Video Article

# Using Pharmacological Manipulation and High-precision Radio Telemetry to Study the Spatial Cognition in Free-ranging Animals

Timothy C. Roth*[1], Aaron R. Krochmal*[2], William B. Gerwig, IV[1], Sage Rush[3], Nathaniel T. Simmons[2], Jeffery D. Sullivan[4], Katrina Wachter[5]

[1]Department of Psychology, Franklin and Marshall College

[2]Department of Biology, Washington College

[3]University of Pennsylvania

[4]School of Forestry and Wildlife Sciences, Auburn University

[5]Morsani College of Medicine, University of South Florida

*These authors contributed equally

Correspondence to: Timothy C. Roth at timothy.roth@fandm.edu, Aaron R. Krochmal at akrochmal2@washcoll.edu

URL: http://www.jove.com/video/54790
DOI: doi:10.3791/54790

Keywords: Environmental Sciences, Issue 117, behavior, ecology, field study, memory, radio telemetry, reptile, scopolamine, space use, turtle

Date Published: 11/6/2016

Citation: Roth, T.C., Krochmal, A.R., Gerwig, IV, W.B., Rush, S., Simmons, N.T., Sullivan, J.D., Wachter, K. Using Pharmacological Manipulation and High-precision Radio Telemetry to Study the Spatial Cognition in Free-ranging Animals. *J. Vis. Exp.* (117), e54790, doi:10.3791/54790 (2016).

## Abstract

An animal's ability to perceive and learn about its environment plays a key role in many behavioral processes, including navigation, migration, dispersal and foraging. However, the understanding of the role of cognition in the development of navigation strategies and the mechanisms underlying these strategies is limited by the methodological difficulties involved in monitoring, manipulating the cognition of, and tracking wild animals. This study describes a protocol for addressing the role of cognition in navigation that combines pharmacological manipulation of behavior with high-precision radio telemetry. The approach uses scopolamine, a muscarinic acetylcholine receptor antagonist, to manipulate cognitive spatial abilities. Treated animals are then monitored with high frequency and high spatial resolution *via* remote triangulation. This protocol was applied within a population of Eastern painted turtles (*Chrysemys picta*) that has inhabited seasonally ephemeral water sources for ~100 years, moving between far-off sources using precise (± 3.5 m), complex (*i.e.*, non-linear with high tortuosity that traverse multiple habitats), and predictable routes learned before 4 years of age. This study showed that the processes used by these turtles are consistent with spatial memory formation and recall. Together, these results are consistent with a role of spatial cognition in complex navigation and highlight the integration of ecological and pharmacological techniques in the study of cognition and navigation.

## Video Link

The video component of this article can be found at http://www.jove.com/video/54790/

## Introduction

Cognition (herein defined as "all processes involved in acquiring, storing, and using information from the environment" [1]) is central to an array of complex navigation tasks[2]. For example, Sandhill cranes (*Grus canadensis*) show a marked improvement in migratory precision with experience[3], and sea turtle species imprint on their natal beaches as hatchlings and return as adults[4-6]. Similarly, successful migration, dispersal, and foraging hinge on an animal's ability to gather information about their spatial environment[7,8]. Some animals appear to learn navigational routes in relation to specific landscape features[9] and may use spatial cognition when moving between nesting and foraging areas[10]. Recent work on Eastern Painted turtles (*Chrysemys picta*) suggests a critical period in navigation, where successful navigation of upland habitat as adults hinges on juvenile experience within a narrow age range (< 4 years old[11-13]). Though together these studies demonstrate the progress that has been made in understanding the role of learning in navigation[4-6, 14-16], the mechanisms that underlie such behaviors and the full role of cognition in navigation remain enigmatic, especially in vertebrates[8, 17, 18].

Field investigations into the role of cognition in navigation are rare[2, 8, 18], due largely to the methodological difficulties involved in monitoring, manipulating, and tracking wild animals. For example, the large spatial and temporal scales on which many animals navigate often preclude investigating both type of information that those animals potentially learn and how that information is acquired. Experimenters often face the logistical difficulties of detecting and locating animals when monitoring behavior over such large areas and time frames, thereby limiting the type of data that can be collected and the conclusions that can be drawn. Although the use of animal-mounted global positioning system (GPS) recorders may improve the probability of detection of widely ranging animals, spatial data collected by these means are generally of very coarse resolution and lack a detailed behavioral component. Consequently, the data that can be collected under such circumstances are of limited value for examining subtle variation in behavior among different groups or experimental treatments. Similarly, the direct, controlled manipulation of target behaviors is often prohibited by the spatial and temporal scales typical of navigation behaviors, as well as by inherent logistical constraints of field studies. Finding animals in their natural habitat, catching and manipulating them, and then collecting behavioral data without inadvertently producing spurious behaviors are major challenges of working with animals in the field. Therefore, the design of experiments on free-ranging animals is often constrained and the ability to conduct rigorous, controlled field experiments on the role of cognition in navigation is limited.

The present study circumvents many of the previous difficulties of investigating the relationship between cognition and navigation in the field by using a novel combination of pharmacological manipulation and high-resolution tracking of freely navigating animals under field conditions. Scopolamine, a muscarinic acetylcholine receptor (mAChR) antagonist, has been shown to block spatial memory formation and recall by blocking cholinergic activity in the brains of a variety of vertebrate taxa[18-24]. Scopolamine can be used effectively on free-ranging animals under field conditions[11, 18] and has a marked but temporary effect (*e.g.*, 6 - 8 hr in reptiles). Methylscopolamine, a mAChR antagonist that does not cross the blood-brain-barrier[19-21], can be used to control for the possible peripheral effects of scopolamine and for non-cognitive aspects of behavior[11]. Pharmacology allows for the precise manipulation of cognition by directly affecting receptors, and high-precision radio telemetry allows for the observation of the resulting effects on behavior. Measurements taken *via* remote triangulation with both high spatial (± 2.5 m) and temporal (15 min) resolution allow for the precise documentation and quantification of animal behavior relative to the experimental manipulation of cognition.

This study[11] was conducted between May and August 2013 and 2014 at Chesapeake Farms, a 3,300 acre wildlife management and agriculture research area in Kent Co., MD, USA (39.194°N, 76.187°W). The protocol involves five main steps: (1) capturing and handling animals (2) affixing radio transmitters (3) preparing the pharmacological agents (4) monitoring and manipulating animal movements, and (5) analyzing spatial data. The study described herein focused on the Eastern painted turtle (*Chrysemys picta*). Turtles in the focal population engage in annual overland movements in which they leave their home ponds and navigate to alternative aquatic habitats using one of four very precise (± 3.5 m), complex, and highly predictable routes[11, 12]. Pharmacological manipulation of animals in this system paired with high-resolution radio telemetry sheds light on the role of cognition in freely navigating wild animals.

## Protocol

All procedures involving animal subjects were approved by the Institutional Animal Care and Use Committees of Franklin and Marshall and Washington Colleges and followed all local, state, and federal regulations.

# 1. Capture and Handling

1. Place hoop traps in the target body of water that is known to contain turtles. Identify water depth ensuring that 4 - 5 inches of the trap remains above water to allow trapped turtles to surface and breathe. Be certain to expand and secure hoop traps to their maximum length with the cross beams to prevent trap collapse. Stake traps into the bed of the body of water to prevent drift.
2. Bait traps with several dead fish, chicken livers, or chicken necks, a can of cat food, and/or a can of vegetables[25].
3. Check traps twice daily and remove turtles. When removing turtles from traps, hold animal by the side and use caution to avoid injury from claws or beak.
    1. Release by-catch. Reset traps if continued trapping is desired. Assess state of bait. If the bait had been consumed, add more. Pull traps to shore if additional trapping is not desired.
4. Determine the sex and age of turtles if desired[26, 27].
5. Place turtle in a holding bag and measure body mass with a spring scale to the nearest g.
6. Transport turtles to the lab in climate-controlled transport boxes with a small amount of water. House animals singly in aquaria with untreated pond water held at approximately 25 °C and deep enough to cover the head only.

# 2. Affixing Radio Transmitter

1. To maximize transmitter life and output, choose the largest radio transmitter possible that does not exceed 5% of the animal's body mass[28].
2. Identify the location of transmitter placement on the carapace approximately halfway between the midline and lateral edge of the carapace approximately 1/3 of the length up from the rear edge of the carapace.
3. Prepare area by removing mud, debris, and algal growth with a dry cloth. Swab area with 70% isopropanol.
4. Attach transmitter with a small amount of 5 min epoxy. Orient the transmitter to maximize contact with the surface of the carapace. Position the antenna so that it trails behind the animal parallel to the long axis of the body.
5. Once suitably positioned, cover the entire transmitter and approximately 1 cm of the surrounding carapace surface with 5 min epoxy.
6. Return the turtle to the aquarium and allow the epoxy to cure overnight.

# 3. Pharmacological Preparation

Caution: Scopolamine hydrobromide and scopolamine methylbromide are potent acetylcholine antagonists. When working with these drugs, consult the Materials Safety Data Sheet, use proper personal protective equipment (*e.g.*, gloves, fume hood), and follow laboratory safety protocols to avoid accidental contact.

1. Using sterile and antipyrogenic supplies, compound a stock solution of scopolamine hydrobromide. Weigh out the desired quantity of the drug on an analytical balance. Mix the scopolamine with injection saline in a conical tube to the concentration of 1 mg/ml. Vortex solution until dissolved. Ensure that chemical purity of the base chemicals meets or exceeds United States Pharmacopeia (USP) formulation when possible[11].
2. Repeat step 3.1 with scopolamine methylbromide.
3. Process solution through a 0.22 µm pore nylon or mixed cellulose esters syringe filter into a sterile sealed serum vial.
4. Store at room temperature. Use within 24 hr.

## 4. Track Turtle Movements Using Radio Telemetry[11, 12]

1. Move to the general location of the target animal, remaining at least 25 m from the animal. Using a directional Yagi antenna and setting the receiver gain at medium, scan the horizon to determine the rough direction and location of the animal. When receiving interference or a weak signal, find a new position. Increase elevation or hold Yagi aloft when possible to enhance signal.
2. Once a suitable location is found to take a bearing, record your position with a GPS.
3. Using the null/peak method[28], determine the bearings of the left and right nulls.
    1. Identify the direction of the strongest signal. Turn the gain down as far as possible while still receiving a detectable signal. Use the attenuator switch on the receiver if so equipped. Move the antenna to the left and record the compass bearing at which the signal is lost.
    2. Repeat the previous step for the right bearing.
4. Repeat step 4.3 from at least two additional locations for the same animal.
   NOTE: These additional points should be taken in such a manner as to surround the animal.
    1. When a set of bearings is taken from a great distance or with unavoidable interference, take at least two additional bearings to increase accuracy. Collect sets of bearings used to estimate a single location as quickly as possible, particularly if the animal is moving.
       NOTE: Alternatively, multiple personnel can coordinate to take multiple independent bearings on the same animal simultaneously.
5. Record locations of animals digitally or by hand every 10 - 15 min using above steps.
6. Manipulation of cognitive processing
    1. Once the unmanipulated animal's path is documented (a within-subjects control), provide a dose of either scopolamine or methylscopolamine. Using the mass collected in step 1.5, calculate the amount of drug to be given to the animal to achieve a reptile-specific final dose of 6.4 mg/kg of scopolamine or 6.8 mg/kg of methylscopolamine[19, 20].
    2. Deliver the drug directly into the peritoneum *via* the caudal peritoneal sinus using a 1.0 ml syringe with a 22 gauge needle. Ensure that the total volume delivered does not exceed 1 ml.
    3. Release animal as soon as possible at its site of capture.
    4. Continue monitoring the animal's movement every 10 - 15 min until it reaches its projected destination.

## 5. Spatial Analysis

1. Calculate estimates of animal location.
    1. For each set of null bearings, bisect the angle (by hand or *via* software) to find the resultant transmitter bearing. Repeat for all bearings at a given time point.
    2. Using triangulation software according to manufacturer's protocol, estimate the animal's position and associated error with the multiple transmitter bearings. Convert position estimates to x/y coordinates if software provides different output.
2. Repeat steps 5.1 for all sets of bearings.
3. Calculate the spatial precision of movement.
    1. Calculate the cumulative geometric mean (*i.e.*, mean path) of unmanipulated animals (negative control) as they move towards their goal[12].
    2. For each individual in the treatment and positive control groups, calculate the cumulative number of points that overlap successively larger swaths from the geometric mean line in 5 m intervals until 100% of all points have been included[11, 12].
    3. Calculate the mean and standard error at each swath distance for all individuals in each group.
    4. Statistically analyze the data, comparing among the treatment groups either for the interval to accommodate 100% of the points or the points of overlap at a given interval, depending upon the question.

## Representative Results

Using the above protocol, the role of cognition in navigation was assessed in a population of Eastern painted turtles (*Chrysemys picta*) that has experienced seasonal ephemeral water sources for ~100 years. This population inhabits a mix of ephemeral (drained annually and rapidly - in several hr) and permanent aquatic habitats (**Figure 1**). Previous studies suggest that after their ponds are drained, resident turtles navigate to alternative water sources with high precision (± 3.5 m) using complex, predictable routes learned before 4 years of age[11-13] (**Figure 1**).

This study showed that the processes used by these turtles are consistent with spatial memory formation and recall[11]. Scopolamine blocked cholinergic activity in the brains of animals (including spatial memory formation and recall[19-21]) during navigation. Experienced adults treated with scopolamine diverted from their precise historic routes while naïve juveniles lacking experience (and thus memory) were unaffected by scopolamine, suggesting no effect of scopolamine on perceptual or non-spatial cognitive processes[11] (**Figures 1** and **2**). Further, neither adult nor juvenile navigation was affected by the methylscopolamine control. Adult animals (*i.e.,* those with previous experience at the site) injected with scopolamine lost their ability to follow the historic paths and the juveniles that use local cues to navigate and those adults injected with the drug that does not cross the blood-brain barrier were unaffected. Therefore, navigation in adults in this system seems to be cognitive in nature. Together, these results are consistent with the idea that turtles have a critical period during which they must learn paths and use cholinergic-dependent cognitive systems (spatial memory) to navigate as adults[11-13].

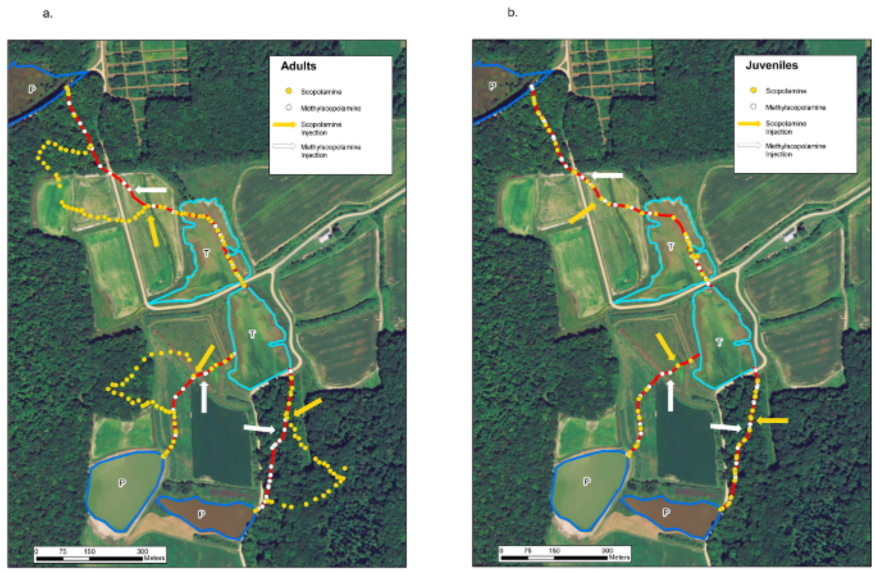


**Figure 1. Navigation is Based on Cognitive Processing in Adult Turtles.** Representative movements of **(a)** experienced adults and **(b)** naïve juveniles (1 - 3 years) from temporary (T) to permanent (P) ponds while treated with either scopolamine or methylscopolamine. All adults receiving scopolamine (a, yellow, n = 9) drifted dramatically away (over 200 m) from the traditional routes (red, p< 0.001), while all naïve juveniles treated with the drug (b, yellow, n = 7) maintained movement exactly within traditional routes (p > 0.999). All control adults (a, white, n = 9) and control naïve juveniles (b, white, n = 6) followed traditional routes (p > 0.999). Each line of points represents one individual. All turtles from all groups maintained high precision prior to injection (p > 0.999). Data from Roth and Krochmal[11]. Please click here to view a larger version of this figure.

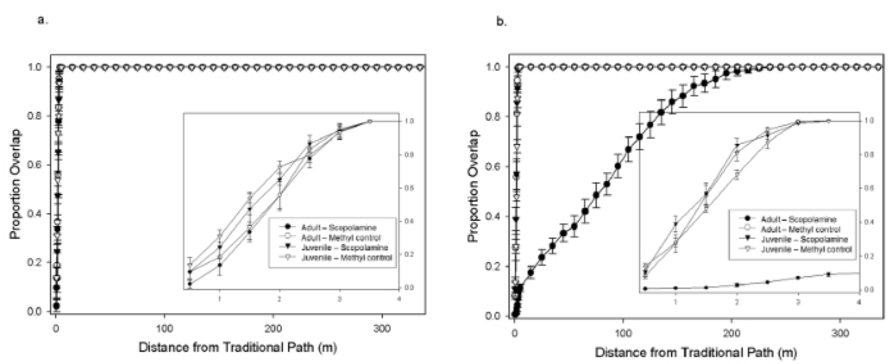


**Figure 2. The Precision of Navigation is a Function of Cognitive Processing in Adult Turtles. a)** All turtles demonstrated high precision of movement prior to injection of the treatment (scopolamine) or control (methylscopolamine; $p > 0.999$). **b)** After injection, adults in the scopolamine treatment deviated significantly ($p < 0.001$) from their traditional routes. In contrast, all other groups continued to navigate with high precision (± 3.5 m; $p > 0.999$). Inserts show detail of overlap from 0.5 - 3.5 m. Points are means ± SEM. Data from Roth and Krochmal[11]. Please click here to view a larger version of this figure.

## Discussion

The protocol presented here allows the experimenter to document and quantify the role of cognition in navigation. Manipulating cognition in the field has proven difficult, as most approaches leave experimenters unable to know which specific aspects of the animal's behavior are being manipulated. However, the protocol presented here allows the experimenter to accurately manipulate and thus assess the role of cognition in navigation. The technique further allows experimenters to monitor animal navigation in real-time with exceptionally high spatial and temporal resolution, thereby empowering researchers to clearly document the behavioral ramifications of experimental manipulation of cognition in wild animals.

Within this context, radio telemetry provides the ability to precisely monitor animal movements over great distances, yielding both high-quality spatial and behavioral data. Although this application of telemetry is by no means new[28], the majority of studies use this technique to address coarse questions in ecology and behavior (*e.g.*, habitat use, home range size, *etc.*). The frequent monitoring of the animal location (4 - 5 times per hr) described here combined with fine scale spatial analyses provide a more detailed behavioral component to an animal's location in space. Note that the optimal tracking distance will be a function of the transmitter strength and the equipment sensitivity. Generally it is best practice to remain at least 25 m away from the animal to avoid disturbing it, although when the animal is located in open vegetation, the distance needed to avoid such disturbance might be larger.

In the current application, high-precision radio telemetry offers unique advantages over the use of animal-mounted GPS recorders. Transmitters can be smaller, are less expensive, and have longer battery life than GPS units[28]. Moreover, the temporal resolution of remote triangulation *via* radio telemetry is far superior to animal-mounted GPS. Temporally, animal-mounted GPS units are limited by battery life (*i.e.*, a finite number of measurements can be taken, thereby constraining their frequency). High-precision tracking with GPS would require a large battery to obtain high frequency position over a long period of time. The substantial mass of these batteries preclude them from use in small animal-mounted GPS units[28]. Furthermore, high-precision radio telemetry is not constrained by expensive data retrieval costs, or limited by on-board memory storage. However, radio telemetry is not optimal for tracking animals with particularly large movement ranges (*e.g.*, during long-distance migration), in deep water or fossorial species, or those in steep montane habitats. In addition, high-precision radio tracking can be very time intensive and requires a relatively large field crew, particularly for fast-moving species; therefore, this approach may not suitable for all questions.

Pharmacological manipulation with scopolamine and methylscopolamine offers specific advances for the study of cognition in a natural setting. Behavior can be hard to interpret, especially under field conditions, thereby limiting the scope of potential inquiry. Scopolamine allows the manipulation of specific receptors that influence cognitive processes, enabling the researcher to ask questions specifically about the manipulation of cognition. Furthermore, as scopolamine easily crosses the blood-brain barrier and methylscopolamine does not, researchers can control for peripheral effects of scopolamine thereby dissociating cognitive-based from non-cognitive behaviors. These benefits of pharmacological manipulation allow for the generation and subsequent testing of clear behavioral predictions and afford the use of complex experimental designs under field conditions. However, scopolamine is a very general acetylcholine antagonist that can have unintended effects on other behavioral, sensory, and cognitive systems[21-24]. Therefore, it is possible that the use of scopolamine can produce effects that can interfere with the interpretation of complex behaviors (*e.g.*, pupil dilation, thermal sensitivity[21-24, 29, 30]); no such confounding effects have been detected in this or previous studies[11-13, 19, 20].

Common problems encountered while radio tracking include weak signal, loss of signal, and interference. To combat a weak signal, increase gain, change antenna orientation, move closer to the animal (being careful to avoid disturbing the anima), and elevate the antenna[28]. If the signal is lost completely, search with the gain and antenna as high as possible in an outwardly spiraling area restricted search[28]. Interference can be combated by decreasing gain, using the attenuator or noise cancelling filter (if so equipped), and changing antenna orientation. If interference cannot be overcome by these means, future work at the study site should focus on bandwidths that are not affected by interference.

Overall, pharmacological manipulation in conjunction with high-precision telemetry provides unique insight into the role that cognition plays in the origin and manifestation of navigation. The novelty of this unique method allows researchers to better understand the underlying neurological mechanisms that give rise to cognition in navigation. Moreover, these techniques can be used for additional studies of cognition in the wild with particular applicability to spatially explicit behaviors (*e.g.*, navigation, migration, foraging, and dispersal)[11-13, 33], the evolution of cognition[1, 7], and conservation (*e.g.*, translocation, reintroduction)[31, 32]. This technique is useful for a wide range of taxa in a wide range of habitats and will be vital to understanding phylogenic patterns in cognition.

## Disclosures

The authors have nothing to disclose.

## Acknowledgements

This research was funded by Washington College's Provost's Office, Middendorf Fund, Hodson Trust, and Franklin and Marshall's Hackman Fund and College of Grants. We thank E. Counihan, S. Giordano, F. Rauh, and A. Roth for assistance in the field. We thank M. Conner, R. Fleegle, and D. Startt at Chesapeake Farms, and Chino Farms for permission and access. The Washington College GIS Program helped with the preparation of maps.

## References

1. Shettleworth, S.J. Cognition, *Evolution and Behavior.* New York: Oxford University Press. (2010).
2. Bingman, V.P., & Cheng, K. Mechanisms of animal global navigation: comparative perspectives and enduring challenges. *Ethol. Ecol. Evol.* **17**, 295-318 (2005).
3. Mueller, T., O'Hara, R.B., Converse, S.J., Urbanek, R.P., & Fagan, W.F. Social Learning of Migratory Performance. *Science.* **341**, 999-1002 (2013).
4. Putman, N.F., *et al.* An inherited magnetic map guides ocean navigation in juvenile Pacific salmon. *Curr. Biol.* **24,** 446-450 (2014).
5. Lohmann, K.J., Putman, N.F., & Lohmann, C.M.F. Geomagnetic imprinting: a unifying hypothesis of natal homing in salmon and sea turtles. *Proc. Natl. Acad. Sci., USA*. **105,** 19096-19101 (2008).
6. Fuxjager, M.J., Davidoff, K.R., Mangiamele, L.A., & Lohmann, K.J. The geomagnetic environment in which sea turtle eggs incubate affects subsequent magnetic navigation behaviour of hatchlings. *Proc. R. Soc. B.* **281,** 1218-1225 (2014).
7. Shettleworth, S.J. The evolution of comparative cognition: is the snark still a boojum? *Behav. Processes.* **80,** 210-217 (2009).
8. Fagan, W.F., *et al.* Spatial memory and animal movement. *Ecol. Lett.* **16,** 1316-1329 (2013).
9. Collett, T.S., & Graham, P. Insect Navigation: Do Honeybees Learn to Follow Highways? *Curr. Biol.* **25,** 240-242 (2015).
10. Menzel, R., *et al.* Honey bees navigate according to a map-like spatial memory. *Proc. Natl. Acad. Sci., USA*. **102,** 3040-3045 (2005).
11. Roth, T.C., & Krochmal, A.R. Pharmacological Evidence is Consistent with a Prominent Role of Spatial Memory in Complex Navigation. *Proc. R. Soc. B.* **283,** 20152548 (2016).
12. Roth, T.C., & Krochmal, A.R. The role of age-specific learning and experience for turtles navigating a changing landscape. *Curr. Biol.* **25**, 333-337 (2015).
13. Krochmal, A.R., Roth, T.C., Rush, S., & Wachter, K. Turtles outsmart rapid environmental change: the role of cognition in navigation. *Comm. Integr. Biol.* (2015).

14. Thorup, K., *et al.* Evidence for a navigational map stretching across the continental U.S. in a migratory songbird. *Proc. Natl. Acad. Sci., USA* **104,** 18115-18119 (2007).
15. Lohmann, K.J., Lohmann, C.M.F., & Putman, N.F. Magnetic maps in animals: nature's GPS. *J. Exp. Biol.* **210**, 3697-3705 (2007).
16. Collett, M., Chittka, L., & Collett, T.S. Spatial Memory in Insect Navigation. *Curr. Biol.* **23**, 789-800 (2013).
17. Foden, W., *et al.* Species susceptibility to climate change impacts. In: Vié JC, Hilton-Taylor C, Stuart SN (eds). *The 2008 Review of The IUCN Red List of Threatened Species.* IUCN. Gland, Switzerland (2008).
18. Kohler, E.C., Riters, L.V., Chaves, L., & Bingman, V.P. The Muscarinic Acetylcholine Antagonist Scopolamine Impairs Short-Distance Homing Pigeon Navigation. *Physiol. Behav.* **60**, 1057-1061 (1996).
19. Powers, A.S., Hogue, P., Lynch, C., Gattuso, B., Lissek, S., & Nayal, C. Role of Acetylcholine in negative patterning in turtles (*Chrysemys picta*). *Behav. Neurosci.* **123,** 804-809 (2009).
20. Petrillo, M., Ritter, C.A., & Powers, A.S. A role for Acetylcholine in spatial memory in turtles. *Physiol. Behav.* **56**, 135-141 (1994).
21. Klinkenberg, I., & Blokland, A. The validity of scopolamine as a pharmacological model for cognitive impairment: A review of animal behavioral studies. *Neurosci. Biobehav. Rev.* **34,** 1307-1350 (2010).
22. Pradhan, S.N., & Roth, T. Comparative behavioral effects of several anticholinergic agents in rats. *Psychopharm.* (Berlin) **12,** 358-366 (1968).
23. Harvey, J.A., Gormezano, I., & Cool-Hauser, V.A. Effects of scopolamine and methylscopolamine on classical conditioning of the rabbit nictitating membrane response. *J. Pharmacol. Exp. Therap.* **225**, 42-49 (1983).
24. Evans, H.L. Scopolamine effects on visual discrimination: modifications related to stimulus control. *J. Pharmacol. Exp. Therap.* **195**, 105-113 (1975).
25. Dreslik, M.J., & Phillips, C.A. Turtle communities in the upper midwest,, USA. J. *Freshwater Ecol.* 20:149-164 (2005).
26. Sexton, O.J. A method of estimating the age of painted turtles for use in demographic studies. *Ecology.* **40,** 716-718 (1959).
27. Wilson, D.S., Tracy, C.R., & Tracy, C.R. Estimating age of turtles from growth rings: a critical evaluation of the technique. *Herpetologica*. **59**, 178-194 (2003).
28. Kenward, R.E. *A Manual for Wildlife Radio Tagging.* Academic Press, London (2000).
29. Jones, D.N.C., & Higgins, G.A. 1995 Effect of scopolamine on visual attention in rats. *Psychopharm.* **120,** 142-149 (1995).
30. Araujo, J.A, Nobrega, J.N., Raymond, R., & Milgram, N.W. Aged dogs demonstrate both increased sensitivity to scopolamine impairment and decreased muscarinic receptor density. *Pharmacol. Biochem. Behav.* **98**, 203-209 (2011).
31. Greggor, A.L., Clayton, N.S., Phalan, B., & Thornton, A. Comparative cognition for conservationists. *Trends Ecol. Evol.* **29,** 489-495 (2014).
32. Roth, T.C., & Krochmal A.R. Cognition-centered conservation as a means of advancing integrative animal behavior. *Curr. Opinion Behav. Sci.* **6**, 1-6 (2015).
33. LaDage, L.D., Roth, T.C., Cerjanic, A.C., Sinervo, B., & Pravosudov, V.V. Spatial memory: Are lizards really deficient? *Biol. Lett.* **8,** 939-941 (2012).


 