contributes to GABAR subunit-subunit interfaces. We studied gating properties and surface expression of wild type (wt) α6β2γ2 and mutant α6(Q237R)β2γ2 receptors expressed in HEK293T cells. Transient currents were evoked using ultrafast application of 400-μsec pulses of saturating GABA (1 mM) applied to excised outside-out macropatches. Peak amplitudes of α6(Q237R)β2γ2 currents were not reduced but α6(Q237R)β2γ2 currents had slower activation (~1.9 msec), slower and more desensitization (~60 %), and faster deactivation (~2-fold) than wt currents. On-cell α6(Q237R)β2γ2 and α6β2γ2 single channel currents had similar mean open durations, but opening and burst frequencies were significantly higher for mutant than for wt currents. We assessed surface and total cellular expression of wt and mutant receptors using flow cytometry. Co-expression of α6(Q237R) with β2 and γ2L subunits resulted in no significant reduction in total or surface expression of any subunit. We concluded that this mutation alters gating, but not trafficking, of α6β2γ2 GABARs.

1481-Pos Board B391

A GABA-A ligand Gated ion Channel Screen - results and Best Practices

Cristian Ionescu-Zanetti, Qin Chen.

The $GABA_A$ receptors belong to a family of ligand-gated ion channels mediating fast synaptic transmission. They are drawing great attention in pharmaceutical field to their potential roles in the development of new therapeutics affecting anxiety, sleep disorders, and muscle relaxation. However, ligand gated ion channel screening has been hampered by the lack of suitable high throughput electrophysiology platforms. While some studies have shown that it is possible to record the GABA tail current and use that information during a screen, such methods have and inherently lower signal to noise ratio and cannot be used on faster desensitizers.

Here we present a the use of a novel electrophysiology screening platform integrating a microfluidics network for the study of $GABA_A$receptor pharmacology. This platform features fast (<100ms) solution exchange coupled with simultaneous data recording. A novel assay could monitor GABA response in real time, and obtain a 3 point EC_{50} dose curve within 1 minute.

The $GABA_A$ α1β3γ2 expressing HEK cells from Millipore were used for this study. The channel was targeted with agonists, including GABA and muscimol, inhibitors (picrotoxin, bicuculline, and gabazine), and positive modulators, including diazepam, zolpidem and chlordiazepoxide. The positive modulators produced concentration dependent augmentation of the GABA EC_{20} response. The pharmacology data determined using this method was consistent with the literature values obtained using other platforms. Statistical data for inter and intra-plate reproducibility, current stability, and Z-values, is used to validate this approach.

1482-Pos Board B392

Assigning Functional States to Structural Models of Nicotinic-Receptor Type ion Channels

Giovanni Gonzalez-Gutierrez, Claudio Grosman.

Aromatic-aromatic interactions are a prominent feature of the crystal structure of ELIC a bacterial member of the nicotinic-receptor superfamily of ion channels where five pore-facing phenylalanines come together to form a structure akin to a narrow iris that occludes the transmembrane pore. To identify the functional state of the channel that this structure represents, we engineered phenylalanines at various pore-facing positions of the muscle acetylcholine receptor, including the position that aligns with the native phenylalanine 246 of ELIC, and assessed the consequences of such mutations using electrophysiological and toxin-binding assays. From our experiments, we conclude that the interaction among the side chains of pore-facing phenylalanines leads to the formation of a non-conductive conformation that is unresponsive to the application of acetylcholine and is highly stable even in the absence of ligand. Moreover, electrophysiological recordings from a GLIC channel (another bacterial member of the superfamily) engineered to have a ring of phenylalanines at the corresponding pore-facing position suggest that this novel refractory state is distinct from the well-known desensitized state. It seems reasonable to propose, then, that it is in this peculiar non-conductive conformation that the ELIC channel was crystallized. It seems also reasonable to propose that, in the absence of rings of pore-facing aromatic side chains, such stable conformation may never be attained by the acetylcholine receptor. Incidentally, we also noticed that the response of the proton-gated, wild-type GLIC channel to a fast change in pH from 7.4 to 4.5 (on the extracellular side) is only transient, with the evoked current fading completely in a matter of seconds. This raises the possibility that the crystal structures of GLIC obtained at pH 4.0 and pH 4.6 correspond to the (well-known) desensitized state.

1483-Pos Board B393

Conformational Dynamics in a Nicotinic Receptor Homologue Probed by Simulations

Hugues Nury, Frédéric Poitevin, Catherine Van Renterghem, Toby Allen, Jean-Pierre Changeux, Pierre-Jean Corringer, Marc Delarue, **Marc Baaden**.

Recently discovered bacterial homologues of eukaryotic pentameric ligand-gated ion channels (LGICs) are increasingly used as structural and functional models of signal transduction in the nervous system. The available structural knowledge of LGICs increased lately with two crystal structures of bacterial homologs in distinct conformations. We crystallized the receptor from the bacteria Gloeobacter violaceus (GLIC), which is gated by protons, at acidic pH [1]. The structure reveals an open pore and molecular dynamics simulations suggest that the protein is stable on a 20 ns timescale when embedded in a lipid bilayer [1]. It can undergo large motion at neutral pH, and we will present a one-microsecond long molecular dynamics simulation of the GLIC channel pH stimulated gating mechanism [2]. The crystal structure of GLIC obtained at acidic pH in an open channel form is equilibrated in a membrane environment and then instantly set to neutral pH. The simulation shows a channel closure that rapidly takes place at the level of the hydrophobic furrow and a progressively increasing quaternary twist. The observed transitions suggest a possible two-step domino-like tertiary mechanism that takes place between adjacent subunits.

Further simulations are underway to better understand the influence of side-chain protonation states on the open state of this receptor at acidic pH, as well as its interactions with general anesthetics.

[1] N. Bocquet et al., Nature 457, 111-114 (2009)
[2] H. Nury et al., PNAS 107, 6275-80 (2010)

1484-Pos Board B394

Structural Rearrangements Underlying Mg2+ Dependent Gating in Cora

Olivier Dalmas, Eduardo Perozo.

The recent structures of CorA from Thermotoga maritima have provided an excellent model for a molecular understanding of Mg2+ transport. So far all crystal structures, obtained at high divalent cation concentrations, appear to be in a ''closed'' conformation. Macroscopic currents recorded from oocytes macropatches show that Mg2+-binding to the cytoplasmic side may act as a gating factor, defining CorA as a Mg2+-activated, Mg2+-channel. The role of the putative Mg2+ sensor was tested by engineering a salt bridge between residue D253 and D89 at the interface of the each monomer. The presence of a positive charge at either residue was sufficient to lock CorA in a closed conformation independent of the free [Mg2+].

Using site-directed spin-labeling EPR spectroscopy, we were able to detect Mg2+-dependent conformational transitions with an apparent KD close to 2 mM, a concentration in the physiological range of free [Mg2+] in cells. The structural rearrangements associated with CorA gating were measured on 106 positions along the stalk helix and TM1 segment, near the fivefold axis of symmetry. Strikingly, a significant increase in the spin-spin dipolar coupling at the tip of the stalk helix was detected from CW-EPR and DEER measurements. Mapping these EPR-determined conformational changes on the CorA crystal structure suggest an explicit structural mechanism for CorA opening, where an umbrella-like closing motion of the stalk helix would lead to an expansion of the pore immediately after the kink. This conformational wave propagate to the pore forming helix TM1 that rotates, ultimately increasing the diameter of the permeation pathway. This mechanism is supported by an increase in both, NieddA accessibility and probe mobility with a concomitant decrease in spin-spin coupling along the permeation pathway. Altogether, these results suggest a plausible molecular mechanism of gating in Magnesium channel related to CorA.

1485-Pos Board B395

Estimation of ion Channel Kinetics from Macroscopic Recordings

Luciano Moffatt.

A sizeable portion of the kinetic information present on macroscopic recordings is discarded by standard statistical analyses based on least-squares minimization. A more general approach that uses all the kinetic information present in the recording consists in maximizing the likelihood function, the probability of obtaining the data as a function of the parameters of the kinetic model.

The exact likelihood function can be calculated only for a very small number of channels. Approximations proposed for preparations above 30 channels work fine when the acquisition time is smaller than the time the preparation needs to change its state, but this is not usually the case on experimental recordings. To overcome this limitation we developed the Integrated Macroscopic Recursive algorithm an approximation that can be applied to experimental data.

This algorithm assumes for each measurement an a priori knowledge of the possible state of the ensemble of channels at the beginning and at the end of

each measurement. This knowledge is modeled by a multivariate normal distribution of the ratio of channels in each possible pair of starting and ending states, the later predicted by the kinetic model under test. By using Bayes theorem we calculate the posterior distribution that result after taking into account the current measurement and we calculate the partial likelihood of each measurement. The distribution of channels at the end of the measurements is then used to calculate the prior distribution of starting state-ending state pair of the next measurement interval.

We present a reliable approximation to the likelihood function that opens the door to several possibilities: a) estimate the kinetic parameters that best represent the experimental data with their error rates, b) to choose between alternative kinetic models and c) to optimize the experimental protocols.

1486-Pos Board B396

Effect of a Temperature Increase in the Non-Noxious Range on Proton-Evoked ASIC and TRPV1 Activity

Maxime G. Blanchard, Stephan Kellenberger.

Acid-sensing ion channels (ASICs) are neuronal H+-gated cation channels, and the transient receptor potential vanilloid 1 channel (TRPV1) is a multimodal cation channel activated by low pH, noxious heat, capsaicin, and voltage. ASICs and TRPV1 are both expressed in sensory neurons. It has been shown that raising the temperature increases TRPV1 and decreases ASIC H+-gated current amplitudes. To understand the underlying mechanisms, we have analyzed ASIC and TRPV1 function in a recombinant expression system and in dorsal root ganglion (DRG) neurons at room and physiological temperature. We show that in this range, the temperature does not affect the pH dependence of ASIC and TRPV1 activation. A temperature increase induces, however, a small alkaline shift of the pH dependence of steady-state inactivation of ASIC1a, ASIC1b, and ASIC2a. The decrease in ASIC peak current amplitudes at higher temperatures is likely in part due to the accelerated open channel inactivation kinetics and for some ASIC types to the changed pH dependence of steady-state inactivation. The increase in H+-activated TRPV1 current at the higher temperature is at least in part due to a hyperpolarizing shift in its voltage dependence. ASIC and TRPV1 currents of DRG neurons are similarly regulated by temperature as the cloned channels, with the exception that the decrease in peak ASIC current amplitudes at 35°C is more pronounced in DRG neurons. The H+-evoked depolarization measured under current-clamp was significantly reduced at 35°C for a sub-population of ASIC channels, without however affecting the number of action potentials. Our study shows that the contribution of TRPV1 relative to ASICs to H+-gated currents in DRG neurons increases with higher temperature and acidity. Still, ASICs remain the principal pH sensors of DRG neurons at 35°C in the pH range >=6.

1487-Pos Board B397

The Role of the Cytoplasimic Domain in pH-Dependent Gating by the KCSA Channel

Minako Hirano, Toru Ide.

Ion channels are membrane-spanning proteins that regulate ion flow across the membrane by responding to environmental changes. These changes are sensed by a sensor region, which then conveys the information to the pore region, which induces channel opening or closing. However, the mechanism for this conveyance remains unknown.

The KcsA channel is a representative potassium channel activated by protons (changes in pH). It is a tetramer with four homologous subunits, each of which has two transmembrane segments which form a transmembrane pore, an α-helix at the N terminus, and a cytoplasmic domain made of 35 amino acids at the C terminus. Studies have argued the region that senses protons and regulates gating is entirely within the transmembrane segments. However, we have found that the cytoplasmic domain may also be involved in these two functions.

Here, we show that the charged amino acids in the cytoplasmic domain, which makes up about half of this domain's amino acids, play an important role in pH-dependent gating. To investigate their effects, we made these amino acids neutral and measured gate activity. The mutant channel could be activated at what is normally inactive pH. This suggests that charges on the cytoplasmic domain generate electrostatic repulsion or attraction within the tetramer and influence KcsA channel activation in response to pH changes.

1488-Pos Board B398

Molecular Interactions Involved in KCSA pH Gating

Ameer N. Thompson, JASON MCCOY, David Posson, Crina M. Nimigean.

The bacterial potassium channel KcsA is gated by high concentrations of intracellular protons, allowing the channel to open at pH < 5.5. Replacing key ionizable residues from the N and C termini of KcsA with residues mimicking their protonated counterparts with respect to charge renders the channel open up to pH 9.0 (Thompson et al. 2008). We proposed that these residues function as the proton-binding sites. At neutral pH they form a complex network of inter- and intrasubunit salt bridges and hydrogen bonds near the bundle crossing, stabilizing the closed state. At acidic pH, these residues change their ionization state, thereby disrupting this network, favoring channel opening. While our previous work identified a network of residues involved in pH sensing, it did not rigorsously dissect the interactions that govern channel opening. To this end, we performed a series of single and pairwise mutations of the residues in the pH sensor. Using electrophysiology and X-ray crystallography we hope to gain a deeper insight into the mechanism of proton dependent gating in KcsA.

1489-Pos Board B399

Binding of Isoflurane to Glic Alters the Structure and Dynamics of the Protein

Dan Willenbring, Lu Tian Liu, Yan Xu, Pei Tang.

General anesthetics are thought to interact with proteins to produce anesthesia. Most of the experimental efforts to date have been focused on searching for discrete anesthetic binding sites. How exactly anesthetics work after the events of anesthetic binding is rarely explored. With the recent availability of two crystal structures for the bacterial Gloeobacter violaceus pentameric ligand-gated ion channel (GLIC), which is sensitive to a variety of general anesthetics, we performed multiple replica molecular dynamics simulations for more than 100 ns with and without the general anesthetic isoflurane. Multiple sites within the protein, primarily in the extracellular domain and at the interface of extracellular and transmembrane domains, provided favorable binding environments for isoflurane, showing little isoflurane translational displacement over the course of the simulations. Isoflurane caused changes to the structure and dynamics of GLIC when compared to the control simulation. Subunit-subunit interactions in the extracellular domain of the pentamer were disrupted, resulting in increased flexibility of the subunit pairs, as measured by Gaussian network model analysis. Salt-bridge linkages between and within the extracellular region of subunits containing isoflurane binding sites were altered by the presence of isoflurane, reflecting a change in the tertiary and quaternary structure of the channel. Changes were not isolated to the extracellular domain. The subunits containing more isoflurane binding sites also showed a greater change in tilt angle of the second transmembrane helices. Taken together, anesthetic binding at subunit interfaces can lead to changes in quaternary structure and global dynamics, causing allosteric changes in channel motional characteristics critical to gating. Supported by the NIH (R01GM56257, R01GM66358, R37GM049202, and T32GM075770), and the NSF through TeraGrid resources provided by PSC, NICS and TACC (TG-MCB050030N).

Acetylcholine Receptors

1490-Pos Board B400

Ligand-Induced Internal Molecular Dynamics of Nicotinic Acetylcholine Receptor Analysis by Diffracted X-Ray Tracking

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The nicotinic acetylcholine receptor (nAChR) is a pentameric ligand-gated ion channel in the central and the peripheral nervous system. The gating mechanism of nAChR is still unclear since the structure of nAChR in the presence of acetylcholine (ACh) has not been determined yet. Additionally, single molecular dynamics of nAChR in the presence of acetylcholine was not observed.

In this study, diffracted X-ray tracking (DXT) was applied for nAChR system. DXT is a method to track the X-ray diffraction spot from the gold nanocrystal labeled on an individual single protein and enables us to observe intermolecular dynamics of the protein in real time and real space. Dynamic twisting motions upon gating of KcsA, a pH sensitive potassium channel, were successfully revealed by the DXT [Shimizu *et al*. (2008). Cell *132*, 67-78]. At first step of new experiments, acetylcholine-binding protein (AChBP) was used as a model system of nAChR. AChBP is a structural and functional homologue of extracellular ligand-binding domain of nAChR. We investigated internal motions of AChBP by DXT in the absence and the presence of ACh, and found that ACh significantly activated the motion of AChBP. This result indicates that the ligand binding may initiate vigorous molecular fluctuations in AChBP, which was also confirmed by molecular elasticity measurement of AChBP with atomic force microscopy (AFM).

In order to apply the DXT for the full length of nAChR, technological innovations will be incorporated into the DXT, such as efficient preparations of gold nanocrystals and speedup of the time resolution of DXT (5-10 μs). Additionally, we can control the orientation of adsorbed nAChR. The improvement of our technology and experimental results will be discussed.