described in this pioneering work from John Landers and his colleagues are going to drive the identification of novel ALS genes in the future. Translating these findings to disease risk for individual patients will however be a tremendous challenge and caution should be taken before any individual variant identified using this approach can be implicated in ALS.

REFERENCES

Chesi, A., Staahl, B.T., Jovičić, A., Couthouis, J., Fasolino, M., Raphael, A.R., Yamazaki, T., Elias, L., Polak, M., Kelly, C., et al. (2013). Nat. Neurosci. *16*, 851–855.

DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., et al. (2011). Neuron *72*, 245–256.

Johnson, J.O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V.M., Trojanowski, J.Q., Gibbs, J.R., Brunetti, M., Gronka, S., Wuu, J., et al.; ITALSGEN Consortium (2010). Neuron *68*, 857–864.

Johnson, J.O., Pioro, E.P., Boehringer, A., Chia, R., Feit, H., Renton, A.E., Pliner, H.A., Abramzon, Y., Marangi, G., Winborn, B.J., et al.; ITALSGEN Consortium (2014). Nat. Neurosci. *17*, 664–666.

Kim, H.J., Kim, N.C., Wang, Y.D., Scarborough, E.A., Moore, J., Diaz, Z., MacLea, K.S., Freibaum, B., Li, S., Molliex, A., et al. (2013). Nature *495*, 467–473.

Ling, S.C., Polymenidou, M., and Cleveland, D.W. (2013). Neuron *79*, 416–438.

Renton, A.E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., et al.; ITALSGEN Consortium (2011). Neuron *72*, 257–268.

Smith, B.N., Ticozzi, N., Fallini, C., Gkazi, A.S., Topp, S., Kenna, K.P., Scotter, E.L., Kost, J., Keagle, P., Miller, J.W., et al. (2014). Neuron *84*, this issue, 324–331.

Tischfield, M.A., Cederquist, G.Y., Gupta, M.L., Jr., and Engle, E.C. (2011). Curr. Opin. Genet. Dev. *21*, 286–294.

Turner, M.R., Hardiman, O., Benatar, M., Brooks, B.R., Chio, A., de Carvalho, M., Ince, P.G., Lin, C., Miller, R.G., Mitsumoto, H., et al. (2013). Lancet Neurol. *12*, 310–322.

van Blitterswijk, M., DeJesus-Hernandez, M., and Rademakers, R. (2012a). Curr. Opin. Neurol. *25*, 689–700.

van Blitterswijk, M., van Es, M.A., Hennekam, E.A., Dooijes, D., van Rheenen, W., Medic, J., Bourque, P.R., Schelhaas, H.J., van der Kooi, A.J., de Visser, M., et al. (2012b). Hum. Mol. Genet. *21*, 3776–3784.

Wu, C.H., Fallini, C., Ticozzi, N., Keagle, P.J., Sapp, P.C., Piotrowska, K., Lowe, P., Koppers, M., McKenna-Yasek, D., Baron, D.M., et al. (2012). Nature *488*, 499–503.

Chasing the Trace

Adam Santoro[1,2] and Paul W. Frankland[1,2,3,4,*]
[1]Program in Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, ON M5G 1X8, Canada
[2]Institute of Medical Science, University of Toronto, Toronto, ON M5S 1A8, Canada
[3]Department of Psychology, University of Toronto, Toronto, ON M5S 3GM, Canada
[4]Department of Physiology, University of Toronto, Toronto, ON M5S 1A8, Canada
*Correspondence: paul.frankland@sickkids.ca
http://dx.doi.org/10.1016/j.neuron.2014.10.001

Event memories are stored in hippocampal-cortical networks. In this issue of *Neuron*, two studies, Cowansage et al. (2014) and Tanaka et al. (2014), tag active cells during memory encoding and optogenetically manipulate the activity of these "engram" cells during subsequent recall to reveal how hippocampal and cortical cell ensembles interact during retrieval.

While there is plenty of debate in the memory field, the classical view is that memory traces for events are laid down in cell ensembles across distributed hippocampal-cortical networks. The hippocampus is considered necessary, at least temporarily after encoding, for successful retrieval of these event memories via reinstatement of the patterns of activity within these cortical ensembles. According to this view, the hippocampus contains indices or pointers to cortical cell assemblies that collectively represent a given event (e.g., Eichenbaum, 2000).

Observations of retrograde amnesia following hippocampal damage in human patients (such as H.M.), as well as in experimental animals, provide broad support for this view (Squire et al., 2004). However, they tell little about how hippocampal and cortical cell ensembles interact to support memory retrieval. Two studies published in the current issue of *Neuron*, Cowansage et al. (2014) and Tanaka et al. (2014), shed light on this interaction. Both studies used a genetic strategy to tag active cells at the time of memory encoding with light-sensitive opsins and then optogenetically manipulate the activity of these "engram" cells during retrieval.

In the first study, Tanaka et al. (2014) used a Fos-driven reporter mouse to tag active cells as mice learned an association between a shock and a context. The formation and maintenance of contextual fear memories engages distributed networks, and, as expected, training tagged ensembles of cells throughout the hippocampus and cortex. Usually, when mice return to the original training context, they exhibit conditioned fear responses, including freezing behavior, indicating that they recognize this as the place in which they previously received a shock. By expressing the inhibitory opsin (ArchT) in tagged cells in the CA1 region of the hippocampus, Tanaka et al. (2014) examined the impact of silencing engram cells

just in this region (rather than the whole hippocampal-cortical network) on fear memory retrieval (Figure 1). When these tagged CA1 cells were silenced, mice no longer froze in the conditioning context. Using a comparable tag and manipulate approach, a recent study showed that silencing engram cells in either the dentate gyrus or CA3 regions of the hippocampus similarly impaired retrieval of a contextual fear memory (Denny et al., 2014).

While these data suggest that reactivating ensembles of hippocampal cells that were active at the time of encoding is required for successful retrieval, there is an alternative explanation. Perhaps silencing any population of hippocampal cells (and not necessarily the engram cells) produces a more general disruption of hippocampal function and impairs retrieval. Tanaka et al. (2014) designed a clever experiment to test this idea. They first tagged CA1 cells with the inhibitory opsin when exposing mice to a different environment. They subsequently fear conditioned these mice in the regular training context while silencing this population of tagged cells (and presumably preventing these tagged cells from becoming part of the contextual fear memory engram). They then placed mice back into the fear conditioning context and silenced this population of "excluded" cells. Silencing had no effect on the retrieval of the fear memory, indicating that their disruption was specific to the target memory and did not affect other contextual memories presumably stored in the hippocampus.

Why don't the mice remember? While fear memories like these are thought to be represented in distributed hippocampal-cortical networks, silencing just the engram neurons in the CA1 was sufficient to impair retrieval. According to the classical view, preventing reactivation of a hippocampal index should prevent reactivation of the entire hippocampal-cortical network. The tagging system used by Tanaka et al. (2014) allowed them to address this issue in ways not possible previously. After CA1 silencing on the retrieval test, they evaluated the likelihood of reactivation for tagged engram cells in the cortex. While overall activation levels (measured by expression of the immediate-early gene Fos) were similar with or without hippocampal silencing, silencing CA1 encoding cells led to a selective reduction in reactivation of tagged cells in the cortex.

Therefore, these data suggest that silencing CA1 engram cells induces retrieval failure because, at least in part, it prevents the reactivation of engram cells in the cortex. If so, is it possible to bypass the hippocampus (and its index) and artificially express a fear memory by directly driving these cortical engram cells? This is the question addressed by Cowansage et al. (2014). Using a similar tag-and-manipulate approach, they first fear conditioned mice and, as Tanaka et al. (2014) observed, found that engram cells were tagged throughout the hippocampus and cortex. Now, instead of turning off tagged cells in the CA1, Cowansage et al. (2014) directly reactivated engram cells in the retrosplenial cortex after placing mice in a neutral context (where freezing isn't normally observed). Artificial reactivation of this component of the memory trace induced freezing. This suggests that reactivation of tagged retrosplenial cells alone was sufficient to artificially express the fear memory.

Focal stimulation of a population of engram cells in the retrosplenial cortex was effective in retrieving the memory possibly because it led to activation of a much broader network of engram cells in the cortex. Indeed, the retrosplenial cortex reciprocally connects with many hippocampal and cortical regions and, during contextual fear memory retrieval, activity of the retrosplenial cortex is coordinated with activity in many other cortical regions (Wheeler et al., 2013). Consistent with the idea that the retrosplenial cortex acts as a hub within a broader memory network, Cowansage et al. (2014) found that "natural" and "artificial" expression of a fear memory activated similar populations of cells in subregions of the amygdala and entorhinal cortex. It is worth noting that the artificially expressed memory is weaker than naturally expressed fear memories, with freezing levels hovering between 15%–20% following activation of retrosplenial engram cells. This is not surprising given that it is unlikely that all engram cells were reactivated. Furthermore, optical stimulation cannot recapitulate the precise temporal patterning observed during the natural retrieval of fear memories, leading to further information loss.

While the hippocampus is necessary for retrieval of contextual fear memories soon after encoding, eventually these types of memories may be expressed independently of the hippocampus (Frankland and Bontempi, 2005). Cowansage et al. (2014) next asked whether hippocampal activity was necessary for artificial recall of a fear memory, when tested just a few days after encoding. To do this, they pharmacologically inhibited the hippocampus while mice "naturally" expressed a contextual fear memory or "artificially" expressed a contextual fear memory (induced by driving retrosplenial engram cells). As expected, shutting down the hippocampus blocked natural recall of the memory. However, shutting down hippocampal activity had no effect on artificial expression of the memory. This surprising finding suggests that driving cortical engram cells alone is sufficient for memory expression, even soon after training. Presumably, whereas under normal conditions the only way to reactivate cortical engram cells is via reactivating the appropriate indices or pointers in the hippocampus, accessing cortical engram cells directly circumvents this requirement.

The creative application of this "tag-and-manipulate" approach allowed these studies to interrogate this classical model of consolidation in ways not previously possible. In particular, the ability to selectively manipulate cells that were active during encoding allowed Tanaka et al. (2014) to provide support for the widely held view that the successful retrieval depends upon reinstatement of cortical patterns of activity that occurred at the time of learning via activation of hippocampal indices. Complementing this discovery, Cowansage et al. (2014) showed that it is nonetheless possible to completely bypass the hippocampus and artificially express a fear memory by targeting engram cells in the cortex directly.

However, as elegant as these studies are, one major issue remains unresolved. What is the nature of the index? This is a fundamental issue that divides opinion. At one extreme, hippocampal cell ensemble activity is thought to provide little more than an index, with

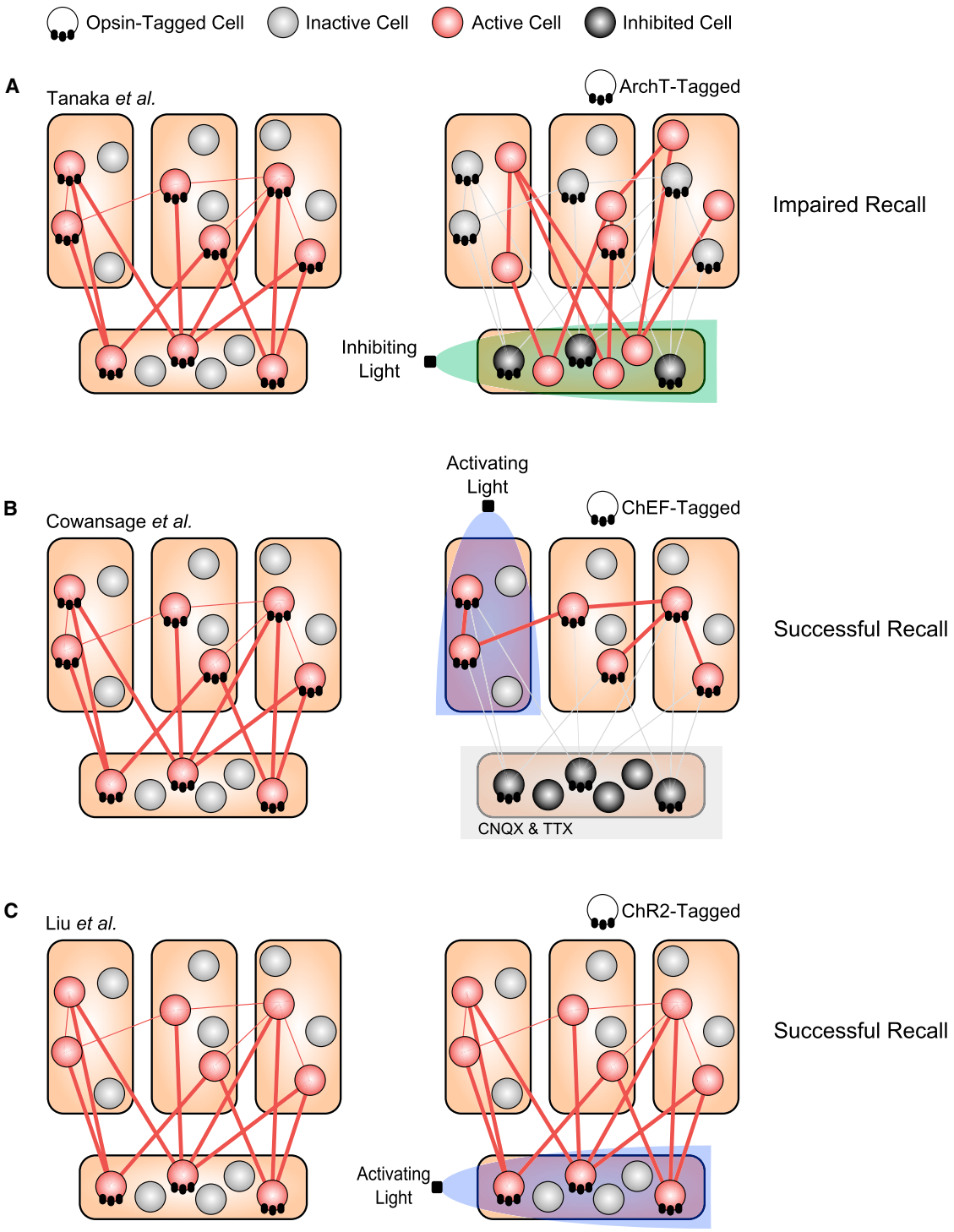

Figure 1. Opsin-Mediated Reactivation or Inhibition of Memory Engram Cells

(A) Tanaka et al. (2014) demonstrate that reactivation of tagged CA1 cells is necessary for the recapitulation of cortical engram cells. When the tagged CA1 cells are inhibited, a different population is recruited in the cortex, impairing memory retrieval.

(B) A memory successfully expresses via optogenetic reactivation of a distinct cortical ensemble (i.e., the retrosplenial cortex), even when pharmacologically inhibiting the hippocampus (Cowansage et al., 2014). Importantly, the engram cells in other cortical regions reactivate during this artificial retrieval.

(C) Previous results demonstrate that reactivation of tagged hippocampal cells is sufficient for successful memory retrieval, presumably via reactivation of cortical engram cells (Liu et al., 2012).

memory content stored in the cortex (Teyler and DiScenna, 1986). According to this account, memories expressed via activation of hippocampal indices or, artificially, via direct activation of cortical engram cells should not differ in quality. That is, the same content is being accessed, albeit via different routes. Alternatively, others argue that, along with containing an index, the information in the hippocampus necessarily includes at least some content that is not present in the cortex (for example, contextually dense or highly spatial details) (Winocur and Moscovitch, 2011). Therefore, according to this account, whether or not the hippocampus contributes to expression does make a difference in the quality of the retrieved memory. A fear memory expressed via activation of hippocampal indices should retain its contextually rich and detailed nature. In contrast, direct activation of cortical engram cells will lead to expression of a fear memory that is necessarily less detailed and more gist-like in quality. In fear conditioning studies, memory quality has most often been assessed by comparing freezing levels in trained versus similar contexts. However, since the artificial recall is already assessed in a neutral context in the Cowansage et al. (2014) study, these types of context generalization experiments are not possible here. This particular debate is destined to continue, and it is our hope that the creative application of new tools will also shed light on this question.

REFERENCES

Cowansage, K.K., Shuman, T., Dillingham, B.C., Chang, A., Golshani, P., and Mayford, M. (2014). Neuron *84*, this issue, 432–441.

Denny, C.A., Kheirbek, M.A., Alba, E.L., Tanaka, K.F., Brachman, R.A., Laughman, K.B., Tomm, N.K., Turi, G.F., Losonczy, A., and Hen, R. (2014). Neuron *83*, 189–201.

Eichenbaum, H. (2000). Nat. Rev. Neurosci. *1*, 41–50.

Frankland, P.W., and Bontempi, B. (2005). Nat. Rev. Neurosci. *6*, 119–130.

Liu, X., Ramirez, S., Pang, P.T., Puryear, C.B., Govindarajan, A., Deisseroth, K., and Tonegawa, S. (2012). Nature *484*, 381–385.

Squire, L.R., Stark, C.E., and Clark, R.E. (2004). Annu. Rev. Neurosci. *27*, 279–306.

Tanaka, K.Z., Pevzner, A., Hamidi, A.B., Nakazawa, Y., Graham, Y., and Wiltgen, B.J. (2014). Neuron *84*, this issue, 347–354.

Teyler, T.J., and DiScenna, P. (1986). Behav. Neurosci. *100*, 147–154.

Wheeler, A.L., Teixeira, C.M., Wang, A.H., Xiong, X., Kovacevic, N., Lerch, J.P., McIntosh, A.R., Parkinson, J., and Frankland, P.W. (2013). PLoS Comput. Biol. *9*, e1002853.

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

Keeping an Eye on Cortical States

James F.A. Poulet[1,2,*]
[1]Department of Neuroscience, Max-Delbrück Center for Molecular Medicine (MDC), Berlin-Buch, Robert-Rössle-Str. 10, 13092 Berlin, Germany
[2]Cluster of Excellence NeuroCure, Neuroscience Research Center, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany
*Correspondence: james.poulet@mdc-berlin.de
http://dx.doi.org/10.1016/j.neuron.2014.10.005

Membrane potential recordings in awake mice have correlated cortical state with locomotion and whisker movements. In this issue of *Neuron*, Reimer et al. (2014) now reveal that pupil dilation in stationary mice equally signals a change in cortical state and an enhancement of visual processing.

Sensory processing and perception are not simply a passive detection of stimuli by the nervous system; in animals that are awake and behaving, it is an active process and a highly integrative one. The peripheries of our sensory systems are constantly engaged, whether we realize it or not: eyes scan, hands manipulate, noses sniff, tongues roll. Although we rarely use them, we even have muscles to move our ears—maybe the vestige of some ancient mechanism to reposition them and capture more sound. When sensory input reaches the CNS, it is integrated with sensory signals of other modalities and a wide range of internally generated signals including copies of motor commands, memories, arousal, and attention. Understanding where, how, and why sensory integration occurs in the brain is a grand challenge for neuroscience.

Nowhere is the integration of external and internal neural signals more apparent than in the mammalian neocortex. The very first electroencephalogram (EEG) recordings of electrical activity from awake animals and the human brain revealed patterns of spontaneous activity that correlated to different behavioral states but seemed unrelated to direct sensory input. This suggested that the neocortex would be a good place to study changes in brain states and their relation to sensory integration, in the hope of finding cellular correlates possibly in identified populations of neurons. This was theoretically possible, but anesthesia was typically used to immobilize the animal. It was a dilemma if you were interested in waking brain states.

The head-restrained mouse preparation came to the rescue and is now in widespread use. This provides the stability