4. F. Grein, A. R. Ramos, S. S. Venceslau, I. A. C. Pereira, *Biochim. Biophys. Acta* **1827**, 145–160 (2013).
5. A. L. Müller, K. U. Kjeldsen, T. Rattei, M. Pester, A. Loy, *ISME J.* **9**, 1152–1165 (2015).
6. H. D. Peck Jr., J. LeGall, J. VanBeeumen, *Philos. Trans. R. Soc. Lond., B* **298**, 443–466 (1982).
7. A. S. Bradley, W. D. Leavitt, D. T. Johnston, *Geobiology* **9**, 446–457 (2011).
8. B. Brunner, S. M. Bernasconi, *Geochim. Cosmochim. Acta* **69**, 4759–4771 (2005).
9. W. D. Leavitt, A. S. Bradley, A. A. Santos, I. C. Pereira, D. T. Johnson, *Front. Microbiol.* **6**, 1392 (2015).
10. T. F. Oliveira *et al.*, *J. Biol. Chem.* **283**, 34141–34149 (2008).
11. S. S. Venceslau, Y. Stockdreher, C. Dahl, I. A. C. Pereira, *Biochim. Biophys. Acta* **1837**, 1148–1164 (2014).
12. K. L. Keller *et al.*, *Appl. Environ. Microbiol.* **80**, 855–868 (2014).
13. D. E. Canfield *et al.*, *Science* **330**, 1375–1378 (2010).
14. F. J. Stewart, O. Dmytrenko, E. F. Delong, C. M. Cavanaugh, *Front. Microbiol.* **2**, 134 (2011).
15. Y. Ikeuchi, N. Shigi, J. Kato, A. Nishimura, T. Suzuki, *Mol. Cell* **21**, 97–108 (2006).
16. K. Parey, E. Warkentin, P. M. H. Kroneck, U. Ermler, *Biochemistry* **49**, 8912–8921 (2010).
17. K. Parey, G. Fritz, U. Ermler, P. M. H. Kroneck, *Metallomics* **5**, 302–317 (2013).
18. Materials and methods are available as supplementary materials on *Science* Online
19. Y. C. Hsieh *et al.*, *Mol. Microbiol.* **78**, 1101–1116 (2010).
20. M. Wagner, A. J. Roger, J. L. Flax, G. A. Brusseau, D. A. Stahl, *J. Bacteriol.* **180**, 2975–2982 (1998).
21. T. Ida *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 7606–7611 (2014).
22. B. D. Paul, S. H. Snyder, *Nat. Rev. Mol. Cell Biol.* **13**, 499–507 (2012).
23. Y. Liu *et al.*, *J. Biol. Chem.* **287**, 36683–36692 (2012).
24. R. W. Nielsen, C. Tachibana, N. E. Hansen, J. R. Winther, *Antioxid. Redox Signal.* **15**, 67–75 (2011).
25. R. H. Pires *et al.*, *Biochemistry* **45**, 249–262 (2006).
26. R. Hedderich, N. Hamann, M. Bennati, *Biol. Chem.* **386**, 961–970 (2005).
27. F. Grein, I. A. C. Pereira, C. Dahl, *J. Bacteriol.* **192**, 6369–6377 (2010).

ACKNOWLEDGMENTS

We thank R. Soares for the mass spectrometry measurements, C. Leitão for HPLC technical support, and H. Santos and L. Gafeira for providing *A. fulgidus*. We also thank J. Wall for plasmids for genetic manipulation of *D. vulgaris*, A. Ramos for technical assistance, A. Masterson for synthesis of the trithionate standard, A. Bradley for detailed discussions, and W. Martin for critical reading of the manuscript. This work was funded by grants from NSF-EAR (1225980 to D.T.J. and I.A.C.P.), from Fundação para a Ciência e a Tecnologia (FCT), Portugal (UID/Multi/04551/2013, PTDC/QUI-BIQ/100591/2008, and PTDC/BBB-BQB/0684/2012) and Deutsche Forschungsgemeinschaft, Germany (Da 351/6-2). A.S. and S.S.V. are recipients of FCT fellowships (SFRH/BD/77940/2011 and SFRH/BPD/79823/2011). W.D.L. acknowledges support by an NSF–Graduate Research Fellowship Program and the Fossett Fellowship from Washington University in St. Louis. The data reported in this paper are provided in the supplementary materials.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6267/1541/suppl/DC1
Materials and Methods
Figs. S1 to S15
Tables S1 to S3
References (*28*–*41*)

2 September 2015; accepted 4 November 2015
10.1126/science.aad3558

ECOTOXICOLOGY

Algal toxin impairs sea lion memory and hippocampal connectivity, with implications for strandings

Peter F. Cook,[1,2]* Colleen Reichmuth,[2] Andrew A. Rouse,[2] Laura A. Libby,[3] Sophie E. Dennison,[4] Owen T. Carmichael,[5] Kris T. Kruse-Elliott,[4] Josh Bloom,[4] Baljeet Singh,[3] Vanessa A. Fravel,[6] Lorraine Barbosa,[6] Jim J. Stuppino,[4] William G. Van Bonn,[7] Frances M. D. Gulland,[6] Charan Ranganath[3]

Domoic acid (DA) is a naturally occurring neurotoxin known to harm marine animals. DA-producing algal blooms are increasing in size and frequency. Although chronic exposure is known to produce brain lesions, the influence of DA toxicosis on behavior in wild animals is unknown. We showed, in a large sample of wild sea lions, that spatial memory deficits are predicted by the extent of right dorsal hippocampal lesions related to natural exposure to DA and that exposure also disrupts hippocampal-thalamic brain networks. Because sea lions are dynamic foragers that rely on flexible navigation, impaired spatial memory may affect survival in the wild.

Domoic acid (DA) is an amino acid neurotoxin that causes neurological symptoms in marine animals, most visibly California sea lions (CSLs, *Zalophus californianus*) (*1*). As a result of environmental change and human impacts on marine systems, the size and frequency of DA-producing *Pseudonitszchia* algal blooms are increasing (*2*), and toxic exposure is widespread in CSLs (*3*). Although exposed CSLs show a reliable and specific pattern of seizures and hippocampal lesions (*4*), the sublethal effects on behavior are unclear. In rodents and humans, the hippocampus is necessary for spatial memory (*5*, *6*). As dynamic central-place foragers (*7*), CSLs may be especially vulnerable to spatial memory deficits, and anecdotal data from postrehabilitation tracking show unusual movement patterns in exposed animals (*8*). Together, these observations suggest that DA exposure in CSLs and resultant hippocampal damage could be associated with impaired spatial memory. In this study, we used controlled behavioral studies, integrated with prerelease veterinary care and structural and functional neuroimaging, to directly test this hypothesis in wild sea lions.

Between April 2009 and November 2011, we studied 30 wild CSLs undergoing veterinary care and rehabilitation (table S1). Drawing from the literature on hippocampal function in rodents, we developed two spatial memory assays and compared performances with hippocampal volumes, measured using in vivo magnetic resonance imaging (MRI). The hippocampus was manually traced (fig. S1), and structural volumes were calculated as percentages of total brain volume for each animal (*9*). Veterinary diagnosis predicted hippocampal volume [repeated measures analysis of variance (ANOVA): $F = 16.25$, df = 1, $P < 0.001$] (*9*), justifying the use of volume as the primary independent variable. Given the magnitude and range of hippocampal volumes across the sample, subsequent analyses treated volume as a continuous variable.

Because lesions are typically unilateral in this population (*3*), and because other species show lateralization of hippocampal function (*10*), right and left hippocampal volumes were regressed separately with behavioral performance. In some species, spatial memory is more reliant on the septal (dorsal) than on the temporal (ventral) hippocampus (*11*). Accordingly, we divided the hippocampi in half by length and conducted follow-up regression analyses with ventral and dorsal hippocampal volumes (*9*). We report these results when they differ from those expected based on analyses of the entire longitudinal extent of the hippocampus. Rodent data suggest that the dorsal third of the hippocampus may be sufficient to support spatial memory (*11*), so in cases where the dorsal half was a significant predictor of behavior, we conducted follow-up analyses that

[1]Center for Neuropolicy, Emory University, Atlanta, GA 30322, USA. [2]Pinniped Cognition and Sensory Systems Laboratory, Institute of Marine Sciences, University of California–Santa Cruz, Santa Cruz, CA 95060, USA. [3]Dynamic Memory Lab, Center for Neuroscience, University of California–Davis, Davis, CA 95618, USA. [4]AnimalScan Advanced Veterinary Imaging, Redwood City, CA 94063, USA. [5]Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA. [6]The Marine Mammal Center, Sausalito, CA 94965, USA. [7]Shedd Aquarium, Chicago, IL 60605, USA.
*Corresponding author. E-mail: pfcook@emory.edu

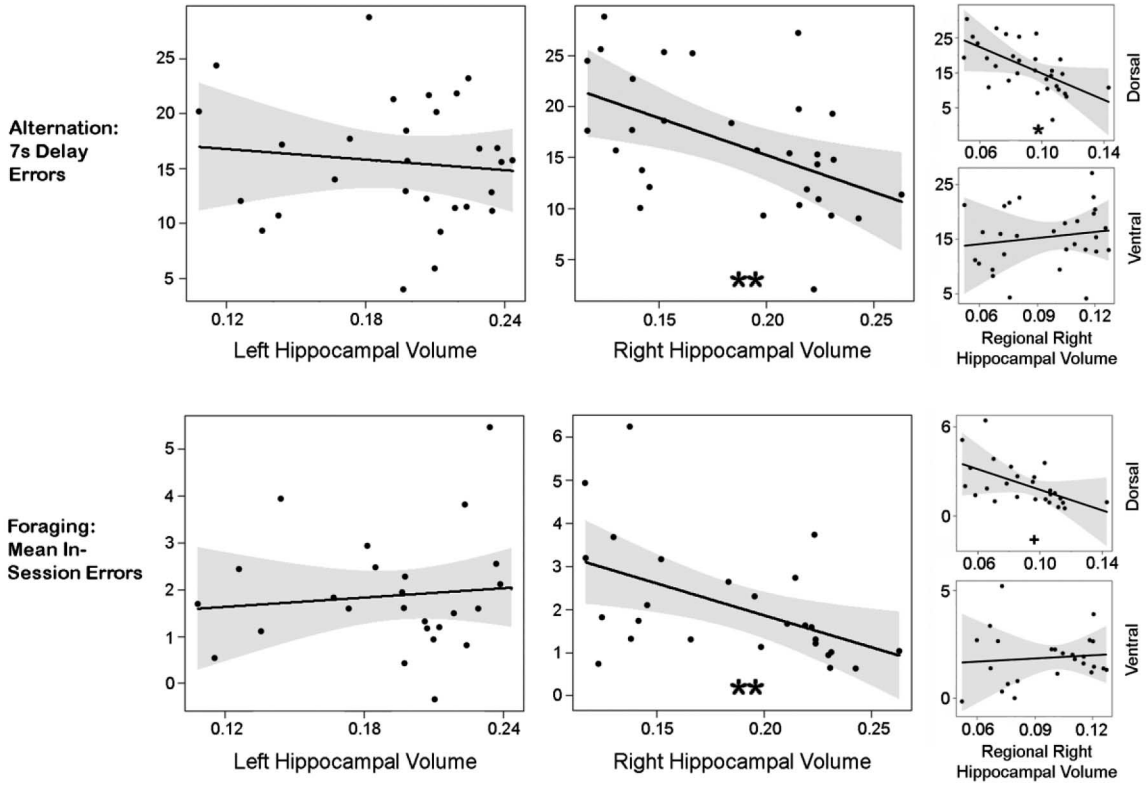

Fig. 1. Spatial memory is related to the integrity of the right hippocampus. The conditional scatterplots show correlations between left and right hippocampal volumes (as a percentage of total brain volume; *x* axes) and performance measures (*y* axes) related to behavioral alternation and foraging tasks, after regressing out other dependent variables. Regression analyses for alternation included no-delay test errors as an independent variable to control for variance in test performance that was unrelated to memory. Alternation, right: $t = -2.82$, df = 27, $P < 0.01$. Alternation, left: $t(0.54) < 1$, df = 27. Foraging, right: $t = -2.66$, df = 23, $P < 0.01$. Foraging, left: $t(0.5) < 1$, df = 23. The insets on the right show correlations of ventral and dorsal right hippocampal volumes with performance measures after regressing out other dependent variables. Alternation, dorsal: $t = -2.05$, df = 27, $P < 0.05$. Alternation, ventral: $t(0.235) < 1$, df = 27. Foraging, dorsal: $t = -1.72$, df = 23, $P < 0.1$. Foraging, ventral: $t(0.33) < 1$, df = 23. Confidence bands for fit lines are shown in gray. Each point represents one animal. $^{+}P < 0.1$; $^{*}P < 0.05$; $^{**}P < 0.01$.

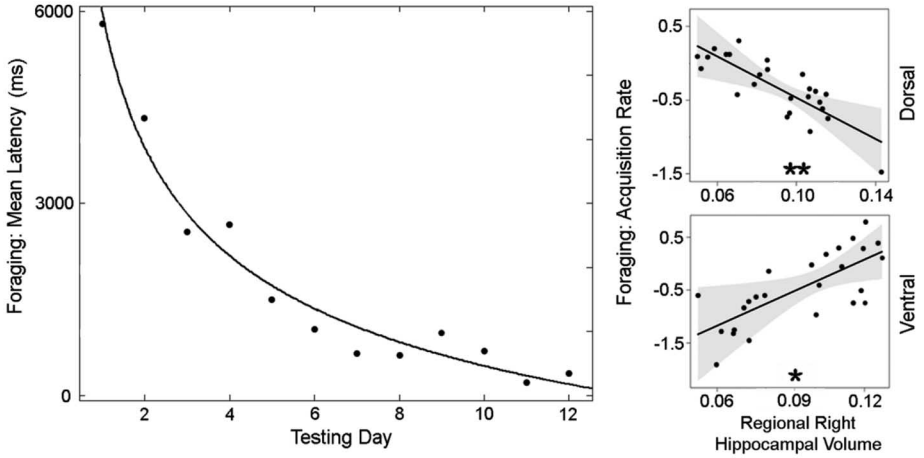

Fig. 2. Dissociable contributions of the dorsal and ventral right hippocampus to long-term spatial memory. Mean latency (to finding a reward) across all subjects is shown as a function of testing day [curve: $y = a*x^b + c$, coefficient of determination (R^2) = 0.97]. The inset conditional plots (right) show correlations between dorsal and ventral right hippocampal volume (as a percentage of total brain volume; *x* axes) and the foraging acquisition rate [log-transformed latency per testing day, *y* axes; represented as the slope of the logged power curve, with the steeper negative slope (faster acquisition) lower on the axis] after regressing out other dependent variables. Dorsal: $t = -3.21$, df = 21, $P < 0.005$. Ventral: $t = 2.44$, df = 21, $P < 0.05$. $^{*}P < 0.05$; $^{**}P < 0.01$.

substituted the volume of the dorsal third. Results were comparable to the dorsal half analyses (*9*).

The first behavioral task involved spatial alternation in a two-choice maze (fig. S2). Delayed alternation performance is impaired by hippocampal lesions in rodents (*12*) and is believed to rely on the role of the hippocampus in representing and sequencing memory for recent navigational episodes (*6*). After training to a baseline success rate of 85% on free-running left-right alternation (movie S1) (*9*), each sea lion was presented with 40 delay trials, in which the animals had to wait for 7 s at the beginning of each trial before entering the maze (movie S2). Delay trials were paired with 40 no-delay comparison trials. Right, but not left, hippocampal volume positively correlated with performance on delay trials. In addition, dorsal, but not ventral, right hippocampal volume positively correlated with performance (Fig. 1).

The second behavioral assessment was a spatial foraging task in which four possible food locations (opaque buckets) were made available once every 24 hours in the animals' enclosure (fig. S3 and movie S3). For each animal, one set location always contained food, while the others did not. At the beginning of a test session, subjects received fish at a central location while the buckets were simultaneously presented. Latency to the correct location across sessions and mean within-session errors (revisits to previously visited locations) were recorded (*9*). Rodent data indicate that within-session errors in similar spatial choice tasks track hippocampal damage (*13*). In our subjects,

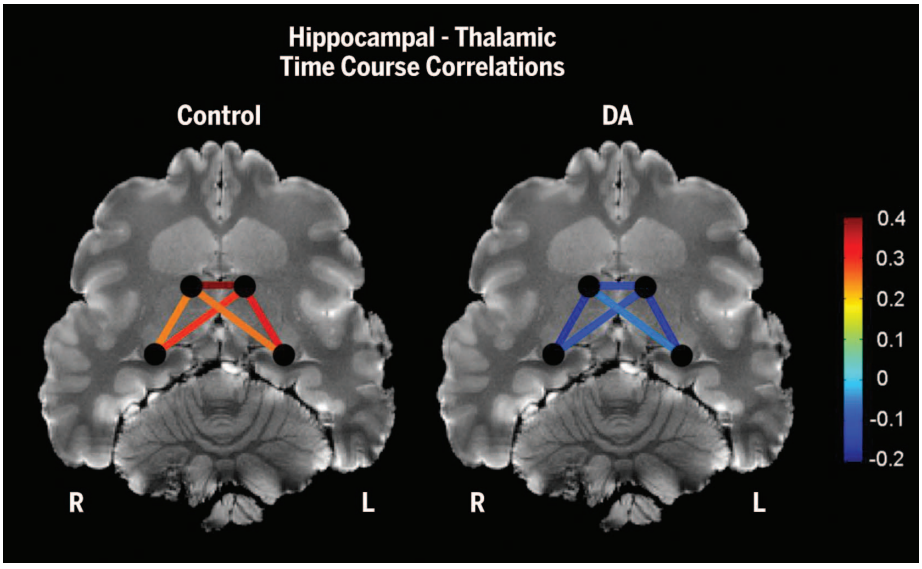

Fig. 3. Altered hippocampal-thalamic connectivity in animals with hippocampal lesions. Shown are maps of hippocampal-thalamic correlation coefficients, averaged for five brains with hippocampal lesions (right) and six without (left). The colors of the lines connecting regions of the brain represent the correlation strength of their respective time courses, with warmer colors indicating higher correlations.

right, but not left, hippocampal volume was positively correlated with within-session performance (Fig. 1).

To assess longer-term spatial memory, an additional performance measure was extracted from the foraging task. Because the mean cross-session learning curve for all animals was fit nearly perfectly by a power function (Fig. 2), the slope of the logged power curve for each animal was used to index the cross-session learning rate (table S3). Neither right nor left hippocampal damage was predictive of learning rate across all sessions (table S4). However, individual learning rates correlated positively with dorsal right hippocampal volumes and negatively with ventral right hippocampal volumes (Fig. 2).

The data reviewed thus far suggest a direct relationship between right hippocampal structure and spatial memory in sea lions. Spatial memory also depends on dynamic interactions between the hippocampus and other brain regions (*14*), with the hippocampal-thalamic axis being particularly relevant (*15*). CSLs with DA toxicosis present with seizures (*3*), which alter hippocampal networks in rodents (*16*). Accordingly, we used functional MRI to examine hippocampal-thalamic functional connectivity (*17*) in 11 CSLs undergoing rehabilitation between August and October 2012 (table S2). Five of the 11 showed volumetric evidence of gross hippocampal lesions (*9*). The other six animals served as a provisional control group. Although their complete ecotoxicant exposure history was not available, independent assessment by a veterinary radiologist and veterinarian found no evidence of neurological abnormality (*9*).

Animals with hippocampal lesions showed reduced hippocampal-thalamic connectivity (repeated measures ANOVA: $F = 22.3$, df = 1, $P < 0.001$) (Fig. 3). Reductions in connectivity were bilateral, with no statistical interaction between group (DA versus control) and laterality [$F(0.003) < 1$, df = 1] (*9*). A subsequent voxel-wise test showed high hippocampal-thalamic connectivity in controls (fig. S5) (*9*).

These data combining behavioral and neural measures in wild sea lions suggest that spatial memory is impaired and hippocampal-thalamic connectivity is disrupted as a result of DA-related hippocampal damage. The functional lateralization matches that found in humans (*10*) and may be consistent with findings of functional cortical asymmetry in sea lions (*18*). Because we examined wild CSLs, the effects of non–DA-related neurological insults were not fully controlled. This limitation is also a strength, because our results directly generalize to wild individuals.

Impairment in short- and long-term spatial memory as a result of hippocampal lesions and altered hippocampal networks probably interferes with foraging in CSLs and could partly explain maladaptive navigational behavior and consequent mortality. Because chronic exposure to DA is widespread in CSLs, these impairments could have population-level consequences, particularly in combination with changing ocean conditions that lead to less reliable foraging conditions (*7*, *19*). In addition, these findings have practical application in the veterinary and rehabilitation setting. Given the negative correlation that we found between navigational memory and the extent of hippocampal damage, in vivo measurements of hippocampal volume in stranded sea lions may be useful markers of prognosis and postrelease outcomes. Specifically, animals with right dorsal hippocampal lesions might be at increased risk in the wild. More generally, these results, obtained from an ecologically valid sample of wild animals that were naturally exposed to DA, may be applicable to other affected species, including sea birds and cetaceans, that are less accessible for neurobehavioral study.

REFERENCES AND NOTES

1. C. A. Scholin *et al.*, *Nature* **403**, 80–84 (2000).
2. M. W. Silver *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 20762–20767 (2010).
3. T. Goldstein *et al.*, *Proc. Biol. Sci.* **275**, 267–276 (2008).
4. P. A. Silvagni, L. J. Lowenstine, T. Spraker, T. P. Lipscomb, F. M. D. Gulland, *Vet. Pathol.* **42**, 184–191 (2005).
5. J. O'Keefe, "Nobel lecture: Spatial cells in the hippocampal formation" (Nobel Media AB, 2014); www.nobelprize.org/nobel_prizes/medicine/laureates/2014/okeefe-lecture.html.
6. N. J. Fortin, K. L. Agster, H. B. Eichenbaum, *Nat. Neurosci.* **5**, 458–462 (2002).
7. M. J. Weise, D. P. Costa, R. M. Kudela, *Geophys. Res. Lett.* **33**, L22S10 (2006).
8. K. Thomas, J. T. Harvey, T. Goldstein, J. Barakos, F. M. D. Gulland, *Mar. Mamm. Sci.* **26**, 36–52 (2010).
9. Materials and methods are available as supplementary materials on *Science* Online.
10. V. D. Bohbot *et al.*, *Neuropsychologia* **36**, 1217–1238 (1998).
11. M. B. Moser, E. I. Moser, *Hippocampus* **8**, 608–619 (1998).
12. W. W. Roberts, W. N. Dember, M. Brodwick, *J. Comp. Physiol. Psychol.* **55**, 695–700 (1962).
13. S. B. Floresco, J. K. Seamans, A. G. Phillips, *J. Neurosci.* **17**, 1880–1890 (1997).
14. H. Eichenbaum, *Nat. Rev. Neurosci.* **1**, 41–50 (2000).
15. D. S. Olton, J. A. Walker, F. H. Gage, *Brain Res.* **139**, 295–308 (1978).
16. J. M. Parent *et al.*, *J. Neurosci.* **17**, 3727–3738 (1997).
17. B. Biswal, F. Z. Yetkin, V. M. Haughton, J. S. Hyde, *Magn. Reson. Med.* **34**, 537–541 (1995).
18. O. I. Lyamin, I. S. Chetyrbok, *Neurosci. Lett.* **143**, 263–266 (1992).
19. S. C. Doney *et al.*, *Ann. Rev. Mar. Sci.* **4**, 11–37 (2012).

ACKNOWLEDGMENTS

This research was conducted under authorization granted to the Marine Mammal Health and Stranding Response Program by the National Marine Fisheries Service under scientific research permit 932-1489-10, and it has been approved by the Institutional Animal Care and Use Committees at the University of California–Santa Cruz (UCSC) and The Marine Mammal Center (TMMC). We thank D. Casper and staff members at both UCSC's Pinniped Laboratory and TMMC for contributions to data acquisition and logistical support. Behavioral data are in the supplementary materials; imaging data are archived on Dryad at http://dx.doi.org/10.5061/dryad.0279k. None of the authors claim any competing financial interests.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6267/1545/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S5
Tables S1 to S5
References (*20*–*23*)
Movies S1 to S3

13 May 2015; accepted 9 November 2015
Published online 14 December 2015
10.1126/science.aac5675

Algal toxin impairs sea lion memory and hippocampal connectivity, with implications for strandings

Peter F. Cook *et al.*

Science **350**, 1545 (2015);
DOI: 10.1126/science.aac5675