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Long-term memory in frog-eating bats

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Long-term memory has clear advantages for animals but also has neurological and behavioral costs[1–3]. Encoding memories is metabolically expensive[1]. Older memories can interfere with retrieval of more recent memories[3], prolong decision-making and reduce cognitive flexibility[2,3]. Given these opposing selection pressures, understanding how long memories last can shed light on how memory enhances or constrains animals' abilities to exploit their niches. Although testing memory retention in wild animals is difficult, it is important because captive conditions do not reflect the complex cognitive demands of wild environments, and long-term captivity changes the brain[4] (Data S1A). Here, we trained wild-caught frog-eating bats (*Trachops cirrhosus*) to find prey by flying to a novel acoustic cue. After they learned the rewarded sound, we released them back into the wild, and then re-captured some of them one to four years later. When re-tested, all eight 'experienced' bats that previously learned the novel prey sounds flew to those sounds within seconds, whereas 17 naïve bats tested with the same sounds showed weak responses. Experienced bats also showed behavior indicating generalization of memories between novel sounds and rewards over time. The frog-eating bat's remarkably long memory indicates that an ability to remember rarely encountered prey may be advantageous for this predator and suggests hitherto unknown cognitive abilities in bats.

The phyllostomid bats are the most extensive adaptive radiation of any mammalian family within the most ecologically diverse mammalian order, Chiroptera. The predatory phyllostomid *T. cirrhosus* is an emerging model in cognitive ecology[5–7], which hunts by eavesdropping on the mating calls of many frog and katydid species, and can discriminate the calls of palatable versus poisonous species[5]. We captured 49 wild adult *T. cirrhosus*, individually marked them, and trained them to fly to a novel, artificial sound (one of two ringtones: "trained-A" or "trained-B")[6]. After training, bats spontaneously generalized the association and flew to other ringtones. We then trained the bats to discriminate between their trained ringtone and three other unrewarded ringtones[6]. Before release, these 'experienced' bats had retrieved rewards in response to flying to their trained ringtone at least 40 times over 11 to 27 days.

We recaptured eight of the 49 experienced bats (seven males and one female) 356–1531 days after their initial release. We retested them on their trained ringtone under the same conditions as their original training[6]. To investigate to what degree bats would generalize the response to similar stimuli, we also played an 'extinguished ringtone' and a 'control sound'. The extinguished ringtone was one of the acoustically similar but unrewarded ringtones used in their discrimination training (ringtone "E" in[6]). The control sound was an acoustically different 1.5-kHz pure tone that the bats had not heard before, selected to assess whether they would generalize the experimental association to any sound from the speaker. As a control group, we presented the same sounds to 17 adult wild-caught naïve bats (13 males and four females) with no prior experience with the experimental sounds. We scored the maximum responses of the naïve and experienced bats using an ordinal scale of increasingly strong

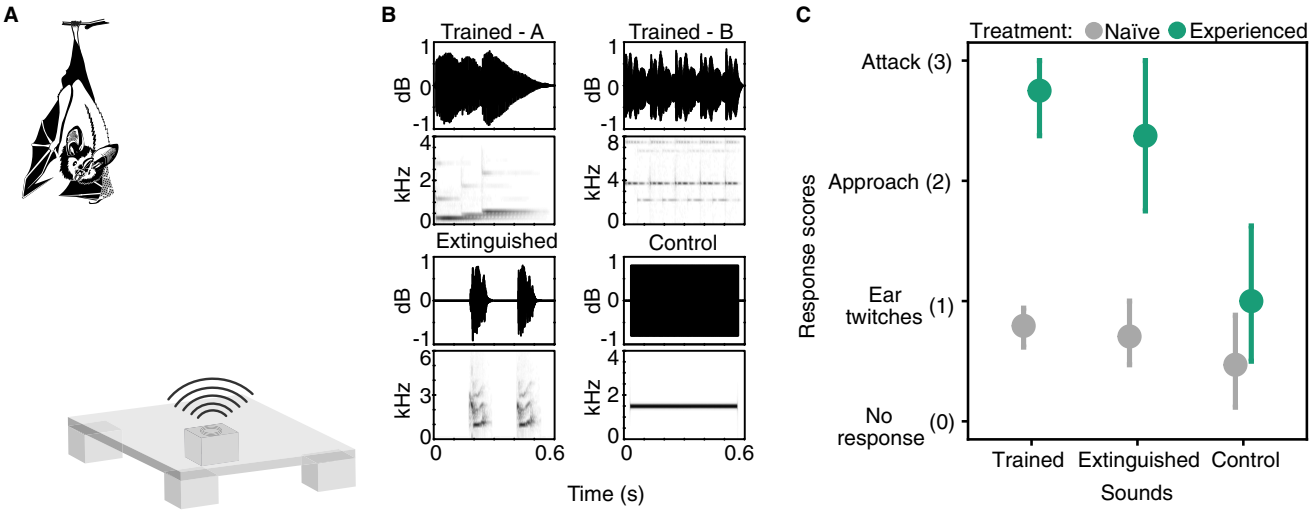

Figure 1. Experienced bats but not naïve bats attacked trained sounds.
(A) Schematic of experimental setup (not to scale). (B) Waveforms and sonograms of the four experimental sounds. Trained-A and trained-B were sounds that experienced bats had previously associated with food, the extinguished sound was heard by experienced bats but was never rewarded, and the control sound was novel for both experienced and naïve bats. (C) Mean responses of naïve (gray) and experienced bats (green) with bootstrapped 95% confidence intervals.

responses: 0 = no response, 1 = ears twitched synchronously with stimulus (Video S1), 2 = approached stimulus within 1 m, and 3 = attacked speaker and retrieved reward.

The experienced bats responded more strongly to the trained sounds than did the naïve bats (Figure 1 and Supplemental information; permutation test: $P < 0.0002$). For example, six of eight experienced bats attacked the trained ringtone and all eight approached it, whereas none of 17 naïve bats attacked and only one approached (Data S1B). Experienced bats also had strong responses to the extinguished sound, with five of eight attacking, and six approaching (Figure 1). The experienced bats' responses to the trained and extinguished sounds were not significantly different from one another but were stronger than their responses to the control sound ($P < 0.001$, Figure 1). The naïve bats, on the other hand, typically only twitched their ears to all sounds (Data S1B). We saw no clear evidence that the experienced bats' responses decreased across the retention times of 356 to 1531 days (Data S1B).

Our results demonstrate remarkably long memories in wild frog-eating bats, with individuals remembering a learned foraging association for up to 4.2 years without reinforcement in the wild. This duration is comparable to that reported for corvids and primates (Data S1A). The observation that six experienced bats also approached the previously extinguished sound (Data S1B) suggests either that they remembered the difference between the sounds but resampled the extinguished sound, or they remembered the general experimental procedure but generalized the trained association to a sufficiently similar sound[8]. They did not exhibit such strong responses to the control sound.

Previous work shows that frog-eating bats approach the calls of allopatric frog species that share acoustic characteristics with local palatable prey and avoid allopatric species' calls that sound like local toxic prey[7]. Approaching sounds similar to ones that were previously profitable is a practicable strategy for finding novel prey while lowering the risks of sampling potentially dangerous prey[7]. Generalization over time may also be adaptive given that older memories are less likely to reflect the current environment[8]. When environmental change increases uncertainty, and especially when sampling costs are low, individuals may benefit from resampling[9], and some sampling is necessary for trial-and-error learning. For example, three of the naïve bats approached novel sounds including the control sound, showing that bats occasionally investigate novel sounds.

Our study highlights that memory experiments with marked individuals at long-term field sites can help researchers link wild memory duration to species-specific ecological traits. Some of the preferred prey species of *T. cirrhosus* are either rare or are explosive breeders that are heard infrequently during much of the year[10]. The ability for this bat to remember previously profitable prey cues over long time intervals would therefore allow them to avoid costly trial-and-error learning when exploiting these seasonal or rare resources. Comparative studies of cognitive ability across diverse taxa could be facilitated by leveraging the existence of marked wild individuals from long-term field studies.

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

Supplemental information including experimental procedures, data file and one video can be found with this article online at https://doi.org/10.1016/j.cub.2022.05.031.

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