of one oxygen atom — derived from the oxygen atom in cpd II — to a biological substrate. Cytochrome P450 enzymes have various metabolic functions, such as the biosynthesis of steroid hormones, vitamins and antibiotics, and are crucial for initiating the removal of potentially toxic xenobiotics ('foreign' compounds in organisms). One subgroup of the cytochrome P450s is the aromatases, which convert the steroid androstenedione to the hormone oestrone. This involves three successive oxidations, each requiring oxygen and NADPH (a naturally occurring reducing agent). The first two oxidations are thought to be mediated by a cpd I entity, whereas the third involves a ferric peroxo compound[10,11]. Because aromatase activity and oestrone levels are elevated in most breast cancers, the enzymes are potential drug targets.

Nitric oxide (NO) synthases[2,12,13] provide interesting examples of how nature can adapt haem-containing active sites to very different roles. As in cytochrome P450s, the active sites of NO synthases contain a haem bound to a cysteine amino acid (the base, B, in Fig. 1b). But their activity is confined to the amino acid L-arginine, which it converts to NO — a signalling molecule vital to the nervous, immune and cardiovascular systems. The chemistry involves two sequential oxidations, each requiring oxygen, protons and NADPH. Each step proceeds via oxy, and follows on to either peroxo[2], hydroperoxo or cpd I intermediates.

In some enzymes, such as haem oxygenases (HOs), ferric hydroperoxide is the oxidizing species[14], and the substrate is the haem itself. HOs are found in many organisms, and in mammals the oxidation products are biologically vital: biliverdin, which acts as an antioxidant; liberated iron(II) ions, which are recycled for use elsewhere (primarily in haems); and carbon monoxide, which is used as a neurotransmitter. Reactions mediated by the enzyme cytochrome *c* oxidase, a member of the haem–copper oxidase (HCO) superfamily, probably also proceed through a ferric hydroperoxo complex, which then undergoes O–O cleavage and formation of cpd II (ref. 15). HCOs facilitate proton pumping across mitochondrial membranes, which generates a proton gradient that is used in the formation of ATP, the cell's energy carrier.

The enzymes discussed above exemplify the extensive involvement in biology of the reaction pathway proceeding from oxy to cpd I and/or cpd II species, through ferric hydroperoxo complexes. The occurrence and precise control of the active-site chemistry in this pathway are essential for proper biochemical functioning. For example, considering the role of ferric hydroperoxo complexes in HOs, improper processing of the reactions in the pathways of other enzymes could lead to unwanted haem degradation. It is therefore crucial to understand fully the manner in which biological hydroperoxo species form, react with different substrates, become protonated and undergo O–O cleavage[2,7–9], and to learn the order of these events[2].

The generation of well-described model compounds such as that reported by Liu *et al.*[1] will enable fundamental insights into haem-enzyme reactions, intermediates and mechanisms to be obtained from chemical investigations[9]. Such biomimetics may also shed light on a vast range of other enzymes that use oxygen, whose reactions are closely related to those of their haem-containing siblings[3]. ■

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NEUROSCIENCE

Astrocytes as aide-mémoires

Mirko Santello and Andrea Volterra

Memory formation is known to occur at the level of synaptic contacts between neurons. It therefore comes as a surprise that another type of brain cell, the astrocyte, is also involved in establishing memory.

Memory is the result of long-lasting changes in synaptic activity usually involving the activation of NMDA receptors (NMDARs) — a special class of receptor for the excitatory neurotransmitter glutamate. Memory formation has always been thought to depend on events occurring exclusively in neurons. But the brain possesses another cell population, glial cells, which include the highly ramified, star-shaped astrocytes. Despite their abundance — they make up 90% of all human brain cells — astrocytes have been relatively overlooked in the search for mechanisms of memory formation because they lack electrical excitability and do not communicate like neurons do. But astrocytes are not silent; they display another type of excitability involving changes in the intracellular concentration of calcium ions (Ca^{2+}). In this issue (page 232), Henneberger *et al.*[1] show that one function of the increase in astrocyte Ca^{2+} is to trigger the release of molecules required for establishing synaptic memory.

Previous studies have observed[2] that the processes extending from different astrocytes do not overlap; that is, each astrocyte occupies an exclusive territory in the brain. This observation forms the basis of the concept[2,3] that each astrocyte territory represents an island made up of many thousands of synapses (about 140,000 in the hippocampal region of the brain, for instance) whose activity is controlled by that astrocyte. Henneberger *et al.*[1] provide the first direct evidence for this proposal. The authors induce long-term potentiation (LTP) of excitatory synapses in the hippocampus using a high-frequency-stimulation protocol, which involves applying repetitive electrical stimuli to the presynaptic fibres. LTP is the sustained increase in synaptic strength associated with memory formation, and the authors monitored this synaptic potentiation locally, in domains roughly corresponding to the territories of individual astrocytes. They did this by recording the electrical signal generated by the ensemble of synapses in the territory, using an extracellular electrode or, alternatively, directly through the astrocyte.

Henneberger and colleagues cleverly use a pipette to record synaptic activity and simultaneously manipulate the cytosol of an individual astrocyte by introducing Ca^{2+}-buffering agents to prevent (clamp) any increase in intracellular Ca^{2+}. Surprisingly, this manoeuvre abolishes the induction of LTP at the surrounding synapses. Moreover, when the authors clamp only one of two neighbouring astrocytes, LTP induction is prevented exclusively at the synapses in the territory of the astrocyte whose rise in intracellular Ca^{2+} is blocked. It occurs normally at synapses in the territory of the non-clamped astrocyte, provided this cell is at least 200 μm away (Fig. 1, overleaf).

LTP can be rescued in the clamped astrocyte territory by adding the amino acid D-serine.

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D-Serine interacts with the so-called glycine-binding site of the NMDAR, permitting its transmembrane channel to open when glutamate binds. Previous studies in astrocyte cultures and hypothalamic slices had suggested that astrocytes can release D-serine through Ca^{2+}-dependent exocytosis[4] (release of substances stored in vesicles), implicating these cells as a possible source of D-serine acting at synaptic NMDARs[5].

To investigate whether D-serine has a similar effect in the hippocampus, Henneberger *et al.*[1] first showed that clamping Ca^{2+} in an astrocyte produces a 25% reduction in the NMDAR current in the surrounding hippocampal synapses. This reduction is reversed by the addition of D-serine, which confirms a causal link between astrocyte Ca^{2+} elevation and occupancy of the NMDAR glycine-binding site.

Intriguingly, if D-serine is added without clamping astrocyte Ca^{2+}, the NMDAR current is enhanced, but if LTP is induced without clamping astrocyte Ca^{2+}, supplying D-serine does not increase synaptic potentiation. The authors propose that these results can be explained by differences in the level of astrocyte activation in the two cases. Indeed, the high-frequency stimulation that induces LTP is a strong synaptic stimulus that also vigorously stimulates a rise in astrocyte Ca^{2+}, leading to massive D-serine release and transient saturation of the NMDAR glycine-binding site. By contrast, the synaptic stimulation used to study NMDAR currents is much milder, and in this case astrocytes show only infrequent oscillations in intracellular Ca^{2+}. These oscillations are probably associated with the release of smaller amounts of D-serine, which only partially saturate the NMDAR glycine-binding site.

Do astrocytes release D-serine directly or do they secrete other substances that trigger D-serine release from neurons[6]? And what is the mechanism of substance release? Henneberger *et al.*[1] address the first question by introducing a blocker of serine racemase, the enzyme responsible for D-serine synthesis, into the astrocyte. They find that this agent inhibits LTP, implying a causal link between astrocyte D-serine synthesis and local LTP induction. However, the serine racemase inhibitor also affects pyruvate production, which can perturb intra-astrocytic glutamate levels. Therefore, we are left with the possibility that glutamate release from astrocytes (which can occur in response to Ca^{2+} elevation[7]) is also needed for synaptic LTP.

The authors addressed the second question by introducing tetanus neurotoxin into the astrocyte to specifically inhibit vesicular exocytosis. This treatment also blocks LTP selectively around the domain of the manipulated astrocyte, suggesting that vesicular exocytosis is the mechanism underlying release of D-serine[4,8] and/or of another astrocytic transmitter.

Henneberger and colleagues' elegant single-cell manipulations[1] enable the study of astrocytes as individual functional units. But the size of the domain that falls under the synaptic influence of a single astrocyte is not clear. Indeed, astrocytes are connected to each other by gap-junction channels, and Ca^{2+}-buffering agents introduced into one astrocyte can pass through these intercellular connections and affect Ca^{2+} levels in neighbouring astrocytes. By contrast, the tetanus neurotoxin is confined to the astrocyte into which it is introduced, and yet it blocks LTP in a larger territory. This suggests either that D-serine diffuses beyond the domain of the releasing astrocyte, or that another transmitter released via Ca^{2+}-dependent exocytosis amplifies the spatial influence of a single astrocyte.

Henneberger and colleagues' work raises many new questions about the role of astrocytes in synaptic transmission. Apart from D-serine, these cells release glutamate and other transmitters[9]. One may wonder if these different molecules are co-released to act together, or are secreted discretely in response to different stimuli and at different cellular locations, each with distinct functional roles. Astrocyte-released glutamate has been shown to activate extrasynaptic NMDARs, in either dendrites or presynaptic terminals[10]. Does D-serine release participate in this extrasynaptic activation? Or is its action confined to synaptic NMDARs? Future definition of the plasma-membrane sites from which D-serine is released and at which it is taken up, compared with those of glutamate, will help to clarify these issues. Ultimately though, Henneberger and colleagues' work[1] conveys an important message — the contribution of glial cells to synaptic functions cannot be overlooked, and any study of synaptic physiology will need to consider glial biology if scientists hope to achieve a comprehensive understanding of brain function. ■

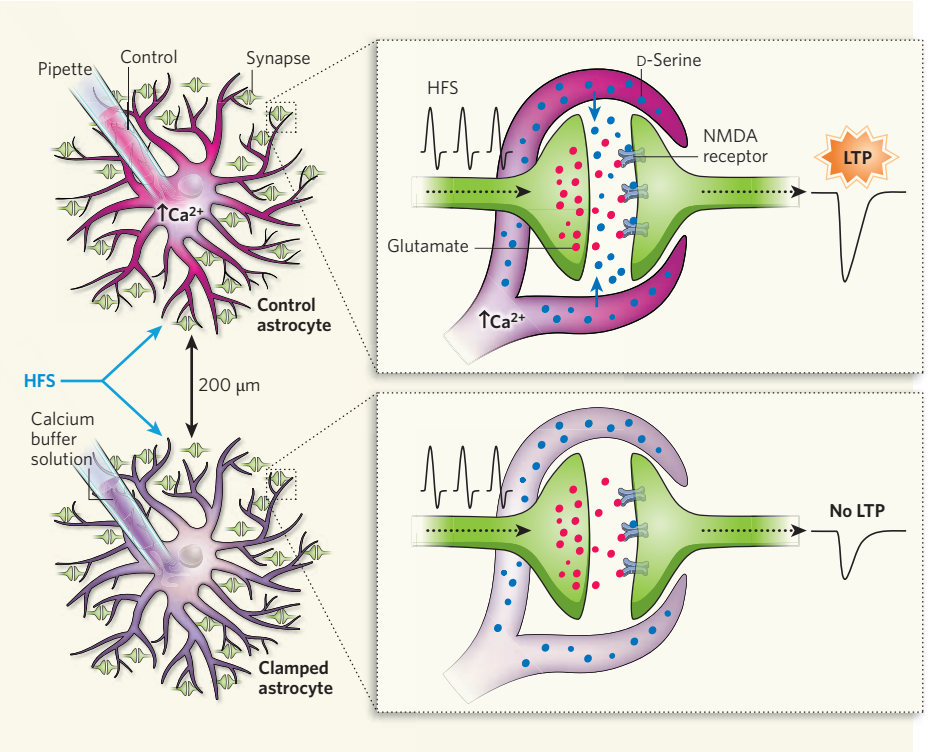

Figure 1 | Astrocytes and synaptic memory. Henneberger and colleagues' single-cell experiments[1] demonstrate that a rise in astrocyte intracellular Ca^{2+} controls the induction of long-term potentiation (LTP) at nearby synapses. The authors induce LTP at hippocampal synapses using high-frequency stimulation (HFS). With a pipette, they load individual astrocytes with a control solution (top cell) or a buffer that prevents an increase in intracellular Ca^{2+} (bottom 'clamped' cell). After HFS, intracellular Ca^{2+} levels increase in the top astrocyte, which releases D-serine. D-Serine binds to NMDA receptors to promote LTP establishment when glutamate is released from the presynaptic terminal. Preventing the rise in intracellular Ca^{2+} in the bottom astrocyte abolishes D-serine release and prevents LTP establishment in its territory, but does not affect LTP in the top astrocyte's domain provided that the two cells are at least 200 μm apart.

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