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Consolidation and Reconsolidation of Memory in Black-Capped Chickadees (*Poecile atricapillus*)

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Multiple phases of protein synthesis are necessary for the synaptic modifications that consolidate long-term memory. The reconsolidation hypothesis supposes that information in long-term memory becomes labile and subject to change when retrieved and must be reconsolidated into long-term memory. The current study used the protein synthesis inhibitor anisomycin to examine memory consolidation in birds and to test the reconsolidation hypothesis. Black-capped chickadees store food and usually remember which of their caches they have emptied and which they have left full. In Experiment 1, anisomycin was injected either immediately and 2 hr after food caching, or 4 and 6 hr after food caching. Inhibition of protein synthesis impaired memory for cache sites 24 and 48 hr later. In Experiment 2, it was hypothesized that long-term memory for food caches becomes labile as predicted by the reconsolidation hypothesis when birds search for caches. Anisomycin was administered immediately after chickadees had searched for their caches. Inhibition of protein synthesis should disrupt memory for caches left full if these sites are retrieved from long-term memory and require reconsolidation. Control birds were later more likely to revisit full caches than caches they had emptied. Birds given anisomycin revisited both kinds of caches and did not distinguish between them. This result shows that reconsolidation of full caches into long-term memory is not necessary following search for cache sites, but also shows that protein synthesis-dependent consolidation is required for updating the status of emptied caches.

Keywords: memory consolidation, reconsolidation, protein synthesis, anisomycin, Black-capped chickadee

The neural basis of memory is one of the fundamental problems in neuroscience. The mechanisms of memory have been the focus of research for over a century, and our understanding of memory has evolved dramatically (Nader, 2003). A little over a century ago, the consolidation hypothesis of memory formation was proposed (Müller & Pilzecker, 1900). Proponents of modern consolidation theory subscribe to the view that memory formation begins with new information encoded initially in an unstable labile state that involves ongoing neural transmission. Such short-term memory (STM) lasts for seconds to hours. As time progresses, memory becomes stabilized and protected from alterations in synaptic transmission by more stable modifications of synaptic architecture (McGaugh, 1966). In this way, information is transformed into a fixed or consolidated state stored in long-term memory (Miller & Matzel, 2000). Only when memory is labile and dependent on ongoing neural transmission is it susceptible to disruption. Furthermore, the severity of such disruption is inversely proportional to how long memory has been in transition from a short-term to a long-term state. This temporal gradient is taken as evidence for a stabilizing consolidation process.

Memory can also become labile, however, when reactivated as shown by Misanin, Miller, and Lewis (1968); Pryzbyslawski and Sara (1997); and Nader, Schafe, and Le Doux (2000a). Nader et al. (2000a) proposed a novel reconsolidation hypothesis; specifically, that retrieval of a stable consolidated memory returns that memory to an active labile state that requires new protein synthesis for restorage. They used an auditory fear-conditioning paradigm in which a footshock ultrasound (US) was associated with a tone conditioned stimulus (CS). Following learning, the protein synthesis inhibitor anisomycin was injected into the lateral and basal amygdala, areas implicated in fear conditioning (Davis, 1997). There were two important findings. The first was that infusions of anisomycin shortly after initial training prevented consolidation of new memories. Second, when the memory trace was reactivated, administration of anisomycin shortly after reactivation also produced amnesia for the tone-shock association. The observed deficits did not recover and were interpreted as permanent amnesia. Moreover, when anisomycin was administered in the absence of reactivation, memory remained intact and performance was unimpaired. This suggests that when reactivated, consolidated memories could return to a labile state susceptible to disruption.

Nader et al. (2000a) investigated the time course of protein synthesis in reconsolidation. Previous studies had demonstrated that protein synthesis inhibition could only disrupt consolidation of

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new memories during a restricted time window shortly after learning. Protein synthesis inhibition outside this time window had no amnesic effect. As with consolidation, protein synthesis inhibition at long intervals postreactivation did not produce amnesia. This supported the idea that reconsolidation, like consolidation, operates in a restricted time window in which protein synthesis is required to establish long-term memory. Reconsolidation suggested that new information and experience could be assimilated into an already existing memory. According to this theory, memory is a dynamic and constructive process, subject to ongoing modification and disruption (Nader, 2003). Nader et al.'s (2000a) results have been corroborated using a variety of paradigms, organisms, and means of intervention. There are conditions, however, in which reconsolidation is not found (Dawson & McGaugh, 1969; Hoeffer et al., 2011; Squire, Slater, & Chace, 1976). The reason for this inconsistency is not clear, but it suggests that reconsolidation is not a universal phenomenon and its occurrence may be bound to specific experimental paradigms (Lewis, 1979; Miller & Springer, 1974).

The current study examines consolidation and reconsolidation in a novel paradigm. We used the protein synthesis inhibitor anisomycin to test consolidation and reconsolidation of memory for food caches in Black-capped chickadees (*Poecile atricapillus*).

Black-capped chickadees store food both in the wild and in captivity (Sherry, 1984). Storage sites are usually partially concealed locations, such as hollow stems, small crevices in bark, and dry leaves. Chickadees may cache up to several hundred food items a day in sites scattered throughout a bird's territory, placing a single food item at each cache site. Chickadees recover these caches with remarkable accuracy using hippocampus-dependent spatial memory (Sherry & Hoshooley, 2007; Sherry & Vaccarino, 1989). Because chickadees retrieve their stored food, they must also remove remembered sites from memory or update the status of these sites in order to distinguish empty from full caches. Experiments show that chickadees do indeed avoid returning to caches they have emptied while continuing to visit intact full caches that they made at the same time (Sherry, 1984).

Protein synthesis is vital to the stabilization of long-term memory (Meiri & Rosenblum, 1998) and is involved in the establishment and modification of neuronal connectivity (Rudy, Biedenkapp, Moineau, & Bolding, 2006). Anisomycin blocks protein synthesis at translation by inhibiting the action of peptidyl transferase on the 60S ribosome, disrupting the proper elongation of proteins (Grollman, 1967). Anisomycin has been shown in many studies to inhibit the consolidation of context-specific long-term memory and even cause the loss of selected memories (Barrientos, O'Reilly, & Rudy, 2002). Despite concerns over the nonspecificity of anisomycin and protein synthesis inhibitors in general (Klann & Sweatt, 2008; Rudy et al., 2006), anisomycin seems to produce specific effects on memory without altering the electrical activity of neurons or synaptic transmission. It does, however, inhibit LTP (Meiri & Rosenblum, 1998).

Anisomycin, therefore, has the potential to disrupt the accuracy of chickadees' cache recovery. Although the effect of protein synthesis inhibition has been examined in the Morris water maze (Meiri & Rosenblum, 1998), it has not been investigated in other models of spatial memory. We used anisomycin to determine whether protein synthesis plays a role in both consolidation and reconsolidation of memory for cache sites in chickadees and tested the hypothesis that when chickadees search for caches, memories for cache sites are reactivated and must be reconsolidated in order for memory for cache sites to persist.

A variety of perturbations have been shown to interfere with consolidation of new memories when applied after initial acquisition including cerebral trauma, electroconvulsive shock, RNA and protein synthesis inhibitors, and a variety of drugs (McGaugh, 2000). Typically amnesia is only produced if perturbation occurs around the time of training or shortly thereafter (Davis & Squire, 1984). Several studies have also demonstrated a second distinct time window in which protein synthesis is necessary for long-term memory consolidation (Bourtchouladze et al., 1998; Epstein, Child, Kuzirian, & Alkon, 2003; Freeman, Rose, & Scholey, 1995; Grecksch & Matthies, 1980; Lattal & Abel, 2004). While studies have examined the possibility of multiple time windows for protein synthesis, the exact temporal boundaries of consolidation remain largely unknown (Alberini, 2007), and some studies using protein synthesis inhibitors have observed only transient amnesic effects (Quartermain, McEwen, & Azmitia, 1972).

The first experiment investigated the susceptibility of newly acquired memories to protein synthesis inhibition during two previously reported time windows for memory consolidation. We also examined the potential for recovery from protein synthesis inhibition induced amnesia.

Experiment 1: Consolidation

Method

Subjects. Black-capped chickadees ($n = 20$; weight 10–13 g) were captured near the University of Western Ontario campus and held in captivity under a Canadian Wildlife Service Scientific Capture permit. All animals were handled and tested according to Canadian Council on Animal Care guidelines and University of Western Ontario animal care protocols. Subjects were assigned to two groups, one given anisomycin at 0 and 2 hr following caching ($n = 5$), another given anisomycin 4 and 6 hr following caching ($n = 5$). Each group was paired with a control group ($n = 5$/group) that received the injection vehicle, 10% β-cyclodextrin PBS.

Birds were individually housed in wire mesh cages (25 cm × 25 cm × 38 cm) on a 14:10 hr light/dark cycle (onset 0700h). Birds were maintained on an ad lib diet of Mazuri Small Bird Diet (PMI Nutrition International LLC, Brentwood, MO) and sunflower seeds ground to a fine powder to prevent food caching in the home cage. Water was available ad lib.

Drug administration. Subjects were administered either anisomycin from *Streptomyces griseolus*, 97% (Sigma Aldrich, St. Louis, MO) in 10% β-cyclodextrin (Sigma Aldrich) dissolved in physiological 0.9% PBS or the injection vehicle, 10% β-cyclodextrin in 0.9% PBS. All injections were given at a dosage of 0.075 mg anisomycin/g body weight. This dosage of anisomycin has been shown to inhibit over 90% of protein synthesis in the brain during the first 2 hrs after injection (Flood, Rosenzweig, Bennett, & Orme, 1973). Drug and vehicle were administered in two injections in the pectoralis muscle. Half of the injection was given in the left and half in the right pectoralis muscle. The same injection protocol was repeated 2 hr later.

Apparatus. Home cages were each equipped with a small door (28 cm × 38 cm) that when opened allowed entry into an

indoor aviary containing four artificial trees. Trees consisted of cut branches approximately 2 m in length held upright in plastic stands. Each tree had a single main trunk with several smaller branches of varying size projecting from it. Each tree contained eight individual potential cache sites randomly located on the tree for a total of 32 sites, each 1 cm deep and 0.5 cm in diameter. For sites with no natural perch nearby, a dowel perch, 0.5 cm in diameter was positioned on the branch below the hole. Sites could be plugged by inserting a small piece of yarn that was attached to the tree branch next to each site. The yarn prevented birds from seeing into the cache site without removing the yarn and provided an objective measure of whether a site had been inspected by a bird. The trees were placed in a different predetermined orientation prior to each trial to vary the spatial arrangement of potential cache sites from trial to trial. Storable sunflower chips were provided during the Storage phase of each trial (see below).

Procedure. Each trial consisted of 4 phases as described below and illustrated in Figure 1. A trial was a complete cycle of caching and cache retrieval that required approximately 48 hr to complete. Each chickadee completed 3–5 trials. For each behavioral measure (see below) a mean was calculated across these 3–5 trails and the mean values from all birds comprised the data for statistical analyses. Behavior was recorded using Noldus Observer XT (Noldus Information Technology, Wageningen, The Netherlands).

Presearch (PS). All chickadees were food-deprived for 3–5 hr (1 hr prior to light offset and 2 or more hr after light onset) and then permitted to enter the aviary. The Pre-Search phase of each trial lasted 5 min. All cache sites were empty, and there was no food available for consumption or storage. A "search" was defined as probing or visually inspecting a cache site. PS data was used to estimate search biases that may have influenced search behavior in later retrieval trials (see details below).

Storage. The storage phase of each trial immediately followed the PS phase. Chickadees were provided with 20 sunflower seed chips for consumption and storage. The number and location of seeds cached was recorded for 15 min. Chickadees were then allowed back into their home cages. Only cached seeds remained in the aviary, while all other seeds and fragments were removed by the experimenter.

Recovery 1 (R_1). R_1 served as the first retention test for memory 24 hrs after caching. Chickadees entered the aviary following 3–5 hr of food deprivation. The only seeds available to the bird were those previously cached in the Storage phase. This phase ran for a maximum of 20 min, or until half of the stored seeds were recovered. Upon retrieval of half of their caches, chickadees were returned to their home cages. All remaining seeds were then removed from cache sites by the experimenter to eliminate the possibility of sensory or olfactory cues in Recovery 2 (R_2).

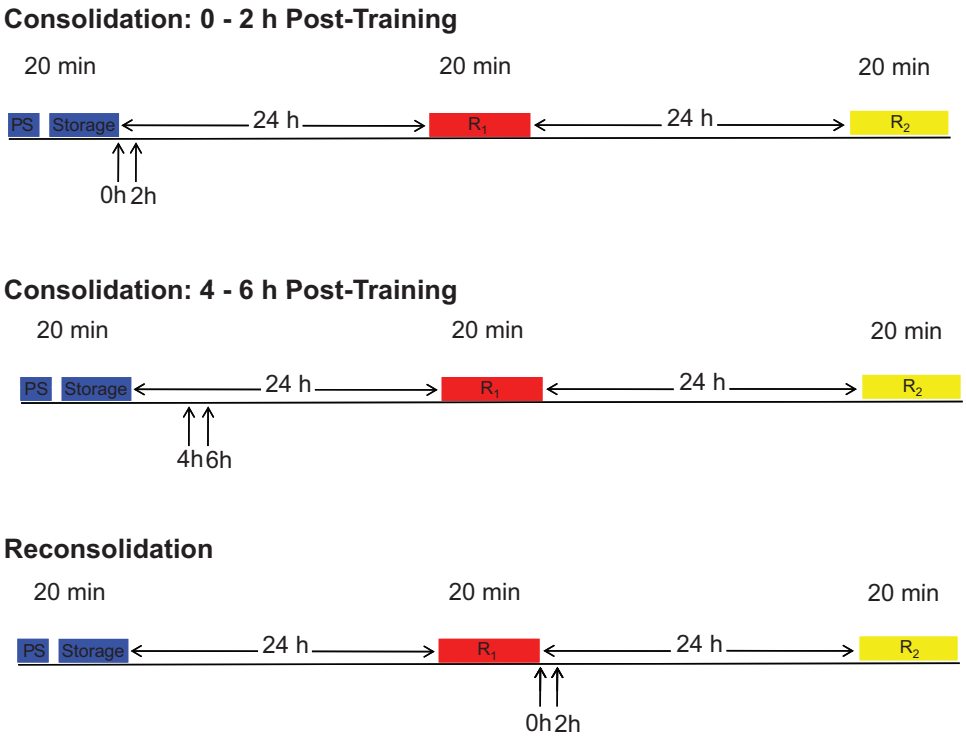

Figure 1. Experiments 1 and 2 followed the same procedure but differed in the point at which anisomycin was administered. Birds were observed in four phases, PS, Storage, Recovery 1 (R_1), and Recovery 2 (R_2). Birds in Experiment 1 (Consolidation) were given anisomycin either 0 and 2 hr (upper) or 4 and 6 hr (middle) following food storing. Birds in Experiment 2 (Reconsolidation) were given anisomycin 0 and 2 hr following Recovery 1 (lower). PS served as a control period for estimating the accuracy of search for caches during R_1 and R_2. See text for details.

Recovery 2 (R_2). R_2 served as a second retention test for cache site memory. Twenty-four hrs after R_1 and following 3–5 hr of food deprivation, the bird reentered the aviary. No previously cached seeds or any other food was available. All cache sites were empty and the bird's searching behavior was recorded for 20 min.

Measures statistics.

R_1 measures. Birds searched in R_1 until they had recovered half of their caches. Two measures of cache site memory were used, accuracy and the number of searches. To estimate accuracy, the number of visits to cache sites was expressed as a percentage of visits to all sites. To standardize for the number of caches a bird made during the Storage phase, this percentage was divided by the number of cache sites. Seventy-five percent accuracy in searching for 6 caches thus becomes a standardized accuracy measure of 12.5. This standardization deals with the fact that birds making a larger number of caches would be more likely to visit a cache by chance. A bird with 10 caches that made only one revisiting error in R_1 would receive a standardized score of 8.3, while a bird with 6 caches that made one revisiting error in R_1 would receive a score of 12.5. Chance accuracy was calculated from the hypergeometric distribution for the total of 32 possible sites, the mean number of caches per trial, and the mean number of searches in R_1 or R_2 phases. The number of searches was defined as the number of searches a subject took to retrieve half of its caches, also divided by the number of cache sites. Both measures were analyzed with analysis of variance (ANOVA) for treatment (anisomycin vs. control). All statistical tests were done with SPSS-statistical package version 17.0, SPSS Inc. An α value of .05 was chosen for statistical significance.

R_2 measures. The same measure of accuracy used in R_1 was used in R_2. In R_2 there were no actual seeds to retrieve. Accuracy in R_2 refers to visits to cache sites from which birds did not retrieve their seeds in R_1. To determine whether anisomycin had any effect on general levels of activity in R_2, we also analyzed the total number of searches to all sites that birds performed during R_2 trials. As with R_1 data, ANOVA was used to compare accuracy and activity measures between anisomycin-injected and control birds. A further analysis was performed on the R_2 data to compare search at empty caches (sites where the bird had collected its stored food in R_1) and full caches (sites the bird left intact in R_1). For PS, this was determined retrospectively from search during PS at those sites that birds later cached food in during the Storage phase. This measure calculated the number of searches at empty and full caches relative to the number of searches in the PS and R_2 phases. Because this measure is relative to the number searches in each phase it should not be influenced by the different durations of the PS and R_2 phases. If, for example, a bird visited 2 cache sites in 3 searches in the PS phase and visited 8 cache sites in 12 searches in the longer R_2 phase, it would receive the same score. Two-way repeated measures ANOVA for treatment (anisomycin vs. control), trial phase (PS vs. R_2), and cache type (empty vs. full) was carried out on these data. This test was performed to detect differences during R_2 in search at caches that were emptied during R_1 and caches that were left full. The comparison between PS and R_2 provides an estimate of how frequently birds returned to cache sites, compared with how likely they were to visit these sites in PS before any food had been stored. PS data provide an estimate of how frequently a bird will visit a particular site by chance or as a result of biases to visit particular trees, branches, or sites. Tukey's post hoc tests were used for further comparison of group means.

Results

Anisomycin 0 and 2 hrs following storage. Birds given anisomycin were significantly less accurate in R_1 than control birds, $F(1, 8) = 27.84$, $p = .001$, and required significantly more searches to recover half of their caches, $F(1, 8) = 7.30$, $p = .027$ (Figure 2). Chance on this per site percent accuracy measure is 3.12, and both control birds and birds given anisomycin exceed this chance level of accuracy.

Similarly in R_2, control birds remained more accurate than anisomycin-treated birds, $F(1, 8) = 27.84$, $p = .001$ (Figure 3). The level of activity, as shown by the total number of searches in R_2, did not differ significantly between anisomycin and control groups, $F(1, 8) = .68$, *ns* (Figure 3).

Two-way repeated measures ANOVA of the relative number of searches during Pre-Storage and R_2 at sites that were empty or full in R_2 showed a significant effect of Trial Phase $F(1, 8) = 105.56$, $p = .001$. As can be seen in Figure 4, birds always searched more at cache sites in R_2 than they had at these same sites in PS. There was also a significant 3-way interaction of Treatment $\times$ Trial Phase $\times$ Cache Type, $F(1, 8) = 9.35$, $p = .01$. Tukey's post hoc comparisons showed that birds given anisomycin and controls

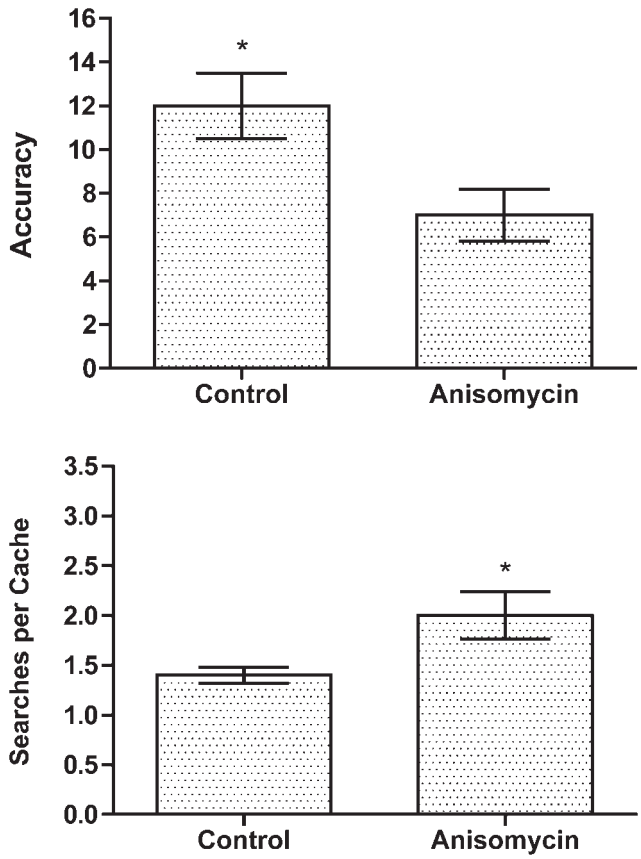

Figure 2. Experiment 1. 0 and 2 hr anisomycin treatment. Upper: Accuracy of memory for cache sites in Recovery 1. Chance equals 3.12. Lower: Searches to retrieve seeds from half of the cache sites in Recovery 1. Error bars equal $\pm$ 1 *SEM*. * Indicates a significant difference between conditions at $p < .05$.

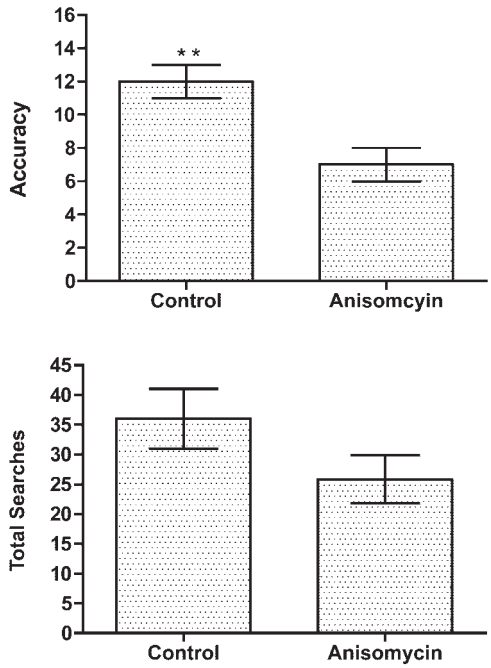

Figure 3. Experiment 1. 0 and 2 hr anisomycin treatment. Upper: Accuracy of memory for cache sites in Recovery 2. Chance equals 3.12. Lower: Total number of searches in Recovery 2. Error bars equal ± 1 *SEM*. ** Indicates a significant difference between conditions at $p < .001$.

revisited both emptied cache sites and full caches more in R_2 than in PS, Anisomycin Empty caches $q(8, 8) = 8.05$, $p < .01$; Anisomycin Full caches $q(8, 8) = 6.56$, $p < .05$; Control Empty caches $q(8, 8) = 7.91$, $p < .01$; Control Full caches $q(8, 8) = 10.69$, $p < .01$; Figure 4. Control birds, however, visited full caches more than empty caches in R_2, $q(8, 8) = 5.68$, $p < .05$, while anisomycin birds did not, $q(8, 8) = 3.36$, *ns* (Figure 4).

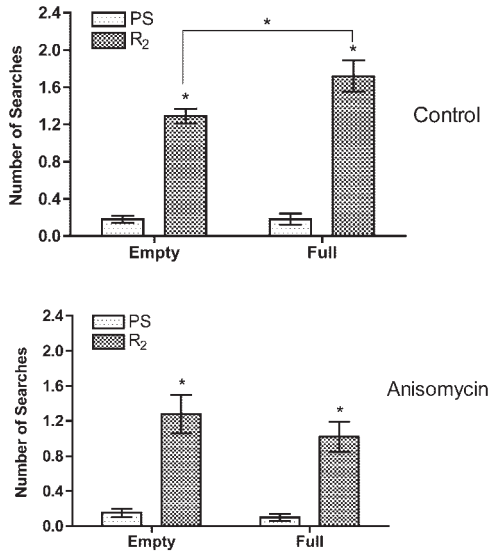

Figure 4. Experiment 1. 0 and 2 hr anisomycin treatment. Mean number of searches at empty and full caches in Recovery 2. A difference between behavior in Presearch (PS) and Recovery 2 (R_2) indicates a tendency to remember and return to cache sites. Upper: Control birds. Lower: Birds given anisomycin. Error bars equal ± 1 *SEM*. * Indicates a significant difference between PS and R_2 at $p < .05$.

Anisomycin 4 and 6 hr following storage. For birds given anisomycin 4 and 6 hr following storage, results were very similar. Birds given anisomycin were significantly less accurate in R_1 than control birds $F(1, 8) = 5.76$, $p = .04$ and required more searches to retrieve half of their caches $F(1, 8) = 14.02$, $p = .01$ (Figure 5). Chance on this per site percent accuracy measure is 3.12, and both control birds and birds given anisomycin exceed this chance level of accuracy.

In R_2, anisomycin and control birds did not differ in accuracy $F(1, 8) = .021$, *ns*, or in their level of activity $F(1, 8) = 2.65$, *ns* (Figure 6).

Two-way repeated measures ANOVA of the relative number of searches at empty and full caches, likewise, showed that anisomycin and control birds revisited both Empty and Full sites significantly more often in R_2 than in PS. The main effect of Trial Phase was significant $F(1, 8) = 93.85$ $p = .001$, as was the three-way interaction of Treatment × Trial Phase × Cache Type $F(1, 8) = 5.73$, $p = .04$. Tukey's post hoc comparisons showed that anisomycin and control birds searched more in R_2 than in PS at both Empty and Full caches, Anisomycin Empty $q(8, 8) = 11.64$, $p < .01$; Anisomycin Full $q(8, 8) = 10.08$, $p < .01$; Controls Empty $q(8, 8) = 10.54$, $p < .01$; Controls Full, $q(8, 8) = 13.84$, $p < .01$; Figure 7. Similar to their behavior when given anisomycin 0–2 hr poststorage, control birds visited full caches

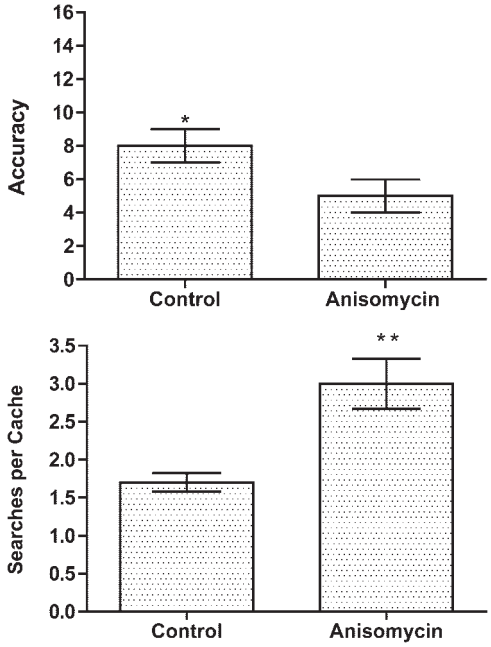

Figure 5. Experiment 1. 4 and 6 hr anisomycin treatment. Upper: Accuracy of memory for cache sites in Recovery 1. Chance equals 3.12. Lower: Searches to retrieve seeds from half of the cache sites in Recovery 1. Error bars equal ± 1 *SEM*. * Indicates a significant difference between conditions at $p < .05$. ** Indicates a significant difference at $p < .01$.

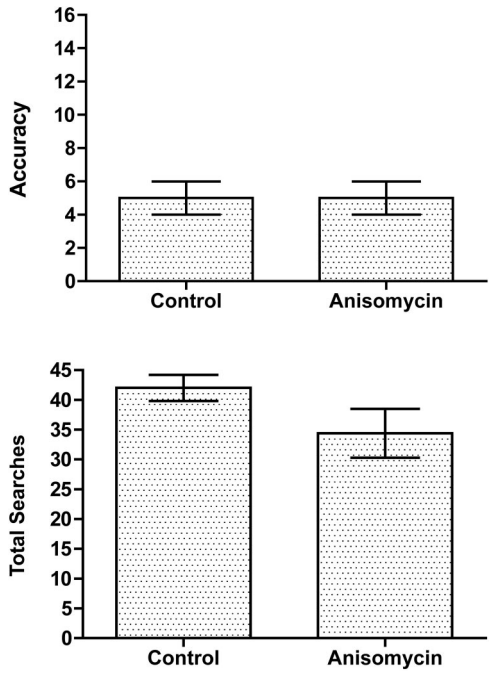

Figure 6. Experiment 1. 4 and 6 hr anisomycin treatment. Upper: Accuracy of memory for cache sites in Recovery 2. Chance equals 3.12. Lower: Total number of searches in Recovery 2. Error bars equal ± 1 *SEM*.

more than empty caches in R_2, $q(8, 8) = 5.69$, $p < .05$, while anisomycin birds did not, $q(8, 8) = 4.11$, *ns* (Figure 7).

Comparisons of anisomycin effects at 0–2 and 4–6 hours post storage. For both the 0- to 2- and the 4- to 6-hr treatments, the effect of anisomycin on accuracy at 24 hr (R_1) and 48 hr (R_2) was analyzed. A repeated measure ANOVA of treatment (anisomycin vs. control), trial phase (R_1 vs. R_2), and time of drug administration (0–2 hr vs. 4–6 h) showed a significant effect of treatment, $F(1, 16) = 4.73$, $p = .045$, and time of drug administration, $F(1, 16) = 12.51$, $p = .003$. There were no other significant main effects or interactions. As can be seen from Figure 8, anisomycin in general reduces cache retrieval accuracy. The accuracy of birds treated 4–6 after caching—both controls and anisomycin-treated birds—is lower than that of birds treated 0–2 hr after caching. Because the accuracy of both controls and anisomycin-treated birds is lower when treated at 4–6 hr, this latter effect is not because of the timing of any protein synthesis inhibition caused by anisomycin.

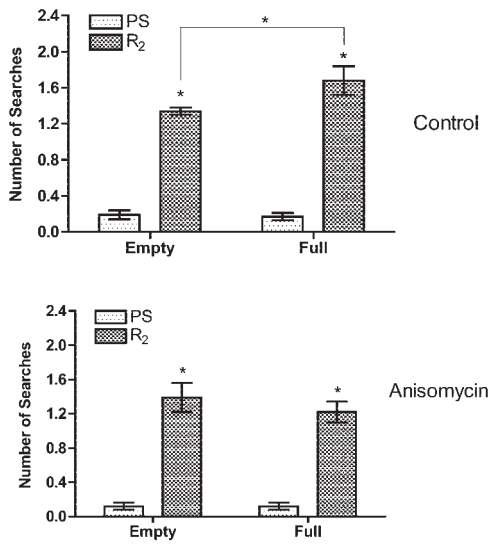

Figure 7. Experiment 1. 4 and 6 hr anisomycin treatment. Mean number of searches at empty and full caches in Recovery 2. A difference between behavior in Presearch (PS) and Recovery 2 (R_2) indicates a tendency to remember and return to cache sites. Upper: Control birds. Lower: Birds given anisomycin. Error bars equal ± 1 *SEM*. * Indicates a significant difference between PS and R_2 at $p < .05$.

Discussion

Birds were significantly less accurate and required more searches to retrieve seeds they had cached 24 hr previously when

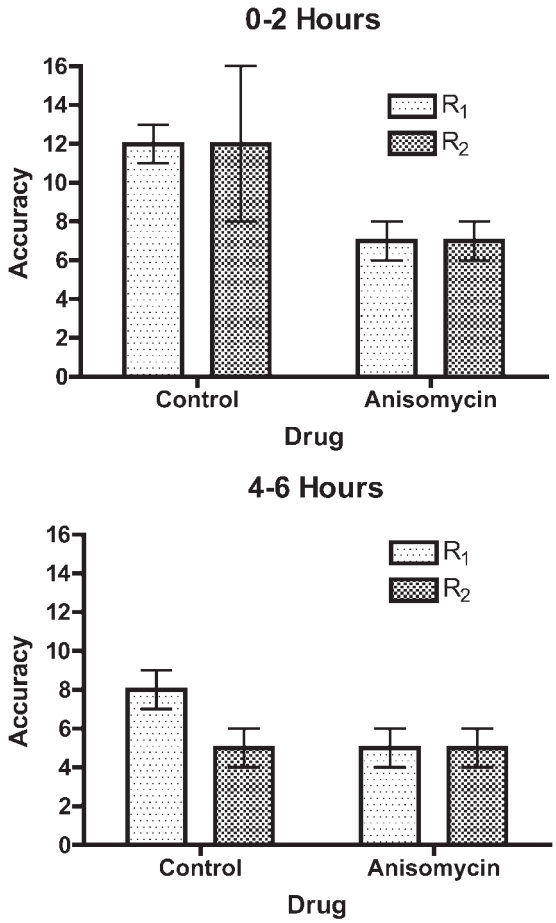

Figure 8. Comparison of accuracy for 0–2 hr and 4–6 hr anisomycin treatments at Recovery 1 (R_1) and Recovery 1 (R_2). Upper: Accuracy for 0–2 hr treatment for both anisomycin and control birds at R_1 and R_2. Lower: Accuracy for 4–6 hr treatment for both anisomycin and control birds at R_1 and R_2. Error bars equal ± 1 *SEM*. Anisomycin significantly reduced cache retrieval accuracy. Performance of the 4–6 hr group (both anisomycin-treated and control birds) was significantly lower than the 0–2 hr group. See text for details.

given anisomycin either 0 and 2 hr or 4 and 6 hr after caching. At 48 hr following caching, birds receiving anisomycin continued to show a level of accuracy comparable to that displayed 24 hr earlier, as can be seen in Figure 8.

The behavior of both anisomycin and control birds was still above the level predicted from PS behavior, however, whether injected 0 and 2 hr following storage or 4 and 6 hr following storage (Figures 4 and 7). Furthermore, their level of searching was above PS levels at both Empty and Full caches. This behavior by control birds is different from that previously described in Sherry (1984) and different from the behavior shown by control birds in Experiment 2 (below). Figures 4 and 7 do indicate, however, significantly more search at full sites than empty sites during R_2 by control birds. One possible explanation for the failure of birds given anisomycin to distinguish empty from full caches is a possible time lag required for protein synthesis to rebound from the effects of anisomycin. If, for example, protein synthesis was reduced during R_1, impairing memory for which caches had been retrieved and which had not, birds given anisomycin might be less able to distinguish empty from full caches, compared with control birds. It would be valuable, in subsequent experiments, to administer anisomycin outside the hypothesized time windows for protein-synthesis-dependent memory consolidation to determine the temporal specificity of anisomycin's effect on memory.

Amnesic treatments rarely block consolidation of memory completely. Systemic injections with anisomycin inhibit 60%–90% of protein synthesis (Alberini, 2007; Flood et al., 1973). There is, thus, the possibility of partial encoding and some sparing of memory (Nader, Schafe, & Le Doux, 2000b). Davis and Squire (1984) state that in order to block memory consolidation protein synthesis inhibition of over 90% is necessary. Although the dose used in the current study (75 mg/kg) has been shown to impair memory consolidation in rats (Flood et al., 1973; Lattal & Abel, 2004) and to inhibit protein synthesis in the brain by more than 90% during the first 2 hr after administration, it is possible that in the current study the protein synthesis was only partially inhibited. Birds given anisomycin showed levels of accuracy that were lower than controls but still above chance. Some memory impairment, however, seems the likeliest cause of the difference observed between anisomycin-treated and control birds in their search behavior.

Experiment 2: Reconsolidation

The theory of reconsolidation supposes that long-term memory becomes labile and subject to modification at retrieval. For information to persist in long-term memory, it must be reconsolidated following retrieval in a process that, like initial consolidation, is protein-synthesis dependent. When chickadees search for cache sites, they retrieve information from memory about spatial location and other properties of caches (Feeney, Roberts, & Sherry, 2009, 2011). If information about recent cache sites is retrieved as a batch, then memory for sites that are not actually harvested as this time may need to be reconsolidated into long-term memory. Experiment 1 showed that birds could relocate in R_2 those caches that were left full following R_1. Inhibition of protein synthesis following R_1 should therefore impair memory for caches that the bird left full if memory for such caches does indeed require reconsolidation.

Method

Subjects. Adult black-capped chickadees (n = 10, weight 10–13 g) were captured near the University of Western Ontario campus and housed, maintained, and tested as in Experiment 1. These birds had not participated in Experiment 1. Subjects were randomly assigned to anisomycin and vehicle control groups, n = 5 per group.

Drug administration. Subjects were administered either anisomycin from *Streptomyces griseolus*, or the 10% β-cyclodextrin PBS injection vehicle as in Experiment 1. Two injections were given, the first immediately after Recovery 1 (see below) and the second 2 hr later.

Procedure. Each trial consisted of 4 phases (PS, Storage, R_1, and R_2) as in Experiment 1 (Figure 1 lower). Each chickadee completed 3–5 trials. For each measure of behavior, a mean was calculated across these 3–5 trails, and the mean values from all birds comprised the data for statistical analyses.

Immediately after the bird returned to its home cage following R_1, it was administered a 0.075 mg/g dose of either anisomycin or 10% β-cyclodextrin PBS. Half of the injection was given in the left pectoralis muscle and half in the right pectoralis muscle. The same injection protocol was repeated 2 hrs later. Twenty-four hours after the second injection and following 3–5 hrs of food deprivation, the bird reentered the aviary. No previously cached seeds or any other seeds were available. All cache sites were open and the bird's searching behavior was recorded for 20 min. Behavior during R_2 was compared with behavior in PS to assess memory for cache sites. Data were analyzed as in Experiment 1.

Results

Figure 9 shows the total number of searches during R_2 by experimental and control birds. Anisomycin had no significant effect on the number of searches in R_2, $F(1, 8) = 2.11$, *ns*, indicating that general activity and search effort was not affected by drug injection.

Figure 10 compares the relative amount of searching during PS and R_2 for the two treatment conditions and two cache types. ANOVA revealed a significant main effect for Cache Type, $F(1, 8) = 35.39$, $p = .01$, showing there were more visits to Full than to Empty caches, and a significant 3-way interaction of Treatment × Trial Phase × Cache Type $F(1, 8) = 12.24$, $p = .01$. Tukey's post hoc tests showed that control birds visited full sites

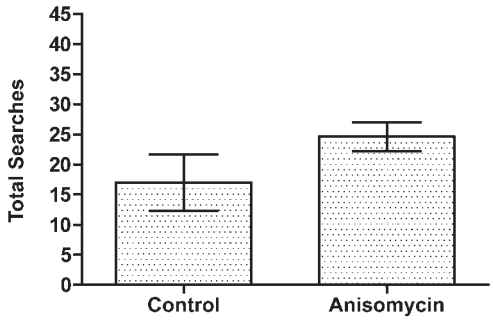

Figure 9. Experiment 2. The total number of searches in Recovery 2 by birds given anisomycin and birds given the vehicle control. Error bars equal ± 1 *SEM*.

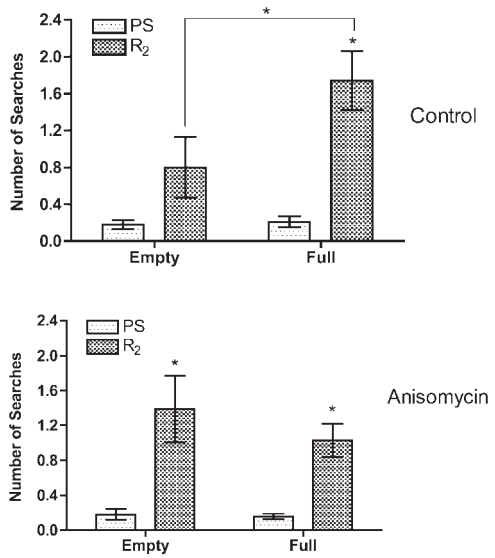

Figure 10. Experiment 2. The mean number of searches at empty and full caches in Recovery 2. A difference between behavior in Presearch (PS) and Recovery 2 (R_2) indicates a tendency to remember and return to cache sites. Upper: Control birds. Lower: birds given anisomycin. Error bars equal ± 1 *SEM*. * Indicates a significant difference between PS and R_2 at $p < .05$.

significantly more in R_2 than in PS $q(8, 8) = 11.72$, $p < .01$, but did not visit empty sites more in R_2 than in PS. This indicates that control birds distinguished full from empty cache sites.

Birds given anisomycin, however, visited both full and empty sites more in R_2 than in PS: empty caches, $q\ (8, 8) = 9.02$, $p < .01$; full caches, $q\ (8, 8) = 6.49$, $p < .01$. Unlike control birds, anisomycin subjects did not treat full and empty sites differently. In addition, similar to Experiment 1, control birds made more visits to full than empty cache sites at R_2, $q(8, 8) = 7.01$, $p < .05$, while anisomycin birds did not, $q(8, 8) = 2.68$, *ns* (Figure 10).

Discussion

The reconsolidation hypothesis predicts that if memory for all cache sites was reactivated, anisomycin administered following retrieval in R_1 would disrupt reconsolidation and subsequent memories for intact cache sites in R_2 should therefore be impaired. The data show that contrary to this hypothesis, chickadees given anisomycin remembered the locations of intact caches. This indicates that memory for all caches, if reactivated as a batch during cache retrieval, does not require protein-synthesis dependent reconsolidation in order to persist in long-term memory. It is possible, too, that memory for only those sites actually retrieved is reactivated during cache retrieval, in which case memory for sites not retrieved would be expected to persist in long-term memory because reconsolidation would be unnecessary for these sites. Because the R1 phase was terminated when birds had retrieved half of their caches, one might expect that memory for some caches not actually retrieved was active when the R1 trial ended. Impairment in memory for intact caches caused in this way might, however, be only a modest effect. What the results do show, however, is that cache sites made at the same time are not reactivated as a batch that then requires reconsolidation.

Unlike control birds, birds given anisomycin failed to distinguish between empty and full cache sites, returning to both empty and full caches in the much the same way during R_2. This suggests that while the inhibition of protein synthesis does not affect long-term memory for all caches, it does interfere with memory for which caches were emptied in R_1. Compared with control birds, empty cache sites were visited by birds given anisomycin as if R_1 had never happened.

Previous studies have induced memory impairments with protein synthesis inhibitors immediately following reactivation of memory in avoidance tasks, contextual fear conditioning, and object recognition (Debiec, LeDoux, & Nader, 2002; Nader et al. 2000a; Taubenfeld, Milekic, Monti, & Alberini, 2001). These results suggest that new protein synthesis is required for the reconsolidation of memories. Reconsolidation is less reliably observed, however, than consolidation (Dudai, 2004). The results of the present experiment did not support the basic prediction of the reconsolidation hypothesis. Our findings resemble previous results which did not detect a labile phase of the original memory caused by retrieval. Berman and Dudai (2001) microinfused anisomycin into the insular cortex in a conditioned taste aversion extinction protocol, both immediately before and after reactivation, and found extinction blocked but the original memory trace unaffected. Vianna, Szapiro, McGaugh, Medina, and Izquierdo (2001) also found that protein synthesis inhibition immediately after reactivation in an inhibitory avoidance task blocked extinction but spared the original memory trace. Other studies have observed a reversal in the memory deficits with the passage of time and with the occurrence of a reminder cue: a memory trace that appeared to be gone could be recovered and reactivated. Such results cannot be due to elimination of the original memory trace but rather an alteration in some other aspect of memory (Lattal & Abel, 2004). The current findings resemble those of Lattal and Abel (2004) in that modification of memory, that is, extinction, could be disrupted by postretrieval manipulations without destroying the original memory trace. Chickadees given anisomycin displayed memory for both empty and full caches in R_2 indicating that original memory traces established during the Storage phase remained intact. New information acquired in R_1 and remembered by control birds, namely that some caches were emptied, was compromised by anisomycin. Updating cache site status as empty is a new memory and for that reason was disrupted by inhibition of protein synthesis.

Dudai's (2004) "weak" version of reconsolidation may provide an account for the current findings. This version supposes that upon reactivation of the original memory trace only the updated, new parts of the modified trace undergo consolidation. This suggests consolidation of a new trace rather than a true reconsolidation of the original trace is what is impaired by inhibition of protein synthesis. This could account for the observation that birds in the anisomycin group, unlike controls, could not distinguish between empty and full caches (the updated memory) but remembered actual cache locations (the original memory trace).

General Discussion

The present study examined the consolidation and reconsolidation of memory in a paradigm in which these processes have not previously been investigated. The results showed that systemic treatment with anisomycin following learning of new spatial locations led to less accurate memory compared with controls. Memory for spatial locations was still more accurate than expected by chance, however, in birds given anisomycin. There was little evidence for the reconsolidation of memory for spatial locations following reactivation of memory. Indeed, the failure of birds to avoid cache sites they had themselves emptied is strong evidence against a reconsolidation process. Rather than disrupting reconsolidation, the effect of anisomycin was to disrupt the incorporation of new information about cache sites, namely that some had been emptied by the bird and contained no food.

The results support the idea that protein synthesis is a component of memory formation in birds as shown by the reduced accuracy of birds in Experiment 1 and their inability to distinguish empty from full caches in Experiment 2. These findings are consistent with studies of spatial memory in rodents using the Morris water maze. Anisomycin given just prior to training—by cannulation into the CA1 region of the hippocampus—produced a dose-dependent increase in latency to locate the platform (Naghdi, Majlessi, & Bozorgmehr, 2003). The results of the present study also show that protein synthesis inhibition immediately following learning and 4 and 6 hr later are equally effective at reducing accuracy of memory for spatial locations.

The processes of consolidation and reconsolidation have both theoretical and practical implications. A better understanding of these mechanisms may aid in the treatment of amnesia, addiction, obsession, phobia, and posttraumatic stress disorder (Dudai & Eisenberg, 2004), as well as elucidating the fundamental mechanisms of memory formation.

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