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

Helmut Kettenmann

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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the ramified phenotype into the ameboid form in response to injury. $P2Y_{12}$ receptor expression decreased to undetectable limits by the end of this period, indicating an inverse correlation with microglial activation. There was a clear correlation between the degree of ramification and $P2Y_{12}$ receptor expression. The authors then went on to confirm this relationship *in vivo* by studying microglial activation for four days after lipopolysaccharide injection into the striatum (a classical trigger for an inflammatory response). They reported a lack of $P2Y_{12}$ expression in the ameboid, activated microglia. These observations indicate that $P2Y_{12}$ receptor expression is restricted to the resting phenotype of microglia and is downregulated during their activation. In line with this observation, the sensitivity of activated microglial cells to respond to ATP is attenuated after activation[5].

Resting microglial cells are highly motile in the normal brain[6,7]. Microglial cell morphology was visualized by two-photon imaging from the cortical surface in a mouse line that expressed green fluorescent protein selectively in microglial cells. Microglial cells moved their processes within tens of minutes, much more rapidly than any movement detected in neurons or astrocytes. Upon application of ATP, the resting microglia rapidly moved their processes toward the source, indicating that purinergic receptors can control the motility of processes. Haynes *et al.* then cross-bred $P2Y_{12}$-deficient mice with mice expressing the fluorescent marker in microglia, allowing them to determine the importance of $P2Y_{12}$ receptors for controlling the movement of resting microglia *in vivo*. They were able to distinguish between the basal motility of microglial processes and the ATP-induced microglial transformation. Although basal motility was not affected, the ATP-induced process orientation was strongly attenuated in the $P2Y_{12}$-deficient mice. This clearly demonstrates that the ATP-triggered process movement is controlled by $P2Y_{12}$ receptors. Moreover, it also shows that basal motility is not influenced by ATP or that it depends on a different type of purinergic receptor. Haynes *et al.* also injured the CNS locally by laser and found that the microglia from mice that were deficient in the $P2Y_{12}$ receptors showed a much slower migration toward to the injury site. Nonetheless, this motility was not completely abolished in these mice, indicating that other purinergic receptors or other factors released from injured tissue also contribute to attracting microglia toward the site of injury.

The authors also observed that ATP-induced membrane ruffling was absent in cultured $P2Y_{12}$ receptor–deficient microglia. Normal microglia, when isolated and subject to serum starvation, revert to a resting state. Application of ATP or ADP to the cultures induces robust membrane ruffling. However, microglia deficient in $P2Y_{12}$ receptors showed no response to ATP or ADP and did not show any polarization toward an ATP gradient. This observation was also validated *in vivo*: Haynes *et al.* placed a microelectrode containing ATP in the cortex of $P2Y_{12}$-expressing or $P2Y_{12}$-deficient mice and followed changes in microglial morphology using time-lapse microscopy. Normal microglia showed an extension of their cellular processes toward the ATP source, whereas $P2Y_{12}$-deficient microglia showed much reduced responses during an equivalent period.

Cultured microglia at rest do not show the classical ramified morphology of microglia *in vivo*. Rather, they resemble a fried egg, with flattened membrane processes, and do not display the same dramatic change in morphology when activated. This has raised a debate about whether microglia in culture are indeed a valid model for microglia *in vivo*. Because Haynes *et al.* showed a strong correlation between process length and $P2Y_{12}$ receptor expression *in situ*, one might not have expected to find the $P2Y_{12}$ receptor on cultured microglia. It is therefore reassuring that ATP stimulation of membrane ruffling in culture is controlled by similar mechanisms as it is *in vivo*, supporting the validity of cultured microglia as an appropriate model to study microglial migration.

Is it only injury that triggers $P2Y_{12}$ receptors in microglia? In slice and culture, astrocytes can release ATP when appropriately stimulated, as could also occur under physiological conditions. ATP release is correlated with a spreading calcium activity termed calcium waves. These coordinated calcium responses are considered an important feedback signal to neurons and are considered to be a part of the cascade by which astrocytes control the brain's oxygen supply[8]. Microglial cells in acutely isolated white matter slices sense the astrocytic calcium wave with a type of response that is typical for activating P2Y receptors[9]. It remains to be explored whether the $P2Y_{12}$ receptors on resting microglia not only serve as a pathology sensor but also are active under normal physiological conditions.

There is an intense debate over whether microglial cells are good guys helping to repair and maintain the brain's environment or whether they are solely bad guys that destroy and eat what is in their way. The latter view is supported by evidence[10] that silencing microglial cells represses the development of experimental autoimmune encephalomyelitis. However, removal of damaged structures may also be beneficial for the system. For example, microglia remove amyloid-β and reduce plaque burden in Alzheimer transgenic mice[11], and impairing microglial invasion prevents the phagocytosis of dendrites in the dentate gyrus of the hippocampus after perforant path lesions[12]. The latter study emphasized the role of the cytokine CCL21 in controlling microglial invasion, indicating that there are other signaling substances besides ATP that instruct microglia in a pathological context. Understanding the mechanisms that govern microglial activation and how it affects response to injury or disease may therefore help devise effective therapies.

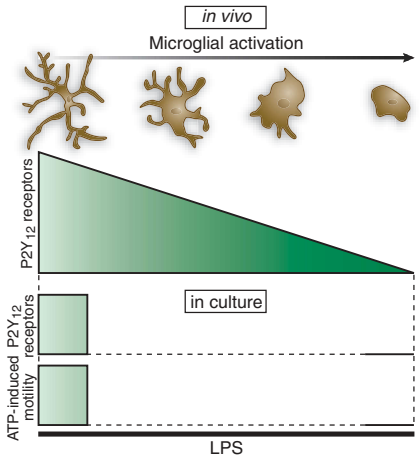

Figure 1 $P2Y_{12}$ receptor expression shows an inverse correlation with microglial activation. *In vivo* (top), $P2Y_{12}$ receptor expression decreases as microglia become activated and transform from highly ramified cells with many processes to an ameboid form. In culture (bottom), activation of microglia with lipopolysaccharide (LPS) also causes a loss of $P2Y_{12}$ receptor expression, even though the cells do not show any dramatic morphological change. The activation in culture also correlates with a loss of ATP-induced motility in cultured microglia, indicating that $P2Y_{12}$ receptors are essential elements for coupling purinergic signaling to microglial motility.

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