

and such measurements will be instrumental for understanding and probing properties of temperature sensors, analogous to the use of gating charge measurement for studying voltage-gated channels or binding assays for ligand-gated channels.

#### 618-Pos Board B398

##### Role of TRPV1 Channels in Glioma Cell Viability and Survival

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High grade primary brain tumors are among the deadliest malignancies known to medicine. Anaplastic gliomas (WHO grade 3) and Glioblastoma Multiforme, or GBM, (WHO grade 4) are prevalent primary brain tumors in adults. Exploration of new avenues in treatment of GBM is absolutely crucial when taking into consideration the short (12-17 months) survival time after the diagnosis. Annually there are around 20,000 new cases, including over 4,000 pediatric (age 0-19) cases. The heat and capsaicin receptor TRPV1 is highly expressed in malignant gliomas, where the channels are thought to play a role in inducing apoptotic cell death. We evaluated TRPV1 expression in different glioma cell lines and determined the correlation of the protein expression with age and sex in the tissue samples obtained from patients with GBM. We found that age dependent correlation of TRPV1 expression is more characteristic for male patients with GBM than for females. However, in both groups TRPV1 expression was higher in patients of age 35-55 years old.

We also investigated the sensitivity of different glioma cell lines to the TRPV1 agonists, including endovanilloid NADA and capsaicin. We found that 50  $\mu$ M of capsaicin inhibited the cell growth and proliferation of U87 but not U251 glioma cells. The presence of TRPV1 on the plasma membrane was higher in U87 cells in comparison to U251, as confirmed by biotinylation, and may account for the effect of capsaicin on the cell viability. These results indicate that TRPV1 could play an important role in regulating  $Ca^{2+}$  homeostasis of primary brain tumors.

#### 619-Pos Board B399

##### Dietary Capsaicin and Exercise: Analysis of a Two-Pronged Approach to Counteract Obesity

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Obesity contributes to diabetes, hypertension and myocardial infarction. Exercise is an effective measure to counteract obesity. Recent research demonstrates a regulatory role of transient receptor potential vanilloid 1 (TRPV1) in high fat diet (HFD)-induced obesity. Here, we evaluated the effects of exercise  $\pm$  dietary capsaicin (CAP, 0.01% of total HFD), an active ingredient in natural chili peppers and a TRPV1 agonist, on HFD-fed wild type and TRPV1<sup>-/-</sup> (TRPV1 knockout) mice. We evaluated the performance of normal chow diet (NCD) or HFD ( $\pm$  CAP)-fed mice on computer-controlled rotarod. Trained mice were exercised for 12 min./day for five days a week. HFD+CAP-fed mice walked on the rotarod for a longer duration of the exercise regimen (630  $\pm$  69 sec.) and showed lesser weight gain after 25 weeks of feeding (11.5  $\pm$  2.1 g) compared to exercised HFD-fed mice (440  $\pm$  215 sec.; 27.5  $\pm$  2.1 g). Both sedentary and exercised HFD-fed groups exhibited similar weight gain, albeit an increase in food consumption shown by exercised HFD-fed group. Also, exercised HFD + CAP-fed mice showed an increased metabolic activity compared to exercised HFD-fed group. Further, NCD-fed WT mice walked for longer duration on the rotarod (704  $\pm$  14 sec) and gained lesser weight at 20 weeks of feeding (4.5  $\pm$  0.7 g) than NCD-fed TRPV1<sup>-/-</sup> mice (665  $\pm$  50 sec.; 7.7  $\pm$  2.1 g). CAP prevented weight gain to a similar extent in both sedentary and exercised wild type mice. Also, Dietary CAP improved the endurance of mice on rotarod and counteracted HFD-induced suppression of muscle coordination. This suggests a novel role of TRPV1 in metabolism and muscle coordination function. Collectively, our data provide evidence for the role of TRPV1 and its activation by dietary CAP and exercise to inhibit HFD-induced obesity.

#### 620-Pos Board B400

##### Proton as a Dual Regulator for TRPV1

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TRPV1 is a pain-sensing polymodal receptor. In the body core where temperature is stable and capsaicin is normally absent, extracellular  $H^+$  (from tissue injury, inflammation, ischemia, etc.) serves as the best-known endogenous ligand. While  $H^+$  is known to strongly activate TRPV1, the underlying mechanism is still unclear. We observe two opposing effects of

$H^+$ : it strongly potentiates activation gating but inhibits ion permeation. The combination of these two effects produces a smaller current upon acidification and a prominent OFF response when  $H^+$  is removed. The permeation effect is voltage-dependent, indicating that  $H^+$  binds in the conducting pore to cause block. Correlations between TRPV1 cryo-EM structures and functional effects of point mutations allow us to investigate the mechanism underlying  $H^+$ -mediated permeation block. Inhibition of ion permeation substantially shifts the macroscopic dose-response relationship for  $H^+$  activation, which needs to be corrected for the study of gating effects.

#### 621-Pos Board B401

##### Unraveling Allosteric Coupling Mechanisms in the TRPV1 Channel

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The TRPV1 channel is a homotetrameric non-selective cation channel that functions in nociceptors as an integrator of external noxious stimuli and endogenous pro-inflammatory signaling molecules. The diversity of its modulators is staggering, including voltage, lipids, protons, cations, temperature, protein kinases, oxidizing and reducing agents and protein toxins. Structural perturbations throughout the receptor and some antagonists have been shown to selectively ablate specific modalities of channel activation without entirely disrupting others, suggesting that most stimuli use distinct molecular mechanisms for regulating the channel. Nevertheless, most stimuli that activate TRPV1 are strongly coupled, exhibiting synergy in both their efficacy and affinity. Here we studied the effects of small extracellular monovalent cations, which had been shown to inhibit channel activity, on temperature-, proton- and capsaicin-dependent activation of the TRPV1 channel. We found that substitution of extracellular sodium or potassium with N-methyl-D-glucamine has a strong effect on channel temperature sensitivity, shifting the threshold of activation to lower temperatures by  $> 20^\circ C$  and causing a 5-10-fold reduction in Q10. However, we also found that both heat and capsaicin can overcome the inhibitory effects of sodium and lead to channel activation in a sodium-independent manner once the cation-inhibitory site has reached saturation, suggesting these stimuli act through distinct mechanisms. In contrast, the potentiating effect of protons at pH  $< 6$  seems to be dependent on sodium concentration. We are currently performing experiments under elevated sodium conditions on proton-associated TRPV1 mutants to determine whether protons modulate the channel by two different mechanisms that cause channel activation or potentiation, and whether sodium is involved in either of these.

#### 622-Pos Board B402

##### Molecular Mechanism of TRPV1 Activation by Capsaicin

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TRPV1 is a capsaicin receptor with exquisite selectivity and sensitivity. How capsaicin binds and drives channel activation remain poorly understood. The TRPV1 cryo-EM structures at apo and capsaicin bounded state show clearly conformational rearrangements near the binding site, however the ligand itself is registered as a small volume that reflects neither the molecule's whole chemical structure nor specific ligand-channel interactions. Using an iterative approach of computational modeling by Rosetta and functional tests such as double-mutant cycle analysis, we found that the vanillyl moiety and amide group of capsaicin form specific interactions with the channel that fix their positions. The hydrophobic tail contributes to binding energy through non-specific hydrophobic interactions but may sample a range of conformations, making it invisible in an averaged structure. State-specific interactions between the ligand and TRPV1 suggest dynamic conformational transitions that may underlie the molecular mechanism of ligand driven activation.

#### 623-Pos Board B403

##### Insight into the Structure of Tetramer hTRPV1 from Homology Modeling, Molecular Docking, Molecular Dynamics Simulation and Virtual Screening

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Transient receptor potential vanilloid type 1 (TRPV1) is a heat-activated cation channel protein, which contributes to inflammation, acute