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Title of the MRP/TFM: Role of Nav1.8 in the regulation of the spontaneous and stimulus-evoked activity of mice corneal cold thermosensitive nerve terminals.

Summary of the Project:

Primary sensory neurons innervating the ocular surface (OS), whose cell bodies are located in the ophthalmic region of TG, play key roles in the detection of thermal, chemical and mechanical stimuli, which are transduced into electrical signals that eventually lead to action potentials that, in turn, reach and are processed by the Central Nervous System leading to sensations. This neural activity is also driven protective reflexes, and contributes to local inflammatory response (e.g., neurogenic inflammation). This pathway contains sensing and amplification steps starting with the sensory transduction. Diverse molecular sensors, such as Transient Receptor Potential channels (TRPs) present in the membrane of the peripheral terminals of primary sensory neurons are activated by exogenous chemical and physical stimuli, and by injury-associated endogenous mediators, leading to cation entry into the nerve terminal and its consequent membrane depolarization. This change in membrane potential is amplified by the opening of voltage-gated Na+ channels (NaV), eliciting action potentials whose instantaneous frequency is the variable that generally encodes the intensity of the stimulus. Conversely, the opening of K+ channels leads to membrane hyperpolarization, which on the one hand counterbalances excitability in resting conditions and, on the other hand, causes action potential repolarization, allowing NaV channels to recover from inactivation. Nevertheless, this a rather simple picture considering the broad variety of molecular mechanisms responsible for the functions of sensory neurons. For instance, primary sensory neurons express several types of NaV channels, including NaV1.1, NaV1.6, NaV1.7, NaV1.8 and NaV1.9. The first three are highly sensitive to tetrodotoxin (TTX) and are known as TTX-S (TTX-sensitive), whereas NaV 1.8 and NaV 19 are weakly sensitive to TTX and are known as TTX-R (TTX-resistant) (Rush et al., 2005). Although it is accepted that TTX-S channels contribute mainly to the fast depolarization phase of the action potential, whereas TTX-R channels contribute to Na+ influx during the whole action potential (Blair and Bean, 2002), the individual functions of these channels are not yet fully elucidated.

One type of primary sensory neurons innervating the OS are cold thermosensitive neurons, whose activity is involved in both detecting cold stimuli acting on the OS to evoke sensations (Gallar et al., 1993; Acosta et al., 2001), and the regulation of spontaneous blinking (Quallo et al., 2015) and basal tearing rate (Parra et al., 2010). Moreover, cold thermoreceptor neurons are the most affected under the pathological condition of chronic tear-deficiency (Kovacs et al., 2016), being increased both their spontaneous and cold-evoked activity as a result of the increased Na+ current and the reduced K+ currents developed under this condition (Kovacs et al., 2016).

The aim of this TFM would be, as one part of the current main project of the lab, to characterize the role of NaV 1.8 on the spontaneous and stimulus-evoked activity of cold thermoreceptor neurons in control (naïve) and tear-deficient animals. For that purpose, we will record “in vitro” from excised corneas the spontaneous and cold-evoked neural activity of the peripheral nerve terminals of corneal cold thermoreceptor neurons in wild type (WT) and Nav1.8 Knockout (KO) mice.

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Methods and technology involved in the MRP/TFM Project:

Focal recording of single cold nerve terminals innervating the cornea of mice will be recorded in the superfused cornea as previously described (Gonzalez-Gonzalez et al., 2017). Excised eyes will be placed in a recording chamber and superfused with saline solution of the following composition (in mM): 128 NaCl, 5 KCl, 1 NaH_2PO_4, 26 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgCl_2$, 10 glucose, pH 7.4, gassed with carbogen (95% O_2, 5% CO_2). The basal temperature of the bath solution will be kept at 34°C (the normal corneal surface temperature) with a home-made feed-back controlled Peltier device that also allows fast change of the chamber temperature. A glass pipette (tip diameter about ~50 µm; filled with physiological saline) gently applied onto the cornea with slight suction will allow focal recording of nerve terminal impulse (NTI) activity. Electrical signals will be amplified with an AC amplifier (Neurolog NL104; Digitimer, Welwyn, UK) and stored at 15 kHz in a computer, using a CED Micro1401 interface and Spike 2 software (both from Cambridge Electronic Design, Cambridge, UK). Ongoing NTI activity at 34ºC and the response of cold thermoreceptor terminals to cold (cooling ramps from 34º to 15ºC) and heat stimulation (heating ramp from 34º to 45ºC), and to perfusion with saline solution containing 100 µM menthol or 200 nM capsaicin will be recorded and analyzed. NTI firing patterns will be analyzed to assess periodicity, instantaneous frequency distribution, bursting pattern and autocorrelation indexes (Gonzalez-Gonzalez et al., 2017). NTI activity of naïve WT and NaV1.8 (KO) mice.

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Member/s of the lab who will act as tutor/co-tutor of the project (if different from the group IP):

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Proposal of Master Research Project / Proyecto fin de Master for the academic year 2023-24

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