

possible cross-talk between CB1 and TRPV1 receptors in the striatum, where the two receptors are partly coexpressed at both the somatodendritic and axonal levels (Fig. 1), particularly in the lateral and rostral caudate putamen¹², and exert profound effects on locomotor activity³. In cells expressing both CB1 and TRPV1, the stimulation of the former can either sensitize or desensitize TRPV1 to the action of its ligands by modulating its phosphorylation state, whereas TRPV1 activation counteracts the effects of CB1 activation by elevating intracellular Ca²⁺ (ref. 3). As indicated by Maccarrone *et al.*⁴, also when the two receptors are not in the same neuron but have complementary distribution (Fig. 1), stimulation of TRPV1 can inhibit CB1 receptor activation by interfering with 2-AG biosynthesis. These cross-talk mechanisms might have functional consequences in other central processes regulated by these two receptor types, ranging from thermoregulation to emotionality. They could be profoundly affected by neurological conditions, such as dopaminergic imbalance and neuromotor degenerative disorders, which modify both endocannabinoid and endovanilloid signaling in the brain³.

However, previous data indicate that this inhibition of 2-AG by anandamide cannot occur just anywhere in the brain. FAAH inhibition or genetic impairment causes elevation of whole brain anandamide levels with no changes in 2-AG (ref. 3). In the ventrolateral periaqueductal gray, administration of URB597 elevates anandamide and indirectly activates TRPV1 receptors without decreasing 2-AG (ref. 13)—in this case, it instead elevates the local concentration of 2-AG, which can be a substrate for FAAH³. In the hippocampus and cerebellum, another form of inhibitory short-term synaptic plasticity, depolarization-induced inhibition of inhibitory transmission (DSI), is not affected by

FAAH inhibitors even though it is still mediated by 2-AG^{8,14} and occurs in neurons, postsynaptic to CB1 receptors, that probably express TRPV1 receptors¹². One may speculate that the TRPV1-mediated inhibition of 2-AG biosynthesis and retrograde signaling would require precise postsynaptic colocalization of DAGL- α and TRPV1, possibly occurring together with glutathione-synthesizing enzymes and juxtaposed to presynaptic CB1 receptors. Because stimulation of presynaptic TRPV1 receptors is coupled to glutamate release and (by enhancing mGluR5 receptor signaling) would counteract the effect revealed in this study, presynaptic TRPV1, and anandamide to activate it, must also be absent (Fig. 1). This combination of prerequisites might not occur too frequently in the brain; this matter should now be investigated by ultrastructural studies.

Another question for further investigation is whether the mechanism described by Maccarrone *et al.* occurs tonically or only after direct or indirect pharmacological stimulation of TRPV1 receptors. Previous experiments with TRPV1 antagonists and *Trpv1*^{-/-} mice suggest that these receptors are tonically activated by endovanilloids in at least three brain regions—the substantia nigra, hippocampus and periaqueductal gray—to strengthen dopamine release, long-term potentiation and descending nociception pathways, respectively^{3,13}. However, in the new study by Maccarrone *et al.*, TRPV1 blockade resulted in no stimulatory effect *per se* on mGluR5 receptor/DAGL- α /2-AG/CB1-mediated inhibition of GABAergic transmission⁴. Stimulation of mGluR5 receptors, unlike muscarinic and purinergic P_{2Y} receptors⁹, seems to be coupled to biosynthesis of 2-AG and not of anandamide¹⁵. For this reason, it may not be sufficient *per se* to enhance anandamide levels. One might then

hypothesize that the concurrent postsynaptic activation of anandamide biosynthesis (which is regulated in a different way from 2-AG biosynthesis³) is a further prerequisite for this phenomenon to occur tonically.

In conclusion, we agree with the authors' statement⁴ that the phenomenon described in this well performed study, although it may not necessarily be very widespread, calls for a "re-conceptualization" of the brain endocannabinoid system, which from now on should not be merely considered as consisting of CB1 receptors and 'interchangeable' endogenous agonists with the same physiological role. Instead, we now need to take into account TRPV1 receptors and the possibility that the different endocannabinoids sometimes act in concert and sometimes do not.

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Crossing borders: sleep reactivation as a window on cell assembly formation

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Learning is believed to be a result of plasticity in synaptic architecture, but few studies have shown this directly. A new paper explores a mechanism that shapes the formation of associative connections between neurons in behaving animals.

The impact of a scientific observation can be judged by how well it spans different levels of description. One successful example of this is

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the observed sensitivity of neurons in area LIP to average movement direction among a group of randomly directed light-point stimuli. The firing of these neurons closely matches the animal's decision-making behavior regarding movement direction, but does not necessarily match the true movement direction¹. As a result, the activity of these neurons extends beyond a

pure sensory response and potentially reflects decision-making processes. Less common are experiments in which the behavior of single neurons reveals details at a more fine-grained level of description, such as anatomical substructure or synaptic plasticity. The work of O'Neill *et al.*² in this issue does just this through the examination of reactivation, a process

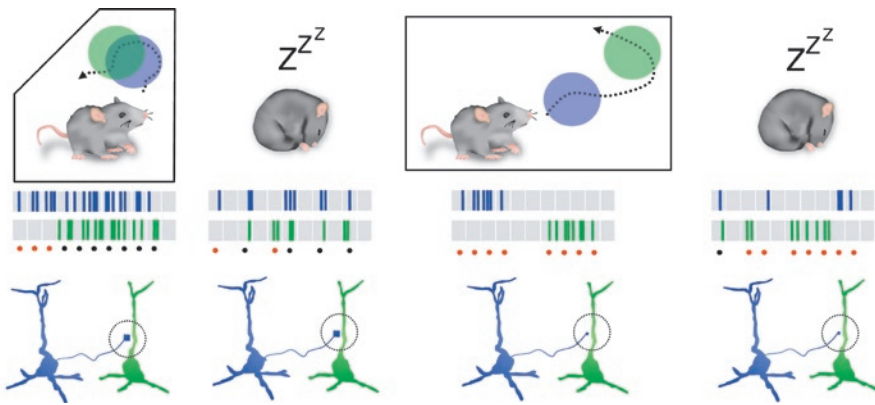


Figure 1 Memory trace reactivation and its dependence on cofiring during waking. Top, stages of the experiment. The place fields for two neurons (blue and green disks) overlap in one environment, but not in another. Middle, spike trains (blue and green ticks) for each neuron as the animal traverses a portion of the environment. Grey boxes mark 100-ms windows during which cofiring events (black dots) and anti-cofiring events (red dots) occur. In sleep sessions following exploration, the relative incidence of cofiring and anti-cofiring roughly matches that observed during the prior exploration period. Bottom, the findings suggest that synaptic efficacy of connections between CA3 and CA1 neurons is determined by the ratio of cofiring and anti-cofiring events.

by which an ‘echo’ of waking hippocampal activity patterns emerges during subsequent sleep. The authors sought to determine the specific features of spike-timing relationships between neuron pairs that maximize reactivation. The result of this analysis sheds new light on the processes that may govern the formation of associative connections between neurons in behaving animals.

The origin of the present work reaches back to Raphael Lorente de N6, who detailed how networks of neurons throughout the brain are organized into closed or ‘reentrant’ circuits³. At the time, he believed his observation to be irrelevant to psychology. Donald Hebb disagreed and made the observation of reentrant circuits the cornerstone of his cell assembly theory, which he argued was “a bridging conception” linking associative processes at the cellular level to those observed at the behavioral level⁴. Hebb also noted that connections between cells in an assembly had to be plastic for the concept to have any relevance to learning and memory.

Other researchers have since elucidated the rules governing the formation and destruction of the connections that Hebb proposed. The short list of likely rules governing associative learning between cells includes the timing of spikes from pre- and postsynaptic neurons⁵, as this can predict the degree and direction of synaptic efficacy change. A second and perhaps more intuitive factor governing plasticity is the frequency of coincident pairings. Generally, increasing the number of coincident pairings increases the robustness of the associative connection. Such rules, however, have been derived primarily *in vitro* or in intact animals using stimuli outside of the normal physiological range. Furthermore, the rules of association seem to be very sensitive to the recent spiking history⁶. Thus, it is difficult, to say the least, to imagine their direct relevance to the complex and often irregular activity patterns obtained from a healthy, awake and

learning brain. The new experimental work of O’Neill *et al.*² makes a substantial advance toward solving this problem.

To investigate Hebbian learning and cell assemblies *in vivo*, the authors focused their physiological investigations on the hippocampus. This choice was obvious for several reasons. First, abundant evidence implicates the hippocampus in episodic and spatial memory formation. Second, long-term potentiation and depression, the hallmarks of associative plasticity in the slice, are consistently observed in various hippocampal subfields⁷ and, by all appearances, operate in intact, behaving animals⁸. Third, hippocampal neurons often show place fields in the form of activity bursts that are highly restricted to particular positions in the environment⁹. The spatial selectivity of these neurons provides an opportunity for analyzing behavior-induced correlations between neuron pairs with overlapping place fields.

The authors’ goal was to identify the rules that govern the preservation of these behavior-induced correlations during the sleep episodes that follow behavior. Correlated hippocampal neural activity reactivates during subsequent sleep¹⁰. During waking behavior, correlated, spatially specific cell activity patterns, called cell assemblies, are driven by a complex interplay among intrinsic network activity, environmental stimuli, self-motion information and theta-band oscillations. Conveniently, most of the extrinsic factors driving such activity disappear during sleep, and thus sleep reactivation may more clearly reflect the strength of connections between members of cell assemblies.

New in the authors’ analyses² was the attention paid to the specific patterns of activity during waking behavior that most effectively produce patterns of co-activity during sleep. For example, they investigated what they termed a cofiring event, defined as a 100-ms interval in which two cells each fire at least one spike (Fig. 1). Anti-cofiring events

were defined as 100-ms intervals in which one, but not both, cells fired. They then determined that the ratio of cofiring to anti-cofiring events during behavior predicted positive and negative shifts in firing-rate correlation between sleep periods before and after behavior. This point has particular significance, as it illustrates that the associative rules operating in behaving animals are not unidirectional; both negative and positive correlations are preserved during rest. This contrasts sharply with Hebb’s rule⁴, which was clearly unidirectional. The results are, however, in better agreement with bidirectional learning rules of synaptic change, such as an associative rule suggested by Jerzy Konorski a year before Hebb’s original postulate¹¹. Furthermore, the results concur with observations from physiological studies that strongly suggest that various patterns of co-activity also weaken synaptic connectivity (including long-term depression¹² and spike timing-dependent plasticity⁵).

Further connecting the present results to physiological observations was a parametric analysis of the temporal interval between cell-pair spiking activity. The authors determined that cofiring events with mean intercell spike latencies of less than 50 ms were most likely to re-occur during subsequent sleep. This narrow temporal window corresponds to the most effective interval for inducing synaptic plasticity¹³.

An exciting implication of this work lies in its promise for bridging the gap between *in vivo* and *in vitro* analysis. Researchers have determined which features of the environment or behavior best correlate with the activity of neurons in behaving animals. *In vitro* work has shown how synaptic strengths depend on the relative timing of pre- and postsynaptic spikes and biochemical components of synapses. How to connect the findings from these very different levels of analysis is still a matter of great debate. *In vitro* physiologists have used naturalistic spike trains to investigate mechanisms of associative plasticity^{14,15}. Sleep

reactivation, as a window onto the changes in synaptic efficacy that occur during waking, offers the *in vivo* physiologist a potential path to meet *in vitro* physiologists halfway. On the basis of the degree of cofiring during sleep after behavior, one could predict which patterns of correlated activity would alter synaptic strengths *in vitro*. Such predictions are best verified *in vitro*, where synaptic efficacy can be measured directly and the cofiring events for connected neurons are not as readily confounded by network activity.

Notably, the impact of variables other than relative spike times will also have to be addressed from both points of view. The present work², for example, demonstrates that exploration of novel environments consistently amplifies, by an unknown mechanism, reactivation strength even

for the same spike-timing relationships between neuron pairs. One can imagine that variables such as overall inhibitory tone, the concentration of neuromodulators such as dopamine, or behavioral variables such as reward obtainment and fear response could impact reactivation strength. Experimental analogies for at least some of these variables may be possible in slice and/or anesthetized preparations. Ultimately, one would hope, the advance made in the present work will lead to a cohesive picture concerning learning and memory (defined at the behavioral level), neural network activity patterns, and the changing architecture of synaptic strengths.

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More to pheromones than meets the nose

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Mice use pheromones to regulate social behavior. New work in mice now identifies the protein in urine that is essential for aggressive behavior, along with the specific subclass of neurons involved in its processing.

Mice provide the ultimate experimental platform in terms of genetic tractability, but unlike primates, olfactory cues regulate almost all aspects of their intraspecies interactions, including reproductive and social behaviors. Olfactory pheromones mediate individual recognition, gender identification, the discrimination of estrus (sexually salient) from diestrus (pregnant or sexually unreceptive) urine, the onset of puberty, the synchronization of the estrus cycle and aggression between male mice. Even information concerning the potential edibility or toxicity of particular foods can be socially transmitted through a mouse colony using olfactory cues and without the need for experience. As a result of this evolutionary reliance on a complex chemosensory landscape, it is not surprising that the systems supporting pheromone processing are complex or that the genes associated with this system are the most diverse in the mammalian genome.

A new paper by Chamero *et al.*¹ in *Nature* significantly advances our understanding

of pheromones in the context of aggressive behavior, which is rarely spontaneous, but occurs in the context of defense or protection of resources. This multidisciplinary study shows that aggression can be precipitated by mouse urinary proteins (MUPs), nonvolatile lipocalins that act via the vomeronasal V2 category of receptor neurons.

Although nonvolatile urinary peptides or protein pheromones have long been known to activate the vomeronasal system, the specific neurons that respond, the identity of their ligands and how specific behaviors are elicited have all been unknown quantities. Not any more. Chamero *et al.*¹ have used their molecular genetic tool kit to identify MUP lipocalins as aggression-promoting pheromones. The aggression activated by these urinary proteins depends exclusively on the vomeronasal organ (VNO) and its central projection, whereas other volatile ligands may activate this behavior via the main olfactory system.

Studies on aggression always raise questions about the specificity of the neural signaling pathway, the receptor types and the context in which the aggression is activated. These issues have been closely engaged in Chamero *et al.*¹, which provides one of the most thorough analyses of the interactions between

mouse urine and the VNO. The authors disaggregated vomeronasal neurons and established them in culture. They then used Ca²⁺ signaling–detection technology to determine whether a potential ligand activated distinct or overlapping neural populations. One distinct population of cells was activated by the high molecular-weight (HMW) urinary protein fraction, whereas a separate distinct population was activated by the low molecular-weight (LMW) protein fraction.

The authors tested whether these urine fractions differed in inducing aggressive behavior by painting each fraction on the back of a castrated male mouse. Such mice are usually not perceived as male and therefore do not initiate an aggressive response. Only the HMW fraction stimulated aggression. Further purification of this HMW fraction suggested that the MUPs alone were sufficient to induce male-male aggression. The major LMW component (dihydrothiazole) did not induce male-male aggression. Denatured (and hence inactivated) urinary proteins were unable to activate vomeronasal neurons. Both recombinant urinary proteins and proteins stripped of their LMW ligands were able to induce male-male aggression. Recombinant

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