activates NR1/NR2D (37%), but does not appear to activate NR1/NR2A (~0%).
Single-channel recordings for NR1/NR2D expressed in HEK293 cells using partial agonists (NH5PG, ethyl-, and propyl-NH5PG) and the full agonist glutamate enable us to determine which states in the process of channel activation can be modulated in an agonist-specific manner. These data show that glutamate, NHP5G, ethyl-, and propyl-NH5PG have strikingly different mean open times for NR1/NR2D (0.747, 0.432, 0.319, and 0.181 ms, respectively). We are currently comparing the rate constants for activation in models fitted to data from different partial agonists. We have also performed molecular dynamics (MD) simulations of the agonist binding domains for NR1/NR2A and NR1/NR2D bound with glutamate or propyl-NHP5G to predict how the agonists interact differentially with receptor subtypes. The synthesis of these lines of investigation will be used to identify structural elements that can be modified using mutagenesis to test working hypotheses on the structural basis for subunit-specific activation of NMDA receptors.

2717-Pos
Gating Effects of a Single-Residue Substitution in the Pore of NMDA Receptors
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NMDA receptors are glutamate-gated ion-channels with high Ca2+-permeability, voltage-dependent block by extracellular Mg2+, and slow gating kinetics. These intrinsic attributes are critical to coincidence detection and synaptic plasticity at excitatory synapses in brain. The GluN1/GluN2A isoform (N1/N2A) predominates in adult brain; it has strong voltage-dependent Mg2+ block and the fastest kinetics of all isoforms. In macroscopic measurements, Mg2+ block generates a region of steep negative slope in the current-voltage (I/V) relationship at membrane potentials negative to −50 mV. In single-channel current records, the binding of one Mg2+ ion precludes conduction by other cations and results in a discrete, resolvable gap. In the presence of strong metal chelators (EDTA) and for receptors that carry a single-residue substitution (N596G) in the N2A subunit (N1/N2AN+1G), the macroscopic I/V plot reverts to its normal linear shape and the Mg2+-dependent gap is absent from single-channel traces. To investigate whether the N+1G substitution in the pore impinges on the receptor's gating kinetics, we recorded single-channel current traces from cell-attached patches of HEK-cells containing only one N1/N2AN+1G receptor and compared these with those recorded from wild-type receptors under similar conditions. Measurements done in the absence of extracellular Mg2+ (1 mM EDTA in the recording pipette) revealed that the N+1G substitution caused a ~2-fold decrease in activity (Po, 0.31 $\pm$ 0.04, n = 10 vs. 0.65 $\pm$ 0.04, n = 12 for wild-type, $p < 0.001$) due to ~2-fold shorter openings and ~3-fold longer closures. Our results indicate that a perturbation in the pore that was intended to render the channels less sensitive to block by extracellular divalent cations also has a significant effect on the gating of NMDA receptors.

2718-Pos
Development of AMPA Receptor Aptamers
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The α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtype of glutamate ion channel receptors plays an essential role in the mammalian brain activities such as memory and learning, whereas the excessive receptor activation has been implicated in neurological diseases such as stroke, epilepsy, and amyotrophic lateral sclerosis. Inhibitors against AMPA glutamate receptors are drug candidates for a potential treatment of these neurological diseases. Using systematic evolution of ligands by exponential enrichment (SELEX), we have successfully identified three different classes of aptamers with nanomolar affinity against the GluR2Qflip receptor, a key AMPA receptor subunit that controls the calcium permeability and mediates excitotoxicity. One class is a group of competitive aptamers, which we selected by using NBQX, a classic competitive inhibitor. The highest potency or IC50 value for one of the aptamers in this class reached 30 nM, rivaling any exiting AMPA receptor inhibitors. We have also identified two other classes of aptamers that are differentially selective to different conformations of GluR2Qflip: one class uniquely inhibits the open-channel conformation whereas the other inhibits the closed-channel conformation. As an initial proof-of-principle experiment, our results suggest the possibility of developing aptamers that are nanomolar affinity, water-soluble and highly selective to both an AMPA receptor subunit and a unique receptor conformation. These aptamers are therefore excellent water-soluble templates for design of better inhibitors and better drug candidates against AMPA receptors.

2719-Pos
A Functional Probe of Ligand Binding and Agonist Efficacy in Ionotropic Glutamate Receptors
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Members of the ionotropic glutamate receptor (iGluR) family (NMDA, AMPA, and kainate receptors) are allosteric ligand-gated ion channels that mediate synaptic plasticity in the mammalian nervous system. Crystal structures of the two-lobed binding domains of these receptors suggest that ligand binding causes the lobes to move toward one another like a clamshell closing. It has also been proposed that the degree of closure is proportional to the degree of receptor activation. Using nonsense suppression methodology to incorporate unnatural amino acids, we have created a functional probe of ligand binding and the subsequent clamshell closure. By altering the electronic (e.g., converting glutamate to nitrohomoalanine) and steric (e.g., converting tyrosine to homotyrosine) properties of binding site residues of the NMDA and AMPA receptors, we have been able to define the requirements for both full and partial agonist binding and to confirm that agonist efficacy is related to the degree of clamshell closure.

2720-Pos
Modal Behavior 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. Cell attached patch recordings were performed with both full and partial agonists on HEK 293 cells stably expressing homomeric GluR3-flip receptor channels. Single channel data were analyzed using QuB software to detect channel conductance states and to determine a simple model of agonist dependent channel activity and underlying modal behavior. 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. The dwell times for the high mode are longer compared to the low mode. 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 full and partial agonists. These data suggests that the mechanisms of channel activation are similar for both full and partial agonists but the transition rates between states differ. Supported by NIH NS049223 and NS063518.