

NMDA receptor subunit gating – uncovered

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Kinetically distinct contributions of NMDA receptor NR1 and NR2 subunits to channel gating have recently been inferred by Banke and Traynelis from single-channel recordings of recombinant NR1–NR2B receptors. The results suggest a new mechanism for receptor activation that will enhance our insight into excitatory synaptic transmission in the brain. In the post-genomic era, this represents an exciting advance in understanding the relationship between structure and function for this unique class of neurotransmitter receptor.

A recent paper by Banke and Traynelis [1] provides, for the first time, insight into the physical mechanism linking binding of glutamate and glycine to NMDA receptor channel opening. NMDA receptor activation produces long bursts of channel openings [2,3] that underlie the slow time course of NMDA-receptor-mediated synaptic currents [3–5] and the ‘coincidence detector’ role of NMDA receptors [6,7]. When viewed using patch-clamp recordings of single-channel activity, the NMDA receptor is an object of beauty and majesty but this fascinating behaviour has yet to be fully described in mechanistic terms: a goal of enormous value because of the insight this gives into excitatory synaptic transmission in the brain. The paper by Banke and Traynelis takes this goal a step closer.

Consider how remarkable receptor activation is. Two small molecules [glycine (molecular weight = 75) and glutamate (molecular weight = 150)] collide with one of the giants of the neurotransmitter world [the NMDA receptor (molecular weight \approx 500 000)] and within milliseconds the receptor channel opens. This is analogous to throwing a grain of sand at the wall of a house and expecting the front door to spring open! The mechanisms of receptor activation have therefore long been a source of fascination and have been particularly enlightened by the use of the patch-clamp technique, which allows the functioning of single protein molecules to be observed in real time.

Nicotinic ACh receptor activation

Del Castillo and Katz [8] described the first physically plausible receptor activation mechanism (Figure 1a), which distinguished conceptually the affinity of the agonist from agonist efficacy: the tendency of the receptor to activate (channel opening) once the agonist was bound. Katz and Thesleff [9] extended this mechanism to include long-lived inactive states of the receptor – receptor desensitization.

The del Castillo–Katz mechanism was formulated before it was known that there are two agonist-binding

sites on muscle nicotinic ACh receptors [10,11]. Subsequently, this scheme has been refined by improvements in recording resolution [12,13] and structural investigation [11] (Figure 1b) to describe ion-channel-receptor activation and the time course of endplate synaptic currents. The revised scheme has also been extended [12,13] to describe receptor activation, the structural mechanism of channel gating [14] and the effects of mutations in ACh receptor structure that result in congenital myasthenic syndromes [15]. These successes have inspired attempts to understand NMDA receptor gating with similar accuracy.

NMDA receptor activation

The first widely applied kinetic mechanism (Figure 1c) was described by Lester and Jahr [16]. However, this mechanism did not well describe some aspects of the single-channel data, such as the channel open time or the likely number of open and closed states (a minimum of five or six closed states and three open states are predicted from most single-channel recordings [2,3]), and was not based on any structural model of the receptor. Structural information concerning the conformational changes in the receptor subunits in response to agonist binding is only beginning to be obtained [17].

As their starting point, Banke and Traynelis used the current structural model of the NMDA receptor as being composed of two glycine-binding NR1 subunits [18] and two glutamate-binding NR2 subunits [19]. They used controlled expression of NR1 and NR2B subunits in a mammalian cell line (HEK-293 cells) to obtain patch-clamp recordings from membrane patches containing a single receptor molecule. This has only rarely been achieved for NMDA receptors [3,20] and is no mean feat: anyone who has tried this will know that patches containing a single receptor molecule are about as rare as the proverbial hens tooth (and just as difficult to extract from the cell...!). Normally, there is no easy way to tell how many receptor molecules are present in a patch of membrane and this can create serious limitations for parts of the data interpretation [21]. Banke and Traynelis obtained recordings from patches containing convincingly only a single receptor. They also made macroscopic recordings (using patches containing many receptors) and related the time course of these responses to the single-channel kinetics. For both conditions, a combination of brief (1–4 ms long) glutamate applications (mimicking synaptic transmission) and longer applications (3 s long, to examine desensitization) were used. These methods provide a powerful approach to investigating the receptor mechanism because, if the mechanism is a good one, then

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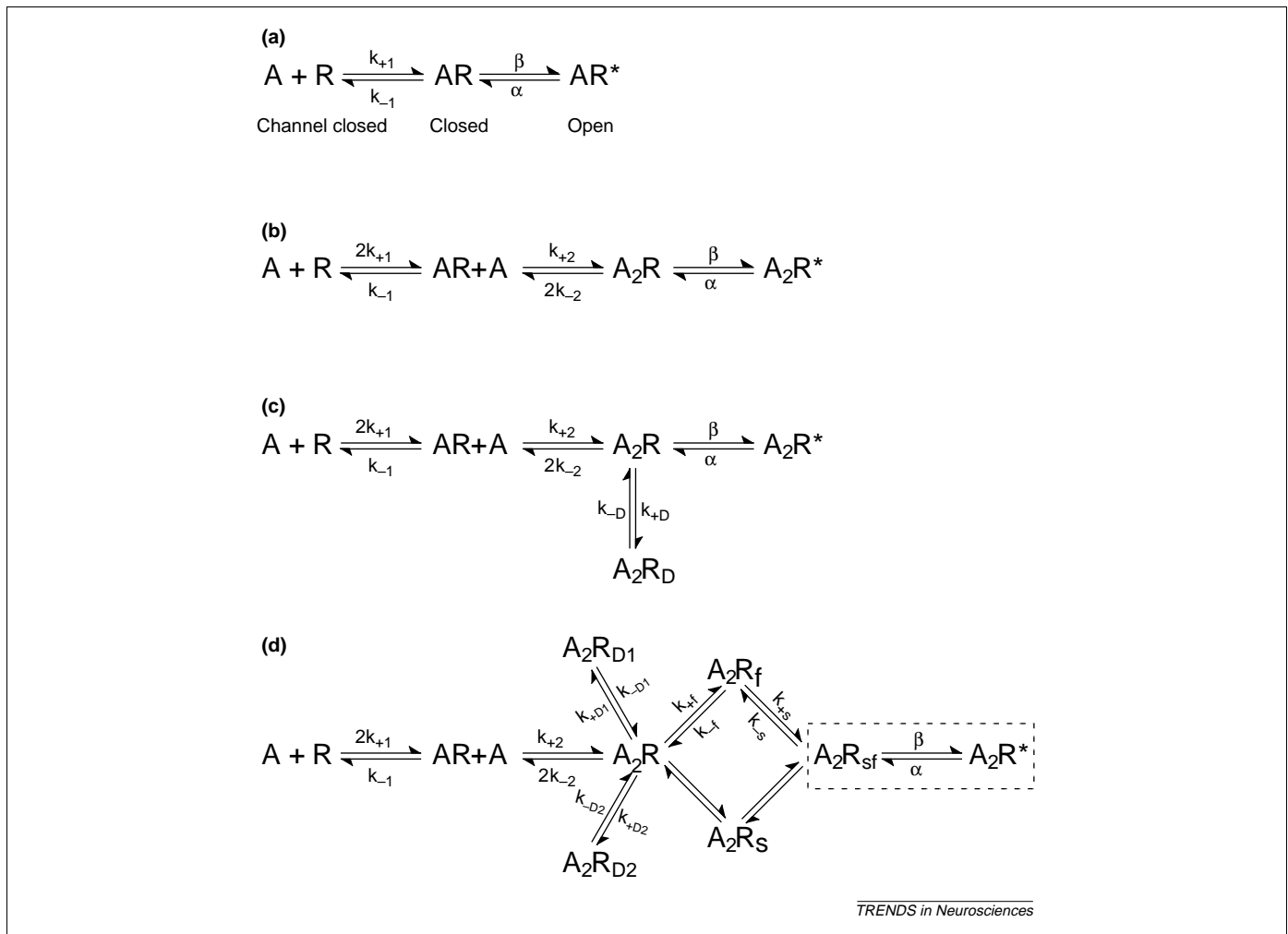


Figure 1. Ion-channel-receptor mechanisms through the ages. **(a)** According to the scheme of del Castillo and Katz [8], the rates of agonist A binding to receptor R and agonist dissociation are described by k_{+1} and k_{-1} , respectively, and β and α are the rate constants for channel opening and closing, respectively. The equilibrium constants $K_A = k_{-1}/k_{+1}$ and $E = \beta/\alpha$ (efficacy) define quantitatively the ability of the agonist to bind to the receptor and then to activate the receptor (asterisk indicates the channel open state). **(b)** Subsequent results led to modification of the del Castillo–Katz scheme, to give a linear mechanism with two agonist-binding reactions, with rate constants k_{+1} and k_{-1} , k_{+2} and k_{-2} , and channel opening and closing rates β and α . There is a factor of 2 before k_{+1} and k_{-2} because the mechanism assumes that either of the two agonist-binding sites can be occupied or vacated first. In addition, the two sites are assumed to be equivalent before agonist binding but the mechanism allows for the possibility of cooperativity in agonist binding if $k_{+1} \neq k_{+2}$ or $k_{-1} \neq k_{-2}$. **(c)** According to the mechanism suggested by Lester and Jahr [16], oscillation into and out of a relatively long-lived shut state, A_2R_D (desensitized state) contributes to the slow kinetics of the receptor, as well as to the decline of the receptor open probability (desensitization) in response to a prolonged glutamate application. The glycine sites on the receptor were assumed to be fully occupied by glycine during the experiment and so only glutamate binding is described by the binding of agonist A to the receptor. **(d)** The mechanism for NMDA receptor activation proposed by Banke and Traynelis [1]. Two new states, A_2R_f and A_2R_s , reflect the fast gating transitions of the NR1 subunits (k_{+f} and k_{-f}) and slower gating transitions of the NR2 subunits (k_{+s} and k_{-s}), which can occur in either order to arrive at the A_2R_{sf} state that is then followed by channel opening (dashed box).

the rate constants that can describe the single-channel data should also describe the macroscopic responses. This is what Banke and Traynelis aimed to achieve and, with some caveats (see following section), they arrived at a mechanism with defined rate constants for the activation of NMDA receptors by glutamate.

To gain insight into the physical basis of their mechanism, Banke and Traynelis used an elegant pharmacological approach that depends on the essence of del Castillo and Katz's work. Del Castillo and Katz [8] studied both high-efficacy 'full agonists' and compared these with 'partial agonists', which only weakly activate receptors once bound. Banke and Traynelis exploit this principle for agonists at the glycine site of the NR1 subunit (which has glycine as a full agonist, and HA-966 and the antibiotic D-cycloserine as

partial agonists) and at the glutamate site of the NR2 subunit (which has glutamate as a full agonist, and NMDA and the neurotoxin quinolinic acid as partial agonists). They hypothesized that if there are differences between the channel kinetics for full and partial agonists (which there are), then this could reflect gating transitions within the glycine-binding or glutamate-binding subunits.

The single-channel recordings in the presence of these different agonists allowed Banke and Traynelis to suggest which aspects of the channel kinetics reflect conformational changes of the NR1 and NR2 subunits. The results show that the time constant of the 10 ms component of the shut-time distribution depends on the efficacy of glutamate-site agonists, whereas the 1 ms shut-time component is associated with the efficacy of

the glycine-site agonists. Banke and Traynelis hypothesize that this reflects the speed of conformational changes in NR1 and NR2 subunits following agonist binding: a rapid conformational change of the NR1 subunits (reflecting their shorter-lived 1 ms shut-time component) and a slower conformational change in the NR2 subunit (reflecting the longer-lived 10 ms component). These data have allowed Banke and Traynelis to propose a new mechanism for NMDA receptor activation (Figure 1d).

Some caveats

Conceptually, should a single reaction be used to describe conformation changes associated with two NR1 (A_2R_f) or two NR2 (A_2R_s) subunits? This is not as unlikely as it might seem. The tetrameric glutamate and cyclic-nucleotide-gated receptors seem to assemble and function as a 'dimer of dimers' [22–25] and so it is reasonable to expect that each dimer could work as a unit.

Is it reasonable to assume that a particular exponential component in the shut-time distribution will be dominated by the lifetime of a single state, when the time constants of components in the shut-time distribution are in fact complex functions of the rate constants linking shut states in the receptor activation mechanism [26]? Further investigation of the differences in rates within the mechanism for full and partial agonists is needed before this can be assessed. The data so far look good but Banke and Traynelis stop short of determining mechanism rate constants for the partial agonist data. For example, if fits to the glutamate-site partial agonist data show that only the rates into and out of the A_2R_s state need to be altered (relative to the glutamate data) to achieve a good fit, this will provide strong support for the new mechanism.

In fitting the mechanism to their data, Banke and Traynelis assume a rapid equilibrium between A_2R_{sf} and A_2R^* states associated with pore opening (enclosed in a dashed box in Figure 1d). This does not detract from the conceptual advance achieved by identifying those aspects of the receptor activation associated with the NR1 or NR2 subunit dimers but undoubtedly future revisions will be required to account for the short-lived closed states and multiple open states, dwell-time correlations and modal gating behaviour observed in most other single-channel data [2,3,20].

At the agonist-binding end of the mechanism, explicit binding reactions will be needed both for glutamate and glycine and for the negative cooperativity between glutamate and glycine binding that results in glycine-sensitive desensitization in whole-cell recordings [27–30]. The implication from desensitization studies is that conformational changes in the NR1 and NR2 subunits influence each other – are these conformational changes related in any way to the receptor activation path suggested by Banke and Traynelis?

Banke and Traynelis have added an extra desensitized state (compared with the Lester and Jahr mechanism [16]) to describe the bi-exponential time course of desensitization. However, the exact connectivity of these two desensitized states is difficult to define (for example, could it be that desensitization occurs from the A_2R_{sf} state that follows conformational changes in the NR1 and NR2 dimers?). Full maximum likelihood fitting of the mechanism to the data in the way most successfully applied so far to the muscle ACh receptor [31,32] might be necessary to answer this.

Banke and Traynelis tested their mechanism to determine whether a single set of rate constants will describe both the single-channel data and the macroscopic responses to high and low glutamate concentrations. The results provide a remarkably good fit of the time course of the responses, the channel open and closed times, and the channel open probability observed in single-channel recordings. Thus, this new mechanism is now begging to be tested with data from other NR2 subunits or NR1 splice variants known from previous work to affect the channel kinetics and open probability [3,20,33].

For the first time, the complex kinetics of NMDA receptor activation have been given the beginnings of a physical basis. As this article illustrates, significant advances in mechanistic understanding appear rarely and so this is a seminal event.

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doi:10.1016/j.tins.2003.10.007

Research Focus Response

Response to Gibb: NMDA receptor subunit gating – uncovered

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We agree with Alasdair Gibb's emphasis on the importance of understanding in detail the functional basis of NMDA receptor activation by full and partial agonists [1]. Kinetic models are not just academic exercises; they also help us to frame questions about how receptors work. Furthermore, quantitative models nicely complement emerging structural studies to help create a clearer picture of function. Our efforts to expand NMDA receptor models to account for single channel properties is an attempt to create a baseline understanding of function from which we can explore the mechanisms that enable ions and exogenous compounds to modulate receptor properties. Our motivation here highlights another important role kinetic models can serve.

We would like to point out that since our paper was published, elegant work with cell-attached patches that contained single NMDA receptors by Popescu and Auerbach [2] has produced some similarities to, as well as some differences from, our current working hypothesis. Some of

the differences are likely to reflect different receptor subtypes (NR2A versus NR2B) and different recording techniques (cell-attached versus outside-out patches). Clearly, we do not yet have the final picture of NMDA receptor function. As in all aspects of science, our current understanding and working models are fluid and will be reshaped and revised as new data emerges. Nevertheless, we feel our studies provide a step forward in thinking about functional features of NMDA receptors. We hope our study stimulates new experimentation that will help further illuminate how the NMDA receptor channel complex operates.

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