for events occurring farther apart in time. Coal-location is not limited to linking memories at encoding. Memory recall may engage a similar process to link new with old memories. We trained mice on event 1, 2 days before event 2. Event 2 memory was enhanced if event 1 was recalled 6 hours, not 24 hours, before event 2 (Fig. 4D). Here, we find that excitatory-inhibitory balance determines whether memories are bound or, alternately, segregated in the LA. More broadly, these principles provide a foundation for understanding how memories are organized within associative networks.

Note added in proof: During final preparation of this manuscript, a notable study showing time-limited coallocation of hippocampal memory traces was published (*25*).

REFERENCES AND NOTES

1. S. A. Josselyn, S. Köhler, P. W. Frankland, *Nat. Rev. Neurosci.* **16**, 521–534 (2015).
2. S. Tonegawa, X. Liu, S. Ramirez, R. Redondo, *Neuron* **87**, 918–931 (2015).
3. J. E. LeDoux, *Annu. Rev. Neurosci.* **23**, 155–184 (2000).
4. S. Maren, *Ann. N. Y. Acad. Sci.* **985**, 106–113 (2003).
5. S. Duvarci, D. Pare, *Neuron* **82**, 966–980 (2014).
6. D. Kim, D. Paré, S. S. Nair, *J. Neurosci.* **33**, 14354–14358 (2013).
7. J. H. Han *et al.*, *Science* **316**, 457–460 (2007).
8. J. H. Han *et al.*, *Science* **323**, 1492–1496 (2009).
9. Y. Zhou *et al.*, *Nat. Neurosci.* **12**, 1438–1443 (2009).
10. A. P. Yiu *et al.*, *Neuron* **83**, 722–735 (2014).
11. L. A. Gouty-Colomer *et al.*, *Mol. Psychiatry* **21**, 364–375 (2016).
12. J. F. Guzowski, *Hippocampus* **12**, 86–104 (2002).
13. M. M. Oh, A. G. Kuo, W. W. Wu, E. A. Sametsky, J. F. Disterhoft, *J. Neurophysiol.* **90**, 2171–2179 (2003).
14. L. T. Thompson, J. R. Moyer Jr., J. F. Disterhoft, *J. Neurophysiol.* **76**, 1836–1849 (1996).
15. L. Monaco, N. S. Foulkes, P. Sassone-Corsi, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 10673–10677 (1995).
16. A. J. Silva, Y. Zhou, T. Rogerson, J. Shobe, J. Balaji, *Science* **326**, 391–395 (2009).
17. Y. Mei, F. Zhang, *Biol. Psychiatry* **71**, 1033–1038 (2012).
18. V. Gradinaru *et al.*, *Cell* **141**, 154–165 (2010).
19. B. N. Armbruster, X. Li, M. H. Pausch, S. Herlitze, B. L. Roth, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 5163–5168 (2007).
20. P. A. Shoemaker, *Front. Comput. Neurosci.* **9**, 12 (2015).
21. P. Sah, E. S. Faber, M. Lopez De Armentia, J. Power, *Physiol. Rev.* **83**, 803–834 (2003).
22. S. B. Wolff *et al.*, *Nature* **509**, 453–458 (2014).
23. A. R. Woodruff, P. Sah, *J. Neurosci.* **27**, 553–563 (2007).
24. S. Trouche, J. M. Sasaki, T. Tu, L. G. Reijmers, *Neuron* **80**, 1054–1065 (2013).
25. D. J. Cai *et al.*, *Nature* **534**, 115–118 (2016).

BEHAVIORAL ECOLOGY

Reciprocal signaling in honeyguide-human mutualism

Claire N. Spottiswoode,[1,2]* Keith S. Begg,[3] Colleen M. Begg[3]

Greater honeyguides (*Indicator indicator*) lead human honey-hunters to wild bees' nests, in a rare example of a mutualistic foraging partnership between humans and free-living wild animals. We show experimentally that a specialized vocal sound made by Mozambican honey-hunters seeking bees' nests elicits elevated cooperative behavior from honeyguides. The production of this sound increased the probability of being guided by a honeyguide from about 33 to 66% and the overall probability of thus finding a bees' nest from 17 to 54%, as compared with other animal or human sounds of similar amplitude. These results provide experimental evidence that a wild animal in a natural setting responds adaptively to a human signal of cooperation.

In 1588, João dos Santos, a Portuguese missionary in Sofala (in present-day Mozambique), often noticed a small bird flying through cracks in the walls of his mission church and nibbling wax from the candlesticks within. This kind of bird, he wrote, had another peculiar habit of leading men to bees' nests by calling and flying from tree to tree. After the men harvested the honey, the birds would eat the wax combs left behind (*1*). We now know this species to have been the greater honeyguide *Indicator indicator* and dos Santos's description to have been accurate. Honeyguides eat beeswax and know where bees' nests are located; humans can subdue the bees and open the nest using fire and tools, thus exposing beeswax for the honeyguides and honey for the humans (*2*). This interaction remains an extremely rare example of mutualism between free-living wild animals and our own species. Here we show that it is also a specific example of reciprocal communication between birds and humans.

Greater honeyguides (Fig. 1A) seeking a human collaborator approach people and give a loud chattering call (audio S1). This call is distinct from their territorial song and is accompanied by referential gestures (*3*): the bird flies from tree to tree in the direction of the bees' nest until its human follower finds the nest (*2, 4*). The honeyguide thus directs a signal of the bees' nest location toward humans, and the honey-hunters use this signal to their mutual advantage. In this study, we ask whether honeyguides in turn exploit specialized signals directed at them by humans. We studied greater honeyguides (hereafter "honeyguides") in the Niassa National Reserve in northern Mozambique. This region has been noted for its honey and beeswax production at least since Arab trading times centuries ago (*5, 6*). The local Yao people still harvest wild honey using traditional methods, and this practice remains economically important.

[1]Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK. [2]FitzPatrick Institute of African Ornithology, Department of Science and Technology–National Research Foundation (DST-NRF) Centre of Excellence, University of Cape Town, Rondebosch 7701, South Africa. [3]The Ratel Trust, Niassa Carnivore Project, Private Bag X18, Rondebosch 7701, South Africa.
*Corresponding author. Email: cns26@cam.ac.uk

First, we confirmed that in northern Mozambique, honeyguides give reliable information to human honey-hunters. To test whether guiding behavior accurately indicates the direction of bees' nests and leads to their successful discovery by humans, we trailed honey-hunters following honeyguides and tracked our movements via GPS. A guiding event was defined as a bout of guiding by an individual bird, sometimes involving consecutive journeys to different bees' nests. Each guiding event probably involved a different individual honeyguide, as the study area was 230 km², and the home ranges of individual honeyguides that we measured using radio telemetry did not exceed 1 km² and overlapped with one another (*7*) (fig. S1). 75.3% of guiding events led to the successful discovery by humans of at least one bees' nest [mean ± SE = 1.00 ± 0.08 nests; range = 0 to 3 nests; *n* = 97 events, excluding controls in the experiment discussed below (*7*)]. 94.6% of nests shown belonged to the honeybee *Apis mellifera*, and the rest to stingless bee species (*7*). Nests were located 0 to 832 m (median = 152 m, *n* = 84 nests, only considering the first nest per guiding event) from the point where guiding began. Figure 1B shows that the birds' initial flight direction accurately signaled the ultimate location of the bees' nest, corroborating a classic study from Kenya (*2*). Overall, 74.5% of bees' nests found by humans (*n* = 149) involved the help of a honeyguide.

Second, we asked whether the signals used by human honey-hunters provide reliable information to honeyguides. Honey-hunters seeking honeyguides often announce their presence with unspecialized sounds such as shouting and chopping wood (*4, 8*). In some parts of Africa, however, humans also make specialized vocalizations used only when hunting honey. In the Niassa National Reserve (and, more widely, in northern Mozambique and adjacent southern Tanzania), Yao honey-hunters seeking and following honeyguides produce a loud trill followed by a grunt: "brrrr-hm" [audio S1; see (*9*) for a melodious whistle used in the same context by the Hadza people of northern Tanzania]. To confirm that "brrrr-hm" is a specialized honey-hunting sound, we interviewed 20 Yao honey-hunters, all of whom reported that they used this specific sound when hunting honey but in no other context. When

asked why, they reported that they learned it from their fathers and that it is the best way of attracting a honeyguide and maintaining its attention. Therefore, this sound has the potential to reliably signal to honeyguides that a prospective human partner is specifically seeking honey and has the tools, skills, and time to open a bees' nest, which many humans do not. A payoff to the bird reliably results from interacting with such a partner, because if a bees' nest is harvested then wax is left behind, either as combs containing no honey or as chewed lumps spat out by honey-hunters.

Finally, we examined whether honeyguides associated this vocal signal with a higher chance of a payoff from cooperation. If so, then honeyguides should be more likely to initiate collaboration with humans producing this honey-hunting sound rather than other sounds. To test this, we carried out 72 15-min experimental transects simulating honey-hunting forays, in which an author and two local honey-hunters walked while playing back one of three acoustic cues every 7 s at consistent amplitude using a calibrated speaker: (i) a control human sound (either the Yao words for "honeyguide" and "honey" or the honey-hunter's name, alternated among transects); (ii) a control animal sound (either the song or the excitement call of the ring-necked dove, *Streptopelia capicola*, alternated among tran-

sects); or (iii) the specialized "brrrr-hm" honey-hunting sound [see (7) for details and audio S2 to S4 for examples]. Each transect used a distinct playback exemplar recorded from a different individual person or bird. Honeyguides have never been confirmed to guide any species besides humans (10). We conducted this experiment during the hot dry season; the average shade air temperature at the end of each transect was 31°C (range = 23° to 38°C). Therefore, we also tested the effects of temperature, trial time relative to sunrise or sunset, and morning versus afternoon [details in (7)].

We were guided by a honeyguide on 30 of 72 transects. Transects accompanied by the honey-hunting call had a 66.7% probability of eliciting guiding from a honeyguide, which was significantly greater than that for transects accompanied by the human control sounds (25%) or animal control sounds (33.3%) (Fig. 2A; planned comparison with controls: estimate ± SE = 1.13 ± 0.38, Z = 2.96, P = 0.0031). The probability of guiding did not differ between the two control treatments (estimate ± SE = 0.25 ± 0.33, Z = 0.76, P = 0.45). The best model also included the time relative to sunrise or sunset as a covariate (probability of being guided weakly decreased closer to the middle of the day: estimate ± SE = −4.34 ± 0.20, Z = −2.13, P = 0.034) and, overall, explained 25% of the variance in probability of being led by a honeyguide.

Once a honeyguide initiated guiding behavior, we followed it while continuing to play back the acoustic treatment, while the honey-hunters searched visually for bees' nests [see (7) for measures taken to encourage and validate equal search effort]. This revealed that honeyguides tended to cease guiding behavior more often when either of the two control sounds was produced, resulting in no bees' nests being found. Of those transects on which we were led by a honeyguide, we found a bees' nest for 81.3% when accompanied by the honey-hunting sound, compared with 66.7 and 50.0% when accompanied by the human and animal control sounds, respectively. Overall, the honey-hunting sound resulted in a 54.2% predicted probability of finding a bees' nest (Fig. 2B; planned comparison with controls: estimate ± SE = 1.21 ± 0.39, Z = 3.14, P = 0.0017) compared with 16.7% for each of the control sounds (planned comparison between controls: estimate ± SE = 0.03 ± 0.39, Z = 0.08, P = 0.94). Thus, production of the honey-hunting sound more than tripled the probability of finding a bees' nest during a standardized 15-min search accompanied by an acoustic cue. This finding experimentally validates the honey-hunters' claims that the honey-hunting sound improves their foraging success.

Honeyguides might respond more to humans producing the honey-hunting sound either because they recognize and prefer it or because they are simply more likely to hear it versus control sounds. If the latter is true, then honeyguide behavior should be predicted by playback amplitude after attenuation in the environment. We measured the mean and maximum amplitudes (in units of A-weighted decibels) of every playback exemplar with a sound-level meter at a distance of 30 m in the natural habitat of these birds
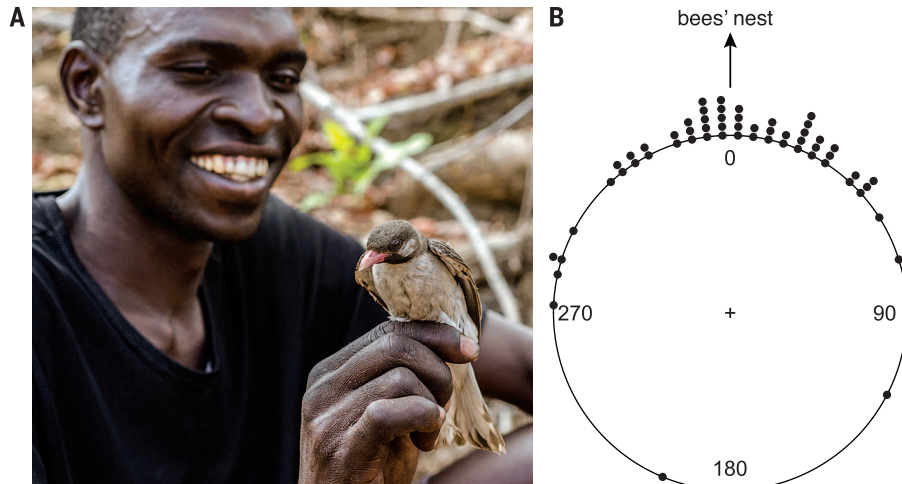

Fig. 1. Greater honeyguides accurately lead humans to bees' nests. (**A**) A Yao honey-hunter and a wild, free-living honeyguide. (This bird was captured using a researcher's mist-net and is neither tame nor habitually captive.) (**B**) Accuracy of honeyguide initial guiding behavior in relation to direction of successfully located bees' nests. Points represent the difference in bearing between initial guiding trajectory over the first 40 m of travel and the ultimate direction of the bees' nest (here set at 0) and are binned into 5° intervals. Each point represents a journey (n = 58 journeys) to a separate bees' nest that was at least 80 m away from the point where guiding began. Sometimes a honeyguide led humans to more than one nest consecutively (n = 50 guiding events). The circular distribution is unimodal (Rayleigh test, P < 0.001) with a mean of 1.7° (95% confidence interval includes zero: 352.3° to 11.1°), showing that honeyguide behavior offers reliable directional information to humans.
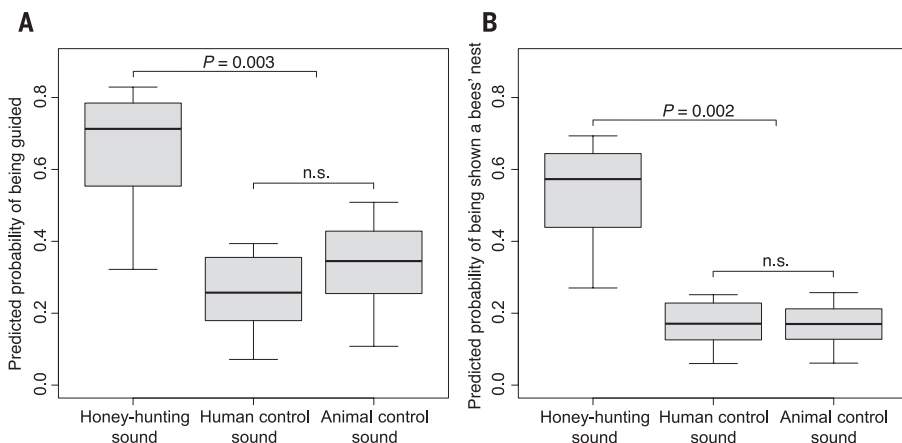

Fig. 2. Probability of a successful mutualistic interaction, in relation to experimentally given acoustic cues. Values are predicted probabilities of (**A**) being guided by a honeyguide and (**B**) being shown a bees' nest on a 15-min search, derived from a logistic model of data from experimental transects and accounting for time of day (minutes from sunrise to sunset). Boxes show medians and quartiles; whiskers show ranges (n = 24 trials per treatment group; P values show planned comparisons; n.s., not significant).

(table S1) and tested whether the amplitudes explained any variance in guiding behavior, either in isolation or in the multivariate models above. In no case did these acoustic measures explain any variance in the probability of being guided or being shown a bees' nest (7). Therefore, the honeyguides' elevated response to the honey-hunting sound is unlikely to be explained by its audibility. Instead, the most parsimonious explanation is that honeyguides associate the honey-hunting sound with successful collaboration. Such partner choice should be adaptive by allowing honeyguides to improve their net benefit from interacting with humans.

These results show that a wild animal correctly attaches meaning and responds appropriately to a human signal of recruitment toward cooperative foraging, a behavior previously associated with only domestic animals, such as dogs (11). Although humans use many species as foraging partners, including falcons, dogs, and cormorants, these involve trained or domesticated individuals that are specifically taught to cooperate. The honeyguide-human relationship is notable in that it involves free-living wild animals whose interactions with humans have probably evolved through natural selection. To our knowledge, the only comparable relationship involves cooperation between artisanal fishermen and free-living dolphins. Several reports exist of men "calling" dolphins to hunt, starting with Pliny the Elder around 70 CE (12). Whether this reflects a similarly specialized communication system to that mediating the honeyguide-human mutualism in Mozambique remains unknown.

How might honeyguides acquire information about honey-hunters' signals of cooperation? Honeyguides are brood-parasitic and reared by insectivorous hosts (4), which suggests that their propensity to locate bees' nests and guide humans to them is likely to be innate. However, the "brrrr-hm" human signal studied here is confined to a specific geographical area, and a different cultural group living 1000 km away uses a different signal which is likely to have the same function (9). Local adaptation is unlikely to account for corresponding honeyguide specialization, given a lack of obvious genetic structure across its range (13). This implies that local refinements to guiding behavior are probably learned, which is supported anecdotally by the belief of many Yao honey-hunters that juvenile honeyguides [which have distinctive yellow plumage (4)] are a separate species (called "namandindi") that, despite beckoning humans in the manner of an adult honeyguide ("sego"), falls quiet in response to the honey-hunting sound. We propose that learning might occur socially from conspecifics in the vicinity of bees' nests, resulting in a local cultural tradition among honeyguides that reflects the customs of their human collaborators.

REFERENCES AND NOTES

1. J. dos Santos, *Ethiopia Oriental* (Convento de S. Domingos de Évora, Évora, 1609).
2. H. A. Isack, H. U. Reyer, *Science* **243**, 1343–1346 (1989).
3. A. L. Vail, A. Manica, R. Bshary, *Nat. Commun.* **4**, 1765 (2013).
4. H. Friedmann, in "The honey-guides" (U.S. National Museum Bulletin 208, Smithsonian Institution, 1955), pp. 1–292; https://repository.si.edu/handle/10088/10101.
5. G. D. Popplewell, T. E. Harcus, *Geogr. J.* **91**, 31–43 (1938).
6. E. Colson, M. Gluckman, *Seven Tribes of British Central Africa* (Manchester Univ. Press 1959).
7. Materials and methods are available as supplementary materials on *Science* Online.
8. H. A. Isack, thesis, University of Oxford, Oxford (1987).
9. B. M. Wood, H. Pontzer, D. A. Raichlen, F. W. Marlowe, *Evol. Hum. Behav.* **35**, 540–546 (2014).
10. W. R. J. Dean, W. R. Siegfried, I. A. W. MacDonald, *Conserv. Biol.* **4**, 99–101 (1990).
11. J. Kaminski, J. Call, J. Fischer, *Science* **304**, 1682–1683 (2004).
12. R.-G. Busnel, *Trans. N.Y. Acad. Sci.* **35**, 112–131 (1973).
13. C. N. Spottiswoode, K. F. Stryjewski, S. Quader, J. F. R. Colebrook-Robjent, M. D. Sorenson, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 17738–17742 (2011).

PROTEIN DESIGN

Accurate design of megadalton-scale two-component icosahedral protein complexes

Jacob B. Bale,[1,2] Shane Gonen,[1,3]* Yuxi Liu,[4]* William Sheffler,[1] Daniel Ellis,[5] Chantz Thomas,[6] Duilio Cascio,[4,7,8] Todd O. Yeates,[4,7] Tamir Gonen,[3] Neil P. King,[1,5]† David Baker[1,5,9]†

Nature provides many examples of self- and co-assembling protein-based molecular machines, including icosahedral protein cages that serve as scaffolds, enzymes, and compartments for essential biochemical reactions and icosahedral virus capsids, which encapsidate and protect viral genomes and mediate entry into host cells. Inspired by these natural materials, we report the computational design and experimental characterization of co-assembling, two-component, 120-subunit icosahedral protein nanostructures with molecular weights (1.8 to 2.8 megadaltons) and dimensions (24 to 40 nanometers in diameter) comparable to those of small viral capsids. Electron microscopy, small-angle x-ray scattering, and x-ray crystallography show that 10 designs spanning three distinct icosahedral architectures form materials closely matching the design models. In vitro assembly of icosahedral complexes from independently purified components occurs rapidly, at rates comparable to those of viral capsids, and enables controlled packaging of molecular cargo through charge complementarity. The ability to design megadalton-scale materials with atomic-level accuracy and controllable assembly opens the door to a new generation of genetically programmable protein-based molecular machines.

The forms and functions of natural protein assemblies have inspired many efforts to engineer self- and co-assembling protein complexes (1–24). A common feature of these approaches, as well as the structures that inspire them, is symmetry. By repeating a small number of interactions in geometric arrangements that are consistent with the formation of regular structures, symmetry reduces the number of distinct interactions and subunits required to form higher-order assemblies (2, 3, 25). Symmetric complexes can be designed to form through self-assembly of a single type of protein subunit or co-assembly of two or more distinct types of protein subunits. Multicomponent materials possess several important advantages, including the potential to control the initiation of assembly by mixing independently prepared components. This property could allow,

[1]Department of Biochemistry, University of Washington, Seattle, WA 98195, USA. [2]Graduate Program in Molecular and Cellular Biology, University of Washington, Seattle, WA 98195, USA. [3]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147, USA. [4]Department of Chemistry and Biochemistry, University of California–Los Angeles (UCLA), Los Angeles, CA 90095, USA. [5]Institute for Protein Design, University of Washington, Seattle, WA 98195, USA. [6]Department of Chemistry, University of Washington, Seattle, WA 98195, USA. [7]UCLA–Department of Energy (DOE) Institute for Genomics and Proteomics, Los Angeles, CA 90095, USA. [8]Department of Biological Chemistry and Molecular Biology Institute, UCLA, Los Angeles, CA 90095, USA. [9]Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, USA.
*These authors contributed equally to this work.
†Corresponding author. Email: neilking@uw.edu (N.P.K.); dabaker@uw.edu (D.B.)

Editor's Summary

Show me a sign of sweetness to come

Communication between humans and domesticated animals is common. Regular communication between humans and wild animals, however, is rare. African honey-guide birds are known to regularly lead human honey-hunters to bee colonies, and the humans, on opening up the nest, leave enough mess for the birds to feast on. Spottiswoode *et al.* show that when the honey-hunters make a specific call, honey-guides are both more likely to come to their aid and more likely to find them a bee's nest. This interaction suggests that the birds are able to attach a specific meaning of cooperation to the human's call––a rare case of mutualism between humans and a wild animal.

Science, this issue p. 387

Article Tools	Visit the online version of this article to access the personalization and article tools: http://science.sciencemag.org/content/353/6297/387
Permissions	Obtain information about reproducing this article: http://www.sciencemag.org/about/permissions.dtl