(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 ± s.e.m.) = 0.20 ± 0.02. This diversity reflects mainly differences in mean closure durations per file: range, 6 - 200 ms; mean closed time = 43 ± 8 ms; with less spread for mean open durations: range, 1.8 - 10.5 ms; mean open time = 4.8 ± 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 ± 0.01 ms) and at least one of three longer components (tau-L = 2.5 ± 0.1 ms; tau-M = 5.0 ± 0.2 ms; or tau-H = 10.0 ± 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 ± 1.3 ms to 1.7 ± 3 ms) and the mean channel closed time increased (12 ± 0.1 ms to 94 ± 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 (ΔN*-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.

**2531-Pos Board B501**

**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

prefers to open to the intermediate and large conductance states. In the presence of the willardiine partial agonists, the channel opens more frequently to the smallest and intermediate conductance states. Kinetic modeling using maximum interval likelihood rate optimization revealed two time constants in each open state and at least three in the closed state for the partial and the full agonists. These data suggest the mode of channel activation is similar for both glutamate and willardiine compounds with varying rates of activation. Supported by NIH NS049223.

**2533-Pos Board B503**

**Energetics of the Cleft Closing Transition and the Role of Electrostatic Interactions in Conformational Rearrangements of the Glutamate Receptor Ligand Binding Domain**

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The ionotropic glutamate receptors are localized in the pre- and postsynaptic membrane of neurons in the vertebrate central nervous system. Activation by the principal excitatory neurotransmitter glutamate allows the ligand binding domain to change conformation, communicating opening of the transmembrane channel for ion conduction. The free energy of the GluR2 S1S2 ligand binding domain (S1S2) closure transition was computed using a combination of thermodynamic integration and umbrella sampling modeling methods. A path that involves lowering the charge on E705 was chosen to clarify the role of this residue. A continuum electrostatic approach in S1S2 is used to show E705, located in the ligand binding cleft, stabilizes the closed conformation of S1S2. Molecular dynamics simulations reveal: (1) in the closed conformation, in the absence of a ligand, S1S2 is somewhat more closed than reported from X-ray structures; (2) a semi-open conformation characterized by disruption of a single cross-cleft interaction differing only slightly in energy from the fully closed S1S2; (3) the fully open S1S2 conformation exhibits a wide energy well, sharing structural similarity to the apo S1S2 crystal structure. Hybrid continuum electrostatics/MD calculations along the chosen closure transition pathway reveal solvation energies, as well as electrostatic interaction energies between two lobes of the protein increase the relative energetic difference between the open and the closed conformational states. By analyzing the role of several cross-cleft contacts and binding site residues we demonstrate how S1S2 interactions facilitate formation of the closed conformation of the ligand binding domain. A molecular model of the full GluR2 receptor is currently being constructed to reflect a consistent physical and biochemical picture based on an evolutionary comparison and all available biophysical data.

**2534-Pos Board B504**

**Hinge and Twist Rigid Body Domain Motions in Ionotropic Glutamate Receptor GluR6 and the Hydrogen Bond Interactions that Switch Them On and Off**

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Ionotropic glutamate receptors are tetrameric ligand-gated ion channels found in pre and postsynaptic cell membranes of the central nervous system. There are three pharmacological classes of ionotropic glutamate receptor, namely N-methyl-D-aspartate (NMDA); alpha-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA); and kainate receptors. Ionotropic glutamate receptors play an important role in neuronal development and synapse plasticity as well as in higher order processes such as memory and learning. Ionotropic glutamate receptors are also implicated in several neurological and neurodegenerative disorders such as epilepsy, Parkinson's and Alzheimer's diseases. Only the structure of the isolated ligand binding domain is known: it comprises two lobes that enclose a ligand binding cleft. Using this isolated domain it has been possible to extract a great wealth of information concerning the relationship between functional and structural states of the receptor. Here, we characterize the intrinsic conformational dynamics properties of the ligand-binding domain of GluR6, a kainate receptor, in the absence of glutamate. Notably, we identify three inter-lobe hydrogen bonds interactions that govern and regulate the opening of the binding cleft via two distinct mechanisms: an hinge-like and a twist-like rigid-body domain motion. The computational studies reveal how the interplay between these interactions promotes either one or the other form of rigid-body motion. Moreover, the pattern of evolutionary conservation of these inter-lobe interactions suggests a putative role in the differential functional properties of the distinct ionotropic receptors classes.

**2535-Pos Board B505**

**Purification and crystallization of iGluR Amino Terminal Domains**

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The glutamate receptor ion channels which mediate excitatory synaptic transmission in the mammalian brain have a unique architecture distinct from that for other ligand gated ion channels. Ten years ago the 1st crystal structure was solved for an AMPA receptor ligand binding domain[1], with members of other iGluR gene families following over the next few years[2-4]. The ligand binding domain is preceded by a large amino terminal domain which controls assembly, but which does not bind neurotransmitter. Despite its key biological role structures of the ATD have not been solved. A major impediment to this is the poor expression of iGluR ATDs in *Escherichia coli*. To address this we screened ATD expression in HEK cells using constructs designed for secretion of soluble proteins and focused on the GluR6 subtype for which we can obtain 4 mg/l of glycosylated protein. The results of crystallization screens and data collection with synchrotron radiation indicate that it will be possible to solve a structure of the GluR6 ATD and explore its role in subtype specific assembly.

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**2536-Pos Board B506**

**Structure And Stability Of Ligand Binding Core Dimer Assembly Controls Desensitization In A Kainate Receptor**

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Ionotropic glutamate receptors couple free energy of agonist binding to opening and desensitization of a transmembrane ion channel. Central to their function is a structural unit formed by a dimer assembly of the ligand binding domains. The rates of transitions between resting, conducting, and desensitized states is controlled by conformational changes in the dimer. The serendipitous discovery that the GluR2 L483Y mutant blocks desensitization by stabilizing dimer assembly has profoundly influenced understanding of AMPA receptor gating. Paradoxically, GluR5-GluR7 subtype kainate receptors have an aromatic amino acid at the equivalent position, but desensitize rapidly and completely. Using a library of GluR6 dimer interface mutants, we used analytical ultracentrifugation to show that for kainate receptors there is a direct correlation between the rate of onset of desensitization and the stability of dimers formed by ligand binding cores, establishing that the gating mechanisms of AMPA and kainate receptors are conserved. Crystal structures for a series of 5 mutants were solved to reveal the underlying molecular mechanisms. Visualized in the crystal structures is a rich complexity of interactions across the dimer interface, illuminating how small sequence differences within the ligand binding domain function to diversify receptor properties. Our results indicate that even following extensive engineering, the stability of kainate receptor dimers is at most half of that of their AMPA counterparts, and that even if it were possible to generate dimers as stable as those for GluR2 L483Y, these would be insufficient to block kainate receptor desensitization. We show this is because the desensitized state in kainate receptors acts as a deep energy well offsetting the stabilizing effects of dimer interface mutants. Our results reveal how receptors with similar structures and gating mechanisms can exhibit strikingly different functional properties.

## Muscle: Fiber & Molecular Mechanics & Structure I

**2537-Pos Board B507**

**Computational Energetic Analysis of Intrafacial Binding Energies in Interpolated Myosin States**

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