Pergamon

Environmental Toxicology and Chemistry, Vol. 13, No. 2, pp. 281–290, 1994

Printed in the USA
0730-7268/94 $6.00 + .00

98

EFFECTS OF FENITROTHION ON MEMORY FOR CACHE-SITE LOCATIONS IN BLACK-CAPPED CHICKADEES

PIERRE MINEAU,*† PETER T. BOAG‡ and RICHARD J. BENINGER§
†National Wildlife Research Centre, Canadian Wildlife Service, Ottawa, Ontario K1A 0H3 Canada,
‡Department of Biology, Queens University, Kingston, Ontario, K7L 3N6 Canada,
§Department of Psychology, Queens University, Kingston, Ontario, K7L 3N6 Canada

(*Received* 11 *January* 1993; *Accepted* 10 *June* 1993)

Abstract—The effect of fenitrothion on food caching in the black-capped chickadee was investigated. Oral doses of fenitrothion calculated to produce peak brain inhibition levels of 50 to 60% had no effect, or possibly even improved the ability of the birds to retrieve existing food-cache memories when administered after cache locations had been memorized. In the more extreme cases, birds were able to remember cache sites even when their physical abilities were still visibly impaired. The same dose of fenitrothion administered to the birds before they were allowed to cache improved cache-recovery performance. This result is consistent with results we obtained at lower brain ChE inhibition levels with physostigmine (reported elsewhere). It is inconsistent with studies that indicate that dosing with anti-ChEs typically gives rise to a U-shaped response with impairment at the higher dose levels.

Keywords—ChE inhibitor Fenitrothion Memory Food caching *Parus atricapillus*

INTRODUCTION

Understanding the extent to which the survival of birds sublethally exposed to ChE-inhibiting insecticides is compromised is an important step in determining the safety of these products in forestry and agriculture [1,2]. Evidence from human poisoning and from laboratory mammal studies [3–7] suggests that both acute and chronic exposure to anti-ChE agents can, among other effects, disrupt cognitive processes such as learning and memory. Gardner et al. [8] and McDonald et al. [9] have argued for a functional link between memory deficits and a reduced density of muscarinic receptors in animals chronically exposed to organophosphates. Effects on stimulus discrimination and reversal were seen in northern bobwhite (*Colinus virginianus*) subjected to dietary intakes of the organophosphates monocrotophos and fenthion, although the effects were inconsistent between the two compounds [10].

Much of the evidence linking acute exposure to anti-ChEs to memory deficits originates from the work of Deutsch and Rogers [11] and Deutsch [12]. They documented memory deficits in rats following intracerebral or intraperitoneal injections of the organophosphate DFP (diisopropyl fluorophosphate) and the carbamate physostigmine at varying times after training.

Despite ample evidence that birds are frequently exposed to anti-ChE agents under current forestry and agricultural practices [13–15], no attempts have been made to look at the possible impacts of acute organophosphate or carbamate exposure on learning or memory in birds under conditions typical of operational pest control programs.

A substantial amount of work on memory in birds has been achieved through the study of food-caching behavior in certain groups, notably the family Paridae (tits or chickadees) [16,17]. Caching behavior is highly repeatable and can be studied in a laboratory environment under controlled conditions. The memory for food caches in the black-capped chickadee (*Parus atricapillus*) has recently been found to be at least in part under cholinergic control (P. Mineau et al., unpublished manuscript). We used this natural behavior to study possible memory effects resulting from acute exposure to the organophosphate insecticide fenitrothion (*O,O*-dimethyl O-(4-nitro-*m*-tolyl) phosphorothioate). This insecticide is the main chemical insecticide currently used for forest insect control in Canada. In New Brunswick alone, the area sprayed with this insecticide in recent years has been as high as 1 to 2 million hectares per year [18]. A wide range of nontarget species, including large numbers of songbirds, are heavily exposed to this product. Although some work has been done on the expected mortality and gross impairment following spraying [18,19], little is known of the potentially more subtle cognitive impacts of sublethal exposure.

METHODS

Subjects

Chickadees were captured in the Ottawa area under permit from the Canadian Wildlife Service and were held for at least one month before any dosing or testing. All procedures involving the birds were authorized by approved animal care committees at Queens University (Kingston, Ontario) and at Health and Welfare Canada (Ottawa, Ontario).

The birds were provided with a standard diet comprising shelled sunflower seeds, raw peanuts, hard-boiled egg, grated carrot (certified pesticide-free produce), Hagen™ (Montreal, Quebec) mynah and soft-billed bird food, gravel, and cuttlebone. Before testing, sunflower seeds were removed from the standard diet and kept for the testing itself. Food was removed from the cages at irregular intervals in the hope of

*To whom correspondence may be addressed.

encouraging caching. Once a week, the standard diet was supplemented with live mealworms. The birds were provided ad libitum with tap water containing Hagen multivitamin drops. The water bowls also served for bathing.

Birds were housed in groups of 12 or fewer in cages measuring 137 × 71 × 61 cm with natural hardwood branches as perches. The light period went from 0700 to 1600 (first group—experiments 1 and 2) or 0900 to 1700 h (second group—experiment 3), and the temperature was held at a constant 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.

Insecticide and dosing procedures

Birds were dosed by inserting small-gauge surgical tubing, affixed to a high-precision 100-μl GC syringe, into the crop. The fenitrothion was dissolved in corn oil, and the solutions were chosen to provide a dose volume of 6 to 8 μl solution per gram bird. The fenitrothion was weighed in volumetric flasks to the nearest 0.0001 g. Birds dosed 48 h following caching or precache were first given three seeds to eat to approximate the status of the other experimental birds, which, because of the experimental design, were dosed after having fed.

Establishment of a dose–response curve

A standard curve was established to relate the level of brain ChE (primarily acetylcholinesterase, or AChE [20]) inhibition to a given dose of fenitrothion under conditions approximating those that would be in effect during the course of subsequent behavioral testing. Birds were fasted overnight but were provided with three to four sunflower seeds 30 min before being dosed. All dosing took place between 0950 and 1140 h over an 11-d period. Serial dosing on different days allowed initial range finding and reduced the overall number of birds. Following dosing, birds were returned to their cages, but food was withheld for 1 h longer. Four hours postdose, the birds were killed and their brains were removed for ChE determination.

Sample treatment

Excised brains were immediately frozen on dry ice and transferred to a freezer at −20°C for overnight storage. All brains were analyzed within 24 h of collection. ChE levels were obtained through the Ellman method as described by Hill and Fleming [21], with the exception that samples were homogenized in a solution of Triton X (three times the brain sample weight of a 1% solution) and centrifuged, and only the supernatants were assayed [22]. Levels are expressed as micromoles of hydrolyzed substrate per minute per gram brain tissue.

Apparatus

We used a test system similar to that initially developed by Sherry [23]. Nine hardwood trees 2.0 to 2.3 m in height were placed on movable stands in an arena that measured 6.1 × 3.0 × 2.9 m and received illumination from banks of overhead fluorescent bulbs (254 lx at 1.2 m from ground). Each tree was drilled with 10 cache sites (approximately 10 mm in diameter, 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 short piece of dowelling (diameter 0.5 cm) was positioned beneath each cache site such that birds would have to stretch upward to look into the cache sites. Natural cavities and other likely natural cache sites were plugged and eliminated wherever possible. Birds gained entry into the arena through a trap door (experiments 1 and 2) or were remotely released from a holding cage within the arena (experiment 3). Bouts were terminated by turning off the lights in the arena, and the birds were hand caught.

Training

Birds were given the opportunity to cache and recover seeds in the arena at least twice before actual testing. Birds to be trained were segregated and their food removed the previous evening. Between about 0900 and 1100, they were released one at a time into the arena. They were given 10 to 15 min to explore, after which a food bowl containing 15 shelled sunflower seeds was remotely uncovered. The birds were allowed 10 to 15 min after finding the bowl to eat and cache, after which they were removed from the arena and returned to their holding cages with food. Birds failing to cache after 30 min were removed and retested on a later day. The birds were again fasted starting about 1200 h and returned to the arena for cache recovery between about 1400 and 1500 h. Before each recovery test the seeds that had been cached were replaced by small seed fragments 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. Birds were deemed to have reached criterion when they consistently cached at least six seeds within the allotted 10-min period and demonstrated the ability to recover twice as many caches as by chance alone in the first 15 site checks.

Procedure

Three different experiments were carried out. In each, the procedure was similar to the training described above, with the following exceptions: first, before recovery, cached seeds were removed and not replaced by seed fragments to rule out any visual cues; second, in experiment 3 (precache dosing of the subjects) birds were allowed to cache only five seeds, after which the bout was terminated. In all cases, close visual inspection of the caches and/or probing were used to ascertain that a bird had indeed checked a cache site. Two observers scored the bouts, which were 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. Birds meeting criterion were randomly assigned to the treated or control groups. A measure of pre- and post-treatment performance was obtained for each individual by subjecting the birds to two full cache–recovery cycles separated by at least 1 d. Control birds were given corn oil only. Data were analyzed by a paired t test. Birds dosed with the insecticide were never reused in subsequent experiments. A few control birds were used in a sub-

sequent experiment after retraining and allowing at least one month between experiments.

Because the number of cached seeds (and therefore the probability of random find) varied between birds in experiments 1 and 2, an index of cache-recovery performance was developed to take this variable into consideration. In addition, in experiments 1 and 2, the initial behavior of the birds when released into the arena from the trap door was found to be erratic, with birds often starting to search cache sites in the vicinity of their first landing. A recovery score for experiments 1 and 2 was then calculated after discarding all site checks up to and including the first successful check. All checks were included in the recovery score calculated for experiment 3. In that experiment, the number of cache sites were restricted to five, and birds were released remotely from a holding cage within the arena, thus giving them the opportunity to orient properly before making the first site check.

To compute our recovery score, the probability that each successful check occurred at random was calculated on the basis of the number of correct and incorrect cache sites left to check. Each cache site was considered only once, and repeat checks were 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 15th site check, the probability of random find would be $(10/90)\cdot(9/86)\cdot(8/76) = 0.00122$. Our recovery score is expressed as the absolute value of the base 10 logarithm of this probability (i.e., 2.91). Therefore, for any 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 one, 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 everything else is equal. A late recovery may achieve a higher score than an early one only if there are fewer seeds left to find, depending on how many cache sites are left to check. The probability of a random find may be lower later 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, which was by paired *t* test, comparing the pre- and postmanipulation performance within each subject dosed either with corn oil alone or with the insecticide. None of the subjects in experiments 1 and 2 was able to find all correct cache sites within the 15 site checks.

For the results of experiment 1, we also plotted the mean bird-specific probabilities that cache sites would be found at random. These probabilities, again computed over the first 15 site checks, were determined by comparing the pre-cache-site checks of each individual against a choice of cache sites made by the same individual at least two tests earlier. The fact that cache sites are recovered at rates higher than chance has been well documented, even when individual preferences for cache-site locations are taken into consideration [16,17].

Yet another measure of memory is the ability of birds to avoid repeating visits to the same site during any given retrieval bout [23,24]. A repeat score was developed, which expressed the number of repeat checks over the total number of site checks performed. The total number of checks (the denominator in this index) was taken to be the smaller of the two numbers of checks obtained in the first 5 min. of either pre- or post-treatment site checking for any given bird, to avoid any bias associated with different checking intensities. The index is the arcsine-transformed proportion. The higher the index, the more repeat visiting to the same cache sites.

In experiments 1 and 2, an activity score was also computed by counting the total number of site checks within the first 5 min of cache recovery.

Experiment 1

The aim of this experiment was to look at the retrieval of memory traces that were either 5 min or 48 h old at the time of dosing. By dosing the first group of birds soon after cache-site selection (5 min after the caching period), we were able to look for any evidence of retrograde effects of the dose on very recent memories. The second group was dosed 48 h after being allowed to cache. Because we initially feared the possible confounding factor of generalized ChE depression on bird performance unrelated to memory, cache recovery was tested 48 h postdose, resulting in a total cache-recovery interval of either 48 or 96 h. Holmes and Boag [25] found that activity levels in zebra finches (*Taenopygia guttata*) dosed to a similar extent with fenitrothion had returned to normal between 24 and 48 h postdose. There were four birds per group, for a total of 16 birds tested in this experiment.

Experiment 2

In this experiment, birds were dosed immediately (5 min) after their caching bout but allowed to recover their caches 4 rather than 48 h later, as in experiment 1. At recovery, most of the birds were noticeably slower and more lethargic, and they had to be provided with more time to make the required 15 site checks. This experiment allowed for a test of retrieval of very recent memories under the highest possible level of brain ChE inhibition. There were five birds per group, for a total of 10 tested birds.

Experiment 3

The final experiment consisted of dosing the birds and subsequently allowing them to cache. Dosed birds were held in their holding cages inside the arena for a minimum of 30 min after dosing and then until they showed interest in a seed left in the cage or were thought capable of locomotion. The cage was then remotely opened and each bird allowed to begin caching. This procedure resulted in intervals between dosing and first cache ranging from 33 to 130 min, with a median

of 59 min. Paired control birds were held for the same amount of time in a holding cage elsewhere and were run immediately after the dosed bird. We attempted to hold the number of cache sites constant at five, but only four caches were obtained in two post-treatment runs, one from each of the dosed and control groups. Birds in this experiment were allowed to recover their caches 24 h after caching. There were five birds per group, for a total of 10 birds tested in this experiment.

RESULTS

Dose–response curve

Fourteen birds received doses of fenitrothion between 5 and 100 mg/kg to establish a dose–response curve for brain ChE levels (Fig. 1). Four controls were dosed with corn oil only. Two birds that were inadvertently killed during capture, as well as netting casualties obtained from a bird-banding club, were added to the control group because their brain values did not differ significantly from those of the birds dosed with corn oil. These additions resulted in control data available for 22 individuals. The mean control brain ChE activity was determined to be 37.4 μmol substrate hydrolyzed per minute per gram (SD 2.77, 95% C.I.s 36.2–38.6).

Based on these data, the dosage level for all experiments was set at 25 mg/kg. The mean inhibition level for the four birds given that dose was 65%. The predicted value of brain inhibition, based on the regression of brain ChE over the logarithm of the dose for the 14 dosed birds, was 63% inhibition with predicted 95% bounds of 30 to 96% inhibition. Observations made on 13 of the dosed birds suggested that the presence of pronounced muscular fasciculation, especially of the wing muscles (often accompanied by prostration), was indicative of an inhibition level >60%, regardless of the actual dose. Six of seven birds that were inhibited >60% exhibited pronounced muscular fasciculation, whereas six birds inhibited 60% or less did not.

Experiment 1

Sixteen birds were used in this experiment: four in each of the control and dosed groups for each of the two cache–dose intervals. Of the four dosed birds with a 5-min cache–dose interval, three had been prostrate and experienced muscle fasciculation, which, as discussed earlier, is indicative of brain AChE inhibition in the 60% range. However, of the five dosed birds with a 48-h cache–dose interval, only one showed these symptoms.

Activity levels, measured as the number of checks performed within the first 5 min of the test, were the same pre- and postdose, indicating that the 48-h interval between dose and recovery was sufficient for gross motor levels to return to normal (Table 1). No significant differences were seen between recovery scores or repeat scores at either the 5-min or the 48-h cache–dose interval (Table 1), although the recovery score just missed showing a significant improvement in the dosed birds with the 48-h cache–dose interval ($P = 0.066$). Plots of the recovery scores computed for the first 15 site checks for the two cache–dose intervals are shown in Figure 2.

Experiment 2

Five birds were used in each of the control and treated groups. Of the five treated birds, four had been exhibiting muscle fasciculation indicative of an inhibition level in the 60% range. At cache recovery, 4 h postdose, treated birds were still measurably affected by the insecticide, as evidenced by a significantly lower activity score (Table 2). Birds appeared slow and more lethargic, and some had balance problems best seen when landing on perches.

However, despite the obvious deficits in their physical abilities, there was no indication of an insecticide effect, as measured by either recovery or repeat scores (Table 2). The recovery scores computed for the first 15 site checks are plotted in Figure 3.

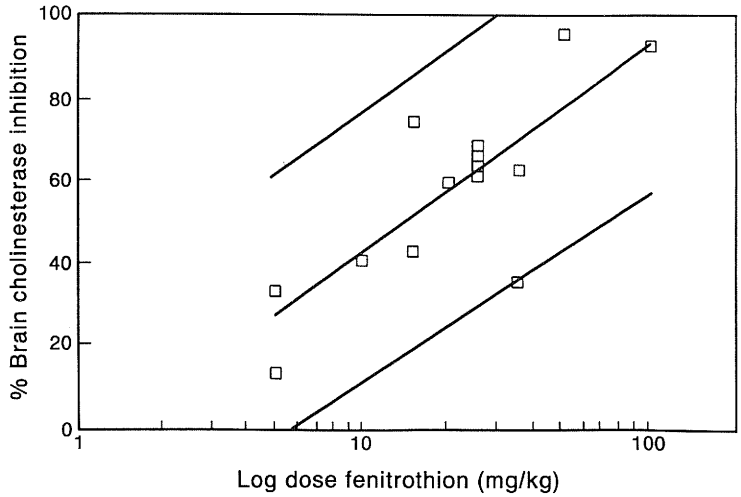
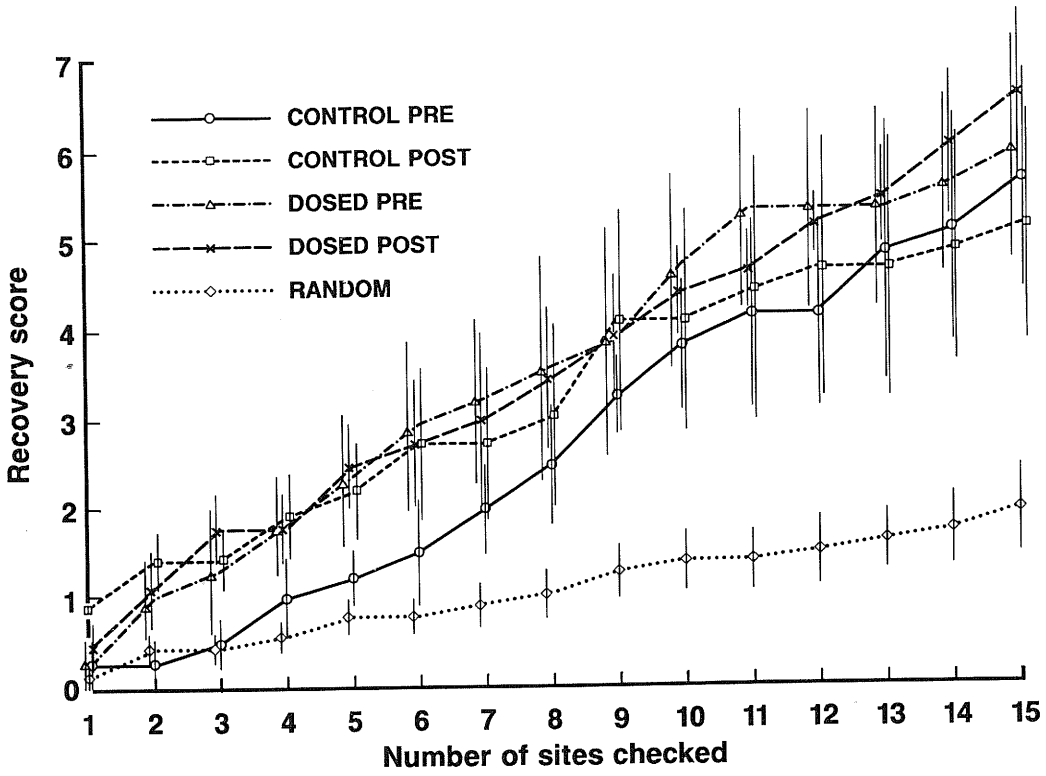
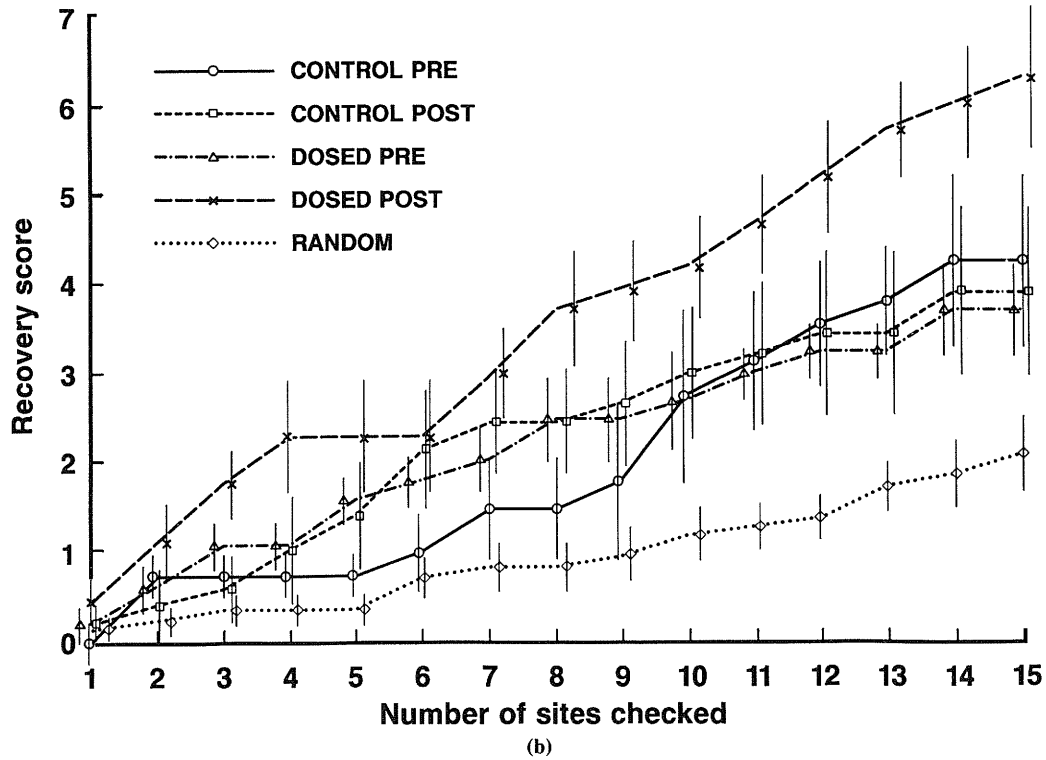


Fig. 1. Log-normal regression of percentage of brain ChE inhibition (and 95% prediction intervals) in the black-capped chickadee against dosage of technical-grade fenitrothion in mg/kg given orally in corn oil. Regression statistics: $F = 21.19$, $R^2 = 0.64$, $P < 0.0006$.



(a)



(b)

Fig. 2. Plot of mean recovery scores (with SES; $N = 4$) for birds dosed with fenitrothion 5 min **(a)** and 48 h **(b)** after seed caching, when seed retrieval was 48 h after exposure to the insecticide. Recovery scores, given the probability of random find, are also shown.

Table 1. Recovery statistics for experiment 1

	5-min cache–dose interval			48-h cache–dose interval		
	Activity level[a]	Recovery score[b]	Repeat score[c]	Activity level	Recovery score	Repeat score
Control pretreatment	26.5 (5.0)	5.61 (1.2)	23.1 (5.3)	47.5 (5.4)	4.25 (0.97)	32.6 (0.99)
P (level (paired *t*)	0.50	0.76	1.0	0.92	0.84	0.087
Control post-treatment	36.0 (9.6)	5.11 (1.3)	23.1 (4.2)	48.5 (14.0)	3.89 (1.1)	26.6 (2.1)
Dosed pretreatment	36.5 (7.6)	5.98 (1.2)	20.2 (8.6)	69.0 (13.0)	3.70 (0.51)	24.6 (3.8)
P level (paired *t*)	0.94	0.68	0.54	0.098	0.066	0.25
Dosed post-treatment	37.2 (6.9)	6.58 (0.91)	24.0 (3.7)	53.5 (10.7)	6.31 (0.80)	30.7 (4.5)

Birds were dosed either 5 min or 48 h postcache and tested 48 h postdose ($N = 4$ birds per group). All birds were tested twice, pre- and post-treatment (treatment = corn oil or insecticide dose). All values expressed as means and SEs.
[a]Number of checks performed in the first 5 min of cache recovery.
[b]Log of the absolute value of the probability that successful checks occurred by chance over the first 15 checks.
[c]Arcsine-transformed proportion of repeat checks in degrees.

Experiment 3

Of the five birds dosed in this experiment, only one showed the heavy muscular fasciculation thought to be indicative of brain ChE inhibition in the 60% range. Yet, even that individual was able to cache seeds just over 2 h after dosing. Birds that had cached their seeds after being dosed with fenitrothion showed a significant enhancement in their recovery scores, measured 24 h later, when compared to their own performance predose (paired t dosed birds = 6.11, $P = 0.0036$; for control [corn oil] birds, paired $t = -1.11$, $P = 0.3295$). The recovery scores computed for the first 15 site checks are plotted in Figure 4. Moreover, there was no indication of any effect of dosing on the repeat visitation rates of subjects (data not shown).

DISCUSSION

It is not unusual for songbirds collected after forestry applications of fenitrothion to register levels of brain ChE depression in the 50 to 60% range. For one spray in Newfoundland, 55% of the birds collected after an application of 210 g a.i./ha were inhibited by more than 50% [18]. The 50% level of inhibition is loosely associated with a plethora of physiological effects and mortality [1]. In our experiments,

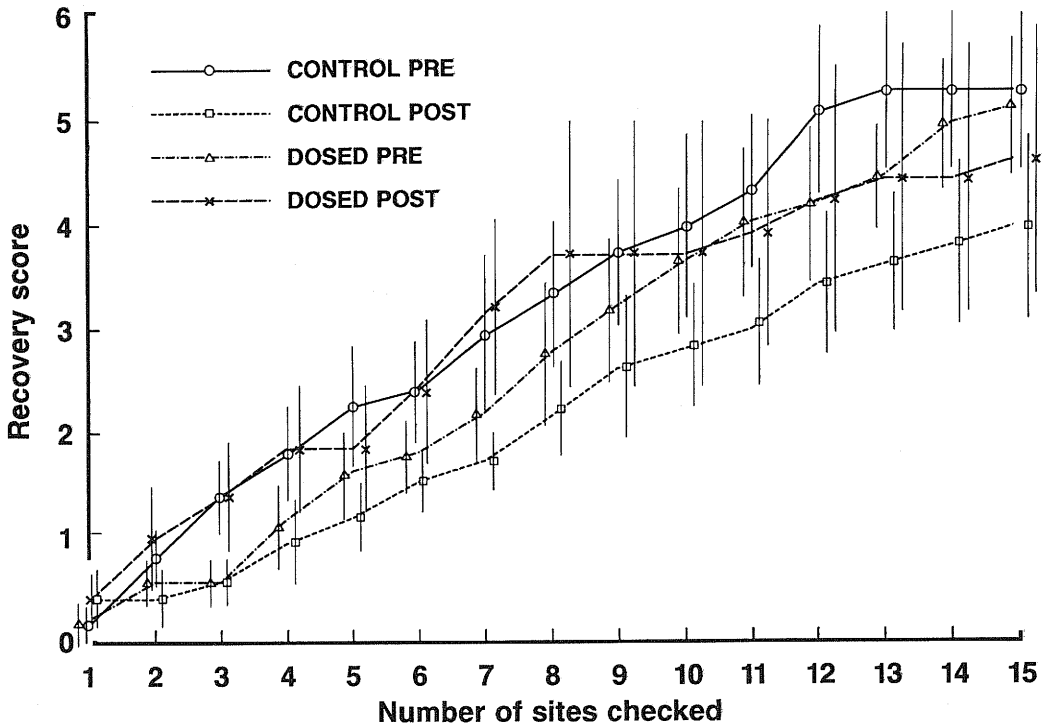


Fig. 3. Plot of mean recovery scores (with SEs; $N = 5$) for birds dosed with fenitrothion 5 min after seed caching, when seed retrieval was 4 h later.

Table 2. Recovery statistics for experiment 2

	Activity level[a]	Recovery score[b]	Repeat score[c]
Control pretreatment	55.2 (14.9)	5.25 (0.72)	31.6 (1.72)
P level (paired *t*)	0.29	0.43	0.48
Control post-treatment	42.2 (8.39)	3.99 (0.91)	28.4 (4.11)
Dosed pretreatment	74.0 (15.4)	5.13 (0.66)	17.4 (7.13)
P level (paired *t*)	0.033*	0.52	0.29
Dosed post-treatment	23.0 (12.7)	4.62 (1.29)	14.1 (6.59)

Birds were dosed 5 min postcache and tested 4 h postdose ($N = 5$ birds per group). All birds were tested twice, pre- and post-treatment (treatment = corn oil or insecticide dose). All values expressed as means and SES.

[a]Number of checks performed in the first 5 min of cache recovery.

[b]Log of the absolute value of the probability that successful checks occurred by chance over the first 15 checks.

[c]Arcsine-transformed proportion of repeat checks in degrees.

*Denotes statistical significance in the difference.

we dosed birds at a level commensurate with the exposure of songbirds in the wild. To dose the birds any higher would not have been justified because individuals dosed beyond this level of inhibition are unlikely to survive in the wild, regardless of any effects of the insecticide on learning or memory.

Deutsch and Rogers [11] and Deutsch [12] looked at the effect of intracerebral or intraperitoneal injections in rats of the organophosphate DFP and the carbamate physostigmine at varying times after training, ranging from 30 min to three weeks. Their results led them to propose a model whereby a memory trace was dependent on a certain level of cholinergic synaptic conductance that initially increased over time, as the memory became more consolidated, then decreased as the memory started to fade. In this model, a certain dose of anti-ChE may "scramble" a strong memory trace by increasing cholinergic synaptic conductance (through its effect of pooling of acetylcholine at the synaptic conductance (through its effect of pooling of acetylcholine at the synapse) beyond the usual working level, whereas the same dose of anti-ChE may not affect, or may even enhance, an old, faded memory by raising a very weak synaptic conductance to within the "retrievable" range. Results consistent with this hypothesis were obtained by Squire et al. [26] and Stanes et al. [27]. Unfortunately, the level of ChE depression that produces these deficits at recall in laboratory rodents has not been measured, so that comparison with our work is difficult. Given that Deutsch and colleagues used an appetitive task to demonstrate memory deficits, it is unlikely that they were inducing a level of ChE inhibition that was any higher than the level of inhibition we used in our experiments.

Without any prior knowledge of the natural rate of forgetting in black-capped chickadees, we opted in experiment 1 to test retrieval for memories that were either 48 or 96 h old under moderate cholinergic stimulation (48 h postdose). There was some indication ($P = 0.066$) of an improved recovery performance in dosed birds for cache-site memories

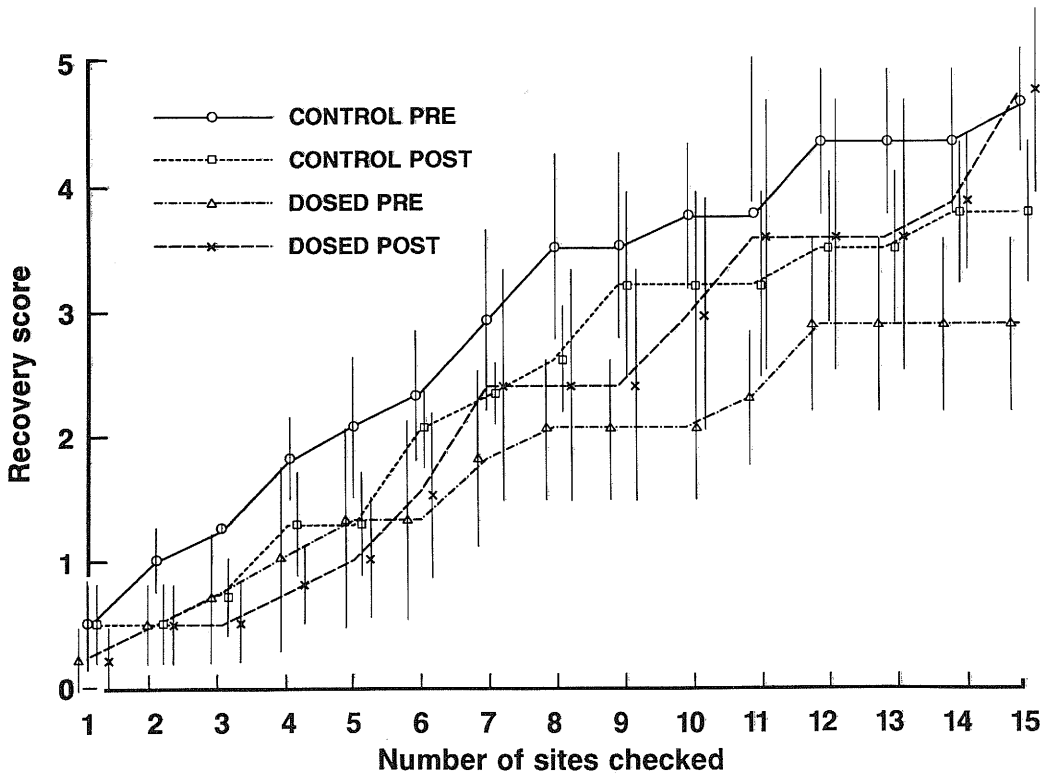


Fig. 4. Plot of mean recovery scores (with SES; $N = 5$) for birds dosed with fenitrothion before seed caching.

that were 96 h old, but no such indication for memories that were 48 h old. Consistent with the theory of Deutsch and colleagues, a prediction of this finding, if real, would be that a certain amount of forgetting of cache-site location had taken place between 48 and 96 h after caching. A post hoc analysis of our data showed this to be the case. For this analysis, all results from pretreatment trials (totally unmanipulated birds) in experiments 1 and 2 were divided into two groups: those that had a cache to recovery period of 48 h or less vs. those for which the recovery period was 96 h. Four birds from experiment 2 that had been run in experiment 1 were removed from the sample so that recovery scores after 15 checks could be compared by regular t test for independent samples. The recovery score after 96 h was significantly lower than that after 48 h or less ($t = 2.4$, $d.f. = 20$, $P = 0.026$). These data are illustrated in Figure 5.

Working with a similar system, Hitchcock and Sherry [28] found a strong trend for a drop in recovery performance between 1 and 14 d postcache, and a definite indication of such a decline between 1 and 28 d postcache. Data on the seed-caching behavior of the closely related marsh tits (*Parus palustris*) in a British woodlot [29] showed that most cache retrievals were within the first 12 h of daylight after storage. Cache sites were seldom visited after 3 d, suggesting the lack of a strong selective pressure for remembering cache sites for more than a few days. Our results are consistent with a short life expectancy of cache sites.

Experiments 1 and 2 also did not provide any evidence of retrograde amnesia, which has been reported for anti-ChEs by some authors [30–33]. Flood and colleagues [33] were able to show that all tested cholinergic agonists (including the anti-ChE physostigmine) produced a U-shaped response, with the higher doses causing long-lasting memory impairments when injected intraventricularly 3 min after training. However, other studies have failed to demonstrate effects of anti-ChEs on the memory consolidation process [34–36].

Experiment 2 was intended to test effects of more pronounced ChE inhibition on memory recall. Retrieval of the caches 48 h postdose in experiment 1 had allowed for some recovery of brain ChE. Holmes and Boag [37] found that zebra finches dosed with fenitrothion to a peak brain inhibition level of 70% at 3 h postdose had recovered to a mean inhibition level of 33% at 48 h postdose. In experiment 2, the shorter interval between dose and cache retrieval allowed birds to be tested at close to peak ChE depression. Consistent with the working hypothesis of Deutsch and colleagues [11,12], testing birds so soon after dosing was expected to raise cholinergic function beyond its usual working limit, thereby causing a deficit in retrieval. However, there was no indication of any such deficit.

Experiment 3 had the birds cache seeds while under the influence of the insecticide. The first unexpected finding was that the birds were able and motivated to cache so soon after dosing. This observation underscores the strength of the food-caching behavior in this species. Furthermore, casual observation of the chickadees after dosing revealed that they recovered extremely rapidly from acute fenitrothion toxicosis, at least relative to the zebra finch, a species studied by

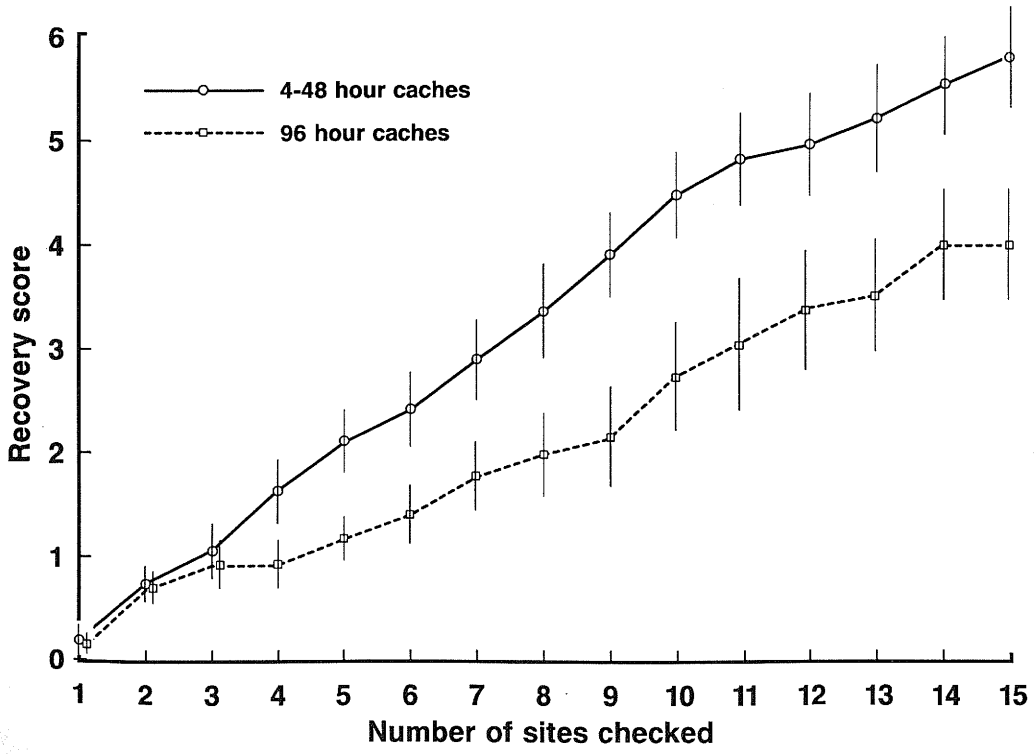


Fig. 5. Plot of mean recovery scores (with SES), computed for the first 15 site checks, for unmanipulated birds, when the cache–retrieval interval was either 96 ($N = 8$) or ≤48 h ($N = 14$).

the senior author [38]. As was the case for the anti-ChE drug physostigmine (P. Mineau et al., unpublished manuscript), dosing with the organophosphate fenitrothion, even at the relatively high level tested here, resulted in enhanced recovery scores 24 h later. It is highly unlikely that this effect resulted from the influence of the insecticide at recall, given the lack of any such effect at either 4 or 48 h in experiments 1 and 2. Therefore, it is likely that the enhanced recovery scores in dosed birds resulted from an improved acquisition or encoding of the original memories for the food caches.

Several authors claim to have shown a reduction in the ability of laboratory rodents to learn a passive avoidance test (learning to avoid entering a favored section of the test environment) when previously dosed with a ChE inhibitor, especially at a high dose [34,35,39,40]. Peak brain AChE depression of 45 to 70% was measured in one of those experiments [35]. However, all of these studies used footshock as punishment, and results should be viewed with caution because at least one anti-ChE insecticide has been shown to reduce the apparent sensitivity of rats to footshocks [41]. Kreitzer and Fleming [10] demonstrated some effects of organophosphates administered in a chronic fashion on the extinction of a behavioral response. A possible parallel measure in our work was the extent of repeat checking of cache sites. No significant effects were seen under conditions of acute dosing.

CONCLUSIONS

Our experiments failed to demonstrate any of a number of possible deleterious effects of anti-ChE administration on learning or recall. Although we tested only a single dose level, it was considered high enough to represent a worst-case exposure. Studies on laboratory rodents have shown learning impairments with doses of anti-ChE below those that cause any measurable deficit in activity level [42].

Reasons for the apparent robustness of cache-location memories in the black-capped chickadee can only be speculated at this time. An explanation of our results consistent with the Deutsch model of cholinergic strength [11,12] is that the individual memory trace for each cache location may depend on such a low level of cholinergic activity that it remains unaffected by administration of the insecticide, even at a high dose. Alternatively, it has been suggested by others that the amnestic effect of anti-ChEs may result from a lowering of the signal-to-noise ratio in the specific memory traces following the general excitation of all cholinergic synapses [42]. If this is the case, the memory traces for food caches in the black-capped chickadee can be said to exhibit an excellent signal-to-noise ratio.

Our results inevitably raise questions about the general applicability of our findings to learning and memory. In wanting to test the effects of pesticides on a paradigm that is obviously relevant to the welfare and survival of a wild species, could we have unwittingly chosen a system that is totally different from other learning and memory processes? The evidence to date favors the view that the memory for food caches, at least in the family Paridae, is not *qualitatively* different from other types of spatial memory [17]. Results from the experiments presented here, as well as the results of similar work with the cholinergically active drugs scopolamine and physostigmine (P. Mineau et al., unpublished manuscript), point to a cholinergic involvement in the memory for food caches. This is also the case with a broad range of both working and reference memory tasks (but especially the former) in primates, rodents, and pigeons [43]. In addition, as demonstrated for spatial and working memory tasks in mammals, the hippocampal complex is important for the memory for food caches in food-storing birds. This finding was shown both through lesioning [44] and through anatomical differences in hippocampal size between storing and nonstoring species [45]. These anatomical differences do argue for at least a quantitative difference between memory for food caches and other types of memory. Whether the memory system for food-cache location is more or less easily perturbed than other memory types by an acute exposure to an anti-ChE insecticide is not known.

Our results did not raise any new concerns with respect to the exposure of birds to anti-ChE insecticides. At doses that were close to lethality, black-capped chickadees were able to resume feeding and successful food caching quickly, even while experiencing poor coordination and other physiological deficits. Our limited observations of a few species appear to evidence large interspecies differences in abilities of birds to recover from severe exposure to anti-ChE agents. This finding raises questions of how well we can predict impacts from exposure to organophosphates and carbamates in the wild and, more specifically, how well we can extrapolate from behavioral deficits measured in artificially constructed paradigms to real-life adaptive behaviors subject to intense selection pressure.

Acknowledgement—Funding was obtained from the World Wildlife Fund and Environment Canada through the Wildlife Toxicology Fund. The senior author thanks the Natural Science and Engineering Research Council of Canada for support while on leave from the Canadian Wildlife Service. Special thanks go to Janette Dean and Jacquie Oblak, who were instrumental in this research. This paper is dedicated to Janette, who died of cancer on May 6, 1993. The following individuals also lent support: Dr. Pierre Thibert, Bill Pearce, Stewart Leich, and other staff of the Animal Resource Division of Health and Welfare Canada; Suzanne Trudeau, Ghiselaine Sans-Cartier, and Brian Collins of the Canadian Wildlife Service; Gord McKinnon and Gary Thomas of Environment Canada; Dr. David Sherry of the University of Western Ontario; and Christine Hitchcock, formerly in the Psychology Department at the University of Toronto.

REFERENCES

1. **Grue, C.E., A.D.M. Hart** and **P. Mineau.** 1991. Biological consequences of depressed brain cholinesterase activity in wildlife. In P. Mineau, ed., *Cholinesterase-Inhibiting Insecticides. Their Impact on Wildlife and the Environment*. Elsevier, Amsterdam, The Netherlands, pp. 151–210.
2. **Mineau, P.** 1991. Difficulties in the regulatory assessment of cholinesterase-inhibiting insecticides. In P. Mineau, ed., *Cholinesterase-Inhibiting Insecticides. Their Impact on Wildlife and the Environment*. Elsevier, Amsterdam, The Netherlands, pp. 277–299.
3. **Namba, T., C.T. Nolte, J. Jackrel** and **D. Grab.** 1971. Poisoning due to organophosphate insecticides. Acute and chronic manifestations. *Am. J. Med.* **50**:475–492.
4. **Levin, H.S.** and **R.L. Rodnitsky.** 1976. Behavioral effects of organophosphate pesticides in man. *Clin. Toxicol.* **9**:391–405.

5. **Russell, R.W.** 1982. Cholinergic system in behavior: The search for mechanisms of action. *Ann. Rev. Pharmacol. Toxicol.* **22**:435–463.
6. **Russell, R.W.** and **D.H. Overstreet.** 1987. Mechanisms underlying sensitivity to organophosphorus anticholinesterase compounds. *Prog. Neurobiol.* **28**:97–129.
7. **Rosenstock, L., W. Daniell, S. Barnhart, D. Schwartz** and **P.A. Demers.** 1990. Chronic neuropsychological sequelae of occupational exposure to organophosphate insecticides. *Am. J. Ind. Med.* **18**:321–325.
8. **Gardner, R., R. Ray, J. Frankenheim, K. Wallace, M. Loss** and **R. Robichond.** 1984. A possible mechanism for diisopropyl fluorophosphate-induced memory loss in rats. *Pharmacol. Biochem. Behav.* **21**:43–46.
9. **McDonald, B.E., L.G. Costa** and **S.D. Murphy.** 1988. Spatial memory impairment and central muscarinic receptor loss following prolonged treatment with organophosphates. *Toxicol. Lett.* **40**:47–56.
10. **Kreitzer, J.F.** and **W.J. Fleming.** 1988. Effects of monocrotophos and fenthion on discrimination acquisition and reversal in northern bobwhite (*Colinus virginianus*). *Environ. Toxicol. Chem.* **7**:237–240.
11. **Deutsch, J.A.** and **J.B. Rogers.** 1979. Cholinergic excitability and memory: Animal studies and their clinical implications. In K.L. Davis and P.A. Berger, eds., *Brain Acetylcholine and Neuropsychiatric Disease.* Plenum, New York, NY, pp. 175–204.
12. **Deutsch, J.A.** 1983. The cholinergic synapse and the site of memory. In J.A. Deutsch, ed., *The Physiological Basis of Memory*, 2nd ed. Academic, New York, NY, pp. 367–386.
13. **Mineau, P.** and **D.B. Peakall.** 1987. An evaluation of avian impact assessment techniques following broad-scale forest insecticide sprays. *Environ. Toxicol. Chem.* **16**:781–791.
14. **Mineau, P.** 1988. Avian mortality in agro-ecosystems. 1. The case against granular insecticides in Canada. In M.P. Greaves, B.D. Smith and P.W. Greig-Smith, eds., *Field Methods for the Study of Environmental Effects of Pesticides. Brit. Crop Prot. Counc. Monogr.* **40**:3–12.
15. **Greig-Smith, P.W.** 1991. Use of cholinesterase measurements in surveillance of wildlife poisoning in farmland. In P. Mineau, ed., *Cholinesterase-Inhibiting Insecticides. Their Impact on Wildlife and the Environment.* Elsevier, Amsterdam, The Netherlands, pp. 127–150.
16. **Sherry, D.F.** 1985. Food storage by birds and mammals. *Adv. Stud. Behav.* **15**:153–188.
17. **Shettleworth, S.J.** 1990. Spatial memory in food-storing birds. *Philos. Trans. R. Soc. Lond. Ser. B* **329**:143–151.
18. **Busby, D.G., L.M. White, P.A. Pearce** and **P. Mineau.** 1989. Fenitrothion effects on forest songbirds: A critical new look. In W.R. Ernst, P.A. Pearce and T.L. Pollock, eds., *Environmental Effects of Fenitrothion Use in Forestry. Impacts on Insect Pollinators, Songbirds and Aquatic Organisms.* Environment Canada, Dartmouth, Nova Scotia, pp. 43–108.
19. **Busby, D.G.** and **L.M. White.** 1991. Factors influencing variability in brain acetylcholinesterase activity in songbirds exposed to aerial fenitrothion spraying. In P. Mineau, ed., *Cholinesterase-Inhibiting Insecticides. Their Impact on Wildlife and the Environment.* Elsevier, Amsterdam, The Netherlands, pp. 211–232.
20. **Walker, C.H.** and **H.M. Thompson.** 1991. Phylogenetic distribution of cholinesterases and related esterases. In P. Mineau, ed., *Cholinesterase-Inhibiting Insecticides. Their Impact on Wildlife and the Environment.* Elsevier, Amsterdam, The Netherlands, pp. 1–18.
21. **Hill, E.F.** and **W.J. Fleming.** 1982. Anticholinesterase poisoning of birds: Field monitoring and diagnosis of acute poisoning. *Environ. Toxicol. Chem.* **1**:27–38.
22. **Fairbrother, A., B.T. Marden, J.K. Bennett** and **M.J. Hooper.** 1991. Methods used in determination of cholinesterase activity. In P. Mineau, ed., *Cholinesterase-Inhibiting Insecticides. Their Impact on Wildlife and the Environment.* Elsevier, Amsterdam, The Netherlands, pp. 35–72.
23. **Sherry, D.F.** 1984. Food storage by black-capped chickadees: Memory for the location and contents of caches. *Anim. Behav.* **32**:451–464.
24. **Sherry, D.F.** 1982. Food storage, memory and marsh tits. *Anim. Behav.* **30**:631–633.
25. **Holmes, S.B.** and **P.T. Boag.** 1990. Effects of the organophosphorus pesticide fenitrothion on behavior and reproduction in zebra finches. *Environ. Res.* **53**:62–75.
26. **Squire, L.R., S.D. Glick** and **J. Goldfarb.** 1971. Relearning at different times after training as affected by centrally and peripherally acting cholinergic drugs in the mouse. *J. Comp. Physiol. Psychol.* **74**:41–45.
27. **Stanes, M.D., C.P. Brown** and **G. Singer.** 1976. Effects of physostigmine on Y-maze discrimination retention in the rat. *Psychopharmacologia* **46**:269–276.
28. **Hitchcock, D.L.** and **D.F. Sherry.** 1990. Long-term memory for cache sites in the black-capped chickadee. *Anim. Behav.* **40**: 701–712.
29. **Stevens, T.A.** and **J.R. Krebs.** 1986. Retrieval of stored seeds by marsh tits *Parus palustris* in the field. *Ibis* **128**:513–525.
30. **Stratton, L.O.** and **L. Petrinovitch.** 1963. Post-trial injections of an anticholinesterase drug and maze learning in two strains of rats. *Psychopharmacologia* **5**:47–54.
31. **Miller, L., W.G. Drew** and **D.F. McCoy.** 1971. Effects of post-trial injections of scopolamine and eserine on acquisition of a simultaneous brightness discrimination. *Psychol. Rep.* **29**:1147–1152.
32. **Illyutchenok, R.Y.** and **A.G. Yeliseyeva.** 1972. Cholinergic mechanisms of memory: Analysis of the amnestic effects of anticholinergic drugs. *Int. J. Psychobiol.* **2**:177–192.
33. **Flood, J.F., D.W. Landry** and **M.E. Jarvik.** 1981. Cholinergic receptor interactions and their effects on long-term memory processing. *Brain Res.* **215**:177–185.
34. **Dilts, S.L.** and **C.A. Berry.** 1967. Effect of cholinergic drugs on passive avoidance in the mouse. *J. Pharmacol. Exp. Ther.* **158**:279–285.
35. **Reiter, L.W., G. Talens** and **D. Woolley.** 1973. Acute and subacute parathion treatment: Effects on cholinesterase activities and learning in mice. *Toxicol. Appl. Pharmacol.* **25**:582–588.
36. **Cox, T.** and **N. Tye.** 1974. Effects of physostigmine on the maintenance of discrimination behavior in rats. *Neuropharmacology* **13**:205–210.
37. **Holmes, S.B.** and **P.T. Boag.** 1990. Inhibition of brain and plasma cholinesterase activity in zebra finches orally dosed with fenitrothion. *Environ. Toxicol. Chem.* **9**:323–334.
38. **Mineau, P., K.M.S. Sundaram, A. Sundaram, C. Feng, D. Busby** and **P.A. Pearce.** 1990. An improved method to study the impact of pesticide sprays on small song birds. *J. Environ. Sci. Health* **B25**:105–135.
39. **Bures, J., Z. Bohdanecky** and **T. Weiss.** 1962. Physostigmine induced hippocampal theta activity and learning in rats. *Psychopharmacologia* **3**:254–263.
40. **Bohdanecky, Z.** and **M.E. Jarvik.** 1967. Impairment of one-trial passive avoidance learning in mice by scopolamine, scopolamine methylbromide, and physostigmine. *Int. J. Neuropharmacol.* **6**:217–222.
41. **Sideroff, S.I.** and **J.A. Santolucito.** 1972. Behavioral and physiological effects of the cholinesterase inhibitor carbaryl (1-naphthylmethyl carbamate). *Physiol. Behav.* **9**:459–462.
42. **Sweeney, J.E., E.S. Bachman** and **J.T. Coyle.** 1990. Effects of different doses of galanthamine, a long-acting acetylcholinesterase inhibitor, on memory in mice. *Psychopharmacology* **102**:539–560.
43. **Beninger, R.J., B.A. Wirsching, K. Jhamandas** and **R.J. Bogman.** 1989. Animal studies of brain acetylcholine and memory. *Arch. Gerontol. Geriatr.* Suppl. **1**:71–90.
44. **Sherry, D.F.** and **A.L. Vaccarino.** 1989. Hippocampus and memory for food caches in black-capped chickadees. *Behav. Neurosci.* **103**:308–318.
45. **Sherry, D.F., A.L. Vaccarino, K. Buckenham** and **R. Herz.** 1988. The hippocampal complex of food-storing birds. *Brain Behav. Evol.* **34**:308–317.