across organ systems, with the potential to inform future strategies for modifying symptoms of disease.

REFERENCES

Buffington, S.A., Di Prisco, G.V., Auchtung, T.A., Ajami, N.J., Petrosino, J.F., and Costa-Mattioli, M. (2016). Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. Cell *165*, 1762–1775.

Han, W., Tellez, L.A., Perkins, M.H., Perez, I.O., Qu, T., Ferreira, J., Ferreira, T.L., Quinn, D., Liu, Z.W., Gao, X.B., et al. (2018). A neural circuit for gut-induced reward. Cell *175*, 665–678.e23.

Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J.A., Chow, J., Reisman, S.E., Petrosino, J.F., et al. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell *155*, 1451–1463.

Hung, L.W., Neuner, S., Polepalli, J.S., Beier, K.T., Wright, M., Walsh, J.J., Lewis, E.M., Luo, L., Deisseroth, K., Dölen, G., and Malenka, R.C. (2017). Gating of social reward by oxytocin in the ventral tegmental area. Science *357*, 1406–1411.

Kaelberer, M.M., Buchanan, K.L., Klein, M.E., Barth, B.B., Montoya, M.M., Shen, X., and Bohórquez, D.V. (2018). A gut-brain neural circuit for nutrient sensory transduction. Science *361*, https://doi.org/10.1126/science.aat5236.

Kim, S., Kim, H., Yim, Y.S., Ha, S., Atarashi, K., Tan, T.G., Longman, R.S., Honda, K., Littman, D.R., Choi, G.B., and Huh, J.R. (2017). Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. Nature *549*, 528–532.

Nicolini, C., and Fahnestock, M. (2018). The valproic acid-induced rodent model of autism. Exp. Neurol. *299* (Pt A), 217–227.

Peça, J., Feliciano, C., Ting, J.T., Wang, W., Wells, M.F., Venkatraman, T.N., Lascola, C.D., Fu, Z., and Feng, G. (2011). Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. Nature *472*, 437–442.

Sgritta, M., Dooling, S.W., Buffington, S.A., Momin, E.N., Francis, M.B., Britton, R.A., and Costa-Mattioli, M. (2018). Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. Neuron *101*, this issue, 246–259.

Vuong, H.E., and Hsiao, E.Y. (2017). Emerging roles for the gut microbiome in autism spectrum disorder. Biol. Psychiatry *81*, 411–423.

Engram Excitement

Zimbul Albo[1] and Johannes Gräff[1,*]
[1]Brain Mind Institute, School of Life Sciences, École Polytechnique Fédérale Lausanne (EPFL), CH-1015 Lausanne, Switzerland
*Correspondence: johannes.graeff@epfl.ch
https://doi.org/10.1016/j.neuron.2018.12.033

Engram cells can encode and switch between multiple mnemonic functions, but how they intrinsically do so is unknown. Pignatelli, Ryan, and colleagues show that upon memory recall, the engram's excitability is transiently elevated, allowing its bearer to adapt to changing environments.

What is memory? Asking this age-old question to a hundred neuroscientists in one room would probably yield as many answers. Even so, converging evidence over the past years suggests that engram cells may provide a physiological substrate for learning, memory storage, and retrieval (Josselyn et al., 2015). Originally defined by the biologist Richard Semon as "the enduring though primarily latent modification in the irritable substance produced by a stimulus …" (Semon, 1904), engram cells have in the meantime been shown to be able to store learned information in latent form (Kitamura et al., 2017), which can be reactivated by recall and alter behavior when being artificially stimulated (Tonegawa et al., 2015). By this virtue, engram cells fulfill three out of four of Semon's original criteria of an engram—namely content, dormancy and persistence (Josselyn et al., 2015).

They also partly fulfill the fourth criterion, ecphory, which refers to the process that "… awakens the mnemic trace or engram out of its latent state into one of manifested activity …" (Semon, 1904). However, this criterion was so far only met under artificial, i.e., optogenetically driven, activation studies of engram cells (Ryan et al., 2015), leaving it unclear whether the process of ecphory occurs under natural circumstances. In this issue of *Neuron*, Pignatelli et al. (2019) illustrate that, upon memory recall, memory engram cells show enhanced intrinsic excitability and that such excitability forms the basis for behavioral flexibility in changing environments.

To do so, Pignatelli et al. (2019) used contextual fear conditioning and a previously established engram labeling technique of their group. This technique consists of a doxycycline (DOX)-inducible double-transgenic system achieved when single-transgenic cFos-tTA mice are stereotaxically injected with viral vectors containing a DOX-sensitive tTA-responsive element coupled to channelrhodopsin (TRE-ChR2-EYFP). Here, this system was used to label cFos-positive engram cells in the dentate gyrus (DG) at encoding of contextual fear memories, during which DOX was removed from the animals' diet. 1 day after encoding, fear memories were recalled by context exposure, and the engram cells' ($EYFP^+$) intrinsic electrophysiological properties compared to those of non-engram cells ($EYFP^-$) by *ex vivo* patch clamp recordings of *a posteriori* biocytin-identified cells.

The authors found that, compared to non-engram cells, engram cells showed increased membrane resistance—indicative of a higher depolarization state—and decreased rheobase—indicating that fewer current steps are needed to reach the action potential threshold. Together,

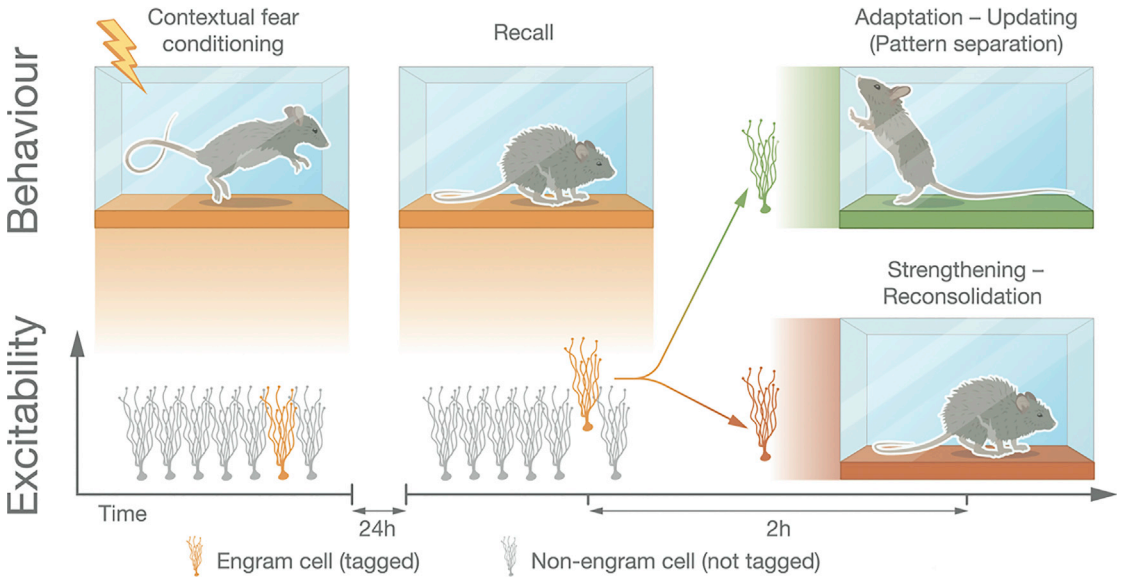

Figure 1. Enhanced Engram Excitability Allows for Behavioral Adaptation to Environmental Changes
Engram cells tagged at contextual fear conditioning show increased excitability for a period of 2 h post memory recall. During this time, which coincides with the so-called reconsolidation window, the animals can recognize a slightly modified context through pattern separation and thus adapt to an environmental change by updating the engram's mnemonic content (top). Conversely, if no environmental change is present, the engram's original mnemonic content would be strengthened through memory reconsolidation (bottom). Note that for simplicity, only the findings for pattern separation are shown; for the findings on pattern completion, the reader is referred to the text. Illustration by Gaia Codoni, medicalwriters.com (Zürich, Switzerland).

these measurements testify to a state of reduced polarization within the engram cell and therefore to enhanced excitability (Figure 1). Interestingly, similar findings were obtained when the animals were made to simply recall a neutral context that had not previously been paired with a foot shock. This increased excitability lasted for 2 h and returned to baseline in a protein-synthesis-dependent manner after that period. Based on these grounds, the increased engram excitability bears striking resemblance to the ecphory process that "… awakens the mnemic trace (…) out of its latent state" (Semon, 1904).

But how does this awakening then transition "…into [a state] of manifested activity"? In other words, how does engram excitability transpire at the behavioral level? For this, Pignatelli et al. (2019) took advantage of their experimentally determined time course of engram excitability and used a behavioral protocol that assesses the animals' capability for pattern separation, a role traditionally assigned to the DG (Neunuebel and Knierim, 2014). Behaviorally speaking, pattern separation refers to the ability to distinguish between two closely matching environmental configurations. In the protocol used in Pignatelli et al. (2019), animals were fear conditioned to context A, and their memory was tested one day later in a degraded context AB, for which all but the visual cues were different than in context A. This testing occurred either 5 or 60 min after a short recall in context A (i.e., still during increased engram excitability), 3 h after it (i.e., after the closing of the recall-induced period of engram excitability), or without recall. The authors found that only the animals with a pre-exposure to A that had occurred 5 or 60 min before were not freezing to AB and thus successfully performed pattern separation. In light of their parallel time courses, it is thus likely that the engram's enhanced excitability contributes to the animal's capability for pattern separation and thus to their ability for behavioral adaptation (Figure 1).

To expand these findings, the authors additionally tested for pattern completion. In contrast to pattern separation, pattern completion refers to the process of using partial cues to fully retrieve previously learned information (Neunuebel and Knierim, 2014). As previously learned information, Pignatelli et al. (2019) used purely contextual memories, which the animals formed on the first day and were made to recall by a 3-min context exposure on the second day. Thereafter, the animals were subjected to a so-called immediate shock procedure, which consists of a very short fear-conditioning phase (context plus electrical shock exposure for less than 10 s). *Per se*, this procedure is known to be too short to lead to a pairing of the unconditioned stimulus (the shock) to the conditioned stimulus (the context) and would usually fail to instigate the conditioned response (freezing). The immediate shock procedure was applied either 5 or 60 min after the recall in A (i.e., still during increased engram excitability), 3 h after it (i.e., after the closing of the recall-induced period of engram excitability), or without recall. When tested 1 day later, the authors found that only the animals undergoing the immediate shock procedure within 2 h post recall displayed significantly enhanced freezing, indicative of improved pattern completion. Together with the findings on pattern separation, these behavioral results provide compelling evidence that engram excitability is a pre-requisite for behavioral adaptation.

Interested to decipher the mechanisms underlying such engram excitability, Pignatelli et al. (2019) pharmacologically tested its susceptibility to a variety of

inward-rectifier potassium channels and found a specific effect of ML133, a blocker of $Kir_{2.1}$, which had previously been shown to regulate DG granule cells' electrophysiological properties. ML133 indeed proved to be capable of significantly reducing inward current in DG granule cells, and $Kir_{2.1}$-specific current inversely correlated with the membrane resistance in engram cells. Furthermore, $Kir_{2.1}$ downregulation at the protein level was manifest in engram cells post-recall, but not in non-engram cells. Based on these grounds, $Kir_{2.1}$ is a plausible cellular mechanism responsible for mediating engram excitability, but is it also underlying the behavioral manifestations thereof?

To assess this, and thereby to provide causal and not only correlational evidence on the impact of increased engram excitability on behavioral flexibility, Pignatelli et al. (2019) eventually proceeded to gain-of-function experiments expressing $Kir_{2.1}$ specifically in engram cells (by using a TRE-$Kir_{2.1}$-RFP containing virus). These "$Kir_{2.1}$-engram" mice were fear conditioned in context A as before, and their memory was recalled one day later. When assessed 5 min later, the authors found decreased input resistance and increased rheobase in $Kir_{2.1}^{+}$ as compared to $Kir_{2.1}^{-}$ cells, demonstrating that $Kir_{2.1}$ is causally implicated in the increased excitability post-recall of DG engram cells. What is more, "$Kir_{2.1}$-engram" mice were no longer capable of recognizing context AB 5 min post recall in context A; likewise, they no longer froze to context A following the immediate shock procedure described above. Thus, the gain of function of $Kir_{2.1}$ in engram cells not only attenuated their intrinsic excitability induced by memory recall, but it also abolished the animals' abilities for both pattern separation and completion and thus their behavioral adaptability.

These findings are likely to have far-reaching consequences. First, they deepen our understanding of the functioning of engram cells at a hitherto unachieved level of specificity by bridging the gap between mnemonic content of neuronal ensembles and channel physiology. Second, they illuminate a fundamental phenomenon in the lifetime of each memory—namely, a transient period of lability following recall (Nader and Hardt, 2009). This lability is protein synthesis-dependent and serves the purpose of memory strengthening when similar situations are encountered at recall and encoding—i.e., the memory can re-consolidate—or memory weakening when altered situations are encountered—i.e., the memory can be updated (Figure 1). Behavioral and molecular evidence circumscribe this period of memory lability from 10 min to 6 h post recall, which is often referred to as the reconsolidation window. Pignatelli et al. (2019) found the engram cells to be in a heightened state of excitability until 2 h post recall, which falls precisely within the reconsolidation window. What is more, it was during this short post-retrieval period only that animals were found to be capable of pattern separation and completion. Although intrinsic neuronal excitability has been speculated and shown to underlie behavioral adaptation with less sophisticated tools previously (Zhang and Linden, 2003), the current study for the first time provides engram-specific evidence for an animal's ability to adapt to environmental contingencies that have changed since the time of encoding.

There is no doubt that such behavioral flexibility is of prime importance, both at shorter and longer temporal scales. So do, for example, extinction paradigms to reduce long-lasting traumatic memories work better when carried out within the reconsolidation window, which has recently been linked to the activity of recall-induced fear engram cells in the DG (Khalaf et al., 2018). Furthermore, it is also easy to imagine that such heightened behavioral flexibility can increase an animal's chance for survival: in the absence of engram excitability, the animal would not be able to either distinguish or complete incongruent pieces of information about the current environmental situation from those present at encoding, which can be a serious problem if such information is life-threatening.

Granted, these scenarios currently remain pure food for thought, as neither memory extinction protocols nor survival rates have been explicitly tested by Pignatelli et al. (2019) Likewise, although $Kir_{2.1}$ was shown to determine engram excitability as well as behavioral flexibility, it is unlikely that this inward rectifying channel is the sole mediator of the observed phenomena. Lastly, as the current study only focused on the DG—as did most engram studies thus far—it remains to be determined whether similar processes apply in other brain areas. This is of particular relevance for pattern completion, which has been traditionally assigned to hippocampal area CA3 (Neunuebel and Knierim, 2014), and for older memories, which are stored and recalled differently than fresh ones at the engram level (Kitamura et al., 2017).

These open questions notwithstanding, the current study not only fosters our understanding of the functioning of engram cells, but also identifies a cellular process underlying memory malleability upon recall, an indispensable feat in continuously changing environments.

REFERENCES

Josselyn, S.A., Köhler, S., and Frankland, P.W. (2015). Finding the engram. Nat. Rev. Neurosci. *16*, 521–534.

Khalaf, O., Resch, S., Dixsaut, L., Gorden, V., Glauser, L., and Gräff, J. (2018). Reactivation of recall-induced neurons contributes to remote fear memory attenuation. Science *360*, 1239–1242.

Kitamura, T., Ogawa, S.K., Roy, D.S., Okuyama, T., Morrissey, M.D., Smith, L.M., Redondo, R.L., and Tonegawa, S. (2017). Engrams and circuits crucial for systems consolidation of a memory. Science *356*, 73–78.

Nader, K., and Hardt, O. (2009). A single standard for memory: the case for reconsolidation. Nat. Rev. Neurosci. *10*, 224–234.

Neunuebel, J.P., and Knierim, J.J. (2014). CA3 retrieves coherent representations from degraded input: direct evidence for CA3 pattern completion and dentate gyrus pattern separation. Neuron *81*, 416–427.

Pignatelli, M., Ryan, T.J., Roy, D.S., Lovett, C., Smith, L.M., Muralidhar, S., and Tonegawa, S. (2019). Engram cell excitability state determines the efficacy of memory retrieval. Neuron *101*, this issue, 274–284.

Ryan, T.J., Roy, D.S., Pignatelli, M., Arons, A., and Tonegawa, S. (2015). Memory. Engram cells retain memory under retrograde amnesia. Science *348*, 1007–1013.

Semon, R. (1904). Die Mneme als erhaltendes Prinzip im Wechsel des organischen Geschehens (Leipzig: Wilhelm Engelman).

Tonegawa, S., Liu, X., Ramirez, S., and Redondo, R. (2015). Memory Engram Cells Have Come of Age. Neuron *87*, 918–931.

Zhang, W., and Linden, D.J. (2003). The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nat. Rev. Neurosci. *4*, 885–900.