs. All of the drug points are significantly different from saline [$df(1,11)$, $p<0.05$], with the exception of ATS in the last two-hour block. The ATS group had nearly recovered by the end of the session, whereas responding of the ATM group was still depressed below both saline and ATS levels eight hours postinjection.

Effects of all treatments on sensitivity are shown in Table 2 which presents mean A′ values for all drug doses and times since injection. Numbers in parentheses are standard errors of the means. Figure 2 shows the effect of drug dose on A′ for the second two-hour block. The horizontal dashed lines indicate the 95% confidence intervals for saline controls. The top frame shows performance during the zero-delay trials, and the bottom frame, the four-sec-delay trials. Sensitivity was reduced below saline levels by all doses of both drugs. There were no systematic differences between the two drugs on zero-delay trials. The effect of dose on ATM performance was not statistically significant, whereas the 3.2 mg/kg dose of ATS decreased A′ significantly more than 0.8 mg/kg [$df(1,10)$, $p<0.05$]. The performance of one animal was completely suppressed for the entire session by 3.2 mg/kg of either ATM or ATS.

Figure 3 shows the time course of saline and 3.2 mg/kg of each drug on A′. Following saline administration, performance improved slightly across the eight-hour session regardless of delay [$df(3,33)$, $p<0.05$]. Time in the session did not significantly affect A′ for either drug on zero-delay trials, or ATM on four-sec-delay trials, but did have a significant effect on ATS performance on four-sec-delay trials [$df(3,30)$, $p<0.05$]. Both ATS and ATM decreased A′ significantly from saline performance for the first six hours of the eight hour session, regardless of delay [$df(1,10)$, $p<0.05$], and ATS performance was significantly depressed below saline during the last two-hour block as well. ATS produced a significantly larger effect than ATM for the first two hours of the session. As Table 2 indicates, lower doses resulted in qualitatively similar results, with smaller performance decrements and shorter recovery times. Analyses of response bias, β'', either towards the lighted lever or lever position, showed no significant changes as a result of administration of either drug.

DISCUSSION

The present study showed that both atropine sulfate and atropine methyl nitrate reduced the frequency of responding of rats on a delayed discrimination task in a dose-dependent fashion. Both drugs achieved peak effects between two and four hours postadministration. Thus, ATM can have behavioral effects qualitatively similar to ATS at certain times and dosages [cf. (7,15)]. However, the mechanisms involved are not clear, and may differ for the two salts. Effects on response frequency persisted much longer for ATM than for ATS, consistent with reports in the literature that ATM is a more potent antimuscarinic drug than ATS (11,29).

Effects of the drugs on sensitivity, however, were quite different, with ATS producing greater performance decrements than ATM on four-sec-delay trials. ATM effects were indistinguishable from those of ATS on zero-delay trials, and similar, though smaller than those of ATS on four-sec delay trials. These findings were unexpected in light of numerous reports that quaternary anticholinergic drugs do not produce appreciable effects on qualitative aspects of performance of rats or mice in radial arm mazes (7,15), and monkey data showing no effect of ATM on errors in a repeated acquisition of response sequences task (14). Explanations for this effect include the possibility that ATM penetrated the blood-brain barrier in behaviorally-active concentrations (30–32), or that the effects on sensitivity were, at least in part, secondary to the activation of peripheral mechanisms such as cardiac, ocular, or gastrointestinal changes induced by ATM. The findings that the ATM effects on sensitivity were not highly dose- or delay-related (Table 2, Fig. 2), argue in favor of such nonspecific peripheral mechanisms.

ATS effects on sensitivity, on the other hand, were both dose- and delay-related, suggesting a prominent central effect. This finding is consistent with reports in the literature from primate subjects (3, 5, 21), and rodents using spatial tasks (7,15), that anticholinergic compounds specifically affect memory processes. The comparable effects of atropine and other cholinergic drugs (7,15) on spatial memory tasks in rodents and under the nonspatial task studied here, as well as in nonhuman primates, support the view that common cholinergic mechanisms underlie both spatial and nonspatial memory.

ACKNOWLEDGEMENTS

The authors thank R. F. Genovese and G. J. Kant for helpful comments on the manuscript.

REFERENCES

1. Aggleton, J. P.; Hunt, P. R.; Rawlins, J. N. P. The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. Behav. Brain Res. 19:133–146; 1986.
2. Aggleton, J. P. One-trial object recognition by rats. Q. J. Exp. Psychol. 37B:279–294; 1985.
3. Aigner, T. G.; Mishkin, M. The effects of physostigmine and scopolamine on recognition memory in monkeys. Behav. Neural Biol. 45:81–87; 1986.
4. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, D. L. The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–417; 1982.
5. Bartus, R. T.; Johnson, H. R. Short-term memory in the rhesus monkey: Disruption from the anticholinergic scopolamine. Pharmacol. Biochem. Behav. 5:39–46; 1976.
6. Drachman, D. A. Memory and cognitive function in man: Does the cholinergic system have a specific role? Neurology 27:783–790; 1977.
7. Eckerman, D. A.; Gordon, W. A.; Edwards, J. D.; MacPhail, R.C.; Gage, M. I. Effects of scopolamine, pentobarbital, and amphetamine on radial maze performance in the rat. Pharmacol. Biochem. Behav. 12:595–602; 1980.
8. Grier, J. B. Nonparametric indexes for sensitivity and bias: Computing formulas. Psychol. Bull. 75:257–262; 1971.
9. Herz, A.; Teschemacher, H.; Hofstetter, A.; Kurz, H. The importance of lipid-solubility of the central action of cholinolytic drugs. Int. J. Neuropharmacol. 4:107–218; 1965.
10. Hursh, S. R. Economic concepts for the analysis of behavior. J. Exp. Anal. Behav. 34:219–238; 1988.
11. Innes, I. R.; Nickerson, M. Atropine, scopolamine, and related drugs. In: Goodman, L. S.; Gilman, A., eds. The pharmacological basis of therapeutics. 5th ed. New York: Macmillan; 1975:514–532.
12. Iversen, L. L. The cholinergic hypothesis of dementia. Trends Pharmacol. Sci. Feb. Suppl.: 44–45; 1986.
13. Jarrard, L. E. Selective hippocampal lesions and behavior: effects of kainic acid lesions on performance of place and cue tasks. Behav. Neurosci. 97:873–889; 1983.
14. Levy, A.; Elsmore, T. F.; Hursh, S. R. Central vs peripheral anticholinergic effects on repeated acquisition of behavioral chains. Behav. Neural Biol. 40:1–4; 1984.
15. Levy, A.; Kluge, P. B.; Elsmore, T. F. Radial arm maze performance of mice: acquisition and atropine effects. Behav. Neural Biol. 39: 229–240; 1983.
16. Mishkin, M.; Delacour, J. An analysis of short-term visual memory in the monkey. J. Exp. Psychol. [Anim. Behav.] 1:326–334; 1975.
17. Olton, D. S.; Feustle, W. A. Hippocampal function required for non-spatial working memory. Exp. Brain Res. 41:380–389; 1981.
18. Olton, D. S.; Samuelson, R. J. Remembrances of places past: spatial memory in rats. J. Exp. Psychol. [Anim. Behav.] 2:97–116; 1976.
19. Overstreet, D. M. Behavioral plasticity and the cholinergic system. Prog. Neuropharmacol. 8:133–151; 1984.
20. Parkinson, J. K.; Elsmore, T. F. A delayed discrimination procedure for rats. Bull. Psychonom. Soc. 27:49–51; 1989.
21. Penetar, D. M.; McDonough, J. H. Effects of cholinergic drugs on delayed match to sample in rhesus monkeys. Pharmacol. Biochem. Behav. 19:963–968; 1983.
22. Pontecorvo, M. J. Effects of proactive interference on rats' continuous nonmatching-to-sample performance. Anim. Learn. Behav. 11:356–366; 1983.
23. Raslear, T. G. Perceptual bias and response bias in temporal bisection. Percept. Psychophysiol. 38(3):261–268; 1985.
24. Rothblat, L. A.; Hayes, L. L.; Kromer, L. F. Effects of septo-hippocampal damage on spatial and nonspatial tests of working memory. Soc. Neurosci. Abstr. 12:743; 1986.
25. Snapper, A. G.; Kadden, R. M. Time-sharing in a small computer based on a behavioral notation system. In: Weiss, B., ed. Digital computers in the behavioral laboratory. New York: Appleton-Century-Crofts; 1973:41–97.
26. Spencer, D. G.; Lal, H. Effects of anticholinergic drugs on learning and memory. Drug Dev. Res. 3:489–502; 1983.
27. Spencer, D. G.; Pontecorvo, M. J.; Heise, G. A. Central cholinergic involvement in working memory: effects of scopolamine on continuous nonmatching and discrimination performance in the rat. Behav. Neurosci. 6:1049–1065; 1985.
28. Wallace, J.; Steinert, P. A.; Scobie, S. R.; Spear, N. R. Stimulus modality and short-term memory in rats. Anim. Learn. Behav. 8:10–16; 1980.
29. Weiner, N. Atropine, scopolamine, and related antimuscarinic drugs. In: Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, F., eds. The pharmacological basis of therapeutics. 7th ed. New York: Macmillan; 1985:130–144.
30. Wenger, G. R. Effects of physostigmine, atropine and scopolamine on behavior maintained by a multiple schedule of food presentation in the mouse. J. Pharmacol. Exp. Ther. 209:137–143; 1979.
31. Witkin, J. M.; Gordon, R. K.; Chiang, P. K. Comparison of *in vitro* actions with behavioral effects of antimuscarinic agents. J. Pharmacol. Exp. Ther. 242:796–803; 1987.
32. Witter, A.; Slanger, J. L.; Terpstra, G. K. Distribution of ^{3}H-methylatropine in rat brain. Neuropharmacology 12:835–841; 1973.