CHEMICAL TOOLS

Memories are made of this

Scientists at the University of California in San Francisco have developed a photoreactive antagonist for a neuronal ion-channel receptor that allows precise temporal and spatial inactivation of the receptor, promising to elucidate its role in memory formation.

Finding the molecular basis of memory formation has been a long-standing quest in the scientific community, and substantial progress has been made in identifying the key players. A research group at the University of California in San Francisco (UCSF) under the direction of Pamela England specifically targeted one of these players, the α-amino-3-hydroxy-5-methyl-4-isooxazole receptor (AMPAR). Despite their unwieldy name, these receptors have the straightforward task of facilitating ion flux into the neuron. The UCSF team developed a new photoreactive antagonist for AMPARs that allows them to study the receptor's regulation during memory formation.

Memory formation involves a strengthening of the communication between neurons. To initiate this communication, the neurotransmitter glutamate is released from a presynaptic cell, diffuses across the gap (synapse) between the neurons and binds the AMPARs on the postsynaptic cell, causing the receptor to open and ions to flow into the cell (**Fig. 1a**). In an experimental model system of learning and memory, hippocampal long-term potentiation (LTP), a long-lasting increase in the strength of the communication is observed after high-frequency stimulation (HFS) of presynaptic cells in the hippocampus. Following HFS, the postsynaptic cells have essentially 'increased their memory': that is, stimulation of the presynaptic cell produces more current in the postsynaptic cell than before HFS. The key question is, what has changed at the synapse to lead to this increased ion flow?

Extensive research in recent years has shown that the change occurs in the postsynaptic neuron. The number of AMPARs at the synapse increases, but what is not clear yet is whether these receptors are trafficked from extrasynaptic sites on the cell membrane or intracellular vesicles. The photoreactive antagonist that England presented in a recent paper in the *Journal of the American Chemical Society* will shed light on the source of AMPARs at the synapse (Chambers *et al.*, 2004). England started with the known antagonist, 6,7-dinitroquinoxaline-2,3 dione (DNQX), and replaced one of the nitro groups (NO_2) with a highly photoreactive azido group ($-N_3$), yielding 6-azido-7-nitro-1,4-dihydroquinoxaline-2,3-dione (ANQX) (**Fig. 1b**). Upon exposure to ultraviolet light, the azido group loses dinitrogen ($-N_2$) to form a highly reactive nitrene (:N), and this photoactivated antagonist then irreversibly binds to and inhibits AMPARs.

England is excited about ANQX"One of the key changes [in memory formation] is an increase in the number of AMPARs at the synapse. With our tool plus focused light we have unparalleled temporal and spatial resolution. It will allow us to test where the receptors come from and how fast they turn over." .

This new antagonist will circumvent problems that have hampered previous studies using overexpressed recombinant AMPARs, which could not discriminate between trafficking to synaptic or extrasynaptic sites on the plasma membrane or required long periods of time to monitor trafficking of receptors to the plasma membrane. Inactivation of endogenous receptors, literally with a flash of light, allows for rapid measurement of trafficking kinetics, and it also allows for a comparison of the kinetics before and after HFS to determine whether memory formation increases the rate of receptor trafficking.

When asked about future applications for ANQX, England said, "The approach is applicable to any system that light can penetrate into." Powerful techniques such as two-photon illumination can easily penetrate hippocampal slices. Next, England's team

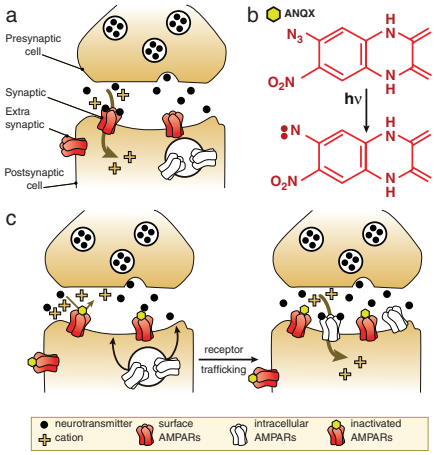

Figure 1 | AMPAR trafficking in neurons. (**a**) Scheme of neuronal signal transduction. (**b**) Upon light activation of ANQX, the azido group ($-N_3$) is converted to nitrene (:N). (**c**) Assay to determine AMPA receptor trafficking pathways.

wants to specifically inactivate the receptor at defined membrane sites in hippocampal slices. By inhibiting both synaptic and extrasynaptic receptors, the researchers will be able to measure the amount of intracellular receptors that are shuttled to the synapse via exocytosis (**Fig. 1c**). Conversely, if only synaptic receptors are inhibited, they can determine whether extrasynaptic receptors move along the plasma membrane to the synapse. Exocytosis and intramembrane movement constitute two radically different ways in which the cell may regulate AMPAR numbers at the synapse, and this new antagonist will allow researchers to distinguish between the two.

Elucidating the exact trafficking pattern of AMPARs during memory formation will be an important piece in the puzzle of understanding the molecular basis of learning.

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RESEARCH PAPERS
Chambers, J.J. *et al.* Photochemically knocking out glutamate receptors in vivo. *J. Am. Chem. Soc.* **126**, 13886–13887 (2004).