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**Figure 1** Schematic diagram of the molecular fingerprint for LTD that Heynen *et al.*<sup>1</sup> show is induced by MD in rodents.

that MD occludes the synaptic expression of LTD. If MD uses the same cellular mechanisms as LTD, then the magnitude of LTD should be reduced in the deprived area of visual cortex following MD. Consistent with this prediction, the magnitude of LTD was significantly reduced in the cortex contralateral to the deprived eye.

On the basis of both mimicry and occlusion, the authors conclude that MD induces LTD in visual cortex, providing the first proof of a molecular basis for the long-term plasticity induced *in vivo* by visual deprivation. Recently, Feldman and colleagues reported that whisker deprivation leads to LTD in corresponding cortical barrels in the somatosensory cortex<sup>12</sup>. It will be interesting to see if this deprivation-induced LTD in somatosensory cortex is mediated by the same molecular mechanisms as

Heynen and colleagues show for MD in the visual cortex. Taken together, these results now provide the long-awaited evidence that should quiet skeptics who held that LTD is simply a slice phenomenon resulting from artificial patterns of electrical stimulation.

Although this paper elegantly demonstrates that MD induces LTD, the authors emphasize that it does not unequivocally show that the mechanisms of LTD are the only mechanisms underlying MD. Indeed, there are likely to be additional mechanisms that contribute to the effects of MD; for example, there are multiple forms of LTD, and there are examples in which altering LTD has no effect on ocular dominance plasticity and vice versa<sup>13</sup>. Nevertheless, the primary implication of this paper is that LTD is a compelling model that will help to reveal the

cascade of cellular events that occurs from the decreased activity in visual cortex resulting from MD to the expression of deprivation-induced synaptic depression and subsequent structural changes. Indeed, one of the most exciting remaining mysteries in this process is how MD initiates the structural changes that underlie its effects on ocular dominance plasticity<sup>14</sup>. To date, LTD has not been shown to result in a loss of connectivity in the central nervous system. Now that there is a strong link between MD and LTD *in vivo*, future work is sure to focus on possible mechanisms whereby LTD could initiate the synapse elimination that follows MD. Finally, given the defects in LTD and synapse elimination that occur in several forms of developmental disorders, including fragile-X syndrome<sup>15</sup>, it is clear that understanding the molecular basis of experience-dependent plasticity will have relevance far beyond the question of how MD leads to blindness.

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## Crystallizing our understanding of partial agonists

David S Weiss & Yongchang Chang

**A powerful combination of X-ray crystallography and single-channel current measurements provides new insights into the mechanism by which the binding of agonists opens the AMPA-type glutamate receptor in the central nervous system.**

Out on a distant domain, perhaps tens of angstroms above the lipid bilayer, an amino

acid docks into its binding pocket. The binding perturbs this multimeric membrane protein, initiating a rapid sequence of events that culminates in the opening of an integral ion pore that passes some  $10^7$  ions per second. Such excitatory chemical transmission is the predominant means by which neurons in the central nervous system communicate, yet despite decades of intensive

research, fundamental aspects of the activation process remain an enigma.

One unresolved issue relates to the mechanism of action of partial agonists. Partial agonists are ligands that can activate the receptor, but not as well as a full agonist. Understanding the actions of partial agonists has long promised to take us closer to unraveling the molecular basis of activation. In this

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issue, Jin *et al.* deliver on this promise with an exciting new study of the AMPA-type glutamate receptor, using a combination of structural and functional techniques<sup>1</sup>.

The glutamate receptor is generally assumed to be in equilibrium between states (structural conformations) in which the pore is either open or closed. Agonists shift the equilibrium toward the open state, antagonists shift the equilibrium toward the closed state, and partial agonists shift the equilibrium somewhere in between. But such a notion is far from an understanding of the underlying mechanism. To explore this issue, Jin *et al.* used a series of compounds that differ only by the size and electronegativity of a single atom at one particular position, but differ substantially in their ability to activate the receptor. This structural consistency in the ligand is absolutely critical as it ensures the agonists are binding to the same site and activating the receptor through the same mechanism or pathway.

Using an approach previously developed in the same laboratory<sup>2</sup>, the authors determined high-resolution crystallographic structures of the isolated glutamate binding site of the AMPA receptor complexed with each of the agonist molecules. This analysis revealed distinct structural conformations for each case. More specifically, they observed a correlation between the size of the substituent at the single position of the agonist molecule and the degree of separation of the two lobes that form the agonist binding cleft. They also noted a trend in that the larger the substitution, the smaller the maximum current amplitude in response to a saturating agonist concentration. The authors speculate, as they did in a previous study<sup>3</sup>, that less binding cleft closure translates to less pull on the gate and therefore less activation.

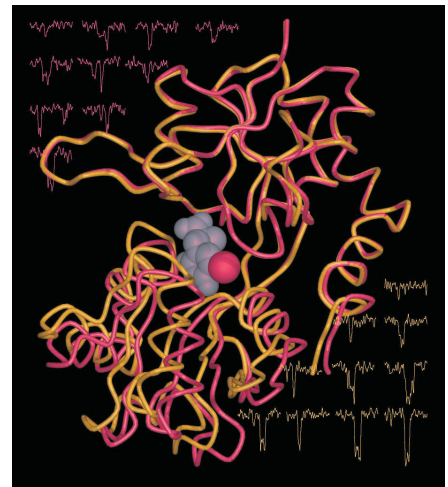
To complement the high-resolution structural images, they then turned to high-resolution functional analysis via the patch clamp technique (Fig. 1). This method allows one to record the activity of individual glutamate receptor currents. When the authors looked at these single-channel currents, they confirmed what others have reported, that a single glutamate receptor can exist in several distinct conductance states<sup>4,5</sup>. Although these states are all open, they differ in the rate at which ions flow through the pore. What was interesting was that no matter which agonist was used to gate the pore, the receptors still populated the same three conductance states. Furthermore, the less efficacious the agonist, the greater the relative proportion of smaller single-channel current amplitudes, thus accounting for the observed differences in the macroscopic current amplitudes. As a further test, they estimated the coupling efficiency between the binding and gating

in each individual subunit from the single channel and macroscopic currents. Assuming each subunit can be gated independently, a simple binomial analysis with the derived coupling efficiency could account for the distribution of single-channel conductance levels as well as the maximum current amplitudes in the presence of the different agonists. Finally, there was excellent agreement between the coupling efficiency and the extent of binding domain closure, giving further credence to their proposed mechanism of action of partial agonists.

In addition to differences in the maximal amplitude, this series of agonists also had a differential sensitivity for activation (concentration of agonist necessary to activate half the receptors, or EC<sub>50</sub>) and desensitization. Although the authors did do radioactive ligand displacement assays for each of the partial agonists, the relationship between those data and receptor affinities is difficult to interpret. Furthermore, the potency profile of the series of agonists seems to correlate with the charge of the substituted atom rather than size. Whether or not their simple model can account for the differential sensitivities remains to be seen.

We have always assumed that partial agonists are 'partial' because they are less efficacious than full agonists. These data, however, refine our thinking in that we now believe the efficacy is to be considered in the gating of the individual subunits rather than a final concerted conformational change of the entire tetramer—which brings up another important point. In addition to providing insight into partial agonist actions, the data speak to the molecular mechanism of activation itself. Previous studies indicate that each conductance state reflects the successive activation of an individual subunit by an agonist molecule<sup>6,7</sup>. In this scenario, there would be four possible conductance states (neglecting the unbound, closed state) for the cases when 1, 2, 3 or 4 agonist molecules are bound to the receptor. Jin *et al.*<sup>1</sup> provide some of the strongest functional evidence, and certainly the first structural evidence, for such an activation mechanism.

Can these data be extrapolated to the other major neurotransmitter-operated ion channel family, consisting of the nicotinic acetylcholine, GABA, glycine and serotonin type-3 receptors? Although time will certainly tell, we suspect not. There is no evidence indicating the nACh receptor family can be activated in a subunit-independent manner as is the AMPA receptor. In fact, the ligand binding sites of this receptor family are located at subunit interfaces<sup>8–10</sup> rather than within each subunit as for the AMPA receptor, supporting the prevailing



**Figure 1** Color-matched examples of the structures and the single-channel recordings evoked by two partial agonists.

notion that the final step in activation, opening of the pore, represents a concerted change of the entire pentameric assembly.

Heralded by structural resolution, our understanding of the mechanisms underlying voltage-activated ion channel activation and permeation has grown by leaps and bounds in the past several years<sup>11–13</sup>. A similar revolution is underway for neurotransmitter-operated ion channels with the structural determination of the acetylcholine binding protein<sup>14</sup>, the glutamate binding site of the AMPA receptor<sup>2,3</sup> and the nicotinic acetylcholine receptor transmembrane domains<sup>15</sup>. As exemplified by the Jin *et al.* study<sup>1</sup>, the next phase is the direct correlation between crystal structures and currents, which promises to bring a thorough understanding of these complex integral membrane proteins within reach.

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