The Venus flytrap is a marvel of plant electrical, mechanical and biochemical engineering. The rapid closure of the Venus flytrap upper leaf in about 0.1 s is one of the fastest movements in the plant kingdom. We found earlier that the electrical stimulus between a midrib and a lobe closes the Venus flytrap upper leaf without mechanical stimulation of trigger hairs. The Venus flytrap can accumulate small subthreshold charges, and when the threshold value is reached, the trap closes. Thigmonastic movements in the sensitive plant Mimosa pudica L., associated with fast responses to environmental stimuli, appear to be regulated through electrical and chemical signal transductions. The thigmonastic responses of Mimosa pudica can be considered in three stages: stimulus perception, electrical signal transmission, and induction of mechanical, hydrodynamical and biochemical responses. We investigated the mechanical movements of the pinnae and petioles in Mimosa pudica induced by the electrical stimulation of a pulvinus, petiole, secondary pulvinus, or pinna by low electrical voltage and charge. Both voltage and electrical charge are responsible for the electro stimulated closing of a leaf. The mechanism behind closing the leaf in Mimosa pudica is discussed. The hydroelastic curvature mechanism closely describes the kinetics of Mimosa pudica leaf movements.

#### 2777-Pos

# Isoform- and Species-Specific Proteolysis of Cardiac Pacemaker Channels Jianying Huang, Han-Gang $\,\mathrm{Yu}.$

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Proteolysis of cardiac pacemaker channels affects biophysical properties of functional channels. Hyperpolarization-activated channels HCN2 and HCN4 can form homomeric or heteromeric functional pacemaker channels in cardiac ventricles. Employing Western blot and immunoprecipitation techniques with antibodies against N- or C- terminus of HCN2 or HCN4, respectively, we investigated protein expression patterns of endogenous HCN2 and HCN4 in cardiac ventricles of small (mouse, rat) and large (sheep, canine) animals and human. Using an antibody against N-terminus of HCN2, more full length protein at 100kD and less cleaved bands around 50kD were detected in small than in large animals. An additional cleaved band around 60kD was exclusively expressed in human. HCN2 C-terminal antibody could not detect any full length protein in all species tested. A 75kD cleaved band was detected in mice, rat, canine and substantially higher in sheep heart ventricles. A 60kD band was observed in human only. Using an N-terminal HCN4 antibody, the full length protein signals (at 160kD) were present in sheep and canine only. The cleaved bands near 100kD predominated in small animals but absent in large animals. With a C-terminal HCN4 antibody, the full length protein was observed in mice, barely detectable in rat, and clearly present in sheep, canine and human. A cleaved band around 100kD predominated in all animals. A minor cleaved band around 50kD appeared in all tested species except human. Overall, there was less HCN2 and more HCN4 proteolysis in small than in large animal cardiac ventricles. Endogenous myocardial HCN2 and HCN4 underwent intensive proteolysis at both N- and C- termini in an isoform- and species-specific pattern. In conclusion, results obtained from HCN2 and HCN4 protein expression in small animals may not be directly applied to large ones including human.

# 2778-Pos

# Mood Stabilizers Activate TREK-1, but not TREK-2

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### 2779-Pos

Color Shifted Channelrhodopsins- Towards Red Light Optogenetics Franziska Schneider, Matthias Prigge, Satoshi Tsunoda, Peter Hegemann. Humboldt University, Berlin, Germany.

Channelrhodopsins (ChRs) are microbial type rhodopsins functioning as light-sensitive cation channels in microalgae. Since channelrhodopsins

depolarize membranes in the light, they are used as optogenetic tools for generating action potentials in neurons by blue light flashes.

Recently we identified two channelrhodopsin variants in the colonial alga Volvox carteri. One of them named Volvox Channelrhodopsin 1 (VChR1) shows a red shifted action spectra with an absorption maximum at 548 nm. Although there is strong demand for a red-absorbing channelrhodopsin, application of VChR1 has been very limited due to its low expression level in neurons. Now we report about the expression of hybrids comprising fragments of VChR1, VChR2 and Chlamydomonas ChR2 with improved expression level. In addition we identified residues involved in color-tuning. Our goal is to provide ChR variants that in total cover the complete visible spectrum all the way from 400 to 600 nm.

#### 2780-Pos

# **Bifunctional Properties of Channel rhodopsin 2**

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Ever since its first characterization (Nagel *et al.*, 2003), Channelrhodopsin-2 (ChR2) has been used extensively in the light-activated control of neural cells in culture as well as in living animals. Here we describe its dual function as proton pump (in-line with for example Bacteriorhodopsin) and light-gated inward rectifying cation channel. Pump currents could be measured both in electrofused giant HEK293 cells and on planar lipid membranes.

We also present the determination of the wildtype (WT) and mutant single channel conductances under different conditions by means of stationary noise analysis. Whole cell recordings of a HEK293 cell line stably expressing the truncated ChR2 (amino acid residues 1-315), which behaves identically to the full length protein (Nagel *et al.*, 2003), or of semi-stable mutant ChR2 cell lines showed additional noise upon illumination. This noise is related to the opening and closing of the channel. From power spectra, the single channel conductance of was obtained (e.g. 91  $\pm$  25 fS for WT ChR2, -60 mV applied and 200 mM Guanidine $^+$  in the bath solution). The inward rectification could be observed on the scale of the single channel (bath: 200 mM Guanidine $^+$ , 0 mV to -60 mV applied). Also, a saturation of the single channel conductance could be observed at high Guanidine $^+$  concentrations.

#### 2781-Pos

## P2R in Eosinophils and Possible Role in Migration

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ATP and other nucleotides can be released from cells through regulated pathways or following the loss of plasma membrane integrity. These nucleotides act on P2 family of receptors that are divided in P2X ionotropic receptors and G protein-coupled P2Y receptors. Such receptors have been characterized in many rat immune cells, one exception are eosinophils which are involved in several pathological and physiological processes.

The eosinophils were obtained from peritoneal lavage of wistar rats followed by a purification step of Metrizamide density-gradient centrifugation. Firstly, we have performed an immunofluorescence characterization using antibodies against P2XR and P2YR. The cells were positives for P2X 1.2.4 and 7 and [[Unsupported Character - Codename ­]]P2Y 1,2 and 4. Our next step was to verify whether those receptors were functional using patch clamp recording which showed that ATP ( $1504 \pm 283 \text{ pA/pF}$ )and ATP $\gamma$ S ( $1231 \pm 164 \text{ pA/pF}$ ) were the most potent agonists where the others elicited little  $(\alpha,\beta)$  me ATP, ADP, BzATP,  $\beta,\gamma$  me ATP, 2me SATP) or no response (UDP, cAMP, adenosine). After that we have tested the participation of these receptors in eosinophils migration in vitro (1 or 2h) using a transwell chamber in order to investigate their possible physiological role. ATP and other agonists were able to increase migration, an effect which could be blocked by suramin, a general blocker of P2R. In keeping with this idea, we tested whether they are implicated in the migration of eosinophils using an inflammation model of rat allergic pleurisy. Our results suggest an increase of eosinophils migration induced by ATP. Corroborating with the transwell results, suramin also blocked migration.

As far as we are concerned, this study was the first to demonstrate that rat eosinophils express P2X and P2Y which can increase migration of eosinophils in *vitro* and *in vivo*.

### 2782-Pos

# The First Transmembrane Domain of a Drosophila Innexin is Loosely Packed

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A tryptophan-scanning technique was applied to the first transmembrane domain (M1) ofthe *Drosophila* gap junction protein ShakB(lethal) variant A