SYNAPTIC PLASTICITY

Step by step consolidation

Memories are encoded in the hippocampus and then transferred to the cortex for long-term storage in a process called systems consolidation. Mechanisms that strengthen synapses are thought to drive this consolidation; however, where and when this plasticity occurs and how it contributes to memory formation are unclear. Using an innovative optogenetic approach to manipulate synaptic long-term potentiation (LTP), Goto et al. now reveal roles for synaptic plasticity at three key stages of systems consolidation.

LTP is associated with dendritic spine growth, or structural LTP (sLTP). sLTP requires the reorganization and stabilization of the spine's actin cytoskeleton and is supported by the actin-binding protein cofilin (CFL). Reasoning that impairing CFL's function would impair sLTP, the authors fused CFL to a 'photosensitizer' protein, SuperNova (SN). When exposed to particular wavelengths of light, SN inactivates its associated fusion protein, a phenomenon known as chromophore-assisted light inactivation (CALI).

The authors expressed CFL–SN in pyramidal neurons in cultured mouse hippocampal slices and induced sLTP by 'uncaging' glutamate close to individual dendritic spines. As expected, glutamate uncaging reduced actin turnover and drove spine enlargement. CALI of CFL–SN induced 10 min after glutamate uncaging reversed these effects, thus 'erasing' the sLTP. sLTP erasure occurred when CALI was performed up to 30 min after (but not 50 min after or 1 min before) sLTP induction. CALI of CFL–SN also eliminated high-frequency stimulation-driven LTP of excitatory post-synaptic potentials (EPSPs). The effects of CALI were transient: both sLTP and LTP of EPSPs could subsequently be re-induced in the same spines or neurons.

Goto et al. used this tool to examine the effects of LTP erasure on memory formation in vivo. Over a series of daily trials, mice learned to avoid the dark side of a partitioned chamber, where they would receive a foot shock. By coupling CFL–SN expression in hippocampal region CA1 with light delivery via optical fibres implanted above CA1, the authors induced CALI in a spatially and temporally specific manner during task performance. They discovered that CALI of CFL–SN within a 20-min window immediately after shock delivery impaired later memory recall.

During periods of rest or sleep, replay of hippocampal activity patterns that occurred during learning occurs and is thought to aid consolidation. The authors found that CALI of CFL–SN in the hippocampus from 2–8 h after training impaired memory recall. This effect was specific to sleep periods, with LTP erasure during wakefulness having no effect on memory.

Having identified two phases of hippocampal plasticity that are important for consolidation, the authors sought to determine how each type of plasticity shapes

> "…synaptic consolidation relies upon at least three successive phases of synaptic plasticity…"

BEHAVIOURAL NEUROSCIENCE

Bodily balancing of fear

Fear is essential for survival, but animals need to maintain a balance in their fear level so as to avoid risky experiences but still benefit if non-threatening opportunities arise. In the body, fear manifests as heart rate changes, but the neural mechanisms that regulate fear via interoceptive feedback are not well understood. Klein et al. now show that the insular cortex — which processes bodily feedback to the brain — maintains fear balance by integrating interoceptive signals to enhance or weaken extinction learning, depending on the animal's fear level.

The authors used an auditory fear conditioning (FC) paradigm in which mice were trained to associate a conditioned stimulus (CS+) — a tone — with an unconditioned stimulus (US) — a mildly aversive or highly aversive foot-shock (weak or strong FC, respectively), which induced freezing behaviour. The duration of freezing when the tone was presented without the shock in the FC context (recall) or in a novel context (extinction) indicated the extent of the fear response. Extinction learning to reduce the fear response was achieved by presentation of the tone alone until freezing behaviour diminished.

Optogenetic inactivation of insular cortex during extinction learning revealed a bidirectional effect that depends on the intensity of FC experienced, with extinction being enhanced (weak FC) or impaired (strong FC) by inactivation of the insular cortex. Freezing duration during recall was used to assess the fear levels of individual mice (high or low), which revealed that extinction learning depended on the individual fear level only when insular cortex was inhibited. Thus, insular cortex gates extinction learning according to an individual's fear level.

Next, the authors used fibre photometry to measure the responsiveness of insular cortex to the CS+. This was

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positively correlated with freezing across all mice, with insular cortex activity increasing during FC and recall with the certainty that the CS+ predicted an aversive outcome. By contrast, during extinction learning, insular cortex activity and the fear levels in individual mice were negatively correlated. When the mice were divided into low-fear and high-fear groups, CS+-evoked responses in insular cortex during extinction revealed that, consistent with the insular cortex inhibition experiments, low-fear mice exhibited increased insular cortex activity, whereas it was decreased in high-fear mice.

As freezing duration distinguishes high-fear from low-fear mice, the authors reasoned that if freezing behaviour slows the heart rate, this could alter

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memory representations. Calcium imaging was performed using a head-mounted microscope as the mice explored the test chamber. The authors examined the effects on neuronal activity of learning (shock) and/or CALI of CFL–SN induced 2 min or 2–8 h after shock. They discovered that the early ('online') LTP is crucial for the establishment of selective neuronal firing related to the learning context, whereas the 'offline' LTP that occurs during sleep later in the same day enables these neurons to fire in a synchronized manner during memory recall.

No memory erasure was seen when CALI of CFL–SN was induced in the hippocampus more than a day after the shock; however, erasing LTP in the anterior cingulate cortex during sleep periods on day 2 of the task did impair memory, indicating a role for LTP in a third consolidation step: memory transfer to the cortex.

This study showcases a new tool to investigate synaptic plasticity in vivo and shows that synaptic consolidation relies upon at least three successive phases of synaptic plasticity that take place first in the hippocampus and then in the cortex.

Katherine Whalley

ORIGINAL ARTICLE Goto, A. et al. Stepwise synaptic plasticity events drive the early phase of memory consolidation. *Science* **374**, 857–863 (2021)

interoceptive information received by insular cortex and might therefore underlie its differential responses during extinction learning. Simultaneous pulse oximetry and fibre photometry confirmed that heart rate reductions occurred during freezing behaviour, and, importantly, that such reductions were strongly correlated with decreased insular cortex activity only after the first CS+ presentation during FC training, recall and extinction. These data indicate that peripheral interoceptive signals influence the onset of a higher fear state.

As freezing onset coincided with these changes, the authors compared freezing responses induced by CS+ presentations in high-fear and low-fear mice. During extinction, CS+ evoked insular cortex activity was negatively correlated with freezing across all animals, but comparison of high-fear and low-fear mice showed that they exhibited increased and decreased insular cortex activity, respectively, when freezing.

High-fear mice responded to the CS+ by freezing for longer and more frequently, so stronger negative interoceptive feedback to insular cortex might explain the difference from low-fear mice. To test this theory, the authors interfered directly with the main interoceptive pathway using vagus nerve stimulation during extinction learning. When the vagus nerve was stimulated, CS+ presentation to high-fear mice evoked increased insular cortex activity, whereas low-fear mice responded with decreased insular cortex activity. As with insular cortex inhibition, this manifested in high-fear mice as impaired extinction and in low-fear mice as enhanced extinction. The authors concluded that interoceptive signals related to fear expression (freezing-related heart rate decelerations) are sent via the vagus nerve to insular cortex to gate fear extinction learning.

In summary, fear is maintained in balance by insular cortex, which gates extinction learning according to fear level, informed by peripheral fear-related interoceptive signals.

Jake Rogers

ORIGINAL ARTICLE Klein, A. S. et al. Fear balance is maintained by bodily feedback to the insular cortex in mice. *Science* **374**, 1010–1015 (2021)

IN BRIEF

SLEEP

DNA damage drives sleep

DNA damage increases during wakefulness and decreases during sleep, but how this is regulated is not completely understood. In the zebrafish dorsal pallium, DNA damage induced by neuronal activity or mutagenesis induced sleep and the recruitment of DNA repair mechanisms involving the DNA damage response (DDR) proteins Rad52 and Ku80. Moreover, the DDR initiator Parp1 was increased following sleep deprivation, and Parp1 administration promoted sleep in both larval and adult zebrafish. Last, inhibition of Parp1 decreased DNA repair during sleep. Overall, these findings identify DNA damage as a homeostatic regulator of sleep.

ORIGINAL ARTICLE Zada, D. et al. Parp1 promotes sleep, which enhances DNA repair in neurons. *Mol. Cell* https://doi.org/10.1016/j.molcel.2021.10.026 (2021)

TECHNIQUES

New model jellyfish?

The jellyfish *Clytia hemisphaerica* is shown to be a genetically tractable organism that has anatomically distinct subassemblies of neurons that regulate behaviour and can be manipulated experimentally — for example, cell-type-specific conditional ablation and whole-organism calcium imaging. The authors identify a subnetwork of peptidergic neurons that are spatially and functionally organized to regulate the transfer of food from the tentacles to the mouth without involvement of a brain. They suggest that this species has potential for the systems-level study of neural function, behaviour and evolution.

ORIGINAL ARTICLE Weissbourd, B. et al. A genetically tractable jellyfish model for systems and evolutionary neuroscience. *Cell* https://doi.org/10.1016/j.cell.2021.10.021 (2021)

SYNAPTIC TRANSMISSION

Push the bouton

As spines and presynaptic boutons are closely connected, increased spine size creates mechanical pressure on the presynaptic bouton. Ucar et al. transiently pushed presynaptic boutons in rat brain slices with a glass pipette and found that this increased both glutamate release and SNARE complex assembly. Glutamate release was SNARE-dependent but calcium-independent. Glutamate uncaging increased glutamate release only when the adjacent spine extended and pushed the presynaptic boutons, revealing a mechanical mechanism that regulates glutamate release.

ORIGINAL ARTICLE Ucar, H. et al. Mechanical actions of dendritic-spine enlargement on presynaptic exocytosis. *Nature* https://doi.org/10.1038/s41586-021-04125-7 (2021)

DEVELOPMENTAL NEUROSCIENCE

Interspecies differences

The hippocampal–entorhinal system has lifelong neurogenic potential in many mammalian species and remains incompletely understood. In this paper, single-nucleus transcriptomes from five hippocampal–entorhinal subregions were profiled in samples from adults of three species (humans, macaques and pigs) and from young adult mice. The authors found both shared and divergent expression of transcriptomically defined markers for various neurons and glia across species. Notably, neuroblast markers in doublecortin-positive cells (indicative of neurogenic potential) were undetectable in humans, but were readily detectable in the other species. Overall, these findings reveal species-specific differences in the neurogenic potential of cells in the hippocampal formation.

ORIGINAL ARTICLE Franjic, D. et al. Transcriptomic taxonomy and neurogenic trajectories of adult human, macaque, and pig hippocampal and entorhinal cells. *Neuron* https://doi.org/10.1016/j.neuron.2021.10.036 (2021)