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PERSPECTIVES

Intracellular determinants of hippocampal cell assembly formation during associative learning

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The search for the neuronal substrate of memory, or engram, is an important part of current neuroscience research. The influential theory of Donald Hebb proposes the formation of cell assemblies, whose synchronous activity would represent the memory trace. Many studies have been carried out to determine whether such cell assemblies exist and the mechanisms behind their formation. Hebb's original theory suggests that coactivation of cell assembly members during memory formation reinforces the synaptic connections between them, facilitating in turn the restoration of the synchronous activity during memory recall. Studies in rodents using extracellular recordings to monitor the spiking activity of large ensembles of neurons in the hippocampus, a key structure for memory formation, observed increased reactivation of the cells activated during exploration (Wilson & McNaughton, 1994). These reactivations occurred preferentially during slow wave sleep-associated sharp-wave ripple events. Subsequent work established correlations between the frequency of co-activation and the strength of the encoded memory. The mechanisms behind this increase in synchrony, however, have remained difficult to investigate with extracellular recording techniques. Indeed, extracellularly, one can only record the output firing of neurons, and not the variations of synaptic inputs and intrinsic properties at the origin of this output.

In a study in this issue of the *Journal of Physiology*, Liu and colleagues have tackled this problem via the *tour de force* of simultaneously recording the membrane potential of pairs of CA1 pyramidal cells *in vivo* in anaesthetized rats before and after associative learning (Liu *et al.* 2017). Previous studies have shown that associative learning is hippocampus-dependent if there is a delay between the conditioned stimulus (here a flash of light) and the unconditioned stimulus (here a mild foot shock), a protocol called trace fear conditioning. The authors observed increased synchrony between the output firing of CA1 pyramidal cells after conditioning, but only transiently (at day 1 but not day 5 after conditioning), in agreement with a role of the hippocampus in the early phase of memory consolidation. Interestingly, replay of neuronal activity during sharp-wave ripples is usually observed for no longer than 1 day after the initial exploration. This increase in synchronous firing was accompanied by an overall increase in the synchrony of the membrane potential dynamics between pairs of recorded neurons, suggesting a general modification of activity at the network level. By analysing more finely the changes in membrane potentials after conditioning, the authors observed a preferential synchronization of large excitatory events without apparent changes in their amplitude. These large events were also more likely to trigger action potential firing. These results are at odds with the classical Hebb theory, which postulates an increase in the strength (and hence amplitude) of excitatory connections as a substrate for increased firing synchronization.

Liu and colleagues suggest that such an increase in input synchronization could result from an increase in the activity of local interneurons, which is observed after this type of conditioning (McKay *et al.* 2013). This hypothesis is intriguing and requires further experimental support. If true, it would suggest that membership of different neurons to a given assembly would be in part determined by the fact that they share inputs from a common interneuron. An alternative hypothesis is that the effect observed on input synchrony in the CA1 region could reflect changes occurring one synapse upstream in the CA3 region. Indeed, the CA3 area is more likely to implement the mechanisms of plasticity described by Hebb because recurrent connections between neurons are more frequent there. Replicating these results in area CA3 would be very important in that regard.

Interestingly, Liu and colleagues also observed intrinsic excitability changes in CA1 pyramidal neurons after conditioning, and in particular a decrease in the threshold for action potential discharge. This could explain why, in the absence of amplitude changes, the large excitatory events are more often associated with spiking after conditioning. It is interesting to note that CA1 pyramidal cells involved in the coding of a novel spatial environment also have a lower discharge threshold (Epsztein *et al.* 2011). However, in this case, the threshold difference was already observed at the beginning of exploring the new environment and not following learning. This suggests that mechanisms of cell assembly formation to code a new environment and the mechanisms enabling the association between two stimuli could be different.

This work begins to shed light on the intracellular mechanisms of cell assembly formation. However, numerous questions remain. For example, what is the influence of brain state in this process? So far, reactivation of cellular assemblies, leading to memory formation has been studied during passive states, such as immobility periods or slow wave sleep, during which the activity of the interneuronal networks is profoundly modified (Somogyi *et al.* 2014). It would be interesting to see whether these changes are specific to this state of vigilance or generalizable to other states. For this, these experiments should be replicated in the absence of anaesthetics. Moreover, the authors observe an increase in the synchrony of action potentials, but action potentials may arise either in isolation or in the form of simple or complex bursts. Burst discharge is associated with more synchronous inputs than isolated spikes, and some recent work has shown that burst discharge but not single spike discharge is important for contextual fear memory acquisition in the hippocampus (Xu *et al.* 2012). Thus, it would be interesting to determine the synchrony of excitatory events during these different types of discharges, to see if observed changes in synchrony are more specific to burst *versus* single spike discharge.

Altogether, this important work is a first step in the fine-scale analysis of the cellular mechanisms involved in the formation

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of cellular assemblies, likely to underlie the memories of our everyday life events. Although challenging, it is important to continue such *in vivo* experiments because they preserve inter-structure connections and large neuromodulatory systems, such as acetylcholine, important for memory formation.

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Additional information

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