

(A - D) results in receptors with distinct gating properties, contributing to the diversity of excitatory post-synaptic currents. Additionally, the NR1/NR2A-isoform can itself respond with distinct kinetics due to modal gating. To investigate whether the NR1/NR2B-isoform also gates with modal kinetics, we recorded steady-state single-channel activity from cell-attached patches of HEK293-cells transfected with NR1 and NR2B, in the continuous presence of saturating agonists. Single-channel records ( $n=37$ ) revealed a variety of gating patterns illustrated by a 25-fold range in measured equilibrium open probability: range, 0.02 - 0.49;  $P_o$  (mean  $\pm$  s.e.m.) =  $0.20 \pm 0.02$ . This diversity reflects mainly differences in mean closure durations per file: range, 6 - 200 ms; mean closed time =  $43 \pm 8$  ms; with less spread for mean open durations: range, 1.8 - 10.5 ms; mean open time =  $4.8 \pm 0.3$  ms. Kinetic analyses revealed that each record had 2-4 open and at least 5 closed components in the respective interval duration distributions. As with NR1/2A-receptors, in all NR1/2B-records we observed sporadic gating changes due to sudden changes in mean open durations, indicative of modal behavior. We identified three gating regimes, each having at least two open time components: a ubiquitous brief component ( $0.27 \pm 0.01$  ms) and at least one of three longer components ( $\tau-L = 2.5 \pm 0.1$  ms;  $\tau-M = 5.0 \pm 0.2$  ms; or  $\tau-H = 10.0 \pm 1.0$  ms). In contrast to NR1/2A-channel behavior where modal gating allowed characterization of all observed channels, for the NR1/2B-receptor we also observed gating patterns which differ in mean duration of closures. These data and analyses reveal the variety of mechanisms generating the previously observed diversity of macroscopic NR1/2B-responses.

#### 2528-Pos Board B498

##### Effect of Protons on the NR1/NR2A NMDA Receptor Kinetics

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NMDA receptors are glutamate-activated ion-channels that mediate fast excitatory transmission, synaptic plasticity and excitotoxicity. They assemble as heterotetramers of two NR1 and two NR2 subunits. Multiple isoforms with distinct kinetics, pharmacology and physiologic roles are differentially expressed in the central nervous system. Of these the NR1/NR2A and NR1/NR2B isoforms are most abundant. They are both inhibited by physiological proton concentrations but so far, the kinetic mechanism of proton inhibition has been characterized only for the NR1/NR2B isoform. To determine the mechanism of proton inhibition of NR1/NR2A receptors we recorded single-channel currents from cell-attached patches of HEK 293 cells transfected with NR1-1a, NR2A and GFP. The patch pipette contained saturating concentrations of glutamate and glycine and several proton concentrations in the range: pH 6.5 to 8.5. These records confirmed that protons do not change the channel's conductance and act solely by decreasing channel open probability ( $IC_{50} = 7.3$ ). Kinetic analyses of our single-channel data showed that with increasing proton concentrations (pH 8,  $n = 5$  vs. pH 6.5,  $n = 4$ ) the mean channel open time decreased ( $7 \pm 1.3$  ms to  $1.7 \pm 0.3$  ms) and the mean channel closed time increased ( $12 \pm 0.1$  ms to  $94 \pm 16$  ms). To identify the rate constants affected by proton-binding we used best fit kinetic models to our single channel data. Results showed that similar to the mechanism previously reported for NR1-1a/NR2B receptors, protons inhibit NR1-1a/2A receptors by increasing the stability of two pre-open conformations. The rate constants we report here will help understand the role of protons in regulating synaptic transmission, plasticity and neuroprotection.

#### 2529-Pos Board B499

##### A LRET Based Method To Studying Intersubunit Conformational Changes In The Ligand Binding Domain Of A Functional AMPA Receptor

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Ionotropic glutamate receptors are the main excitatory neurotransmitter receptors in the mammalian central nervous system. During activation of the receptor, an agonist binds to an extracellular domain initiating a sequence of conformational changes leading to the opening of a cation-selective channel, which subsequently closes during desensitization. Structures of the isolated ligand binding domain of the AMPA subtype of the receptor have provided the first clues of the structural movements within the ligand binding domain; however, these structures lack the crucial functional portion of the protein, the transmembrane segments. Additionally, these limited structures do not reveal the structural changes associated with desensitization, unless artificially decoupled with a disulfide bond. In order to determine how the agonist controls receptor activation and desensitization, it is necessary to investigate the changes in the ligand binding domain in the presence of the transmembrane segments. We have modified a functional AMPA receptor ( $\Delta N^*$ -AMPA) to serve as an

LRET based probe that allows us to measure distance changes of the ligand binding domain in the presence of the transmembrane segments. This receptor has been modified such that fluorophores can be introduced at defined sites to serve as a readout of intersubunit distance measurements associated with the apo, activated, and desensitized state. These investigations suggest that the apo state in the presence of the transmembrane segments is decoupled, and during activation, the interface is coupled due to the driving force of cleft closure, thereby stabilizing the open channel, and then the interface decouples thus leading to desensitization.

#### 2530-Pos Board B500

##### Partial Agonism And Lobe Orientation In The Glutamate Receptor, GluR2

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Ionotropic glutamate receptors (iGluRs) mediate the majority of excitatory synaptic transmission in the vertebrate CNS. iGluRs are ligand-gated ion channels and their complete structure is unknown; however, studies on the soluble constructs of ligand binding cores (S1S2) have provided considerable insight into structure, function and dynamics. A number of X-ray structures of these constructs bound to various ligands have been determined, all showing a bilobal structure open to different degrees depending on the bound ligand. Some structures of GluR2 S1S2 suggested a direct correlation between the degree of lobe closure and the efficacy of channel opening. However, significantly different structures were obtained in several cases for the same ligand at different crystallization conditions. The measurement by NMR spectroscopy of residual dipolar couplings (RDCs) in partially aligned proteins provides a means of orienting protein domains in solution. We used this method to determine the domain orientation of GluR2 S1S2 bound to partial agonists and an antagonist. The precision required and the limited stability of S1S2 made it necessary for us to develop a somewhat novel approach. The main limitation for achievable precision is the presence of "structural noise" in X-ray structures. We refined several structures using NH RDCs measured in 5 alignment media for S1S2 bound to glutamate. These structures were then shown to exhibit reduced structural noise when used with RDCs measured with other ligands. This allowed us to calculate the difference in the lobe orientation between glutamate and any other ligand with high precision. The results indicate that the degree of lobe closure is not necessarily correlated to the efficacy of a ligand, and that in some cases, the lobe orientation is likely to be highly dynamic.

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##### Microsecond-to-second Timescale Motions In The Ligand Binding Domain Of Glutamate Receptor 2

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Within the central nervous system, AMPA-type glutamate receptors mediate fast synaptic transmission and deactivate on the millisecond timescale. In this study, we characterize backbone amide nuclear spin dynamics associated with conformational and hydrogen exchange events in the ligand binding domain of glutamate receptor 2 and obtain a novel view of the backbone motions occurring over five orders of magnitude of timescale, spanning microsecond-to-second timescale motions. Most notably, we find that hydrogen exchange rates of particular residues in the ligand binding site provide important clues about the sequence of events leading to ligand detachment from the ligand binding domain. These results thus provide insights into the mechanism of receptor deactivation.

#### 2532-Pos Board B502

##### Functional Characteristics of iGluR3 AMPA Receptor-Channels in Cell Attached Recordings

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Ionotropic glutamate receptors (iGluR's) are ligand gated ion channels that mediate most of the fast excitatory neurotransmission in the CNS. Aberrant function of glutamate neurotransmission can lead to epilepsy and other neurodegenerative disorders. The extracellular ligand binding domain is a bilobal structure that binds an agonist and induces channel activation. Data from single channel recordings from homomeric AMPA receptor subtype (GluR3) in cell-attached patches were analyzed using QuB software to examine preliminary kinetic models of agonist dependent channel activity. Cell attached recordings were performed with both full and partial agonists on stably transfected HEK 293 cells. Amplitude analysis uncovered three conductance states, 15 pS, 27 pS, and 40 pS, in the presence of the full agonist, glutamate, as well as the partial agonists, fluorowillardiine, chlorowillardiine and nitrowillardiine. Different modes of activation ranging from low to high open probability exist for this channel. In the presence of the full agonist, glutamate, during a high mode of activation, the channel