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Jody L. Lewis

*University of Wisconsin–Stevens Point, jody.lewis@uwsp.edu*


Alan C. Kamil

*University of Nebraska-Lincoln, akamil1@unl.edu*

Kate E. Webbink

*Field Museum of Natural History, Chicago, kwebbink@fieldmuseum.org*

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## Changing Room Cues Reduces the Effects of Proactive Interference in Clark's Nutcrackers, *Nucifraga columbiana*

Jody L. Lewis, Department of Psychology, University of Wisconsin–Stevens Point

Alan C. Kamil, School of Biological Sciences, University of Nebraska–Lincoln

Kate E. Webbink, Field Museum of Natural History, Chicago, Illinois

Corresponding author — Jody L. Lewis, Department of Psychology, University of Wisconsin–Stevens Point, Stevens Point, WI 54481; email Jody.Lewis@uwsp.edu

### Abstract

To determine what factors are important for minimizing interference effects in spatial memory, Clark's Nutcrackers, *Nucifraga columbiana* were tested for their spatial memory for two serial lists of locations per day. In this experiment two unique landmark sets were either different between List 1 and List 2 or the same. We found that Nutcrackers were most susceptible to interference when the landmark sets were the same. This study suggests that repeatedly testing animal memory in the same room, with the same cues, can hamper recall due to interference.

**Keywords:** *Nucifraga columbiana*, Clark's nutcrackers, proactive interference, memory, landmarks

In 2006, we found that Clark's Nutcrackers, *Nucifraga columbiana* are susceptible to proactive interference when tested for their memory of spatial lists (Lewis & Kamil, 2006). We studied spatial list learning by serially presenting several locations in an open field and testing the bird's memory for each location by allowing the bird to choose among the correct location and five other possible locations (i.e., the cluster method; see Lewis & Kamil, 2006). Nutcrackers made more errors when recalling a list of spatial locations if they had previously been tested with a spatial list the same day (Lewis & Kamil, 2006). Despite the nutcracker's impressive memory abilities (e.g., Bednekoff, Kamil, & Balda, 1997; Balda & Kamil, 1992; Tomback, 1980; Vander Wall & Hutchins, 1983), they are still susceptible to interference in the laboratory like many other nonhuman species such as rats and pigeons (e.g., Cohen, Sturdy, & Hicks, 1996; Grant, 1981; Hoffman & Maki, 1986; Roberts & Dale, 1981).

Proactive interference is thought to be primarily responsible for memory failure or what we think of as “forgetting.” Memory researchers argue that forgetting involves, not the loss (or decay) of memories, but the inability to correctly retrieve past information (Loftus & Loftus, 1980; Roediger & Guynn, 1996). Errors in memory are typically due to retrieval failure, which can occur when an individual has difficulty distinguishing between old information and new information. Researchers argue that the act of remembering is simply learning to discriminate a current, target memory from past, competing memories (Capaldi & Neath, 1995;

McGeoch, 1932; Spear, 1971).

Interference effects, in animal laboratory studies, are typically caused by intrusion errors (e.g., Sands & Wright, 1980a, 1990b). To test for intrusion errors, the researcher periodically includes a previously rewarded item as one of the incorrect alternatives in the choice phase of the study. Animals make errors by choosing the sample that occurred prior ( $N - 1$ ) to the current sample ( $N$ ). Clark's Nutcrackers are also susceptible to intrusion errors in spatial memory and make more errors within a List 2 cluster when it contained a location that was previously correct during testing of List 1 (Lewis & Kamil, 2006).

Although previous studies have shown that proactive interference can be minimized when the interval between old and new information is increased (e.g., Gordon & Feldman, 1978; Cohen, Reid, & Chew, 1994, Cohen et al., 1996; Grant, 1981), this has not been replicated with Clark's Nutcrackers (Lewis, 2005). However, another effective way to decrease the effects of proactive interference is to change the environmental stimuli between trials (e.g., Wright, Urcuioli, & Sands, 1986; Zentall, 1970). Other studies have also shown evidence that proactive interference can build up during a study when researchers repeatedly run trials in the same small rooms, with the same visual cues (e.g., Brodbeck, Burack, & Shettleworth, 1992). Zentall (1970) suggests that providing an animal with cues that help them to distinguish between trials can reduce interference by providing the animals with task appropriate instructions. He argues that unlike humans, who can use verbal

instructions, animals may be confused about which previously acquired information is to be tested on a given trial.

We predicted that changing the environmental cues between List 1 and List 2 would decrease the effects of proactive interference found in our previous experiments (Lewis & Kamil, 2006). Specifically, we hypothesized that changing the landmark array should help to decrease intrusion errors made during recall of List 2 clusters that contained a location that was previously correct in List 1.

## Method

### Subjects

We tested five wild-caught Clark's Nutcrackers (unknown age and sex), all of which had been tested in previous spatial memory tasks testing proactive and retroactive interference (Lewis & Kamil, 2006). All birds were housed in a 22°C room with a 14:10-h light:dark cycle. Each bird was kept in an individual cage and was maintained on a diet of turkey starter, sunflower seeds, pellets, mealworms, and pine nuts at 85%–90% of their free feeding weight. They were given *ad libitum* access to water and grit.

### Apparatus

We conducted this experiment in the same testing room as our prior studies (Lewis & Kamil, 2006). The 4.4 × 2.7-m room consisted of a raised plywood floor that contained 176 holes (16 × 11). Each 9-cm diameter hole was separated by 23 cm (center to center) and contained a plastic cup filled with sand. Each location could be left open for probing or closed with a plaster cap. The birds entered the room through a porthole, and the experimenter entered through a door on the east side of the testing room.

For this experiment, we chose 14 landmarks that were different from the 2006 Lewis and Kamil study. We placed 12 landmarks on the floor (approximately 30–50 cm from each other) and attached two painted Styrofoam landmarks to the walls. We made landmarks from several different materials such as wood, brick, PVC pipe, and paint cans, and we painted the landmarks in a variety of different colors. Each landmark could easily be removed and placed back into its original position. Each landmark was attached to its respective surfaces with Velcro strips so they were less likely to be moved throughout the day. We also traced a line around the base of each landmark and drew an arrow on the bottom of each landmark (pointing east), so that it could be placed back in the same exact location and orientation in the room between lists.

We matched pairs of similar landmarks (similarity was based on height, length, and materials). For example, we had two landmarks made of Styrofoam that were made to be mounted on the wall, and we had two landmarks that were very tall and two landmarks that were short but long. We flipped a coin to determine which landmark in the matched pair would be part of landmark Set A or Set B. We chose 12 locations on the floor and two locations on the wall to serve as landmark locations. For each location, we flipped a coin to determine whether a landmark from Set A or Set B would be placed in that location. To be sure that the landmarks

from each set were evenly distributed throughout the room, we did not allow more than two landmarks of the same set to be immediately adjacent to each other. A diagram of the testing room can be seen in Figure 1, which shows that Set A consisted of different landmarks in different locations from Set B.

Although scoring was completed during each trial, we videotaped all trials using a closed circuit camera (Panasonic WVBL200) mounted in the center of the ceiling. The camera was attached to a Sony Trinitron TV and Sharp VC-A410 VCR outside the room.

### Procedure

**Acclimation and training.** All of the birds had been previously trained on the basic experimental procedures and on list learning and cluster testing (see Lewis & Kamil, 2006). We ran this study in October 2004; 2 months had passed between the end of the interference experiments and the beginning of this experiment. We gave the birds one trial a day (i.e., list) for 4 days to refresh the birds on the list learning procedures (see Lewis & Kamil, 2006) and to acclimate them to the new landmarks. During the 4 days, we tested each bird with a random order of each landmark set twice. A list consisted of three stages: study, retention, and recognition.

In the study phase, we gave the bird a serial presentation of five randomly chosen locations (we chose locations without replacement). To present the five locations, the light above the holding cage was turned off, and the bird was encouraged to enter the room through the porthole. The bird then saw only one open hole in the room, and we allowed the bird to dig in the cup to retrieve a portion (one third) of a seed buried about 1 cm deep in the sand. After the bird had consumed the seed, we turned the lights off, and the bird reentered the holding cage. After the bird returned to the holding cage and the porthole was closed, the room lights were turned back on, and the experimenter swept up any sand that may have spilled out of the first hole, closed it with a plaster cap, and opened another randomly chosen hole in the room and baited it with a seed. We repeated this procedure for each of

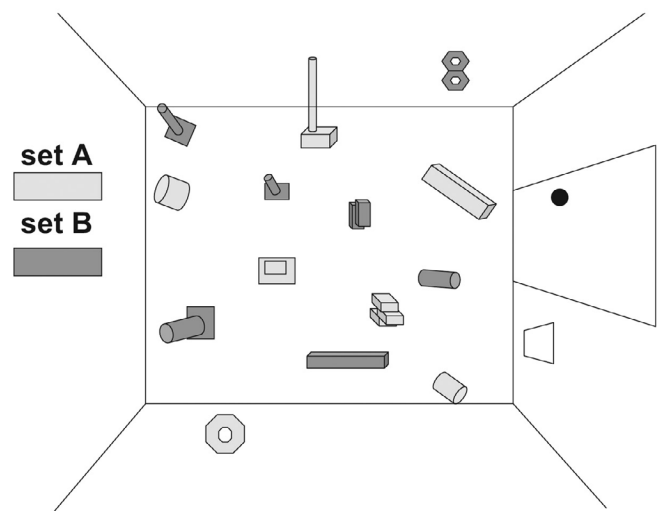


Figure 1. Diagram of the testing room with landmark Sets A and B.

the five locations in the list (there was approximately 30 s between each presentation). After the bird had been shown all five locations, it waited in the holding cage for 5 min (retention phase) before the recognition phase.

During the recognition phase, we tested the birds' memory for each location in the list. In order to control for output interference, each location in the list was tested individually and in a different (random) order than the order given in the study phase. We randomized the testing order by using a 5 (trial)  $\times$  5 (study position) Latin square. We tested the accuracy of each bird's memory by allowing them to search for the correct (seeded) location within a cluster of six possible holes. Each cluster was a  $2 \times 3$  pattern of holes. For each cluster within a list, we randomly chose the orientation of the cluster (north-south vs. east-west) and the position of the correct location (Location 1-6). We also included the restriction that clusters within a list could not touch (one row and one column of holes had to surround each cluster). Testing clusters of holes allowed us to measure the bird's accuracy in a choice test between the correct location and adjacent locations. In order to discourage random search, we limited the birds to four visits per cluster before the lights were turned off. We defined a visit as any time the bird's beak touched the sand within a cup, and we did not record revisits to holes. We determined the number of errors a bird made within a cluster by counting the number of choices the bird made before it found the correct location. If birds were digging at random, the mean number of visits within a cluster would be 2.3. This is the mean probability of making zero, one, two, three, or four mistakes within a six-hole cluster.

**Testing.** We tested each bird for its memory of two lists of locations per day. We tested Nutcrackers' memory for locations in List 1 (control clusters), which never contained a correct location from List 2. During the recognition phase in List 2, we tested the birds with two types of clusters. Three clusters in List 2 (unique clusters) did not contain an interfering location from the previous list, but contained the correct location and five other possible incorrect locations that were unique for that day. The remaining two clusters (repeat clusters) contained the correct location, the interfering location (a location that had been rewarded during the recall of List 1 but was not rewarded during the recall of List 2), and four other possible incorrect locations. The purpose of the repeat clusters was to test for intrusion errors. If Nutcrackers make intrusion errors due to proactive interference, they should make more errors within repeat clusters than in unique clusters (Lewis & Kamil, 2006).

We tested the birds 5 days a week. On each testing day, the birds received List 1, a 5-min retention interval, and then List 2. During a testing day, we randomly assigned each bird in the study to receive one of the four landmark conditions (the fifth bird received the same condition as one of the other birds that day). The four conditions were testing with landmark Set A during both lists (same condition), landmark Set B during both lists (same condition), landmark Set A during List 1 and landmark Set B during List 2 (different condition), and landmark Set B during List 1 and landmark Set B during List 2 (different condition). Each bird received 10 trials of each condition over the course of the study.

## Results

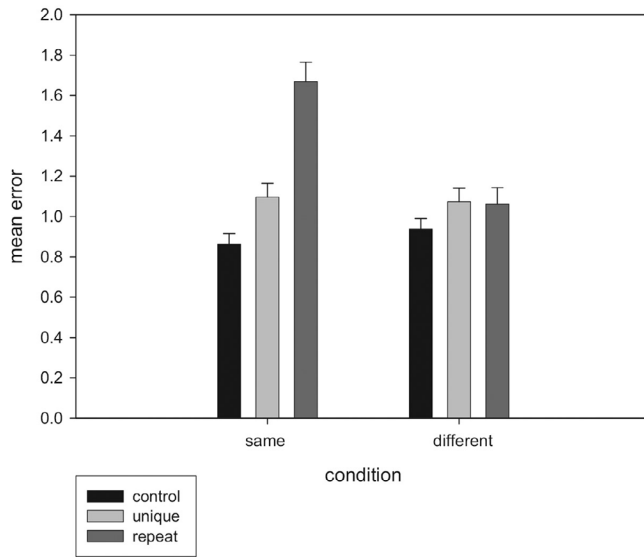
We found no significant difference in performance as a function of landmark set (A or B) that allowed us to pool the data from the two landmark set into two levels of the independent variable landmark (same and different). For the following analyses, we averaged the data for all five clusters and all 10 trials within each list. Using a landmark set (A/B)  $\times$  bird repeated-measures ANOVA for List 1 data, we found no significant difference in mean error per cluster between Set A ( $M = 0.92$ ,  $SD = 0.21$ ) and Set B ( $M = 0.88$ ,  $SD = 0.21$ ),  $F(1, 4) = 0.40$ ,  $p = .56$ . For List 2 data, we also found no significant difference between Set A ( $M = 1.25$ ,  $SD = 0.22$ ) and Set B ( $M = 1.15$ ,  $SD = 0.28$ ),  $F(1, 4) = 2.88$ ,  $p = .17$ .

Nutcrackers made more errors per cluster during List 2 when the landmark sets were the same between List 1 and List 2 than when the landmark sets were different between List 1 and List 2. We used a Landmark (same/different)  $\times$  List (1/2) repeated-measures ANOVA using mean error per cluster as the dependent measure. We found no main effect of landmark set on mean error within clusters,  $F(1, 4) = 2.10$ ,  $p = .22$ . However, we found a main effect of list as birds made significantly more errors in List 2 than in List 1,  $F(1, 4) = 31.70$ ,  $p = .005$ . We also found a significant Landmark  $\times$  List interaction,  $F(1, 4) = 18.86$ ,  $p = .01$ . Fisher's least significant difference (LSD) pairwise comparisons revealed that during recognition of List 1, Nutcrackers made approximately one error per six-hole cluster in both the same ( $M = 0.87$ ,  $SD = 0.50$ ) and different ( $M = 0.95$ ,  $SD = 0.52$ ) landmark conditions. However, during the recognition phase of List 2, Nutcrackers made more errors when the landmarks were kept the same between List 1 and List 2 ( $M = 1.34$ ,  $SD = 0.58$ ) than when the landmarks were different ( $M = 1.11$ ,  $SD = 0.58$ ).

The poor performance during the recognition phase of the List 2 different condition was due to interference from List 1. Nutcrackers made more mistakes in List 2 repeat clusters (which contained a location that had previously been correct during testing of List 1) than in List 1 control and List 2 unique clusters (which did not contain interfering information from List 1). Using mean error per cluster as the dependent variable, we ran a Landmark set (same/different)  $\times$  Cluster type (control/unique/repeat) repeated-measures ANOVA and Fisher's LSD pairwise comparisons. The main effect of whether the landmarks were the same or different for Lists 1 and 2 approached significance,  $F(1, 4) = 5.81$ ,  $p = .07$ . There was a main effect of cluster type,  $F(2, 8) = 24.0$ ,  $p = .001$ , and a significant Landmark  $\times$  Cluster type interaction,  $F(2, 8) = 7.41$ ,  $p = .02$ . Figure 2 shows that there were no differences in mean error between control, unique, and repeat clusters when the landmarks were different between List 1 and List 2, but when the landmarks were the same between List 1 and List 2, birds made significantly more errors in repeat clusters than in either control and unique clusters, which did not differ from each other.

The interference seen in List 2 repeat clusters was due to intrusion errors (mistakenly visiting a location during List 2 that had been correct during list 1). When probing a repeat cluster for the first time, Nutcrackers had three possible choices: correct (rewarded) location in List 2, the interfering location (intrusion error), or another incorrect location. Nutcrackers were more likely to make intrusion errors in repeat





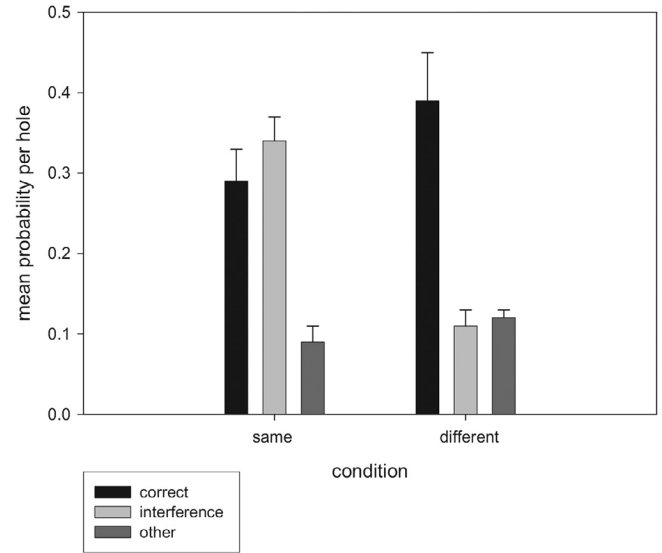
**Figure 2.** Mean error per cluster in for control, unique, and repeat clusters for same and different conditions. Error bars represent standard error.

clusters when the landmarks were the same between List 1 and List 2 than when the landmarks were different between List 1 and List 2. We ran a Landmark set (same/different)  $\times$  Choice (correct/interference/other) repeated-measures ANOVA using the mean number of first visits each bird made as the dependent measure. There was no main effect of whether birds were given the same or different landmark sets,  $F(1, 4) = 4.26$ ,  $p = .11$ , however, there was a main effect of choice,  $F(2, 8) = 33.76$ ,  $p = .001$ . There was also a significant Landmark  $\times$  Choice interaction,  $F(2, 8) = 7.75$ ,  $p = .01$ . We used Fisher's LSD to analyze the pairwise comparisons. Figure 3 shows that when the landmark sets were different between List 1 and List 2, birds were more likely to make its first visit to the correct location than any other location in the cluster. However, when the landmark sets were the same between List 1 and List 2, Nutcrackers were equally likely to make their first choice to both a correct and an interfering location both of these choices were greater the other incorrect locations.

## Discussion

By presenting the Nutcrackers with the same landmarks between List 1 and List 2, we replicated results of our prior study, which demonstrates that Clark's Nutcrackers are susceptible to proactive interference in tests of spatial memory (Lewis & Kamil, 2006). However, we also demonstrated that changing the landmark array between List 1 and List 2 decreases the effects of proactive interference typically seen during the recognition of List 2. When we changed the landmarks between lists, Nutcrackers made fewer errors in List 2 because they made fewer intrusion errors in repeat clusters (i.e., they were less likely to visit locations that were correct for List 1). This suggests that changing the landmarks helped Nutcrackers to distinguish between old and new information.

These results suggest that animals perform better on memory tests when potentially conflicting information is learned in different contexts than when learned in the same context.



**Figure 3.** Mean probability of visiting a correct location, interference, or other incorrect location during the first choice within repeat clusters for same and different conditions. Error bars represent standard error.

This agrees with past research on proactive and retroactive interference with rats in a radial arm maze. Rats' performance on a radial maze declines when they are exposed to extra visits to arms within the same maze (Hoffman & Maki, 1986; Cohen et al., 1996). However, this interference can be reduced if rats are tested in a separate maze than the test maze (Beatty & Shavalia, 1980; Cook, & Brown, 1985; Maki, Brokofsky & Burg, 1979). Roberts (1981) systematically varied the similarity between the context of the original and the interpolated maze. He found no evidence of interference when rats were tested in different mazes that were beside or on top of one another. However, he did find effects of interference when the rats were forced to make choices into arms in the same maze. This research suggests that interference effects are caused by the stimulus similarity between the contexts of the target and interfering information and that changing context can help animals to segregate conflicting spatial memories. The context may help to provide the animal with "instructions" on which location is correct on a given trial (Zentall, 1970).

The organism's "context" could include any visual, auditory, olfactory, or tactile stimulus in the environment. We argue, however, that the most effective memory cues will depend on the biology of the organism one is studying. For example, changing the patterns, colors, and objects in and outside a radial maze only weakened, but did not eliminate, interference for rats (Cohen et al., 1994). In our studies, however, we were able to minimize the effects of interference by changing the visual cues. This may be because birds have better vision than nocturnal rodents and may be more effective at making associations in their environment with visual stimuli, whereas rats learn more effectively with gustatory or olfactory stimuli (e.g., Eichenbaum, Fagan, & Cohen, 1986; Shettleworth, 1998; Wilcoxon's, Dragoin, & Kral, 1971). In addition, landmarks may serve as the most salient memory cue for food storing animals like because they rely heavily on landmarks to recover stored food (e.g., Barkley & Jacobs, 1998; Gould-Beierle & Kamil, 1996; 1999 Vander Wall, 1982).

The results of our study, in conjunction with Brodbeck et al. (1992) and Barkley and Jacobs (1998), suggest that testing food storing animals in the same testing room, using the same landmarks repeatedly over trials, can cause a build-up of proactive interference that will hurt the animal's performance on the task. Providing the animal with trial-unique stimuli should decrease the detrimental effects of interference.

For example, Brodbeck et al. (1992) found that providing food-storing animals with unique sets of landmarks over multiple trials can reduce the effects of proactive interference from previous trials. Using a one-trial associate task, they found that chickadees will make fewer errors when remembering a baited location when a unique set of feeders are placed in unique locations during each trial. Conversely, birds performed only at chance levels when they had to remember a baited location and were given the same set of feeders in the same locations during multiple trials.

The importance of trial-unique landmark configurations may also help to explain the results of Barkley and Jacobs' (1998) study. They found that Merriam's kangaroo rats (*Dipodomys merriami*) retrieved more caches during long delays when they cached food in a room provided with a large number of landmarks versus no landmarks, but the number of landmarks and the trial unique configuration of the landmarks were confounded in this study. Each time the kangaroo rats cached and recovered in an environment with landmarks, the array was changed (which was not possible in the 0 landmark condition). This suggests that decreased performance on the 0 landmark condition could have been due to proactive interference from previous trials that were conducted in the same context. Furthermore, many of the errors kangaroo rats made were to locations the animal had used as a cache site during previous trials. This suggests that proactive interference effects during cache recovery may cause animals to make mistakes by revisiting depleted locations (i.e., intrusion errors).

This study provides a reminder to those who study animal memory, that proactive interference is also likely to build whenever stimuli are repeatedly reused in a study. Not only is proactive interference likely to build when researchers reuse the same rewarded items repeatedly in the same study (Sands & Wright 1980a, 1980b; Wright, Katz & Ma, 2012), but animals are also most susceptible to proactive interference when they are repeatedly tested in the same environment with the same contextual stimuli. In order to obtain an animal's true performance on a memory test, researchers should provide trial unique stimuli. The type of stimuli that should be changed will depend on the particular species one is working with. For food storing animals, such as the Clark's nutcracker, changing the landmarks between trials should help to decrease the effects of interference and increase performance on memory tasks.

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