then tested the GLIC-ELIC chimera with crotonic acid and picrotoxin. Crotonic acid inhibits GLIC with an IC50 of 110µM; our data indicate it binds to the extracellular domain. Picrotoxin (IC50 = 2-6µM) blocks the GLIC pore(4); it likely cannot access the ELIC pore(5), but may bind to the extracellular domain (IC50 = 96µM). These compounds were less potent than expected in the chimera (IC50 > 300µM). Overall the data suggest that domain specific effects may not be accurately reproduced in complex chimeras with intercommunicating domains, such as an orthodromic binding site and a pore in ligand-gated ion channels.


2170-Pos Board B307
Role of the Transmembrane α-Helix M4 in the Potentiation of Pentameric Ligand-Gated Ion Channels
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Glycine receptors are ligand-gated chloride channels that mediate much of the fast synaptic inhibition in the spinal cord and brainstem. They can exist as homomers (formed by five α1 subunits), or as heteromers (formed by 3 α1 subunits and 2 β subunits). One of our current lines of work is to understand the effect on glycine receptors of a range of agonist molecules with structures systematically changed from that of glycine. In this study, we selected two agonists, β-alanine and D-alanine, for detailed single channel analysis. By fitting kinetic schemes to cell-attached data we were able to get insight into the binding and gating of the glycine receptor in response to the different agonists. We also used concentration jump experiments in the outside-out configuration to validate the fitted data. Molecular dynamics simulations also allowed us to see how the agonists docked into the binding site. While binding and gating rate constants remained relatively similar between all agonists, the ability of the agonists to stabilise the pre-open flipped conformation varied in a manner similar to that reported by Lape et al. (Nature, 2008. 454 722-729). The less effective agonist D-alanine proved also to have a lower binding affinity than the other two agonists. Molecular docking studies were able to confirm that both D-alanine and β-alanine bind in a similar fashion to glycine. However, β-alanine shows a lot more mobility in the binding site, and this perhaps accounts for the large amount of heterogeneity in single channel recordings with this agonist.

2172-Pos Board B309
Evolution of Pro-Loop Channels: A Fresh Look at the Former Cys-Loop Family
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Cys-loop neurotransmitter-gated ion channels were a well-known superfamily of synaptic receptors. A small revolution occurred when Tasneem et al. identified prokaryotic members of the family that lacked both the eponymous cysteines and obviously the neuronal context of their eukaryotic relatives. A decade later, a new foray into the phylogeny and evolution of the family brings more surprises still, including related channels in various unicellular eukaryotes, as well as Cys-less members in a number of Metazoan species. This prompts a significant rewrite of the evolutionary history of the more aptly named “Pro-loop” channels, while leaving many more questions open.

2173-Pos Board B310
Electromagnetic Fields Inhibit Cys-Loop Receptor Function by Inducing a Novel Conformational State
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The fact that the general population is increasingly exposed to electromagnetic fields (EMFs) due to the advances in technology raises concern about their potential health effects. There are many gaps in knowledge, particularly at the molecular level, still needing to be filled before better health risk assessments can be made. We therefore studied the influence of EMFs on two Cys-loop receptors: muscle nicotinic (AChR) and 5-HT3A receptors. The transient exposure of cells expressing these receptors to EMFs (15 Hz-120 kHz) significantly decreases the peak current and increases the rise time of macroscopic currents elicited by the agonists. The peak current decreases as a function of EMF frequency (IC50 = 54 kHz for AChR). The effects on both receptors are qualitatively similar but more profound for 5-HT3A, indicating different sensitivity to the EMF within the receptor family. To understand the molecular basis leading to the macroscopic changes, we compared single-channel properties before and after the exposure to EMFs. Single-channel amplitude, open duration, duration of activation episodes (clusters) and open probability within clusters are not affected by the EMF. However, EMF leads to a profound decrease of the number of clusters as a direct function of frequency. The analysis reveals that EMFs induce a novel, non conductive, conformational state that arises from the closed resting state through a frequency-dependent transition. Thus, the stabilization of this novel state by EMF sequesters receptors from the activation pathway. Simulations of macroscopic and single-channel currents on the basis of a scheme including this new state well reproduce our experimental data. The identification of a novel conformational state induced by EMF enhances our understanding of receptor function, in general, and of the mechanisms by which EMFs affect neuronal excitability, in particular.

2174-Pos Board B311
Super-Resolution Imaging and Single Particle Tracking of Serotonin 5HT3A Receptor in Biomimetic Membranes
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The serotonin 5HT3 receptor is a member of the Cys-loop super family of ligand gated ion channels (LGIC). Like all members of this family, 5HT3 is composed of five independent subunits. The receptor has a large extracellular ligand gated domain for the activation of ligand gated ion channels. A small revolution occurred when Tasneem et al. identified prokaryotic members of the family that lacked both the eponymous cysteines and obviously the neuronal context of their eukaryotic relatives. A decade later, a new foray into the phylogeny and evolution of the family brings more surprises still, including related channels in various unicellular eukaryotes, as well as Cys-less members in a number of Metazoan species. This prompts a significant rewrite of the evolutionary history of the more aptly named “Pro-loop” channels, while leaving many more questions open.

The serotonin 5HT3 receptor can be efficiently incorporated into planar supported biomimetic membranes and the orientation can be controlled. The mobility of 5HT3 is greatly affected by both the orientation of the LGIC and the composition of the biomimetic assembly. We have used single molecule fluorescence imaging to track the mobility of individual proteins within the supported lipid bilayer and have used c-terminal labeling to determine the orientation of individual proteins. We have recently constructed a low temperature super-resolution microscope and have carried out experiments in frozen assemblies in order to identify the individual subunits that compose 5HT3A (the homopentamer of 5HT3).