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sensory neurons can be targets of LPS, raising the possibility that they may also contribute to trigger and regulate innate immune responses.

1428-Pos Board B379

The Brite Side of TRPV1: Novel Role in Browning of White Adipocytes

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Obesity foreshadows metabolic syndrome. Sedentary life style and high calorie intake lead to obesity. Therefore, a strategy to prevent obesity and facilitate weight-loss is an urgent need. In an effort to achieve this goal, we report that dietary capsaicin (CAP; 0.01%) inhibited weight gain in high fat diet (HFD; 60% calories from fat)-fed wild type mice but not those genetically lacking transient receptor potential vanilloid 1 ($TRPV1^{-/-}$), without modifying *ad libitum* food or water intake. HFD inhibited TRPV1 expression, activity and facilitated fat accumulation in white adipose tissue (WAT) while dietary CAP antagonized the effects of HFD. Furthermore, introduction of CAP in diet suppressed HFD-induced weight gain in wild type mice. Analyses of mechanisms by which CAP antagonized HFD-induced obesity revealed that HFD suppressed TRPV1 expression and activity in adipocytes and CAP ablated this. Also, CAP significantly increased the expression of (1) brown fat marker genes - uncoupling protein-1, bone morphogenetic protein 8b and peroxysome proliferator activated receptor gamma (PPAR) coactivator-1 (PGC-1); (2) sirtuin 1 (SiRT1; NAD-dependent protein deacetylase - a gene that increases fat metabolism) and (3) PRDM-16 (a gene that regulates browning of white fat and promotes energy expenditure) in WAT of wild type but not $TRPV1^{-/-}$ mice. Consistently, dietary CAP increased metabolic activity of wild type mice. The increase in SiRT1 was associated with a concurrent decrease in acetylated PPAR in inguinal and epididymal adipose tissues, which is important for the recruitment of PRDM-16 to PPAR to induce browning of white adipose tissues. CAP also increased the expression of SiRT1 and PRDM-16 in brown adipose tissue. Collectively, we demonstrate that dietary CAP antagonizes obesity by stimulating the browning of WAT. Our work uncovers the emergence of TRPV1 agonists as new drug candidates to combat obesity.

Ligand-gated Channels I

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Protons Potentiate GluN1/GluN3A Glycinergic NMDA Receptor Currents

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GluN1 and GluN3 subunits of the N-methyl-D-aspartate receptor family form tetrameric cation-permeable channels that are gated by glycine alone and are insensitive to glutamate. They are expressed primarily during early development and their role in cellular physiology is unknown. One hypothesis states that their tonic activation by basal levels of brain glycine controls cellular excitability. Notably, GluN1/GluN3A receptors are selectively up-regulated following ischemia, while 'classical' GluN2A- and GluN2B-containing receptors are down-regulated and also strongly inhibited by ischemic acidification. We found that extracellular protons strongly potentiated peak glycine-elicited currents from recombinant GluN1/GluN3A receptors (Ipk, 10-fold at pH 6.8 versus pH 8.0) with a half maximal effect in the physiologic range (EC50 = pH 7.1 $\pm$ 0.03). The time-course of current desensitization was also significantly prolonged (2-fold at pH 6.8 versus pH 8.0) and the recovery from desensitization was accelerated (2-fold at pH 6.8 versus pH 7.4). In addition, extracellular protons decreased receptor sensitivity to the agonist glycine (EC50, 48 $\pm$ 6 μM at pH 7.4 versus 83 $\pm$ 3 μM at pH 6.8) and to the endogenous potentiator $Zn2+$ (EC50, 32 $\pm$ 3 μM at pH 7.4 versus 185 $\pm$ 26 μM at pH 6.8). Importantly, we found that extracellular acidification during glycine-elicited steady-state activity produced a large transient influx of positive charge (Ipk, 8-fold at pH 6.8 versus pH 7.4) and increased steady-state activity (Iss/Ipk, 0.03 $\pm$ 0.01 at pH 7.4 versus 0.5 $\pm$ 0.2 at pH 6.8) which depolarized the membrane substantially (-23 ± 5 mV at pH 7.4 versus -13 ± 3 mV at pH 6.8). Taken together, these results indicate that small pH fluctuations potently modulate GluN1/GluN3A receptor currents and that protons may play a novel positive modulatory role at GluN1/GluN3A receptors in vivo by increasing cellular excitability.

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Investigations of the Structural Mechanism of Modulation of the NMDA Receptor

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The NMDA receptor, one of the three main types of glutamate receptors, is involved in learning and memory formation. Upon activation by the agonists, the NMDA receptor forms a cation selective pore. The receptor is an obligate heterotetramer typically composed of GluN1 and GluN2 subunits. The GluN2 subunits can be one of four subtypes (A-D). Each subunit is organized into distinct domains, the intracellular carboxy-terminal domain, the transmembrane pore forming region, and extracellularly the agonist binding domain and the amino-terminal domain (ATD). The ATDs of the NMDA receptor contain the binding site for a number of modulators. Inhibitors and potentiators bind the ATDs with specificity for a particular GluN2 subtype. Zinc inhibits the receptor and has highest affinity for the GluN2A subtype and intermediate affinity for the GluN2B subtype. The synthetic compound ifenprodil inhibits receptors that contain the GluN2B subtype, and spermine potentiates receptors that also contain the GluN2B subtype. Extensive studies have focused on the mechanism of zinc inhibition, and previous work from our lab and others has shown that zinc inhibition proceeds via a cleft-closure conformational change. To determine if the mechanism employed by zinc was a common mechanism of inhibition, we used luminescence resonance energy transfer to map the conformational changes that the receptor undergoes upon binding of ifenprodil in GluN2B inhibition. Additionally, we monitored the conformational changes when the potentiator spermine binds. Interestingly, spermine potentiation of agonist-evoked current in GluN1-GluN2B containing receptors seems to proceed through an opposite structural mechanism to inhibition; both the GluN1 and GluN2B ATDs seem to be stabilized in an open conformational state. Additionally, the data suggest that the lower lobe of the GluN2 ATD twists in addition to moving towards or away from the upper lobe of the ATD.

1431-Pos Board B382

Effects of External and Internal Ca^{2+} on Unitary NMDA Receptor Properties

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N-Methyl-D-aspartate (NMDA) receptors are glutamate- and glycine-gated channels widely expressed throughout the central nervous system. When active, they generate electric and biochemical signals by fluxing Na^{+} and Ca^{2+} into the post-synaptic cell. The NMDA receptor-mediated Ca^{2+} transient initiates internal cascades that result in synaptic plasticity and excitotoxicity. In turn, Ca^{2+} regulates channel activity directly and indirectly through allosteric and 2nd messenger mechanisms that are poorly understood. We investigated the effects of external and internal Ca^{2+} concentrations on recombinant GluN1/GluN2A (N1/2A) receptors using single-channel current recordings, statistical analysis, and kinetic modeling. Increasing concentrations of extracellular Ca^{2+} reduced the unitary channel conductance from 75.9 $\pm$ 1.4 pS in 0 mM $[Ca^{2+}]_e$, to 55.2 $\pm$ 1.6 pS in 1.8 mM $[Ca^{2+}]_e$, and to 11.8 $\pm$ 0.7 pS in 75 mM $[Ca^{2+}]_e$. Importantly, receptors lacking the intracellular C-terminal domain (CTD) of the N1 subunit ($N1^{\Delta}$/2A) but not the 2A subunit ($N1/2A^{\Delta}$) exhibited higher unitary conductance in the absence of external Ca^{2+} ($N1^{\Delta}$/2A $\gamma = 83.3 \pm 1.0$ pS, $N1/2A^{\Delta}$ $\gamma = 79.2 \pm 1.4$ pS). This is consistent with a role for the N1 CTD in setting channel conductance for Na^{+}. However, in the presence of physiological external Ca^{2+} (1.8 mM), both exhibited $N1^{\Delta}$/2A ($\gamma = 50.4 \pm 1.2$ pS, $p<0.05$) and $N1/2A^{\Delta}$ ($\gamma = 60.8 \pm 2.0$ pS, $p<0.05$) channels had a lower conductance relative to wild-type. Based on these novel results, we asked whether this Ca^{2+}-dependent regulation of conductance depends on calmodulin binding to either the C0 or C1 cassettes of N1. Furthermore, we asked whether local Ca^{2+} influx through the NMDA receptor pore is sufficient for this regulation. Our results help to further unravel the Ca^{2+}-dependent processes that control the properties of individual NMDA receptor channels.

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NMDA Receptor smFRET Studies Reveal Role of Dynamics of the Agonist-Binding Domain in Mediating Agonist Efficacy

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Ionotropic glutamate receptors are tetrameric ligand-gated ion channels which mediate the majority of excitatory neurotransmission in the central nervous system. This family is subdivided into three classes: the AMPA receptor, the kainate receptor, and the NMDA receptor. Extent of cleft closure of the agonist-binding domain is one mechanism by which the agonist mediates channel activity for a number of the glutamate receptor subtypes. However, only an open-cleft or a closed-cleft conformation has seen in crystal structures of the glycine-binding GluN1 subunit of the NMDA receptor, and no partially-closed cleft states have been observed. Here, we have used single molecule

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 GluN1 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-Pos 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 antagonists 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-Pos 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 depending on the flow pattern used. These types of fluidics have 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-Pos 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-incidence detectors. Here we examine the biophysical property of negative co-operativity 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 then when GluN2A is unliganded. We also 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 combing these measures of conformational change with electrophysiology and mutations blocking co-operativity, we aim to elucidate the structural mechanism of negative co-operativity at NMDA receptors.

1436-Pos 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 neurogenerative 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 clamshell 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-hydroxyl-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 stepwise 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.

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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 c-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