Ethology, **72**, 22—30 (1986)

ISSN 0179-1613

Department of Biological Sciences, Wellesley College, Wellesley

Cues Used for Localizing Food by the Gray Squirrel (*Sciurus carolinensis*)

DENISE B. MCQUADE, ERNEST H. WILLIAMS & HOWARD B. EICHENBAUM

With 2 figures

Received: January 11, 1985
Accepted: June 17, 1985

Abstract

We compared the use of olfactory, visual, and spatial cues for learning the location of stored food by gray squirrels (*Sciurus carolinensis*). All experimental cues were extrinsic, that is, they originated from the environment around the food rather than from the food itself. In training trials, artificial caches with one of two odors, one of two colors, and six of 12 spatial locations contained sunflower seeds. In experimental trials, the odors, colors, and sets of spatial locations associated with food were reversed one at a time, so that only two of the three training cues gave evidence of the food rewards. Consequent declines in food localization by the squirrels revealed differential use of particular cue modalities. The data show that squirrels used visual cues the most and olfactory cues the least with this design. These results, along with other evidence, suggest that gray squirrels use spatial memory in food recovery.

Introduction

Though gray squirrels (*Sciurus carolinensis*) use olfaction in recovering food caches (CAHALANE 1942), the range of sensory modalities in food recovery has not been reported. Cue use in gray squirrels is of interest for several reasons. These animals lead arboreal and diurnal lives and have therefore been subject to different environmental pressures than have most rodents; in consequence, gray squirrels may use a different combination of available sensory cues than do other rodents. Cache recovery also has ecological importance because of the dual role of squirrels as seed predator and seed disperser, roles which have produced presumed coevolution between squirrels and some trees (JANZEN 1971; SMITH & FOLLMER 1972). Not knowing how squirrels can recover food has led to open questions in models of caching behavior (STAPANIAN & SMITH 1978, 1984; KRAUS 1983).

U.S. Copyright Clearance Center Code Statement: 0179-1613/86/7201-0022$02.50/0

Olfaction is clearly important to squirrels (CAHALANE 1942; LEWIS 1980), but their biology suggests that they may make significant use of other sensory modalities as well. Squirrels require well-developed spatial abilities for arboreal movement and vision for diurnal activity, and they may learn well the features of their home range. Squirrels cache acorns, their preferred food (BAUMGRAS 1944; DAVIDSON 1963), for recovery in the winter and early spring, and there is a tendency for caches to be concentrated near the den tree (STAPANIAN & SMITH 1978). Furthermore, as STAPANIAN & SMITH (1978) point out, the only advantage a resident squirrel has over naive squirrels in the recovery of its caches is its knowledge or memory of their location. These and other facets of squirrel biology indicate the importance of sensory modalities other than olfaction.

Squirrels may use either (1) intrinsic cues, such as food odor, soil disturbances, or scent marks associated with a cache, which signify reward to both caching and non-caching animals, or (2) extrinsic cues, arbitrary ones which are known only to the caching animals and which distinguish the locale of the cache, such as a rock (visual cue), a plant (olfactory cue), or the place of the cache relative to many local landmarks (spatial cue). We here report on the ability of gray squirrels to use extrinsic visual, spatial, and olfactory cues in the localization of seed rewards.

Methods

Two male and two female adult gray squirrels were captured from suburban wooded areas during Oct., 1981. They were housed individually in cages with a 14-h dark — 10-h light cycle with constant ventilation at approximately 20 °C. One female was unwilling to forage in the experimental arena and was subsequently eliminated from the analysis. For the testing apparatus, we constructed an open field arena, measuring 3 × 2 × 0.5 m, of wood and window screening. All trials were monitored indirectly with a Sony video camera positioned in a corner of the room; the experimenter then viewed the behavioral sequence from an adjacent room and taped each run with a Sony video deck. Squirrels were first allowed to habituate to the arena and to the transfer process. Later, sunflower seeds were localized in artificial caches made of plastic Petri dishes (95 mm) wrapped with black electrical tape. For stabilization, each dish was glued to one half of a red brick.

Each artificial cache provided distinct olfactory, visual, and spatial cues. We used two purified chemical extracts, phenyl ethyl alcohol (PEA) and geraniol (GER), as olfactory cues. These two odors have been used extensively in studies with rats, which easily discriminate them (EICHENBAUM et al. 1980, 1983). Extract was reapplied frequently to filter paper on the covers of the Petri dishes to maintain an odor detectable by a human observer.

To provide local visual cues, the covers of the Petri dishes were covered with either yellow or blue tape, with colors most closely approximating picric yellow and pale cerulean blue in the Muncell series (RIDGWAY 1912). Gray squirrels can distinguish between wavelengths above and below 500 nm (JACOBS 1976), and they should, therefore, be able to discriminate between a yellow greater than 500 nm and a blue less than 500 nm (GROVES & SCHLESINGER 1979). We did not attempt to control for brightness of the colors since it was necessary only for the squirrels to discriminate visually between the two types of colored lids.

The spatial cue was incorporated into the study by choosing 12 standard positions in the arena for the artificial caches and allowing the lighting, walls, and other asymmetrical aspects of the room and arena to serve in recognizing unique locations. The spatial arrangement consisted of four parallel rows with three artificial caches on each row (Fig. 1).

We first tested for initial preferences for certain odors or colors by rewarding all cue combinations (PEA and GER or yellow and blue) and observing the sequence of visitation by the

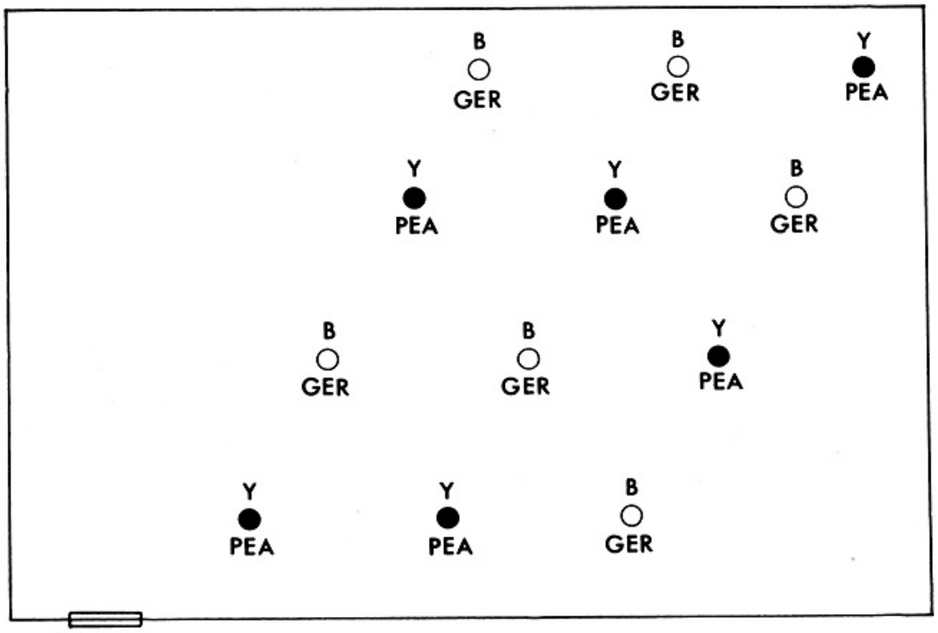

Fig. 1: Overhead view of the open-field arena used in the training and experimental trials. Each position is labelled with the color and odor it was associated with in the training trials (B = blue, Y = yellow; GER = geraniol, PEA = phenyl ethyl alcohol), and closed circles mark the artificial caches with seeds. The arena was 2 × 3 m in size, with the entrance shown in the lower left

squirrels during 17 trials. For all runs (pretraining, training, and experimental), a trial refers to one run by one squirrel on a single day. The order of visitation to the 12 artificial caches was also analysed to determine what preferences existed for the 12 positions. In the subsequent training trials, only half of the artificial caches contained seed rewards, and those caches were always associated with one of the odors (PEA), one of the colors (yellow), and six of the 12 positions (Fig. 1). The three squirrels each ran once daily in that training arrangement for 14 trials.

In the 30 experimental trials, one cue at a time was reversed from the training arrangement. For example, when the odor cues were reversed, the rewarded caches were paired with GER instead of PEA, while the seeds were still located in the same six training positions and still had yellow covers. If the squirrels located the rewarded caches primarily according to the odor with which seeds had been associated during the training runs, they would visit the unrewarded caches with PEA covers first. Experimental trials were also run with visual and spatial cues reversed. Each experimental trial was followed by at least one retraining trial, where the seeds were in the six original positions with PEA/yellow covers. In retraining, if a squirrel failed to score five correct choices in its first six attempts, it was provided subsequent training trials until the five of six criterion was met. The order that the squirrels were run and the order of the experimental trials for each squirrel were randomized.

For each experimental trial we recorded the number of rewards obtained from the first six positions visited. The reward in each artificial cache was six sunflower seeds because squirrels are attracted but do not satiate with this amount. The concrete floor of the arena, painted dull red, was washed daily with Stanley Formula 112 Spearmint Disinfectant Cleaner, and the Petri dishes and bricks were switched in position frequently to guard against recovery by scent marking.

Results

To determine the pretraining bias for either color or either odor, an index C was devised to represent preference in the visitation sequence. C = (sum of ranks of one cue in a visitation sequence — minimum sum of ranks)/(max. sum of ranks — min. sum of ranks); C ranges from 0.0, representing initial choice of all caches with one of two alternative cues, to 1.0, representing initial choice of all caches with the other cue, and a value of 0.5 indicates a lack of preference. The data (Table 1) show a slight overall bias for PEA and yellow, but there is high variance among trials and among subjects. What is important, though, is not that the animals showed an initial bias, but that they learned in the training trials to associate certain cues with food. The bias then changed substantially.

The rank order of visitation to each position was also summed separately to examine bias for position. The squirrels did show higher visitation rates to positions nearer the entry into the arena (Fig. 1; Table 2), with positions 2 and 5 usually being approached first, then positions 1, 3, 6, and 9, and position 12 usually approached last. D is an index similar to C above; 0.0 indicates that a position was visited first on each run, while 1.0 means that it was visited last, and 0.5 results from the position being on average in the middle of the sequence. The preference for certain locations did not influence the results, however, because the visitation sequence to positions that were rewarded was similar to the visitation sequence to unrewarded positions (D = .51).

Squirrels learned the association of cues and rewards quickly (Fig. 2), so that by the 8th trial, each had scored a perfect run. Their performance remained consistently high (at least five of 6 choices correct) through the next six training trials.

In the experimental runs, the visual cue reversal scores were consistently lowest, followed by spatial cue reversal scores, while olfactory cue reversal scores

Table 1: Pretraining bias for the experimental cues. Values of the index C are shown (see text); a value of 0.5 results from equal choice of the two cues, while 0.0 indicates preference for the first cue in each pair (PEA, yellow) and 1.0 avoidance

Subject	Trial	Odor PEA — GER	Color yellow — blue
1	all trials	.35	.55
	1	.17	.72
	2	.28	.56
	3	.61	.36
2	all trials	.43	.42
	1	.44	.44
	2	.22	.25
	3	.64	.56
3	all trials	.36	.37
	1	.53	.42
	2	.33	.31
	3	.22	.39
all subjects		.38	.44

Table 2: Pretraining bias for positions in the experimental arena. Values of the index D are shown (see text); 0.0 indicates that the position was the first one visited on all runs, and 1.0 means that it was visited last on all runs. The data are summed for 6 trials of the first two animals and 5 trials of the third

Position	Subject			All
	1	2	3	Subjects
1	.53	.50	.31	.46
2	.18	.35	.46	.32
3	.39	.53	.44	.46
4	.53	.68	.64	.62
5	.42	.21	.33	.32
6	.32	.58	.60	.49
7	.59	.42	.66	.55
8	.55	.46	.62	.54
9	.55	.42	.33	.44
10	.68	.59	.38	.56
11	.50	.62	.51	.55
12	.76	.64	.71	.70

were the highest (Table 3). A low score indicates that the performance of a squirrel was strongly affected by the reversal of that cue. Scores remained high on retraining trials. Based on a two-way analysis of variance (ANOVA) by cue and by subject, there was no significant interaction ($F = 1.76$; $df = 4,21$; $.20 < p$), and using the interaction term as the error term, there was a significant effect for cue ($F = 16.70$, $df = 2,4$; $p < .05$) but no significant effect for subject ($F = 0.71$; $df = 2,4$; $.50 < p$). Because of the lack of a significant effect by subject, a one-way ANOVA was performed with the data collapsed on only the cue effects. Again

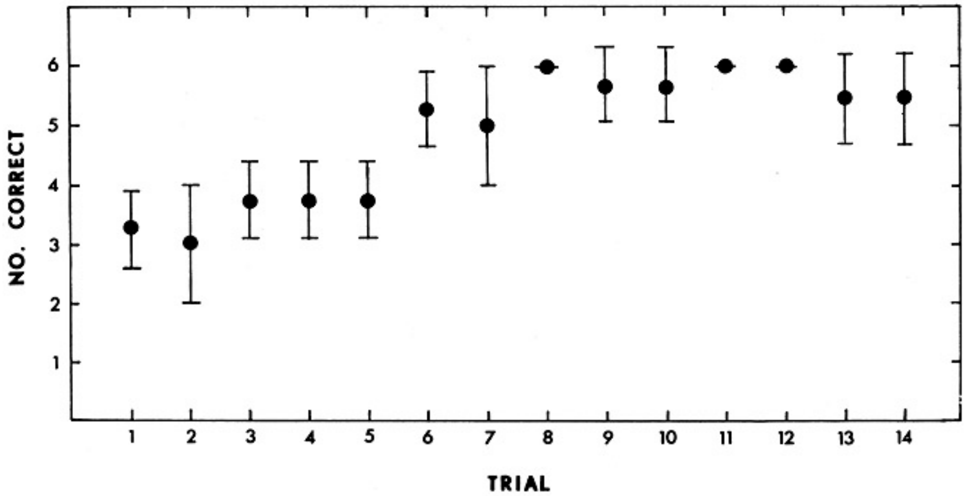

Fig. 2: Mean scores of all squirrels in the training trials. The scores represent the number of correct (rewarded) choices in the first six artificial caches visited. The trials took place over a three- to four-week period

Table 3: Mean number correct of the first six choices by each squirrel during the experimental and retraining runs

Trials	Subject #1	#2	#3	Mean x ± sd (n)
Experimental trials (cue reversed)				
Olfactory	5.5 ± 0.6	5.0 ± 0.8	5.5 ± 0.7	5.3 ± 0.7 (10)
Visual	2.5 ± 0.6	3.0 ± 0.8	2.5 ± 0.7	2.7 ± 0.7 (10)
Spatial	4.3 ± 1.0	3.0 ± 0.8	4.5 ± 0.7	3.8 ± 1.0 (10)
Retraining trials	5.8 ± 0.5	5.5 ± 0.5	5.3 ± 0.7	5.5 ± 0.6 (33)

Table 4: Preferential cue use by the squirrels, based on ANOVAs and the Scheffé multiple contrast test

Cue comparison			p
visual	>	olfactory	$p < 0.01$
spatial	>	olfactory	$p < 0.01$
visual	>	spatial	$p < 0.05$
olfactory	=	retraining	$0.20 < p$

there was a significant cue effect (F = 25.84; df = 2,27; $p < .001$). Subsequent analysis revealed no significant difference between scores with the olfactory cue reversed and scores on the retraining trials (by type of trial: F = 1.48; df = 1,41; $.20 < p$; and by subject: F = 1.54; df = 2,40; $.20 < p$).

The results are summarized in Table 4; there was a significant difference among reversal scores for each pair of the sensory cues. The squirrels, therefore, relied on each sensory modality to a different extent, choosing first visual cues, then spatial cues, and finally olfactory cues from the extrinsic cues provided.

Discussion

The training trials provided the squirrels with the opportunity to associate extrinsic olfactory, visual, and spatial cues with the food rewards. It is clear that the squirrels were able to recover the food with a lapse of one to two days between trials. The experimental trials then indicated how important each cue was to the squirrels in the recovery of the seeds. All of the cues were artificial but distinct, all were extrinsic, all were equally accessable to the squirrels, and all three were present simultaneously. In order from greatest to least use, the squirrels relied on visual, spatial, and olfactory cues in these experiments since low scores indicate that a squirrel continued to follow that cue even when it was not associated with a reward.

Naturally occurring complex odors may also be preferred over isolated components (SCHULTZ & TAPP 1973). Squirrels may be more flexible in learning to associate new colors and spatial locations with food, since these cues would be quite variable in nature, than they are with new odors, especially if they rely on nut odors or scent marks. The odors may have been too strong. Finally, squirrels may not have relied on the olfactory cue because of dependence on another cue during the initial training; such overshadowing or blocking of one cue by another has been demonstrated elsewhere (NIGROSH et al. 1975).

We interpret our results as suggesting that extrinsic olfactory cues are not important in food localization by squirrels, while we accept the observations of others (CAHALANE 1942; BARKALOW & SHORTEN 1973; LEWIS 1980) which point to the importance of intrinsic olfactory cues in cache recovery. Extrinsic visual and spatial cues, on the other hand, are important. There may be a hierarchy in the use of sensory cues by squirrels; memory of location and local visual cues may be used to locate the relative positions of caches, with intrinsic odors used to detect the exact locations for digging. The use of different sensory modalities undoubtedly depends on the availability of different kinds of cues; for example, as CAHALANE (1942), JANZEN (1971), and SMITH & FOLLMER (1972) have remarked, visual cues may be much reduced with complete burial or snow cover.

The described experimental paradigm differs from actual cache recovery in that the specific cues provided do not occur in the natural environment, and the association of food location is made by the experimenter rather than by the squirrel during food burial in its territory. It is possible that experimenter-assigned cues are relatively less salient than marks left by squirrels when making their own caches. For example, several authors (BARKALOW & SHORTEN 1973; STEINER 1975; KIVETT et al. 1976) have suggested that ground squirrels may mark cached nuts with volatile secretions from mouth corner glands and use these later to recover the caches. The present results do, however, reveal the ability to use different extrinsic cues and the relative reliance of those cues under experimentally controlled conditions.

The construction of models for caching behavior, such as that of STAPANIAN & SMITH (1978), requires knowledge of the capabilities and means of recovery by squirrels. It is clear that squirrels can use arbitrary local visual cues and spatial memory for recovering food in a controlled environment. They did not use local olfactory cues in this experiment. However, the evolution of cache recovery implies that the discovery of another's caches would be associated with selection for well-developed olfactory capabilities, while recovery of one's own caches would be associated with selection for spatial memory. For that reason, memory is likely to be important in the field as well and should be investigated further.

Acknowledgments

We thank Elissa KOFF and Lucia JACOBS for discussion; C. C. SMITH, J. P. HAILMAN, and two anonymous reviewers for comments on the manuscript; Donald BROOKS for help in constructing the arena; Douglas HERRMANN and Jonathan VAUGHAN for statistical help; and Win POOR, MIT-DLAM, for loan of the cages. Financial support was provided by Wellesley College and Hamilton College.

Literature Cited

BALDA, R. P., 1980: Recovery of cached seeds by a captive *Nucifraga caryocatactes*. Z. Tierpsychol. **52,** 331—346.

BARKALOW, F. S., & M. SHORTEN, 1973: The World of the Gray Squirrel. J. B. Lippincott Co., Philadelphia.

BAUMGRAS, P., 1944: Experimental feeding of captive fox squirrels. J. Wildl. Mgmt. **8,** 296—300.

BOSSEMA, I., 1979: Jays and oaks; an eco-ethological study of a symbiosis. Behaviour **70,** 1—117.

CAHALANE, V. H., 1942: Caching and recovery of food by the western fox squirrel. J. Wildl. Mgmt. **6,** 338—352.

DAVIDSON, V. E., 1963: Selection of foods by gray squirrels. J. Wildl. Mgmt. **28,** 346—352.

EICHENBAUM, H., R. A. CLEGG, & A. FEELEY, 1983: Reexamination of functional subdivisions of the rodent prefrontal cortex. Exp. Neurol. **79,** 434—451.

— —, K. J. SHEDLACK, & K. W. ECKMANN, 1980: Thalamocortical mechanisms in odor-guided behavior. I. Effects of lesions of the mediodorsal thalamic nucleus and frontal cortex on olfactory discrimination in the rat. Brain Behav. Evol. **17,** 255—275.

GODIN, A. J., 1977: Wild Mammals of New England. John Hopkins Press, Baltimore.

GROVES, P., & K. SCHLESINGER, 1979: Introduction to Biological Psychology. Wm. C. Brown Co., Dubuque.

HAILMAN, J., 1960: Notes on the following response and other behavior of young gray squirrels. Am. Midl. Nat. **63,** 413—417.

JACOBS, G. H., 1974: Scotopic and photopic visual capacities of an arboreal squirrel (*Sciurus niger*). Behav. Evol. **10,** 307—321.

— —, 1976: Wavelength discrimination in gray squirrels. Vision Res. **16,** 325—327.

JANZEN, D. H., 1971: Seed predation by animals. Ann. Rev. Ecol. Syst. **2,** 465—492.

KIVETT, V. K., J. O. MURIE, & A. L. STEINER, 1976: A comparative study of scent-gland location and related behavior in some northwestern nearctic ground squirrel species (Sciuridae): An evolutionary approach. Can. J. Zool. **54,** 1294—1306.

KRAUS, B., 1983: A test of the optimal-density model for seed scatterhoarding. Ecology **64,** 608—610.

LEWIS, A. R., 1980: Patch use by gray squirrels and optimal foraging. Ecology **61,** 1371—1379.

MURIE, J. O., 1977: Cues used for cache-finding by agoutis (*Dasyprocta punctata*). J. Mammal. **58,** 95—96.

NIGROSH, B. J., B. M. SLOTNICK, & J. A. NEVIN, 1975: Olfactory discrimination, reversal learning and stimulus control in rats. J. Comp. Physiol. Psych. **89,** 285—294.

RIDGWAY, R., 1912: Color Standards and Color Nomenclature. Publ. by author, Washington, DC.

SCHULTZ, F. E., & J. T. TAPP, 1973: Olfactory control of behavior in rodents. Psych. Bull. **79,** 21—44.

SHERRY, D., 1984: Food storage by black-capped chickadees: Memory for the location and contents of caches. Anim. Behav. **32,** 451—464.

SHETTLEWORTH, S. J., & J. R. KREBS, 1982: How marsh tits find their hoards: the roles of site preference and spatial memory. J. Exp. Psychol.: Anim. Behav. Proc. **8,** 354—375.

SLOTNICK, B. M., & H. M. KATZ, 1974: Olfactory learning set formation in rats. Science **185,** 796—798.

SMITH, C. C., & D. FOLLMER, 1972: Food preference of squirrels. Ecology **53,** 82—91.

STAPANIAN, M. A., & C. C. SMITH, 1978: A model for seed scatterhoarding: Coevolution of fox squirrels and black walnuts. Ecology **59,** 884—896.

— —, — —, 1984: Density-dependent survival of scatterhoarded nuts: An experimental approach. Ecology **65,** 1387—1397.

STEINER, A. L., 1975: "Greeting" behavior in some Sciuridae, from an ontogenetic, evolutionary and socio-behavioral perspective. Naturaliste Can. **102,** 737—751.

TOMBACK, D. F., 1980: How nutcrackers find their seed stores. Condor **82,** 10—19.

VANDER WALL, S. B., 1982: An experimental analysis of cache recovery in Clark's Nutcracker. Anim. Behav. **30,** 84—94.

Authors' addresses: E. H. WILLIAMS, Department of Biology, Hamilton College, Clinton, New York 13323, U.S.A.; D. B. MCQUADE and H. B. EICHENBAUM, Department of Biological Sciences, Wellesley College, Wellesley, Massachusetts 02181, U.S.A.