RESEARCH HIGHLIGHT

Putting memories in their place

Chia-Hsuan Wang[1] and Thomas J. McHugh[1]✉

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Spatial learning and the rodent hippocampus have served as a powerful model system to study memory, spanning genetic, in vitro and in vivo physiological approaches. Recent work by Pettit et al. bridges the gaps in these fields, reporting that neurons expressing markers of plasticity constitute a more reliable spatial code and may underlie stable spatial memories.

65 years ago, studies on patient H.M., who had his temporal lobe removed bilaterally as a treatment for severe epilepsy, revealed the importance of the hippocampus in the encoding of episodic memories: the who, what, where, when memories that define us as individuals.[1] Subsequent studies revealed the necessity of the hippocampus for rodent spatial learning and the existence of "place cells", neurons in the hippocampus that encode specific locations in space.[2] These findings served as the basis of the theory that the hippocampus constructs a map-like representation of the world for the anchoring of memories.[3] Moreover, the hippocampus has served as the primary region in which the mechanisms and genetics of synaptic plasticity, experience-dependent changes in synaptic strength thought to underlie learning, have been probed.[4] This convergence of data makes fertile ground to test hypotheses of the mechanisms of memory.

More recently, advances in genetic tools such as optogenetics and transgenic mice have allowed scientists to tag and manipulate neurons recruited during the formation of a specific memory, so called engram cells,[5] making feasible to investigate exactly what information is encoded in the neurons recruited during learning. Experimentally, engram cells have been defined as not only being active during memory formation, but also expressing specific genes of the immediate-early gene (IEG) family, such as *cFos*, *arc* and *Npas4*, that have been linked to the onset of synaptic plasticity.[5] Importantly, later optogenetic excitation or inhibition of neurons labeled on this basis has been shown to facilitate or impede memory recall, respectively, solidifying the links between IEGs and functional memory traces.[6] While the majority of these engram experiments have focused on behavioral correlates of their manipulation as tested by context recognition, not spatial navigation, using in vivo physiology Tanaka et al. reported that engram cells can be place cells during the encoding and recall of a novel context,[7] but did not track these neurons across many days.

In a new paper published in *Nature*, Pettit et al.[8] explores these links in a new way, employing in vivo 2-photon imaging of hippocampal activity in transgenic mice which allows cFos expression and neural activity to be simultaneously optically monitored at the cellular level, facilitating the correlation of cFos expression within individual cells with their spatial properties in a familiar virtual reality environment across many days. The red-shifted calcium indicator jRGECO1a[9] was expressed in mouse hippocampus; as neuronal spiking correlates with calcium influx, a transient increase in this fluorescent signal can serve as a proxy for neural activity. Crucially, these mice also express a short-lived version of the green fluorescent protein eGFP under the control of the *cFos* promoter, allowing the levels of cFos induction related to the animals' daily training to be quantified by the strength of signal in the green channel 3 h after behavior. Using a virtual 2 m long circular track, thirsty mice were trained to lick within a non-labeled target reward zone to receive a water reward, a type of learning dependent on the hippocampus. This clever experimental design thus allowed Pettit et al. to characterize differences in the physiological properties between neurons with high cFos induction levels (Fos-high) and those with low cFos induction levels (Fos-low) across many days of behavior.

Consistent with Tanaka et al.,[7] the authors demonstrated that while both Fos-high and Fos-low cells could develop place fields, both the prevalence and properties of place cells differed between these groups. A higher proportion of Fos-high cells were place cells compared to Fos-low cells, and the place fields of Fos-high cells were larger and carried more spatial information. Furthermore, Pettit et al. demonstrated that the place fields of Fos-low cells were concentrated near reward locations, while Fos-high fields were distributed more homogeneously across the track. When these populations were used to decode the animal's location, the estimations were more accurate when the model was trained based on Fos-high place cells compared to on Fos-low place cells, suggesting that while Fos-high cells predominantly constitute a spatial representation of the environment, Fos-low cells may carry more non-spatial information, related to objects, events, or valence.

The hypothesis that the Fos-high cells form a scaffold supporting a spatial map was further strengthened by the observation that the place codes of Fos-high cells were more robust compared to Fos-low cells across multiple time scales. Within the same recording session, the in-field firing rates of pairs of Fos-high cells varied synchronously across trials. That is, if a Fos-high cell fired more on the first trial than on the second when the animal traversed its place fields, it was more likely that other Fos-high cells would also fire more on the first trial, but not on the second. Conversely, these trial-by-trial variations were relatively irregular for pairs of Fos-low cells. Further, across days, the correlations between place cell maps were higher for Fos-high cells than Fos-low cells, suggesting a more consistent representation of the environment. A stable and robust spatial code may be

[1]Laboratory for Circuit & Behavioral Physiology, RIKEN Center for Brain Science, 2-1 Hirosawa, Wako-shi, Saitama, Japan. ✉email: thomas.mchugh@riken.jp

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important for integrating events occurring at different times in the same place and the properties of Fos-high neurons make them a strong candidate for constructing a stable cognitive map.[3]

Given the correlation between the cFos induction level and the robustness of the place code, the authors also examined the role of Fos signaling itself in the establishment of the spatial code. While they could not manipulate the Fos activity in Fos-high or Fos-low cell groups specifically, they instead knocked down Fos pathway activity in random hippocampal cells (Fos-KO) by injecting a low titre virus encoding the Cre recombinase into a mouse strain carrying multiple Fos family member genes marked for Cre-mediated deletion. The authors observed that Fos-KO neurons had lower place cell prevalence and less robust place coding compared to the other cells, suggesting that the inhibition of Fos signaling impacts spatial coding. However, given that Fos is involved in various signaling pathways and cellular events, the direct causality is impossible to determine.

The findings of Pettit et al. reveal that cFos signaling is shared by the mechanisms underlying memory formation and stable spatial representation; however, it is worth noting that the Fos-high cells defined in this study may be fundamentally different from the engram cells labeled and manipulated in earlier experiments.[5,7] cFos induction levels in the hippocampus are known to drop after an environment becomes familiar to the animal,[10] but here, Fos-high cells were defined daily as the top 20% of cFos induced cells on a familiar track, thus it is unclear how they precisely overlap with the population captured by other approaches, and may explain some findings unique from earlier work.[7] A second remaining question is why the stable place cells continue to express higher levels of cFos, typically thought to be an indicator of plasticity, across experiences? Is plasticity required for stability or does this indicate that other, non-spatial, information is being integrated into an existing stable map? Future work in more dynamic environments or with higher cognitive demands may help answer whether Fos-high cells serve as engram cells and whether they integrate non-spatial information over time.

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ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Thomas J. McHugh.