Structural biology

Brain receptor reveals its regional accent

Andrew J. R. Plested

AMPA receptors are a class of protein complexes crucial for neuronal communication. Two complementary studies converge on structures of AMPA receptors found in a brain region called the hippocampus. **See p.448 & p.454**

Dialects distinguish Berliners from Bavarians, and New Yorkers from New Englanders, reflecting differences not only in speakers' geographical location, but also in their culture and history. At first glance, it seems unlikely that such diversity would exist among AMPA receptors — a class of protein complexes activated by the neurotransmitter molecule glutamate during neuronal communication. These crucial receptors are found everywhere in the brain, but the protein subunits that form their central channel structure are encoded by just four genes, suggesting there might be little scope for them to have distinctive molecular 'accents' in different brain regions. However, proteomic studies suggest that AMPA receptors could in fact be large complexes with many extra subunits, which have been challenging to isolate. Yu *et al.*[1] (page 448) and Zhang *et al.*[2] (page 454) provide complementary views of the structure of AMPA receptors from the hippocampus, a brain region central to memory, to show the molecular accents that colour their native 'language'.

At an excitatory synaptic connection between two neurons, glutamate is released by the presynaptic neuron and binds to AMPA receptors in the cell membrane of the postsynaptic neuron. When AMPA receptors are activated, their channels open, allowing a rapid movement of ions into the cell that leads to electrical excitation. Newly assembled AMPA receptors are placed into the cell membrane and, once there, they can be pulled into the postsynaptic density (PSD) — a cluster of proteins in the postsynaptic neuron directly opposite the presynaptic sites from which glutamate is released. Scaffold proteins, such as PSD-95, draw in AMPA receptors depending on the level of the synapse's activity. The strength of a synapse can be changed by adding or removing receptors. The processes that underlie synaptic plasticity (the ability of synapses to change in strength) are candidate cellular mechanisms for learning skills and retaining memories.

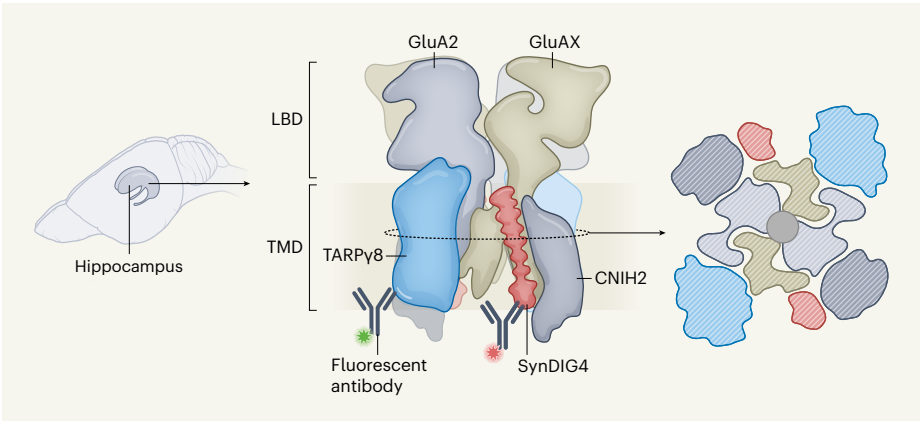

Figure 1 | The structure and composition of native AMPA receptor complexes. AMPA receptors are membrane-bound complexes of multiple different protein subunits, including channel-forming subunits (GluA1–GluA4) and auxiliary proteins such as TARPγ8, CNIH2 and SynDIG4. Yu *et al.*[1] determined the composition of GluA2-containing AMPA receptors purified from a brain region called the hippocampus in mice. They used cryo-electron microscopy to resolve the structures of the complexes through labelling with small, subunit-selective antibody fragments; shown here are the ligand-binding domain (LBD) and transmembrane domain (TMD) of a complex containing two GluA2 channel subunits and two unspecified channel subunits (GluAX), along with three pairs of auxiliary subunits (membrane not shown). The authors also worked out the composition of complexes by labelling individual subunits with antibodies attached to fluorescent dye molecules.

Pioneering work that measured the expression of the four AMPA receptor channel subunits, GluA1–4, in different cell types in the brains of rats[3] explained some, but far from all, variations in the activation properties of AMPA receptors. Hippocampal AMPA receptors are fascinating because of their participation in synaptic plasticity, but what makes them special compared with AMPA receptors in other brain regions? AMPA receptors are decorated with members of an elusive class of more than 30 'auxiliary' proteins[4], which could provide AMPA receptors in different brain regions with a regional molecular accent.

Multiprotein complexes perform many tasks in biology, and characterizing assemblies sourced from native material (that is, derived from biological tissue) is important for understanding the true biological properties of these complexes. However, although previous work has resolved the structures, composition and activation properties of certain combinations of AMPA receptor subunits *in vitro*, native (or native-like) AMPA receptor complexes that contain more than a small number of subunits have largely defied similar classification.

Mining the impressive details of these receptor complexes relies on the ability of modern cryo-electron microscopy to sort and pool samples consisting of a mixture of complexes with different subunit compositions. The two studies used different approaches to purify their complexes, albeit with each approach introducing some bias in the composition of the complexes obtained. Zhang *et al.*[2] fused together DNA sequences encoding the GluA2 subunit and the TARPγ8 auxiliary protein, and expressed this construct and the GluA1 subunit in a cell line forced to co-express the auxiliary protein CNIH2. Yu *et al.*[1] extracted the scarce complexes directly from mouse hippocampi by trapping GluA2, the most prevalent subunit, and by promoting the extraction of complexes including TARPγ8. They did this using a molecule called JNJ-55511118 that binds and blocks the activity of TARPγ8-containing complexes. The authors were able to work out the combination of subunits in each complex using small antibody fragments that labelled specific subunits. In this way, Yu *et al.* were also able to resolve JNJ-55511118 in its binding site in structures of the native receptors, sandwiched between GluA1 and TARPγ8.

The two studies' results are convergent, and each study provides independent support that their structural findings are representative of the composition of native complexes. Yu *et al.*[1] attached fluorescent dye molecules to the native receptors using selective antibodies. They found that the fluorescence of most of the

dye molecules that were attached to TARPγ8 subunits in each AMPA receptor complex could be photobleached (extinguished) in two steps, thus confirming that these complexes each contain two TARPγ8 subunits. A complementary approach by Zhang and colleagues[2] showed that the activation properties of their octameric complexes containing two GluA1, GluA2, TARPγ8 and CNIH2 subunits more closely mimicked those of receptors from the cell bodies of hippocampal pyramidal neurons than did those of lower-order combinations, for example with CNIH2 or TARPγ8 alone.

A further structural analysis of the native complexes isolated by Yu and colleagues[1] signalled the presence of an unexpected α-helix at the periphery, between the GluA1 and CNIH2 subunits. Antibody labelling and photobleaching provided evidence that this helix belongs to the auxiliary protein SynDIG4 (Fig. 1). This unprecedented 10-subunit complex (the octamer plus two putative SynDIG4 subunits), is a thrilling find and a game-changer because it expands the number of possible ways in which the function of AMPA receptors might be modulated by combinations of auxiliary proteins. With hindsight, the structure agrees surprisingly well with previous mass spectrometry data[5] that suggested a peripheral location for this subunit. Curiously, SynDIG4 is predicted to have two membrane helices[6]; the fact that an expected part of this protein (the second membrane helix) is not visible in Yu and colleagues' structures suggests that there is more subunit diversity to be unveiled in AMPA receptors.

The octameric complex generated by Zhang *et al.*[2] yields another gem: the structure of an activated state with an open ion-channel pore. From myriad structures of full-length glutamate receptors, this is only the third bona fide open structure[7,8]. A comparison of the structures of resting and activated receptors shows that a gentle 'flowering' motion of the peripheral subunits accompanies activation (Fig. 2).

CNIH2 has been the subject of much controversy[9], but is now without doubt a key player in AMPA receptor biology — it strongly stabilizes the open state, and the transition characterized by Zhang *et al.* gives two clues to how it does this. First, a triplet of phenylalanine amino-acid residues on the outer face of CNIH2 controls assembly of the complex. Second, an extended helix of CNIH2 bears against the intracellular loops of the channel subunits, the movement of which with gating was previously detected by optical spectroscopy[10].

A substantial fraction of neuronal AMPA receptors are stored inside the cell, or reside in the cell membrane outside synapses; thus, how representative the examined complexes[1,2] are of synaptic receptors is a pertinent question. The caveat that these are not synaptic receptors has regularly rung out in the 30 years since the cloning of these subunits[11]. Yu *et al.*[1] found PSD-95 in their preparation, but whether this means that their receptors were synaptic is unclear, because PSD-95 is also found outside synapses[12]. Unless methods can be developed to mark or capture synaptic receptors, and to work with the doubtless vanishingly small quantities that would be obtained, this gripe will persist.

That said, it would be a surprise, and a substantial waste, if the receptors resolved in these studies were not genuinely synaptic, because they have the requisite 'hooks' to get caught at PSDs. However, it is possible that they could instead be the raw materials from which synaptic receptors are made, perhaps following subunit exchange in the cell membrane. Synaptic receptors themselves might be harder to get at, anchored at their base to the PSD, and pinned by interactions between their extracellular domains and other molecules across the synapse.

Both studies introduce firm rules on how subunits participate in AMPA receptor complexes. However, other subunits, such as CKAMP44 and other TARP auxiliary proteins, should also be present in hippocampal AMPA receptor complexes, and how such complexes compare with those in other brain regions remains to be considered. Specialists will now eagerly await reports of other membrane protein complexes, and those from brain regions such as the cerebellum and cortex. Making sense of the interactions within such complexes requires functional and spectroscopic measurements, as done previously[13]. Time-resolved studies of the activation of native receptors, akin to those on the muscle nicotinic receptor from *Torpedo marmorata* electric ray fish[14], should also now be possible.

Interpreters and expats often battle to follow baroque expressions and local vernacular. But, despite their thick accents, AMPA receptors now say loud and clear: "We have so much still to tell you."

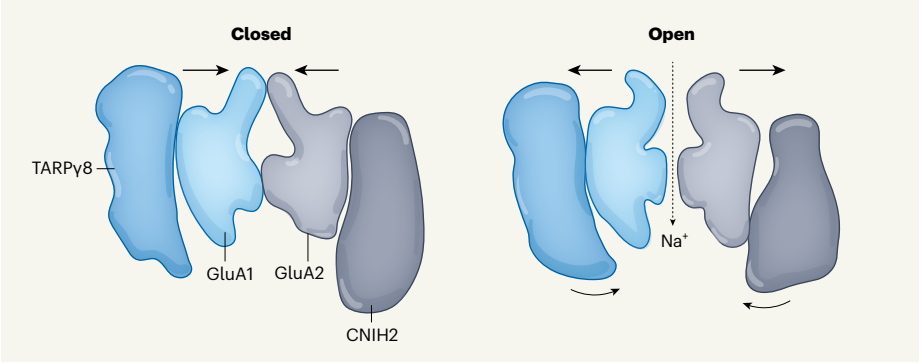

Figure 2 | The opening of the AMPA receptor complex. AMPA receptors include four central, channel-forming subunits (GluA1–GluA4), surrounded by auxiliary protein subunits such as CNIH2 and TARPγ8 that can influence the activation properties of the complex. A cross section of one such receptor is shown here, with 4 out of the 8 subunits depicted. When the neurotransmitter molecule glutamate (not shown) binds to the AMPA receptor, the configuration of the receptor changes to open the channel, allowing ions such as sodium (Na^+) into the neuronal cell. Zhang *et al.*[2] used cryo-electron microscopy of AMPA receptor complexes to show that the activated receptor opens in an outwards 'flowering' motion (black arrows). The closed and open states of the cell-membrane-spanning part of a complex containing GluA1, GluA2, TARPγ8 and CNIH2 subunits are depicted here (the cell membrane is not shown).

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