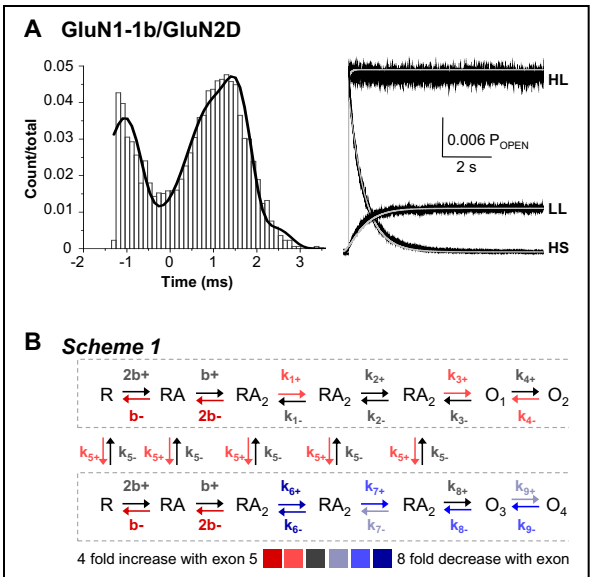
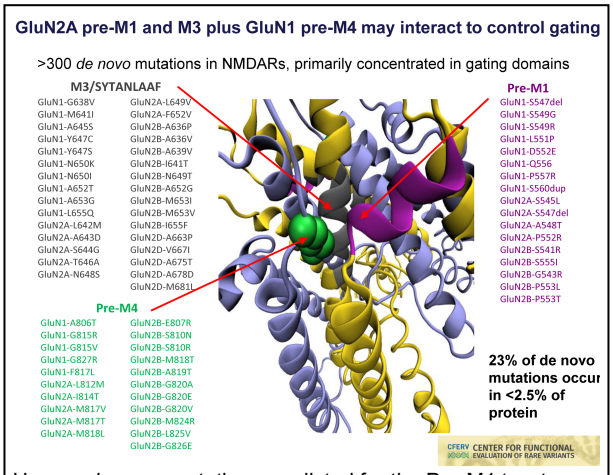
Mechanism by which the NMDA receptor pore opens

NMDA receptors are members of a family of glutamate-gated channels that catalyze the transmembrane flux of cations in response to the binding of the neurotransmitters glutamate and glycine. The architecture of the NMDA receptor is becoming better understood with recent crystallographic and cryo-EM data, which unambiguously show that the NMDA receptors contain a large extracellular region comprising four dimers of eight bilobed clamshell-like domains that are connected by short polypeptide linkers, four of which harbor an agonist binding site deep within a clamshell cleft. The four bilobed domains that make up the agonist binding site are connected by additional linkers to a non-selective cation pore, which shares substantial similarity with an inverted potassium channel. Receptors are tetrameric assemblies of two glycine-binding GluN1 and two glutamate binding GluN2 subunits, which occur as four unique gene products that each endow the receptor with distinct properties. The GluN1 subunit undergoes alternative RNA splicing, which also controls receptor properties (see Figure to right). At the single channel level, a decade of detailed analyses has shown that the complex kinetics that underlie channel function contains at least three conformationally distinct changes that precede pore opening, which include a very fast state that is almost certainly the brief delay prior to explosive pore dilation. Rapid pore dilation gives the channel-mediated unitary currents recorded in excised patches their characteristic square appearance. The other two closed states apparent in recordings of single NMDA receptors reflect unique rate limiting conformational changes, which functional data and structural data suggests may be controlled in part by the different subunits.

We are currently studying the contributions of specific domains on receptor gating, with a focus on the parts of the receptor that must undergo the rate limiting conformational changes we see prior to opening. We utilize two forms of perturbations of receptor function to learn about the relationship between structure and function of key gating elements. These include single channel studies of the mechanism of action of our novel subunit-selective positive and negative allosteric modulators, which bind near the linkers that connect the agonist binding domain to the channel gate and dramatically alter NMDA receptor function. We also have identified a large number of human mutations at three closely spaced interacting elements that we think are involved in channel gating. We are testing specific structural hypotheses about how channel gating proceeds by recording and analyzing the effects of 0, 1, or 2 copies of these mutations in NMDA receptors, as well as studying channel properties in the presence and absence of modulators. These studies will provide a better understanding of how the channel works, which could improve our understanding of how channel mutations contribute to neurological disease and how therapeutically-relevant modulators change receptor function.



A, The fit of a data from a GluN1+exon5GluN2D receptor in an outside-out single with Scheme 1 is given (left panel). The result of least squares fitting of Scheme 1 (right panel) to macroscopic recordings of GluN1+exon5/ GluN2D activated by 1 mM L-glutamate for 10 sec (HL), 1 mM L-glutamate for 10 ms (HS), and 0.0025 mM L-glutamate for 10 sec (LL) is given. Waveforms predicted from the fitted rate constants are superimposed in white **B**, Rates are color-coded based on the change between fitted GluN1+exon5 compared to GluN1-exon5. Dark red indicates a 4-fold increase, light red indicates 1.5-fold increase, gray indicates no change, light blue indicates a 1.5-fold decrease, medium blue indicates a 2-5-fold decrease, and dark blue indicates 8-fold decrease for exon 5. From Vance et al., 2012.



Human *de novo* mutations are listed for the Pre-M1 two turn helix (magenta), the pre-M4 linker (green), and the conserved M3-SYTANLAAF motif (Charcoal). This trio of interactions occurs for GluN1-pre-M1/GluN1-M3/GluN2-pre-M4 (GluN1 yellow and GluN2 blue) and for GluN2-pre-M1/GluN2-M3/GluN1-pre-M4 (*not shown*).