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Improved Comparative Models of Human Gabaar Ligand-Gated Ion Channels Based on Structural Dynamics of GluCl
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Ligand-gated ion channels (LGICs) play a paramount role in the function of the central nervous system, and are critically important to understand effects e.g. of anesthetics, alcohol, or benzodiazepines that all modulate these receptors.
The fact that there are no structures available of human LGICs makes it difficult to relate experimental results to structural properties, although both simulations and electrophysiological studies on bacterial homologs have made it possible to develop the first models of potentiation and inhibition. A recent eukaryotic structure (X-ray structure of GluClα from *C. elegans*; PDB 3RHW) with higher similarity in sequence and function is opening another door to help us model and study human LGICs [1].
However, the presence of co-crystallized ligands in the structure is a major concern for computational studies as they might force the channel to a particular state. As part of our modeling efforts of human LGICs, we therefore performed 1μs simulations of GluCl with and without ligands in order to analyze the stability and the native state of the structure, and how it is affected by removal of the ligand. We found that presence of ivermectin indeed increases the subunit distance. Without ivermectin, subunits are closer and the intersubunit pocket volume drops by 50%. When ivermectin is present, the pore explores more open conformation(s) and the loops at the extracellular domain are more flexible, especially loop C where the neurotransmitters binding pocket is located. Based on our findings we have constructed different GABAaR models using GluClα structure as a main template together with prokaryotic structures (3EAM) to make it possible to model subunits themselves and the relative subunit placement independently.
1. Hibbs, R.E., and E. Gouaux. 2011. *Nature*. 474: 54-60.

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Analysis of the Interactions between GABA(A) Receptors and T3 using Electrophysiology and Molecular Dynamics Simulations
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Disorders of the thyroid cause a multitude of neurological dysfunctions including depression, anxiety, and psychosis. Thyroid hormones have been primarily thought to act via genomic mechanisms throughout the organism; however, recently another mechanism has been proposed for the adult brain. We hypothesize that the thyroid hormone triiodothyronine (T3) acts directly on GABA(A) receptors via a mechanism similar to that of neurosteroids. Previous electrophysiology experiments on expressed recombinant GABA(A) receptors demonstrated an inhibition of GABA responses in the presence of T3, and, at much higher concentrations of T3 alone, a direct stimulation of receptor activity. Current experiments are being conducted to investigate stereoselective effects and interactions with other compounds known to bind directly to the GABA(A) receptor. using a GABA(A) receptor homology model based on the crystal structure of the related Glutamate-gated chloride channel, atomic resolution molecular dynamics simulations were conducted to analyze the possible interaction of T3 and the GABA(A) receptor. In simulations, T3 is stable in binding sites in the transmembrane domain of the GABA(A)receptor in a region that is associated with the activation by neurosteroids. Alchemical free energy perturbation calculations are underway to test the affinity of T3 for the binding site in the GABA(A) receptor. Our results provide strong evidence supporting earlier experimental findings indicating a role of T3 in regulating the activity of GABA(A) receptors in brain, while bringing additional insight into both the molecular binding mode and the mechanism of modulation.

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A Mechanism for Potentiation of the GABA(A) Receptor by Bound Cholesterol
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Effects on a human GABA(A) receptor of cholesterol molecules bound to its transmembrane domain are investigated using modeling and explicit, all-atom molecular dynamics simulations. A new homology model of an α1 β1 γ2 GABA(A)R is constructed based on the crystal structure of the glutamate-gated chloride channel of C. elegans. The receptor is simulated with and without cholesterol molecules directly bound to the intersubunit sites where ivermectin is observed in GluCl. When not bound to cholesterol, the channel closes tightly within about 50 ns following an iris-like motion of helices M2 and M3, as predicted for other Cys-loop receptors. In the presence of directly bound cholesterol, iris motion does not occur and the pore constriction remains wider, although likely still too small for conduction of a solvated chloride ion. The cholesterol molecules are stabilized in the binding sites by hydrogen bonds with M2 Ser15'; however, widening of the pore does not appear to be mediated directly by these hydrogen bonds. Instead, it is consistent with a ''wedge'' mechanism in which cholesterol prevents contraction of the ring of M1-M3 helices, hence keeping the inner, pore-lining M2 ring open.

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The Activation Mechanism of Rat α3 Homomeric Glycine Receptors
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The α3-containing glycine channels (GlyR) are found in discrete areas of the spinal cord and hippocampus, but despite their likely physiological relevance, their kinetic properties are unknown. We investigated the activation mechanism of recombinant α3 rat homomeric glycine receptors. Cell-attached steady-state single channel recordings were obtained at 50 - 10000 μM glycine. Macroscopic synaptic-like glycine-evoked currents were obtained by applications of pulses of glycine (1 ms, 10 mM) to outside-out patches (intracellular chloride concentration 20 mM). Kinetic mechanisms were tested using maximum likelihood fits by the HJCFIT program to sets of idealized single channel records. The adequacy of each mechanism was judged by comparing the predictions of the model with the summary statistics of the single channel data and the time course of macroscopic deactivation.
The single channel open probability of homomeric α3 GlyR was strongly concentration-dependent, with a Hill slope of of 3.7 ± 0.1, much steeper than that of α GlyRs (1.82 ± 0.24, Beato et al., 2004). This suggests that α3 GlyR require all five binding sites to bind glycine in order to reach their maximum open probability. In other homomeric Cys-loop channels, including α GlyR, occupancy of three out of five sites is sufficient.
Other features of a3 GlyR activation were similar to those of other GlyR. In particular, the fully-liganded opening rate constant was 150,000 ± 24,000 s-1 and the overall efficacy was 67 ± 4. The microscopic affinity of glycine for the intermediate shut ''flip'' conformation was 160 ± 24 μM, approximately 5-fold higher than for the resting conformation (890 ± 80 μM; n = 3 sets). This accounted for the apparent cooperativity of the response.
Beato, Groot-Kormelink, Colquhoun & Sivilotti (2004). J Neurosci 24 , 895.

Bacterial Mechanics & Motility

3278-Pos Board B433
The Computational Analysis of Spirochete Motility in Viscous Fluids: Mimicking Host Reservoir Micro-Environments
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Borrelia burgdorferi, the causative agent of Lyme disease, exists in an enzootic life-cycle involving the transmission and acquisition between arthropod vectors, *Ixodes scapularis,* and mammalian reservoirs. While either escaping from or disseminating within the host, the spirochetes encounter both visco-elastic networks of complex polymers and diverse viscous fluids. This study aims to establish practical *in vitro* systems, which accurately replicate the physiological enzootic milieu, to observe and quantify the effects of environmental conditions on spirochete motility.
Gelatin matrices ranging from 2% (wt/vol) to 5% in concentration are utilized to reproduce the biomechanical behavior of numerous visco-elastic environments such as: the extracellular matrix, dermis and organ soft tissues, joint ligaments and tendons. The *in vitro* behavior of *Borrelia* in gelatin matrices intrinsically resembles the pathogen's movements in the chronically infected mouse dermis, and is characterized by four distinct motility states: non-motile, wriggling, lunging and translocating. Bio-physical modeling is used to demonstrate the relationship of transient membrane adhesions on motility state dynamics. Solutions of 1% (wt/vol) to 30% Ficoll, a non-ionic synthetic sucrose polymer, are used to imitate assorted viscous fluids encountered during spirochete infection, like: mammalian blood circulation, tick hemolymph, synovial and cerebrospinal fluids. Examination of spirochetes swimming in a purely viscous fluid simplifies the analysis of flagellar motor energy dissipation, enabling us to probe the speed-torque relationship of borrelial flagellum and motors. Analysis of the *Treponema pallidum* spirochete is included to help elucidate how differing morphological features, such as number of