ION CHANNEL STRUCTURE

A long arm to reach you

NMDA (*N*-methyl-D-aspartate) receptors are unique in that their activation requires the binding of two different agonists — glutamate and glycine. These two agonists bind to different subunits: NR2 subunits harbour the glutamate-binding site, and NR1 subunits are responsible for glycine binding. But despite residing in separate subunits, the binding sites do not seem to be entirely independent. For example, receptors that are formed by NR1 and NR2C subunits have a higher affinity for glycine than heteromers formed by NR1 and NR2A. How do the two binding sites interact? Does binding of one ligand directly affect binding of the second one, as in allosteric proteins such as haemoglobin? Are there allosteric interactions between the NMDA receptor subunits? A recent paper by Regalado *et al.* provides appealing answers to these questions.

The authors started by searching for the regions of NR2 that are responsible for imparting different affinities for glycine to NMDA receptors. They created chimeric subunits by combining segments from NR2A and NR2C, and identified a short amino-terminal stretch of NR2C that conferred properties of this subunit on NR2A. How does this segment work? To answer this question, Regalado *et al.* set out to determine exactly what happens to glycine affinity when glutamate binds, and made a startling observation: the subunits showed negative cooperativity. In other words, glutamate binding led to a reduction in glycine affinity, and the short segment that they had previously identified was crucial for the expression of cooperativity.

The findings of this study raise some intriguing questions. Which part of NR1 mediates its interaction with NR2? The authors provide us with a good lead to answer this question by identifying a mutation in NR1 that abolishes cooperativity. What is the structure of the NR2 segment that mediates the intersubunit interaction? Although we know the atomic structure of other glutamate receptor binding cores, this segment does not have a direct equivalent in other receptors, making it difficult to model. Does it extend like an arm from one subunit to the next to exert its allosteric effect? Regardless of the precise answer, the discovery of allosteric interactions in NMDA receptors reveals a new facet of this fascinating molecule.

Juan Carlos López

References and links

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FURTHER READING Madden, D. R. The structure and function of glutamate receptor ion channels. *Nature Rev. Neurosci.* **3**, 91–101 (2002)

WEB SITES

Encyclopedia of Life Sciences: http://www.els.net/NMDA receptors

NEUROTECHNIQUES

Electronic delivery

The gain-of-function transgenic approach can provide valuable insights into gene function, but controlling when and where a transgene is expressed remains a considerable challenge, because promoters that are truly tissue specific are hard to find. An alternative tactic is to target DNA to specific regions using viral vectors, but it is difficult to restrict the spread of infection. To address these problems, Saito and Nakatsuji have combined *in utero* and *exo utero* surgical techniques with an electroporation-based gene-transfer system, and have managed to achieve stable, targeted transgene expression in the embryonic mouse brain.

Electroporation — the application of a pulse of electric current to make cells transiently permeable to large molecules — is widely used to introduce DNA into cells *in vitro*, and has also been used successfully *in vivo* in chick and cultured mouse embryos. However, because the mouse embryo can be maintained in culture for only a few days, it has not been possible to examine the long-term effects of transgene expression in this model.

In this new study, reported in *Developmental Biology*, Saito and Nakatsuji electroporated DNA constructs into the brains of mouse embryos without removing them from the uterus. The DNA was injected into the appropriate region using a micropipette, then an electric pulse was applied using forceps-like electrodes. Beyond 13.5 days post coitum, the brain was clearly visible through the uterine wall, but for younger embryos, it was accessed by *exo utero* surgery. The DNA was injected into one side of the brain only, so that the other side could act as a control. More than 90% of the embryos survived, and in many cases, transgene expression was maintained for at least six weeks after electroporation. In addition, the authors were able to introduce several constructs into the same cell simultaneously.

Another team has used a similar *in utero* electroporation protocol to label neurons and track their migration in the developing mouse brain, but Saito and Nakatsuji went one stage further by showing that the technique can be used to reveal gene function. They injected constructs that expressed genes for either Hes1 or a constitutively active form of Notch1, both of which are inhibitors of neurogenesis. In both cases, neuronal differentiation was suppressed around the site of injection, indicating that the transgenes were functioning normally.

Saito and Nakatsuji showed that their system can accurately target and restrict the expression of transgenes in the developing mouse brain. In these preliminary experiments, the genes were driven by ubiquitous promoters, but the authors speculate that by using region- or cell-type-specific promoters, they might be able to target expression even more precisely in future.

Heather Wood

References and links

ORIGINAL RESEARCH PAPER Saito, T. & Nakatsuji, N. Efficient gene transfer into the embryonic mouse brain. *Dev. Biol.* **240**, 237–246 (2001)

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WEB SITES

***In vivo* electroporation:** http://www.frontier.kyoto-u.ac.jp/rc01/in_vivo_electroporation.html