High times for memory: cannabis disrupts temporal coordination among hippocampal neurons

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Exogenous cannabinoid receptor agonists impair hippocampus-dependent learning and decrease the power of hippocampal electroencephalographic activity. A new paper shows that cannabinoids desynchronize neuronal assemblies without affecting average firing rates, and that this effect correlates with memory deficits in individuals.

To inhale or not to inhale—American presidential politics aside, this question has sparked debate between parents and teenagers for decades. Youthful proponents point to the thousands of years of recreational and medicinal use of phytocannabinoids, which include the major psychoactive principle of marijuana and hashish, Δ^9-tetrahydrocannabinol (THC). The older and wiser point to the less desirable effects of cannabinoids, including disruption of short-term memory, decreased attention and deficits in verbal expression. The mechanisms by which cannabinoids alter perception and memory have not been elucidated. In this issue, Robbe *et al.*[1] used *in vivo* recordings of population activities and single neurons to demonstrate that THC and the related synthetic cannabinoid compound CP55940 disrupt the synchrony of action potentials between hippocampal neurons, with only a marginal effect on average firing rates. These new findings suggest that reduction in spike timing coordination and the associated decrease in various hippocampal oscillatory population activities are important in marijuana-induced memory deficits. A major implication of these data is that the synchrony of spike timing in neuronal assemblies is a necessary component of proper hippocampal function.

Mammalian tissues express two types of cannabinoid receptor, designated CB1 and CB2. Both of these receptors are G-protein coupled, with the CB1 receptors being present in the central and peripheral nervous systems, whereas CB2 receptors are largely, albeit not exclusively, limited to the immune system. CB1 receptors are among the most abundant G protein–coupled receptors in the brain, and the hippocampal formation has unusually high densities of CB1 receptors[2]. Because the hippocampus is critical for short-term memory, the high expression of CB1 receptors already hints at a potential link between memory deficits and marijuana consumption.

The endogenous ligands for CB1 receptors (endocannabinoids) belong to the eicosanoid family of molecules, and include 2-arachidonoyl glycerol (2-AG) and anandamide. Endocannabinoids are lipophilic compounds that are generated and released on demand, and are metabolized by specialized intracellular enzymes in a highly compartmentalized fashion. They are predominantly synthesized in dendritic compartments following activity-dependent rises in intracellular calcium, or stimulation of metabotropic glutamate or acetylcholine receptors, whereas CB1 receptors are localized at both glutamatergic and GABAergic presynaptic processes. CB1 receptors are present on certain peri-terminal axons at astonishingly high densities[3], enabling endocannabinoids to potently inhibit action potential-evoked GABA and glutamate release by means of CB1-mediated inhibition of N-type presynaptic calcium channels. Thus cannabinoids acting through these presynaptic CB1 receptors can dramatically depress fast synaptic communication in the hippocampal network, leading to a functional decoupling of neurons. Circuit specificity is provided by selective expression of CB1 receptors. For example, within the GABAergic interneuronal subpopulations, CB1 receptors are expressed exclusively in cholecystokinin (CCK)-positive basket and dendritically projecting cells, whereas other major interneuronal classes, such as the parvalbumin-positive basket cells, are devoid of these receptors[4].

What are the network effects of cannabinoid-induced synaptic decoupling of hippocampal neurons? Robbe *et al.*[1] examined the consequences of cannabinoid administration on hippocampal ensemble activity in head-restrained and freely moving rats. *In vivo*, THC reduces the power of local field potentials and depresses the hippocampal and neocortical electroencephalograms at multiple frequencies (reviewed in ref. 1). Therefore, Robbe and colleagues first set out to demonstrate that, as expected, the power of the local field potentials (reflecting the extracellular summation of excitatory and inhibitory synaptic potentials) was decreased by the CB1 (and CB2) receptor agonist CP55940 in a dose-dependent manner. The cannabinoid diminished the power of hippocampal electroencephalographic activity in the theta (4–10 Hz) and fast ripple (100–200 Hz) bands, with a lesser effect on gamma oscillations (30–80 Hz). These effects were blocked by preadministration of the CB1 receptor antagonist SR141716A.

The effect of cannabinoids on these oscillations is significant because neurons form *ad hoc* assemblies defined by synchronous action potential firing. These assemblies are thought to be tasked with the representation, storage and retrieval of information. The oscillatory rhythms are network patterns that arise from the collective action of many neurons, and their behavior-related changes can be studied by examining the recorded current flow in the extracellular space. Hippocampal

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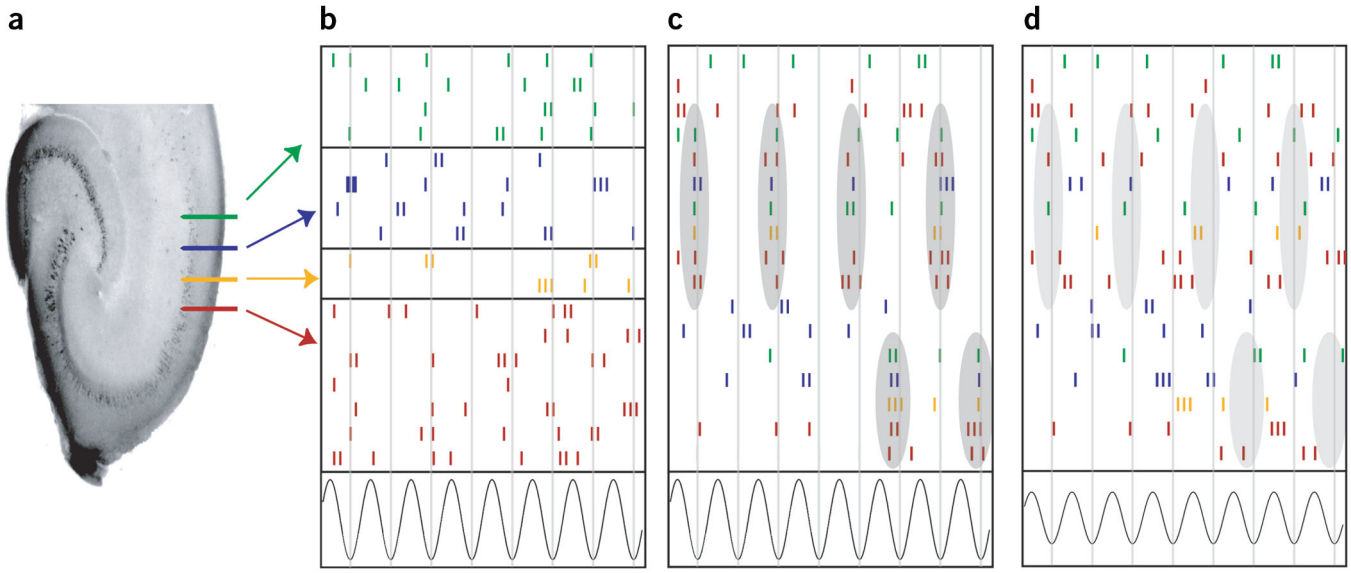

Figure 1 Cell assemblies and the effect of cannabinoids according to Robbe and colleagues[1]. (**a**) Location of the recording electrodes in the pyramidal cell layer within the CA1 region of the hippocampus. (**b**–**d**) Hypothetical, schematic representation of raster plots of 17 simultaneously recorded neurons that are active during a one-second period of spatial exploration. Color code in **b**,**c** refers to locations of the recording electrodes in **a**. Time is left to right, and each spike is represented by a small vertical line. Each electrode records the activity of multiple cells: for example, the green electrode records spikes from four nearby neurons simultaneously. (**b**) When neurons are arranged according to their actual, physical positions in the CA1 layer, no location-dependent temporal groupings (synchrony) are evident in the spiking patterns among the cells. (**c**) However, when the same spike rasters are reordered to highlight synchrony between anatomically distributed cells, the so-called 'cell assembly' organization is now visible. The highlighted subpopulations (cell assemblies) fire together repeatedly during the theta cycles (represented by the sinusoidal waves). (**d**) Exogenously applied cannabinoid ligands, such as the psychoactive component of marijuana, decrease theta power and destroy cell assembly organization. Panels **a**–**c** adapted by permission from Macmillan Publishers Ltd: ref. 7, © 2003.

theta and gamma oscillations are thought to be critical in working memory and the encoding of episodic memories, and in the coordination of neuronal discharges across regions[5]. Ripple oscillations are believed to be important in memory consolidation and the transfer of hippocampal memory traces to the neocortical circuits for long-term storage. Of importance, theta and ripple oscillations occur in clearly distinct behavioral states. Because CP55940 affected both rhythms, these data indicate that the cannabinoids were not simply altering behavioral states. Direct intrahippocampal injections of cannabinoids indicated that the effects observed after systemic drug injections in the previous experiments were likely to be due to hippocampal mechanisms.

Systemic cannabinoid application significantly impaired performance on a hippocampus-dependent delayed spatial alternation task, in which rats are required to learn to alternate right and left turns to obtain water reward. Although the motivation of rats under the influence of cannabinoids might be suspect, the data are consistent with previous measures of cannabinoid effects on hippocampal performance[6]. The decrease in the power of theta oscillations and learning were correlated, providing an important link between the cannabinoid effects on electrical activity and higher cortical function. Learning stopped at the highest cannabis doses (the rats' directional choices were no better than chance), and theta power was reduced by a factor of 2. This suggests a threshold level of neuronal cooperation below which learning may not occur.

A critical observation in the physiological experiments was that the averaged firing rates of pyramidal cells and interneurons were only modestly affected by the highest concentrations of the CB1 ligands, indicating that the cannabinoid effects could not be explained simply by the network-wide suppression of neuronal discharges. If total action potential frequency was not decreased, then what reduced the EEG power? Reduction in extracellular currents induced by synchronous action potential-dependent postsynaptic potentials could occur either as a consequence of a cannabinoid-induced decrease in synaptic transmitter release or decreased synchrony of action potentials. If the EEG effects were due to reduced transmitter release, one might expect a coincident reduction in action potential generation, although the relatively balanced effect of cannabinoids on inhibitory and excitatory transmitter release leaves this question open.

Robbe and colleagues evaluated the effect of cannabinoids on the synchronous action potential firing that define *ad hoc* neuronal assemblies. In a previous paper[7], researchers from the same laboratory demonstrated that these assemblies were not anatomically defined but rather were identified by synchrony of action potential firing across many cycles of theta oscillations (**Fig. 1a-c**). These neuronal assemblies, defined by the transient synchrony of anatomically distributed groups of neurons, are suggested to be critical for information transmission and storage in cortical networks. Significantly, Robbe and colleagues found that it was precisely this kind of temporal coordination of action potentials that was disrupted by cannabinoid receptor ligands (**Fig. 1c**,**d**). Because the cannabinoid-mediated disorganization of cell assemblies and the decrease in hippocampus-dependent memory performance occurred without major changes in average firing rates, these data support the idea that spike timing across neuronal assemblies is an important aspect of physiological functions of the hippocampus. Reduction in neuronal synchronization but not firing rates by exogenous cannabinoids may provide a clue to understanding puzzles such as the finding that exogenous cannabinoids disrupt the induction of hippocampal long-term potentiation (LTP) and impede behavioral learning, whereas

endogenously released endocannabinoids facilitate the induction of LTP[8]. By reducing synchronous firing, exogenous cannabinoids may reduce the associational activation of synapses that induces LTP, whereas the synthesis and release of endogenous cannabinoids may be subject to conditions[9] that do not preclude LTP induction.

Of course, good studies create as many puzzles as they solve. Robbe and colleagues[1] found no effect of the CB1 antagonist (and inverse agonist) SR141716A on network activity and the firing rates of single neurons. This result suggests that endogenous cannabinoids do not interfere with formation of synchronously firing neuronal committees and therefore do not influence learning. However, the finding is not consistent with several reports that SR141716A facilitates memory acquisition and consolidation (see ref. 10 and additional references therein). Indeed, SR141716A potently modulates GABA release in the CA1 region of the hippocampal slice preparations, revealing the existence of a homosynaptic, tonic control of neurotransmitter release mediated by CB1 receptors[9]. Perhaps under some conditions endogenous cannabinoid release is sufficient to affect mechanisms of learning, and these conditions may not have been uniform across all studies that have examined the mnemonic effects of SR141716A. For example, the firing rates of CCK- and CB1-expressing interneurons strongly modulate the efficacy of cannabinoid-mediated presynaptic inhibition both after exogenous application of synthetic CB1 ligands and following the physiological mobilization of endocannabinoids[11]. In addition, the firing of CCK-positive interneurons may be unusually sensitive to subtle changes in behavioral states[12], in contrast to the stereotyped firing of other interneuronal subtypes. Future studies are needed to define the conditions that sculpt the temporal and anatomical extent of endogenous cannabinoid signaling. Who knows? Teenagers waiting to inhale may be surprised to find that they have been enjoying the effects of their brains' own cannabinoids all along.

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Triggering the brain's pathology sensor

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Microglia, the brain's intrinsic immune cells, rapidly sense brain injury and help clear cellular debris. Haynes *et al.* now show that $P2Y_{12}$ receptors are critical for activating microglia and directing them to the site of injury.

Microglial cells—the brain's roaming clean-up crew—phagocytose cells and thus clear cellular debris in the brain. They are the brain's intrinsic immune cells and serve as damage sensors in the brain, as any type of injury or pathological process leads to activation of these cells from their resting state. This transition occurs within hours and causes a dramatic change in appearance. In response to injury, microglia change their highly branched, ramified resting morphology, retracting their processes and eventually transforming into cells with an ameboid appearance. Activated micro-glial cells can then migrate to the site of injury, proliferate and release substances that affect the pathological process. These substances include proinflammatory cytokines, such as tumor necrosis factor-α, and interleukin-6 or interleukin-12, signals for the invading T lymphocytes. Microglia are the antigen-presenting cells of the central nervous system and interact with invading immune cells by way of the major histocompatibility complex type II protein, which then initiates an immune response. Major histocompatibility complex type II is expressed only in activated microglial cells, and we know little about the factors that initiate this activation or that direct microglial cells to the site of injury[1]. In this issue[2], Haynes *et al.* provide an important piece of the puzzle by showing that $P2Y_{12}$ receptors, a subtype of purinergic receptors, are critical to alerting resting microglia to injury and directing them toward the site of action (**Fig. 1**).

Multiple factors act as attractants for microglial cells, drawing them to sites of injury. One important candidate is ATP, which is released from damaged or injured cells. Microglia express a variety of ATP-sensitive purinergic receptors of both P2X (cation channel) and P2Y (G protein–coupled receptor) families. Stimulation of purinergic receptors can trigger IL-1β and IL-10 release or attenuate the release of the proinflammatory cytokines TNF-α or IL-6 by activated microglia (for review, see ref. 3). Moreover, ATP is also a chemoattractant for cultured microglial cells[4]. In culture, P2Y receptors are important in the rapid morphological transformation of microglia triggered by ATP and also for a ruffling movement of the flattened processes. Furthermore, the particular $P2Y_{12}$ receptor is critical for microglial motility *in vitro*[4]. However, whether these results would hold up *in vivo* was unclear. Haynes *et al.*[2] now provide genetic evidence that the $P2Y_{12}$ receptor is a primary site at which ATP acts to induce microglial activation in response to local CNS injury *in vivo*.

Haynes *et al.* first used a $P2Y_{12}$-specific antibody to demonstrate that this receptor was predominantly expressed by microglia in the central nervous system. Macrophages, which infiltrate the CNS after injury, did not express $P2Y_{12}$ receptors when they were in the resting state. $P2Y_{12}$ protein was localized to the cell surface of microglia, including the ramified processes. The authors then asked what happened to $P2Y_{12}$ receptor expression during microglial activation. To study this, they imaged microglia in brain slices (taken from mice expressing GFP in microglia) and examined the change in microglial morphology in response to injury. As expected, microglial cells transformed over a 24-hour period from

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