FRET to examine the dynamics of the NMDA receptor specifically with respect to the cleft closure conformational change of the isolated agonist-binding domain of GluN2A when bound to ligands of varying efficacy. These studies reveal differences in the range of cleft closure states occupied by the agonist-binding domain with the antagonist DCKA-bound form and the full agonist glycine-bound form showing a large range of cleft closure states, while the partial agonists ACBC and L-alanine, as well as the full agonist D-serine, have a much narrower spread in their cleft closure states. Further analysis shows that the fractional occupancy of the isolated domain in cleft-closure states below a threshold does correlate with agonist efficacy, providing a link between agonist-binding domain dynamics, along with cleft closure, and channel activity.

1433-P Sun Board B384 Reduced Curvature of Ligand-Binding Domain Free Energy Surface Underlies Partial Agonism at NMDA Receptors
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NMDA receptors are ligand-gated ion channels that mediate excitatory synaptic transmission in the central nervous system. Partial agonists elicit submaximal channel activation, but crystal structures of the ligand-binding domain (LBD) bound with partial and full agonists show little difference. To uncover the molecular mechanism for partial agonism, here we computed the free energy surfaces of the GluN1 (an obligatory subunit of NMDA receptors) LBD bound with a variety of ligands. The free energy minima are similarly positioned for full and partial agonists, but the curvatures are significantly reduced in the latter case, indicating higher probabilities for sampling conformations with a not fully closed domain cleft. The free energy surfaces for agonists have both shifted minima and further reduced curvatures. Reduced curvature of free energy surface appears to explain well the partial agonism at NMDA receptors and may present a unique paradigm in producing graded responses for receptors in general.

1434-P Sun Board B385 Measurement of Nr1/Nr2B NMDA Receptor Currents on a Microfluidic Benchtop Automated Electrophysiology Platform
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The N-methyl-D-aspartate (NMDA) receptor is a central nervous system glutamate receptor implicated in synaptic transmission and memory function. It is also a prime target in ion channel drug discovery for both academic and pharmaceutical laboratories. The NMDA receptor has interesting biophysical characteristics in that activation of the NMDA receptor requires two coincidental events; the binding of glutamate and depolarization to remove magnesium ions that block the ion conducting pore at resting membrane potentials. We present here studies performed using a microfluidic system capable of recording from 32 experimental patterns at once. Each experimental pattern is self-contained and can deliver 8 unique solutions through individual fluidic channels to the cells in the recording chamber. Multiple solutions can flow past the cells at once providing receptor activation or protection from ligand dependence. This fluidic pattern used has proven to be useful for performing experiments to explore Positive Allosteric Modulators (PAM) on multiple fast ligand-gated channels including nicotinic receptors. Groups of 20 cells are measured simultaneously to mitigate biological variability. Each of the 32 experimental patterns records from 2 groups of 20 cells each, potentially resulting in 32 recordings performed in duplicate. Results of these experiments are measured and compared to results using commercially available automated patch clamp systems. The NMDA receptor cell line was kindly provided courtesy of ChanTest corporation (Cleveland, OH).

1435-P Sun Board B386 The Structural Basis of Negative Cooperativity between Subunits of the NMDA Receptor
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NMDA receptors are crucial signaling proteins in the mammalian central nervous system and play roles in development, synaptic maturation and learning and memory. As such, the biophysical properties of these channels can profoundly shape physiological function. For example, the biophysical property of pore block by magnesium ions endows NMDA receptor with the ability to act as synaptic co-incident detectors. Here we examine the biophysical property of negative cooperativity between the co-agonists glutamate and glycine. It has been previously reported that the glutamate bound to the GluN2A subunit speeds the dissociation of glycine from the GluN1 subunit. Using rapid perfusion methods on outside-out patches, we confirm that glycine dissociates from the GluN1 subunit roughly 3 fold faster when glutamate is bound to GluN2A than when unbound. We then report that glutamate dissociates from GluN2A approximately 2 fold faster when glycine is bound compared to when GluN1 is unoccupied. We hypothesize that this co-operativity arises from long unstructured loops of amino acids, found in the upper ligand binding domain (LBD) lobes of GluN1 and GluN2, interacting between LBD dimers. To test this, we are using fluorescence labelling of unnatural amino acids and luminescence resonance energy transfer experiments to measure conformational changes between LBDs of different dimers under varying occupancy conditions. By combining these measures of conformational change with electrophysiology and mutations blocking cooperativity, we aim to elucidate the structural mechanism of negative cooperativity at NMDA receptors.

1436-P Sun Board B387 Simulated Closing of the NMDA Ligand-Binding Domain
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Glutamate receptors are one of the most prevalent neuroreceptors in the central nervous system, and glutamate is the main neurotransmitter found in the body. Structurally, glutamate receptors are huge, complex, tetrameric, multi-domain proteins that possess several possible drug-binding sites throughout the different domains. Glutamate receptors can be over stimulated by excess glutamate or excitotoxins, causing neurodegeneration and neuronal damage through excitotoxicity. Due to their role in excitotoxicity, glutamate receptors are thought to be involved in many neurodegenerative diseases, such as Alzheimer’s and forms of Parkinson’s.
The mechanism of activation of glutamate receptors occurs when a glutamate molecule binds to the binding site, located in the center of a ‘clamshell’-like ligand binding domain (LBD). Upon binding, the bottom half of the clamps closes over the ligand. This closing movement in turn causes the top half of the transmembrane domain to also move outwards, thus opening the channel of the receptor. Once the channel is open, cations are able to flow across the membrane, thus potentiating nerve transmission. One of the major subtypes of glutamate receptors are the AMPA receptors, named for the additional agonists (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate) that bind to them with high specificity. Using multiple molecular dynamics (MD) sampling techniques we have resolved the detailed closing mechanism of an AMPA receptor clamshell LBD after ligand binding has occurred. A combination of both multiple brute force and nudged elastic band MD methods allowed for the energetic refinement of a pathway observed in unbiased simulations. The closing movement is revealed to be highly asymmetric and possibly step-wise in nature. This closing motion may reveal how the AMPA receptor channel gates upon ligand activation of the receptor, and indicates that there could be intermediate ‘activated’ states.

1437-P Mon Board B388 Effect of Phosphorylation on Structure of C-Terminal Segment of AMPA Receptor
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The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is the primary contributor to fast excitatory transmission in neurons. The AMPA receptor can be divided into four domains. Extracellularly, there are the amino-terminal and ligand-binding domains. The transmembrane domain serves as the actual ion-channel pore and, of course, links the extracellular domains to the cytoplasmic domain. Of these four domains, the structure of the outermost three has been shown in detailed crystal structures of the tetramer. However, very little is known about the structure of the cytoplasmic domain. Although it is widely thought that this segment is highly disordered, it is unknown whether local order (higher levels of secondary and/or tertiary structure) exists in the cytoplasmic terminus, or whether structural changes may occur as conformational shifts in the terminal due to functional modifications. Previous studies have established phosphorylation sites at residues S818, S831, and T840 in the GluA1 subtype receptor. These studies examined a representative membrane-proximal section of the GluA1 e-terminus comprising residues 809-841 in order to consider structural changes brought about by these phosphorylation events. The peptide was examined using circular dichroism (CD) investigation, which showed a conversion to greater helix content in the phosphomimetic sample. CD