NEWS & VIEWS

NEUROBIOLOGY

Triggers for channel opening

Cynthia Czajkowski

Fast transmission between nerve cells relies on specialized ion channels. Probing the structure of these proteins reveals how the binding of a neurotransmitter causes the communication channels to open.

Chemical signalling in the brain involves the rapid opening and closing of channels known as ligand-gated ion channels, which lie in the membranes of nerve cells. Binding of a specific activator (a ligand) to these proteins triggers the opening of an integral pore through the membrane in as little as tens of microseconds[1]. Although we know a fair amount about the structure of ligand-gated ion channels, the mechanisms by which the binding of a ligand triggers channel opening are still under debate. On page 243 of this issue, Lee and Sine[2] identify a network of interacting amino-acid residues in one such protein, and reveal a pathway by which changes at the protein's ligand-binding site can be propagated to its channel region. And on page 248 Lummis and colleagues[3] identify a proline residue that acts as a molecular switch to control channel opening. Together, the two reports provide a compelling description of the structural machinery that couples ligand binding to channel gating.

Communication between nerve cells takes place at junctions called synapses. When a presynaptic cell is activated, it releases neurochemicals (neurotransmitters) across the synapse that bind to ligand-gated ion channels on the surface of the postsynaptic cell. Binding of neurotransmitter causes the channels to open, allowing ions to flood across the postsynaptic-cell membrane and change the cell's activity. So ligand-gated ion channels can be thought of as transducers that rapidly convert chemical signals into an electrical output. Their opening and closing regulate information flow throughout the brain, and mutations in these channels are responsible for a number of 'channelopathies', such as congenital myasthenic syndromes, epileptic disorders and hereditary hyperekplexia.

Lee and Sine[2] and Lummis *et al.*[3] examined the structures of two members of the 'Cys-loop' family of ligand-gated ion channels. This family includes channels that respond to the neurotransmitters acetylcholine, serotonin, γ-aminobutyric acid (GABA) and glycine. The receptors are large transmembrane proteins (molecular weight 300,000) consisting of five similar subunits arranged around a central ion-conducting channel, with each subunit contributing to the lining of the transmembrane channel (Fig. 1a). The neurotransmitter binds to the extracellular interface between two subunits. But what has long puzzled researchers is how the binding of a neurotransmitter, which is around 6 ångstroms long, is translated so rapidly into the opening of an ion channel more than 50 ångstroms away in the transmembrane domain of the receptor.

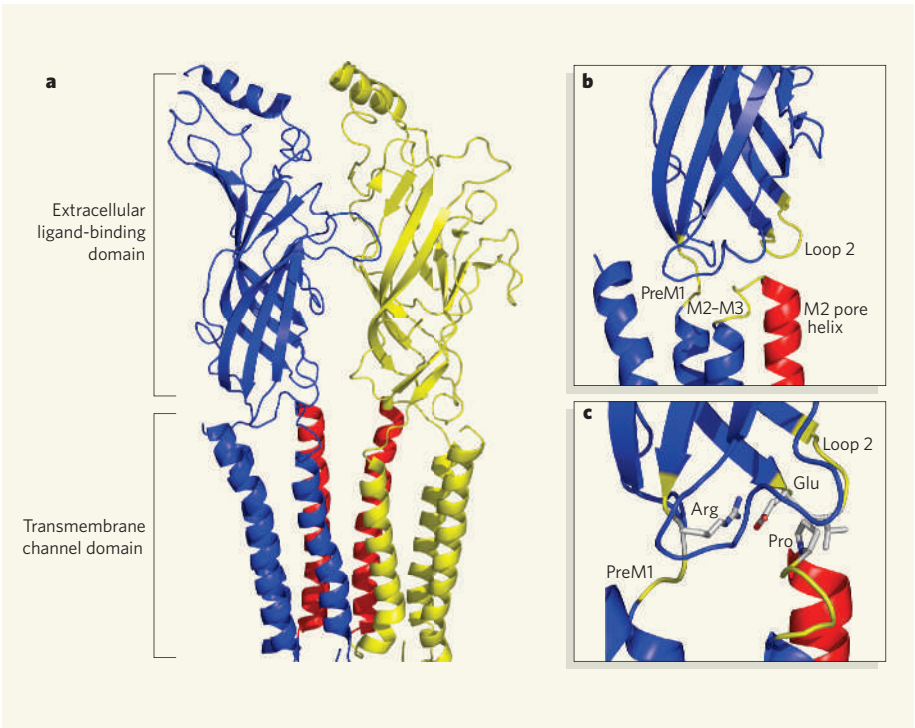

Figure 1 | The pathway that links ligand binding to gating. **a**, Structure of two subunits of the nicotinic acetylcholine receptor. The M2 helices that line the channel pore are shown in red. **b**, **c**, Close-up of the interface between the extracellular ligand-binding domain and the intracellular transmembrane channel domain of one subunit. **b**, Lee and Sine[2] identified several regions — preM1, loop 2, M2–M3 (shown in yellow) — that are interconnected and couple ligand binding to channel opening. The pore-lining helix (M2) is red. **c**, Some of the specific amino-acid residues pinpointed by Lee and Sine that form the activation pathway involved in this coupling (Arg, Glu, Pro). Lummis *et al.*[3] found that the proline residue at the same position in the serotonin 5-HT_3 receptor acts as a switch to control the ion-channel gating.

Lee and Sine[2] set out to answer this question. They used the nicotinic acetylcholine receptor, whose structure was recently refined to 4-ångstrom resolution[4], to identify receptor amino acids that could physically link the binding site to the channel. They then created a series of mutations, by substituting amino acids, to break these potential links, and analysed the mutations' effects, both individually and in combinations, on channel activity. As a result, they identified a set of interacting residues that functionally and structurally link the binding site to the channel.

The interacting residues connect three distinct regions of the receptor — the preM1 region, loop 2 and the M2–M3 loop. These regions lie at the interface between the binding site and the channel, and so are perfectly situated to transmit changes in the binding site to the channel (Fig. 1b). Although previous studies[5–7] have pinpointed these regions as

coupling elements and have even identified some pair-wise interactions, the real novelty of Lee and Sine's work is that it teases apart the functional interactions between these different regions on an atomic scale. Their data provide a detailed molecular scaffold for the idea that the binding of neurotransmitter triggers a wave of conformational changes that propagates from the ligand-binding site to the pore through the membrane[8].

One particularly attractive feature of Lee and Sine's model is the electrostatic interaction that exists between a positively charged arginine residue in the preM1 region and a negatively charged glutamate residue in loop 2 (Fig. 1c). Charged residues occur in these positions in every member of this receptor superfamily, suggesting that it is a common mechanism for linking binding-site changes to loop 2. Loop 2 sits above the extracellular end of the M2 helix that forms the channel, next to the M2–M3 loop, and one can easily envisage how movements in loop 2 could be conveyed to the channel through interactions between residues in these two regions[9–11]. Lee and Sine found several interacting residues, in particular a proline in the M2–M3 region (Fig. 1c). And this is where the work by Lummis *et al.*[3] comes in — their study concentrated on a proline in exactly the same position in the serotonin 5-HT_3 receptor.

Proline residues are unique in that their side chains are covalently bonded to the nitrogen atom of the protein's peptide backbone. The nitrogen atom of a peptide bond is usually involved in secondary interactions with other parts of the protein, helping to hold helices together, for example; but at proline, these secondary interactions are disrupted. In addition, proline is the only natural amino acid for which two different conformations of the peptide bond (*cis* and *trans*) are possible. These properties allow prolines to act as molecular hinges or switches[12].

In an elegant study on 5-HT_3 receptors, Lummis *et al.*[3] replaced the proline in the M2–M3 loop with a series of synthetic amino acids that had different propensities for adopting *cis* versus *trans* conformations. Amino acids that favoured the *cis* conformation resulted in receptors with high apparent affinities for neurotransmitter, producing ligand-induced 'locked' open channels. Amino acids that mostly took on *trans* conformations resulted in unresponsive closed channels. So it would seem that the conformation of the M2–M3 proline is coupled with the conducting state of the channel, pointing to the proline as a gating switch.

Taken with Lee and Sine's work, this conjures up a mechanism in which neurotransmitter binding to the receptor sets in motion a cascade of structural movements that end up flipping the proline to the *cis* conformation. This results in a repositioning of the M2 channel-lining region, such that the channel pore opens.

Although the two studies tell a compelling story of how ligand binding is translated into channel opening, it is probably not complete. The proline residue in the M2–M3 loop of the acetylcholine and serotonin receptors does not occur in the receptors for GABA or glycine, so other mechanisms must be invoked to explain how they open. Changes between *cis* and *trans* proline conformations are generally slow, so we need to determine whether this switch is fast enough to open channels on a sub-millisecond timescale. And because other regions and residues are involved in coupling binding to gating, the activation pathway charted by Lee and Sine will probably have others feeding into it. Finally, ligand-gated ion channels not only open and close in response to binding neurotransmitter, but they also become desensitized (close) in the continued presence of neurotransmitter. How this occurs is still a mystery. ■

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MATERIALS SCIENCE

Erasing electron mass

Charles L. Kane

Two-dimensional graphite could be useful in carbon-based electronic devices. How electrons move in these structures seems best described by relativistic quantum physics, modelling them as if they have no mass at all.

Graphite, the form of carbon found in pencil lead, leaves its mark thanks to weakly coupled layers of atoms that slide easily over one another. A single such layer — a two-dimensional sheet of carbon a single atom thick — is known as graphene. Although graphite has been studied for decades, graphene was only isolated in 2004 after a long struggle. The successful method was astonishingly simple: starting with a graphite crystal, layers of carbon atoms were peeled off one by one with adhesive tape, until a single-layer flake was left[1]. In this issue, Novoselov *et al.* (page 197)[2] and Zhang *et al.* (page 201)[3] investigate the properties of graphene further, showing it to be a remarkable conductor in which electrons mimic the behaviour of massless, relativistic particles.

In graphene, the carbon atoms are arranged in a honeycomb pattern (Fig. 1a), with each atom bound to three neighbours through strong, covalent bonds. This gives graphene exceptional structural rigidity within its layers. Because a carbon atom has four electrons available for bonding, each atom also contributes one unbound electron that is free to wander through the crystal, giving graphene its second distinctive characteristic — excellent conductivity.

The mobile electrons in graphene seem to behave differently, however, from those in two-dimensional semiconductor structures. In semiconductors, an electron can be modelled as a particle that obeys Newton's laws of motion, provided it is ascribed an effective mass, m^*, which takes into account the interaction between the electron and the semiconductor's crystal lattice. Electrons in semiconductors are thus characterized by a quadratic relationship between energy and momentum (Fig. 1b), and their quantum-mechanical behaviour can be described by the non-relativistic quantum theory formulated in the Schrödinger equation.

In contrast, the observations of Novoselov *et al.*[2] and Zhang *et al.*[3] show that, in the honeycomb structure of graphene, the relation between energy and momentum of the conduction electrons is linear (Fig. 1c). This is reminiscent of Einstein's theory of relativity for massless particles — which travel at the speed of light — and suggests that electrons in graphene obey a two-dimensional version of the relativistic quantum theory introduced by Paul Dirac in 1928. Electrons in graphene are, of course, not actually massless, and their typical speed (8×10^5 m s^{-1}) is almost 400 times lower than the speed of light in a vacuum (about 3×10^8 m s^{-1}) — although still much higher than the speed typical of electrons in semiconductors.

The conclusions of the authors[2,3] are based, to a large extent, on their observation of the quantum Hall effect in graphene. This phenomenon is the quantum-mechanical