alanines to abolish Ca^{2+} binding. Unexpectedly, this mutant Doc2 fully rescues the decrease in spontaneous release induced by shRNA knockdown of Doc2 proteins in cultured mouse cortical inhibitory neurons, challenging the legitimacy of Doc2 as the Ca^{2+} sensor for spontaneous release (Pang et al., 2011). What is the effect of this mutant Doc2 in evoked asynchronous release? Unfortunately, Yao et al. do not report this. They also do not report how knockdown and overexpression of Doc2 affect the Ca^{2+} sensitivity of asynchronous release, another important parameter for assessing the Ca^{2+} sensor function.

Groffen et al. (2010), Pang et al. (2011), and Yao et al. (2011) all agree that Doc2 does not affect evoked synchronous release. Groffen et al. (2010) and Pang et al. (2011), however, claim that Doc2 is not involved in asynchronous release either. What could account for this discrepancy? The three studies were based on different experimental approaches. Yao et al. (2011) use both knockdown and knockout approaches, and the results are consistent with each other, providing substantial strength to the data. In Pang et al. (2011), the knockdown efficiency is measured from the entire neuronal culture, but inhibitory neurons constitute only a small fraction of the neuronal population. Hence, it is not obvious if the Doc2 proteins were sufficiently suppressed in inhibitory neurons. Although spontaneous release is reduced in these neurons, asynchronous release may have a different sensitivity to Doc2 reduction. For example, shRNA-mediated knockdown of complexins affects excitatory neurons, but not inhibitory neurons (Maximov et al., 2009), whereas a full genetic knockout has the same effects on both neuronal types (Xue et al., 2008). Alternatively, other Ca^{2+} sensors may compensate for the loss of Doc2 proteins in cortical neurons assayed by Pang et al. (2011). It is also possible that Doc2 is the Ca^{2+} sensor for asynchronous release in excitatory neurons, but not inhibitory neurons. Finally, it is not obvious why Groffen et al. (2010) did not observe a defect in asynchronous release.

The study by Yao et al. is important because it provides a promising candidate for the Ca^{2+} sensor of asynchronous release in many cell types and raises interesting questions about their mechanism of action. It also raises an issue with respect to their counterparts in invertebrates, given that Doc2 proteins are not evolutionarily conserved in many species (Craxton, 2010). Future work may test whether rabphilin, a conserved C2 domain-containing protein that shares a high degree of homology with Doc2 proteins, subserves this role in invertebrates.

REFERENCES

Craxton, M. (2010). BMC Genomics *11*, 37.

Friedrich, R., Yeheskel, A., and Ashery, U. (2010). Mol. Neurobiol. *41*, 42–51.

Geppert, M., Goda, Y., Hammer, R.E., Li, C., Rosahl, T.W., Stevens, C.F., and Südhof, T.C. (1994). Cell *79*, 717–727.

Goda, Y., and Stevens, C.F. (1994). Proc. Natl. Acad. Sci. USA *91*, 12942–12946.

Groffen, A.J., Martens, S., Díez Arazola, R., Cornelisse, L.N., Lozovaya, N., de Jong, A.P., Goriounova, N.A., Habets, R.L., Takai, Y., Borst, J.G., et al. (2010). Science *327*, 1614–1618.

Maximov, A., Tang, J., Yang, X., Pang, Z.P., and Südhof, T.C. (2009). Science *323*, 516–521.

Pang, Z.P., Bacaj, T., Yang, X., Zhou, P., Xu, W., and Südhof, T.C. (2011). Neuron *70*, 244–251.

Rizo, J., and Rosenmund, C. (2008). Nat. Struct. Mol. Biol. *15*, 665–674.

Yao, J., Gaffaney, J.D., Kwon, S.E., and Chapman, E.R. (2011). Cell *147*, this issue, 666–677.

Xue, M., Stradomska, A., Chen, H., Brose, N., Zhang, W., Rosenmund, C., and Reim, K. (2008). Proc. Natl. Acad. Sci. USA *105*, 7875–7880.

Two Routes for Remembering the Past

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Which brain circuits underlie retrieval of distant memories? Goshen et al. (2011) use a powerful optogenetic-based approach to reveal the critical contribution of the hippocampus to remote memory retrieval. In so doing, they provide new evidence toward resolving a long-standing debate in cognitive neuroscience.

The French psychologist T. Ribot was the first to note that there was something different about recent and remote memories (Ribot, 1881). Specifically, memory loss following brain injury tended to affect the remembrance of recent memories more than memories of the distant past. His observation suggested the possibility that memories might be reorganized over time. Findings from humans and animal models confirmed this idea, showing that damage to the hippocampus caused temporally graded memory deficits such that recall of information learned just before the time of hippocampal damage was severely impaired, whereas information learned in the remote past was remembered normally. This phenomenon,

termed temporally graded retrograde amnesia (Squire and Alvarez, 1995), forms the basis of consolidation theory, which states that newly formed memories are initially dependent on the hippocampus, and those memories (including context-rich, detailed memories) gradually become fully dependent on the cortex and independent of the hippocampus. A conflicting body of findings reports retrograde amnesia following hippocampal damage that is equally severe for recently and remotely learned information (i.e., a flat retrograde amnesia gradient). These latter findings led to the development of two related theories: multiple trace theory (Nadel and Moscovitch, 1997) and transformation hypothesis (Winocur and Moscovitch, 2011), which state that remote memories retain rich contextual detail and remain dependent on the hippocampus for as long as the rich memories remain available. The memory trace that eventually develops in cortex is less detailed than the hippocampus-dependent memory, and there is a dynamic interplay between the two types of memory such that one or the other may be dominant at the time of retrieval.

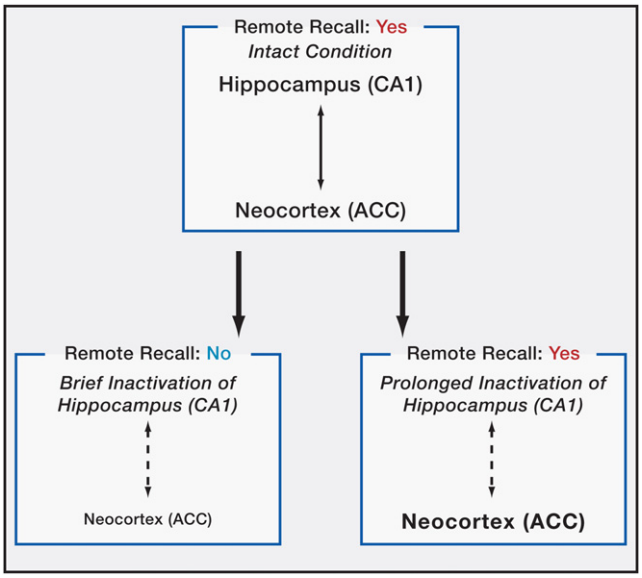

Figure 1. Two Ways to Probe Remote Memories

Top: Illustration of the functional interactions (double-headed arrow) between the hippocampus and the anterior cingulate cortex (ACC) thought to be required for normal remote contextual fear memory. Bottom left: Summary of the effects of brief CA1 inactivation resulting in decreased c-*fos* expression in the ACC (smaller font) and impaired remote contextual fear memory. Bottom right: The effects of prolonged inactivation of CA1 include increased activity in ACC as measured by c-*fos* expression (larger font) and successful remote contextual fear memory. Dashed double-headed arrows illustrate disruption of CA1-ACC interactions.

These conflicting experimental findings (i.e., temporally graded versus flat retrograde amnesia) and their corresponding theories (consolidation theory versus multiple trace theory/transformation hypothesis) have been difficult to resolve experimentally. In this issue of *Cell*, Goshen et al. (2011) present a remarkable set of findings that help address key aspects of this controversy. Until now, the inability to inhibit the hippocampus with temporal precision left open the possibility that compensatory mechanisms that develop during the relatively long inactivation caused by pharmacological agents or lesions could be clouding our interpretation. Using an optogenetics approach, the authors selectively inhibit excitatory CA1 cells in the dorsal hippocampus with precise temporal control. They study remote memory using contextual fear conditioning (FC), which is known to be dependent on the hippocampus. In this paradigm, mice were given a series of footshocks in a particular training context. After the context-shock pairing, remote FC memory was measured as the duration of freezing exhibited in the original training context at least 1 month after the shocks were administered. Now, for the first time, Goshen et al. are able to compare the effects of two types of hippocampal inactivation on remote contextual fear memory (Figure 1). In the first condition, hippocampal inactivation is matched precisely to the 5 min test period (brief inactivation). In the second condition, to mimic the longer inactivation typical in pharmacological studies, the hippocampus is inactivated for 30 min starting 25 min before the test period and including the 5 min test period (prolonged inactivation).

Compatible with predictions of the multiple trace theory/transformation hypothesis, the authors find that remote memory is severely impaired with brief inactivation during the test period. This striking finding suggests that the hippocampus is normally engaged during the retrieval process of even well-established contextual memories. However, the authors show that the hippocampus does not work alone in remote memory retrieval. Both short and prolonged inactivation of anterior cingulate cortex (ACC), an area previously shown to be important for the long-term storage of fear memories (Frankland et al., 2004), also impair retrieval of remote contextual fear memories. By measuring c-*fos* expression, the authors show that normal activity during remote contextual memory retrieval in the ACC depends on an intact hippocampus. This finding suggests that remote memory retrieval requires communication between the hippocampus and the ACC (Figure 1, bottom left).

Consistent with previous pharmacological studies (Kitamura et al., 2009), Goshen and colleagues confirm that prolonged inactivation of CA1 does not affect remote fear memory. However, an interesting new spin on this familiar observation is provided by the finding that brief CA1 inactivation has devastating consequences for remote fear memory. Namely, whereas previous studies had interpreted spared remote memory after prolonged/pharmacological hippocampal inactivation as evidence for a hippocampus-independent, neocortical memory trace, these new findings suggest that the spared memory is instead a striking example of plasticity that can develop after just 25 min of hippocampal inactivation. c-fos studies suggest the basis for a possible compensatory mechanism by showing that with prolonged hippocampal inactivation but not with brief inactivation, the ACC exhibited significant hyperactivation (Figure 1, bottom right).

Taken together, these findings suggest two possible routes for remembering the past. One corresponds to a default (intact) condition where both the hippocampus and ACC, along with the functional

interactions between these two brain regions, are important for normal remote contextual memory retrieval. The other route is compensatory. When the hippocampus is inactivated, ACC activity can increase, enabling a hippocampus-independent route to remembering. This study from Goshen et al. provides new insights that advance our understanding of the brain circuits underlying remote memory and describes exciting new technological tools that can be used for future investigations. A key remaining question concerns the nature of the representation underlying the remote memories along these distinct routes. The multiple trace/transformation theory posits that hippocampal-based remote memories are always richer in contextual detail than neocortically based remote memories (Wiltgen et al., 2010). On the other hand, neuropsychological studies report that the hippocampus-independent remote memories can also be rich in contextual detail (Bayley et al., 2003). How does the neocortex represent remote memories in the presence and absence of the hippocampus? Neurophysiological studies that directly characterize the representation of remote contextual fear memories in the ACC in the default condition (no inactivation) or with brief or prolonged hippocampal inactivation will be required to resolve this question. Given the strong and reciprocal connections between the hippocampus and other medial temporal lobe areas (Lavenex and Amaral, 2000), it will also be important to record from these other areas to characterize their physiological responses during both brief and prolonged hippocampal inactivation.

REFERENCES

Bayley, P.J., Hopkins, R.O., and Squire, L.R. (2003). Neuron *38*, 135–144.

Frankland, P.W., Bontempi, B., Talton, L.E., Kaczmarek, L., and Silva, A.J. (2004). Science *304*, 881–883.

Goshen, I., Brodsky, M., Prakash, R., Wallace, J., Gradinaru, V., Ramakrishnan, C., and Deisseroth, K. (2011). Cell *147*, this issue, 678–689.

Kitamura, T., Saitoh, Y., Takashima, N., Murayama, A., Niibori, Y., Ageta, H., Sekiguchi, M., Sugiyama, H., and Inokuchi, K. (2009). Cell *139*, 814–827.

Lavenex, P., and Amaral, D.G. (2000). Hippocampus *10*, 420–430.

Nadel, L., and Moscovitch, M. (1997). Curr. Opin. Neurobiol. *7*, 217–227.

Ribot, T. (1881). Les Maladies de la Memoire (New York: Appleton-Century-Crofts), [English translation: Diseases of Memory].

Squire, L.R., and Alvarez, P. (1995). Curr. Opin. Neurobiol. *5*, 169–177.

Wiltgen, B.J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M.G., Parivash, S.N., Li, W., and Silva, A.J. (2010). Curr. Biol. *20*, 1336–1344.

Winocur, G., and Moscovitch, M. (2011). J. Int. Neuropsychol. Soc. *17*, 766–780.