Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

A translational model of brief diode laser stimulation for the investigation of heat transduction by TRPV1 and its relevance for human heat pain

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Pain is the vital sense preventing tissue damage by harmful noxious stimuli. The capsaicin receptor TRPV1 is known to be activated by noxious temperatures. However, acute heat pain is only marginally affected in mice after TRPV1 knockout while it is completely eliminated in mice, swine and humans lacking functional TRPV1- carrying fibers. Exploring contribution of candidate signal transduction mechanisms to heat pain in humans needs translational models for stimulation with noxious heat.

In this thesis, a translatable model for *in vivo* and *in vitro* heat stimulation was established and characterized. Focused, non-damaging, short near-infrared laser heat stimuli (wavelength 1470/1475 nm) are used to study the involvement of TRPV1-expressing nerve fibers in the encoding of heat pain intensity. Both lasers and the induced heating of dermal and epidermal nerve fibers as well as cells under the microscope were carefully characterized to demonstrate the translatability and enable comparison to existing models, as described in literature.

Limitations of temperature measurement were overcome by developing a Monte Carlo Multi Layer simulation model of laser-tissue interaction and resulting spatio-temporal heat profiles and peak temperatures. This simulation model was validated with data from literature and compared to own infrared measurements in skin and an agar phantom.

Encoding properties of transient, non-damaging laser heat stimuli of human psychophysics (both sexes) were compared to calcium transients as an indicator of heat-induced activation in native rat DRG neurons and heterologously expressing HEK293 cells using live cell calcium imaging. Heating of dermal and epidermal nerve fibers in humans with laser stimuli of ≥ 2.5 mJ (≥ 25 ms, 100 mW) induced pain that increased linearly as a function of stimulus intensity in double logarithmic space across two orders of magnitude. Thresholds were lower in the hands (2.6 mJ, about 53.3 °C) than in the feet (4.3 mJ, about 55.3 °C). Thresholds in glabrous skin were higher than in hairy skin; however, pain ratings were higher in glabrous skin. Pain thresholds in humans were about five times higher than in cells, suggesting a need for spatial or temporal summation in CNS processing of heat pain. Functional characteristics of TRPV1 in signal transduction were compared between native neurons and a heterologous expression system by assessing thresholds, suprathreshold encoding, strength-duration curves and tachyphylaxis. Thresholds (DRGs 0.56 mJ, HEK cells 0.52 mJ, about 40 °C) and strength duration curves were nearly identical in DRG and HEK cells with a threshold energy of about 0.6 mJ for a 100 µm spot (75 mJ mm^{-2}) when stimulating for about 5 ms, but threshold energy tended to increase with increasing stimulus duration, indicating a rheobase of 24 mW, which indicates the extent of radial heat loss at equilibrium (lateral heat diffusion). Tachyphylaxis upon repetitive stimulation occurred in HEK cells (54 %), DRGs (59 %), and humans (25 %). Thermal gating of TRPV1 is similar in transfected HEK cells and DRG neurons and transfection with TRPV1 is sufficient for induction of large calcium transients by laser stimulation.

The involvement of TRPV1 in signal transduction was compared between native neurons and the heterologous expression system. In DRG neurons and TRPV1-expressing HEK cells, heat sensitivity was limited to capsaicin-sensitive cells. To investigate the importance of TRPV1 for the encoding of non-damaging laser stimuli, the effect of the TRPV1 antagonist capsazepine

on laser heat sensitivity in the expression system was tested alongside the desensitization of TRPV1-carrying nerve fibers in humans using topical capsaicin (8 % patches). Responses to laser heat were significantly reduced by TRPV1 antagonist CPZ in TRPV1-transfected HEK cells (by 48.6 % vs. vehicle). Defunctionalization of TRPV1-carrying nerve fibers in humans abolished heat sensations completely, indicating that TRPV1-expressing nerve fibers encode transient non-damaging heat pain in humans. This hints that possible compensating mechanisms were defunctionalized as well.

Diode laser stimulation provides an experimental model suitable for *in vitro* and *in vivo* heat stimulation, inducing non-invasive, non-damaging, transient activation of heat-sensitive TRPV1 receptors. The developed simulation model provides information about temperature changes, particularly when temperature measurements are limited. Expression of TRPV1 fully conveys laser heat response properties observed in human heat pain and native sensory neurons to non-excitable HEK293 cells. TRPV1-mediated tachyphylaxis is an important modulator of heat pain sensitivity. These findings suggest that TRPV1 expressed in dermal and epidermal populations of nociceptors serves as first-line defense against heat injury.