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The Effects of Physostigmine and Scopolamine on Memory for Food Caches in the Black-Capped Chickadee

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MINEAU, P., P. T. BOAG AND R. J. BENINGER. *The effects of physostigmine and scopolamine on memory for food caches in the black-capped chickadee.* PHARMACOL BIOCHEM BEHAV 49(2) 363-370, 1994.—The possible effects of anticholinesterases on the central nervous system and, in particular, on learning and memory, have generated considerable interest. Food caching in the black-capped chickadee is an excellent natural paradigm of spatial working memory. Its susceptibility to cholinergically active drugs was explored in the present study. Our ultimate objective was to use food caching as a natural paradigm for the study of the consequences in birds of sublethal exposure to anticholinesterase insecticides. Biochemical analyses showed that administration of the anticholinesterase physostigmine (eserine) led to a short-lived effect, with recovery of brain cholinesterase levels already underway 5 min after an intramuscular injection. Birds administered the anticholinergic scopolamine before caching demonstrated significantly impaired recall compared to birds given physostigmine. Birds given saline only had an intermediate performance. Giving the drugs between caching and recovery had no measurable effect. These findings suggest that effects of cholinergic agents on cache recovery in chickadees are comparable to their effects in tests of working memory in mammals.

Memory Anticholinesterase Anticholinergic Chickadee Food caching Physostigmine Scopolamine *Parus atricapillus*

THE role of central cholinergic neurotransmission in learning and memory has been the subject of much research and controversy. One reason for the continuing interest in this field is the apparent link between a reduction of cholinergic activity and memory disorders such as senile dementia and Alzheimer's disease in humans. The field has been extensively reviewed, for example (4,5,7,9,11-13,15,18,20,22,38). Despite the large volume of research, there are still fundamental disagreements on how cholinergically active substances affect the different stages of the learning and memory process; namely, the initial acquisition and encoding of information, as well as its consolidation, retention, and eventual retrieval. As pointed out by Izquierdo (21), the best way to reconcile all of the research evidence is to accept that cholinergic mechanisms are involved in most or all of the memory stages outlined above.

A substantial amount of work on memory in birds has been achieved through the use of food caching behavior in certain groups, notably the family Paridae (tits or chickadees) (31,36). There has been some question as to whether the excellent spatial memory for food caches in food-caching species is qualitatively or quantitatively different from other memory systems (32,35,36). The formation of memories for food caches in the black-capped chickadee (*Parus atricapillus*) has recently been shown to be impaired by lesions to the cholinoceptive hippocampal complex (33), a finding analogous to the impairment of working memory in mammals with hippocampal lesions. Furthermore, food-caching species have been found to have particularly large hippocampal complexes (34).

The aim of the present research was to test whether the learning and memory processes involved in food caching can be affected by the administration of cholinergically active drugs. The anticholinesterase physostigmine and anticholinergic scopolamine were used. Scopolamine has been found to reliably cause amnesia in a variety of tests of working memory

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(5). Our ultimate objective was to use food caching as a natural paradigm for the study of the consequences of sublethal exposure to anticholinesterase insecticides (25). Brain cholinesterase levels at various times after dosing with physostigmine, therefore, were measured in order that they could be compared with levels recorded following exposure of birds to insecticides. It was hypothesized that physostigmine would enhance and scopolamine would impair memory.

METHOD

Subjects

The chickadees were captured under permit from the Canadian Wildlife Service in the Ottawa area between 5 and 10 October 1989, and held for at least 1 month before any drug injections or testing. All procedures involving the birds were authorized by approved Animal Care Committees both at Queens University and at Health and Welfare Canada. The birds were provided with a standard diet comprising shelled sunflower seeds, raw peanuts, hard-boiled egg, grated carrot (certified pesticide-free produce), Hagen™ Mynah bird and soft-billed bird food, gravel, and cuttlefish bone. Prior to testing, sunflower seeds were removed from the standard diet and thereafter kept for the testing itself. Once a week, this diet was supplemented with live mealworms. The birds were also provided ad lib with tap water containing Hagen™ multivitamin drops. The water bowls also served for bathing.

Birds were housed in groups of 12 or less in cages measuring 137 by 71 by 61 cm with natural hardwood branches as perches. The light period lasted from 0900 to 1700 and temperature was held constant at 15°C. They received full-spectrum fluorescent lighting at an intensity of 250 lx measured inside the cages. Following the experiment, all surviving birds were released where they had been captured.

Apparatus

We utilized a test system similar to the one initially developed by Sherry (30). Nine hardwood trees 2.0 to 2.3 m in height were placed on moveable stands in an arena that measured 3.0 by 6.1 by 2.9 m and which received illumination from banks of overhead fluorescent bulbs (254 lx at 1.2 m from ground). Each was drilled with 10 cache sites (approximately 10 mm diameter and 15 mm in depth) positioned at approximately regular intervals on the sides of the trees facing the observation window. The cache sites ranged in height from 23 to 218 cm from the floor. A piece of string with a large terminal knot was attached beside each hole allowing cache sites to be in an open (string hanging free) or closed (knot blocking the hole) position. To check a closed cache site, birds had to pull on the string thereby uncovering the cache site. A short piece of dowelling (0.5 cm diameter) was positioned beneath each cache site for perching. Natural cavities and other likely natural cache sites were plugged and eliminated wherever possible. Birds were released remotely from a holding cage within the arena. Bouts were terminated by turning off the lights in the arena and the birds were hand caught.

Procedure

Birds were initially screened for their ability to uncover cache sites by pulling on the strings. All birds used for testing successfully cached and recovered seeds in the arena at least twice before actual testing began. The experiment began when all birds showed the ability to recover at least 2 of 5 cache sites following 15 or fewer different cache site checks.

Two experiments were carried out. In the first (precache administration), the birds were given the drugs immediately (1–2 min) before release into the arena where they cached. In the second (prerecovery administration), birds were given the drugs 15 min before being released to begin recovery of cached seeds. In both experiments, the interval between caching and recovery was 4–5 h.

A total of 12 birds was tested in two behavioral experiments. For each experiment, six birds were tested in a repeated measures design with three treatments (scopolamine, physostigmine, saline) and three repeated measurements per bird per treatment. In precache administration, one of the birds was eventually excluded from the experiment when it repeatedly failed to cache regardless of treatment. The order of presentation of treatments was alternated among birds with one of each treatment being presented on any given experimental day. Birds were not tested more than once per week. Birds to be tested were segregated and their food removed the previous evening. Caching took place between 0900 and 1100 h. Birds were released remotely from a holding cage within the arena. The 90 cache sites were left open and a bowl with shelled sunflower seeds provided. Birds were allowed to eat seeds to satiety and then to cache five seeds. (The actual number of seeds cached varied between 4 and 8, the mean number being 5.3 for both experiments.) Birds failing to cache after 30 min were removed and retested on a later day (this was a rare occurrence). Birds were returned to the arena for cache recovery between 1400 and 1500 on the same day that the caching took place. Prior to each recovery test, small seed fragments were placed in the bird's chosen cache sites and all sites closed. Seed fragments were used to reduce the likelihood of satiation. Trees were moved around the arena so that any one individual never encountered the same tree configuration for more than one cache-recovery cycle. Recovery bouts lasted from a minimum of 5 min to about 30 min. In all cases, this was sufficient time to ensure that the required number of site checks had taken place.

Scoring Memory Effects

Two observers scored the bouts and these were also videotaped. Site checks and other relevant information were recorded on the audio portion of the tape. The videotape was consulted in cases of disagreement between the observers. Pulling at the strings or probing of the sites around the knot were deemed to be site checks. Our scoring methodology was designed to accomodate the small variation in the number of seeds cached during each experimental bout by basing the score on the probability of random find. To compute our recovery score, the probability that each successful check occurred at random was calculated based on the number of correct and incorrect cache sites left to check. Each cache site was only considered once, repeat checks being excluded from this particular index. The recovery score was calculated as the product of the individual probabilities for each successful check tallied for the first 15 site checks. This number of checks was arbitrarily chosen as the highest reliable number of checks available per bout for all birds. For example, if 10 seeds were cached in 90 possible cache sites and if, during recovery, a seed was found on the first, fifth, and fifteenth site check, the probability of random find would be: $(10/90) \times (9/86) \times (8/76) = 0.00122$. Our recovery score is expressed as the absolute value of the base 10 logarithm of this probability (i.e., 2.91). Therefore, for a given number of site checks, the higher the score, the better the performance. With each unsuccessful

attempt in a recovery sequence, the denominator decreases by 1 but the numerator stays the same. The result is that, when the absolute log of the fraction is taken to compute the recovery score, a higher score is given to an early recovery when all else is equal. A late recovery may achieve a higher score than an early one only if there are fewer seeds left to find and depending on how many cache sites are left to check. Depending on the exact situation, the probability of a random find may, indeed, be lower later on in a trial. The recovery score provides an unbiased assessment of the merit of each find. It is naturally weighted to recognize a successful find as the main contribution to a good recovery score while allowing for some differentiation between an early and late recovery in the trial.

Data are also shown visually as plots of the recovery score computed over the first 15 site checks and averaged for all subjects. Standard error bars are provided to give an indication of the inherent intersubject variability, although these error terms were not used in the statistical analysis of the results (see statistical analysis below).

For the sake of comparison with actual bird performance, recovery scores computed on the basis of seeds being found at random rather than by memory were also graphed. For each bird, five cache sites were selected at random from the sample of cache sites used in the course of the experiments by that particular bird, and this randomly chosen set compared to the site checks recorded for each bird's first saline trial (obviously, cache sites utilized during this trial were excluded from the random selection process). The result is the probability of random find corrected for each bird's preferences for a particular subset of caches.

Two alternate indices of memory performance were also measured: first, the total number of caches recovered and seed pieces eaten in the course of a fixed (5 min) recovery period, and second, the ability of the birds injected prerecovery to avoid revisiting cache sites. Rechecking either an empty cache site or one that the bird has just emptied is inefficient, and it follows that revisits to the same sites are generally avoided (29,30). A repeat score was computed as the proportion of all site checks that were repeats recorded during the first 5 min of the recovery period. All statistics were computed on the arcsine transformed values.

Scoring Other Drug Effects

Several additional behavioral measurements were made to assess possible nonmnemonic effects of drug treatments. Hunger was assessed in the precache injection experiment by computing an arbitrary measure of seed consumption as the number of seeds eaten + (the number of seeds partially eaten/2). Measures of motivation were taken to be the latency to cache or to recover caches while drugged as well as the intensity of site checking (over a 5 min period) when injected prerecovery. In birds injected precache, the latency to establish the first cache site after release into the arena as well as the time interval between first and fifth cache were also compared between treatments (all birds in that experiment cached at least five seeds).

Finally, the general level of activity of birds in different treatment groups was assessed. The activity level of birds dosed precache was measured by looking at the rate of site visiting (the number of times the birds alighted on the perches positioned under each cache site) in the interval between the first and fifth food cache, expressed as the number of site visits per minute during this time period. For prerecovery injections, the total number of site visits excluding site checks was compared between treatments for the first 5 min after release into the arena. Site checks were excluded from this index because they had been used already as a measure of motivation.

Drugs and Injections

Birds were injected intramuscularly in the pectoral muscle, alternating from right to left from injection to injection. We used 30 1/2 gauge needles (outside diameter = 0.028 cm) affixed to a high precision 50 μl gas chromatography syringe. Scopolamine (also known as hyoscine) hydrobromide and physostigmine (also known as eserine) hemisulfate (Sigma) solutions in saline were prepared fresh on every test day in sterile containers and kept on ice and in the dark until used. Concentrations were arranged so that the injection volume was approximately 1 μl per g of bird (the birds ranging from 10–13 g). Doses (expressed here as the salt weight) were chosen that produced only slight observable peripheral drug effects such as the occasional loss of balance.

Measurement of Cholinesterase Activity

A group of nine birds was used to measure the extent of cholinesterase inhibition following injection with physostigmine hemisulfate. Following overnight fasting, birds were dosed in the usual way and then held for either 5, 15, or 30 min, at which time they were asphyxiated with CO_2, their brain removed, and placed in liquid nitrogen. The samples were transferred to a $-80°C$ freezer where they remained for less than a week until analysis. Cholinesterase levels were obtained through the Ellman method as described by Hill and Fleming (19), with the exception that samples were homogenized in a solution of Triton X (3 times the brain sample weight of a 1% solution), centrifuged, and only the supernatants were assayed (16). Data are expressed as micromoles of substrate hydrolysed/min/g brain tissue. Samples were retested after spontaneous enzymatic recovery on a gel column (24).

Statistical Analysis

Data from both the precaching and prerecovery injection experiments were subjected to an analysis of variance (anova) as a two-factor experiment (drug and bird) with repeat testing of the same subjects. An ANOVA was also used to analyze the time course of cholinesterase inhibition following injection with physostigmine. A linear regression of inhibition measurements over time was used to compute 95% prediction intervals for this relationship. The Wilk-Shapiro statistic (results of a rankit plot) was used to ascertain that the recovery scores were normally distributed. Comparisons of the means for a drug effect were performed by Tukey's method. All tests were performed on the software Statistix 3.1.

RESULTS

Setting of Dose Levels

Because it was important that the birds' behavioral performance not be overly impaired while treated with a drug, overt symptomology was used as a rough guide in setting the dose levels. Subjective assessments of the signs of toxicity accompanying various dose levels in preliminary trials led to choosing levels of 1.0 mg/kg scopolamine hydrobromide and 0.1 mg/kg physostigmine hemisulfate. A subjective evaluation of ob-

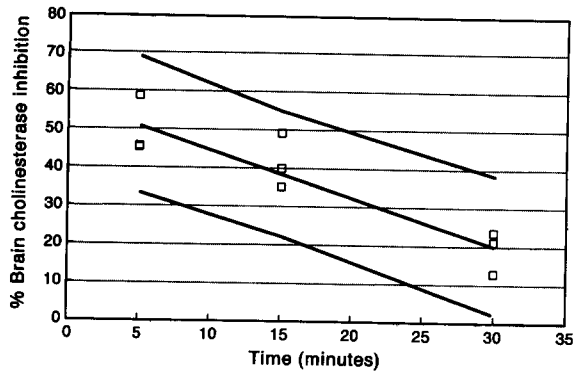

FIG. 1. Percent brain cholinesterase inhibition over time following intramuscular injections of 0.1 mg/kg physostigmine hemisulfate. Normal levels (0% inhibition) assumed to be 37.39 μM substrate/min/g of brain tissue. Mean inhibition levels and 95% prediction intervals are based on a linear regression of inhibition values over time.

served signs of toxicity at these doses is as follows: a slight hyperactivity, an occasional brief loss of balance, and dropping of seeds in scopolamine-treated birds and, in physostigmine-treated birds, frequent bill wiping, occasional lethargy, piloerection resulting in a fluffed appearance, slight wing tremors, and the occasional brief loss of balance. The appearance of these signs was variable, and some individuals receiving either drug appeared symptom free.

Cholinesterase Inhibition Following Dosing With Physostigmine

Individual cholinesterase values are plotted in Fig. 1. Every sample demonstrated significant spontaneous reactivation of the carbamylated enzyme after samples were placed on the gel columns (data not shown). A one-way ANOVA showed a significant effect of time since injection on the raw cholinesterase values, $F(2, 6) = 15.93$, $p < 0.005$. The mean control (saline injected) value for this species was determined to be 37.39 μM of substrate hydrolyzed/min/g of brain tissue (SE = 0.59, $n = 22$) (25). Relative to control, brain levels were, therefore, inhibited by 50%, 41%, and 19% at 5, 15, and 30 min postinjection, respectively.

Cache Recovery and Other Memory Effects

Precache administration of the drugs. Cache recovery scores (as well as the bird-adjusted probability of random find) over the first 15 site checks are plotted for each drug treatment in Fig. 2.

A plot of the recovery scores against their rankits (ranked normal deviates) after 15 site checks (not shown) showed no deviation from normality with a computed Wilk-Shapiro statistic of 0.96. An ANOVA revealed that recovery scores after 15 site checks differed among drug treatments, $F(2, 38) = 3.55$, $p < 0.05$. The scopolamine group performed significantly more poorly than the physostigmine group ($p < 0.05$ on a Tukey's test), although neither group was statistically different from the saline control.

An ANOVA for the number of seeds recovered in the first 5-min period (mean values of 4.1, 4.0, and 2.9 seeds for physostigmine, saline, and scopolamine groups, respectively) also showed a significant drug effect, $F(2, 38) = 3.31$, $p < 0.05$, largely as a result of a significantly lower number of recovered seeds in the scopolamine treatment relative to the other two. However, this difference just missed significance ($0.06 < p < 0.07$) in a post hoc Tukey's multiple means test.

Prerecovery administration of the drugs. Recovery scores were similarly compared among drug treatments when the drugs were administered to the birds prerecovery. The recov-

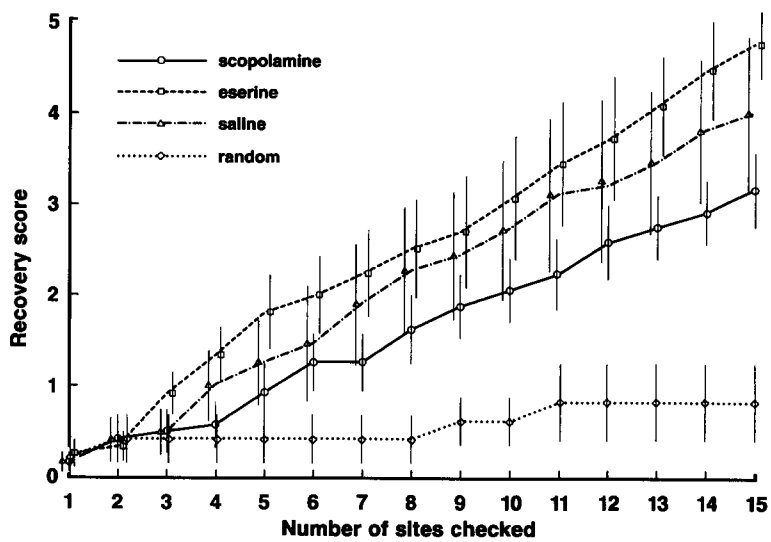

FIG. 2. Plot of mean recovery scores (with standard errors – $n = 5$) for the three drug treatments in birds injected prior to seed caching. See text for an explanation of the recovery score computed for the first 15 site checks. Recovery scores computed for the bird-adjusted probabilities of random find are also plotted.

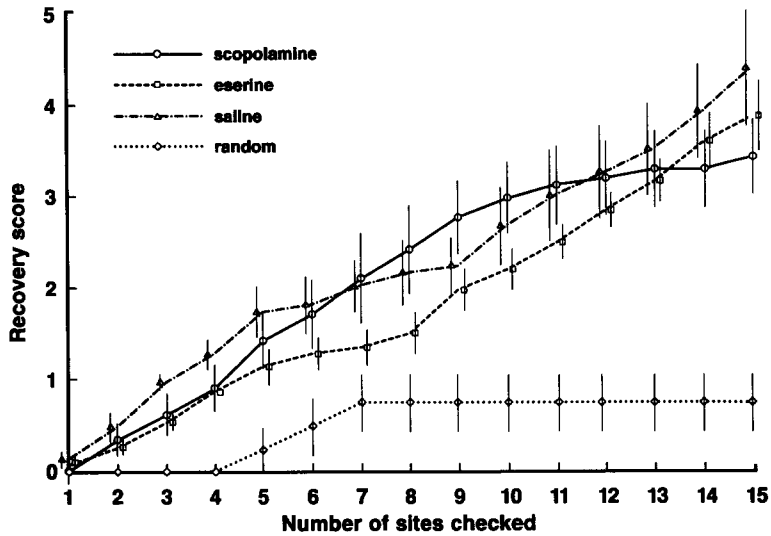

FIG. 3. Plot of mean recovery scores (with standard errors – $n = 6$) for the three drug treatments in birds injected prior to seed recovery. See text for an explanation of the recovery score computed for the first 15 site checks. Recovery scores computed for the bird-adjusted probabilities of random find are also plotted.

ery scores (and bird-adjusted probabilities of random find) computed for the first 15 site checks are plotted in Fig. 3.

Again, recovery scores after 15 site checks were found to be normally distributed with a Wilk-Shapiro stastic of 0.96. The ANOVA gave no indication of any statistically reliable drug effect on recovery scores after 15 site checks, $F(2, 46) = 1.43, p > 0.2$. The same was true for the alternate measure of recovery success: the number of seeds recovered in a fixed (5 min) time period after release into the arena. Mean numbers of recovered seeds were 3.1, 2.8, and 2.5 for physostigmine, saline, and scopolamine groups, respectively, $F(2, 46) = 1.01$, $p > 0.3$).

The ability of birds to avoid rechecking cache sites also did not show any statistically reliable treatment effect. Repeat scores averaged 11.8, 11.3, and 15.4 for the physostigmine, saline, and scopolamine groups, respectively, $F(2, 46) = 0.52$, $p > 0.5$.

Other Drug Effects

Precache administration of the drugs. Reduced hunger might decrease the motivation for accurately committing cache sites to memory. An analysis of food ingestion at the beginning of the caching period is given in Table 1.

Although birds, when injected with scopolamine, appeared to eat less than when injected with either saline or physostigmine, such a treatment effect just missed significance at the 0.05 probability level. Further examination of the data showed that this difference, if real, was not a result of birds injected with scopolamine dropping and abandoning partially eaten seeds but rather, a difference in the numbers of seeds totally consumed by the birds.

Two measures of subject motivation were analyzed (Table 1). Neither the latency to first cache nor the time elapsed between first and fifth cache showed any indication of a statis-

TABLE 1

TREATMENT EFFECTS OTHER THAN EFFECTS ON MEMORY WHEN DRUGS GIVEN PRECACHE

		Motivation:		Activity
Treatment	Hunger: Seeds Consumed	Latency to First Cache (min)	Interval First–Fifth Cache (min)	Rate of Visits in Same Interval (Visits/min)
Physostigmine	2.5	10.1	8.9	5.7
Saline control	2.5	10.6	6.0	5.8
Scopolamine	1.8	7.5	5.8	7.7
F value (drug)	3.02	1.39	1.59	2.03
df	2,38	2,38	2,38	2,38
p	0.061	0.262	0.218	0.145

TABLE 2

TREATMENT EFFECTS OTHER THAN EFFECTS ON MEMORY WHEN DRUGS GIVEN PRE-RECOVERY

	Motivation		Activity
Treatment	Latency to First Site Check (s)	Number of Sites Checked First 5 min	Number of Other Sites Visited in First 5 min
Physostigmine	4.2[a]	20.0	4.7[a]
Saline control	6.0[ab]	15.8	4.2[a]
Scopolamine	10.9[b]	17.9	21.6[b]
F value (drug)	3.60*	1.10	12.7‡
df	2,46	2,46	2,46
p	0.0354	0.340	<0.0001

*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

[ab]Values bearing different superscripts significantly different ($p < 0.05$) as determined by a Tukey's test.

tically valid treatment effect. Finally, the measure of overall activity, namely the rate of site visitation during seed caching, also failed to demonstrate a significant effect (Table 1).

Prerecovery administration of the drugs. Two measures of subject motivation were analyzed in this experiment (Table 2).

There was a statistically significant treatment effect on the latency of first site check. Birds injected with scopolamine showed a significantly longer latency than when injected with physostigmine, although neither treatment was significantly different from the saline controls. However, the total intensity of site checking computed over the first 5 min of the recovery period showed no statistically reliable drug influence.

Qualitative observations of subject behavior were that birds given scopolamine had a tendency to be hyperkinetic. This was borne out quantitatively by an analysis of the overall activity level measured as the number of site visits (excluding checks) during the same 5-min period. Birds given scopolamine changed location significantly more often (over 4.5 times more often on average) than when given either physostigmine or saline. In light of this observation, the somewhat longer latency to first site check in birds given scopolamine can most easily be explained by the general state of hyperactivity experienced by the birds. However, the more critical indicator of motivation, the total number of site checks, was not significantly affected by treatment.

DISCUSSION

The half-life of physostigmine is known to be short (41). However, the depression in brain cholinesterase levels resulting from intramuscular administration of physostigmine to chickadees was even more short lived than expected. Recovery was already underway 5 min after injection. In the first experiment when the drugs were administered before caching, the median time interval between injection of physostigmine and first seed cache was 10 min, and all cache sites were established within 39 min of drug injection. In the second experiment, the median time for first cache recovery was 15 min after physostigmine injections and all cache recoveries had taken place within 26 min of drug administration. We can, therefore, estimate that birds treated with physostigmine had their brain cholinesterase inhibited by 20–40% on average while being tested. We have no comparable measure for the birds treated with scopolamine.

A survey of the literature reveals considerable variation in the time interval between physostigmine administration and the onset of testing. Unfortunately, brain cholinesterase levels of test subjects are seldom reported. Test duration is usually not specified but experimenters commonly wait up to 30 min after an intraperitoneal or intramuscular injection before commencing the test (2,28). In at least one report (2), the combined pretest waiting period and test duration totalled 55 min. If the rapid recovery from brain cholinesterase depression documented here is typical of other species and other routes of administration, there is reason to believe that at least some of the conflicting results obtained by different experimenters (see below) may be due to tests being carried out under different effective drug conditions.

Birds administered scopolamine before caching demonstrated significantly impaired recall compared to birds given physostigmine. This was not unexpected in view of the evidence to date (see below). Key measures of subject motivation such as latency to first cache and total time needed to establish all cache sites were not significantly affected.

Beninger et al. (5) reviewed a number of animal studies that suggested that working memory (the memory for specific events associated with a trial) was more easily affected than reference memory (the memory for aspects of the task that do not vary from trial to trial) by a loss of cholinergic function (administration of cholinergic antagonists or lesions of cholinergic cell bodies). They reviewed evidence that suggested that impairments were not problems of sensory perceptual abilities, motivation, or performance unrelated to memory; including the finding that, at some doses, even difficult reference memory tasks show no impairment despite deficits in working memory tasks. Although scopolamine reliably produces impairments in these test systems, the effect of physostigmine appears to be bimodal, showing an inverted U function (1–3,8) with maximum facilitation generally recorded at intermediate concentrations. Physostigmine has been found to improve long-term memory storage of word lists in human subjects (10), whereas scopolamine has the opposite effect (27).

Our test system was different from the paradigms reviewed above where working memories needed to be elaborated and retrieved while the subject was under the influence of the drug. The only comparable measure in our work was the extent of repeat checking in birds injected prerecovery. To avoid checking sites more than once, the birds had to be able to commit to memory those sites previously visited. However, we did not observe any statistically reliable treatment effect for this measure.

An attempt to look for other test systems with a clear separation of the encoding and retrieval processes leads to a consideration of the many studies utilizing passive shock avoidance as a learning paradigm. Unfortunately, fear-motivated memories associated with these test situations are likely to differ substantially from working memories for spatial or delay matching tasks known to be very sensitive to cholinergic manipulation. Furthermore, there is the danger that drugs administered before the initial learning test will alter the subject's perception of the foot shock and, hence, the memory of it. At least one anticholinesterase insecticide has been shown to reduce the apparent response of rats to foot shock (37).

Despite these drawbacks, it can be seen that in passive avoidance, scopolamine has a reasonably consistent amnestic effect when given pretraining [(6,14,26); but see also (17,23)].

On the other hand, physostigmine administered pretraining consistently inhibits memory for the test conditions accross a wide range of doses (6,14). Similar results have been obtained for the acetylcholinesterase inhibitor galanthamine (41).

No statistically reliable effects from drug injection just prior to cache recovery were found in this study. In other studies, administration of cholinergically active substances prior to recall has been found to have varying effects, depending on the dose and the time between drug administration and recall. To explain these observations, a working model was proposed (12,13) whereby a memory trace is dependant on a certain level of cholinergic synaptic conductance that initially increases over time as the memory becomes more consolidated and then decreases as the memory starts to fade. In this model, a certain dose of anticholinesterase may scramble a strong memory trace by increasing synaptic conductance (through its effect of pooling of acetylcholine at the synapse) beyond the usual working level, whereas the same dose of anticholinesterase may not affect or may even enhance an old faded memory by raising a very weak synaptic conductance to within the retrievable range. Anticholinergic drugs would produce a mirror effect of the anticholinesterase. Several authors have obtained results consistent with this working model (39,40). Unfortunately, the level of cholinesterase depression that produces these deficits at recall in laboratory rodents has not been measured, so that comparison with our work is difficult.

Trying to clearly separate those studies dealing with memory acquisition from the ones assessing retrieval effects may, in the end, not be very realistic. Rusted and Warburton (27) recently suggested that postlearning scopolamine-induced amnesia, generally thought to result from an encoding or consolidation problem, may, in fact, be a result of impaired retrieval because the initial memories could be elicited after prompting. These authors hypothesized that memories may have achieved durable storage, but that there may have been input problems such that an orderly retrieval was difficult.

CONCLUSION

This study supports the hypothesis that the memory for cache site locations in the Black-capped Chickadee is, at least in part, under cholinergic control. The learning and/or memory process could be manipulated by the use of cholinergically active substances at least when they were administered prior to the birds establishing their caches. This finding shows that effects of cholinergic agents on cache recovery in chickadees are comparable to their effects in tests of working memory in mammals.

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