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Short communication

Humans, but not animals, perceive the thermal grill illusion as painful

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HIGHLIGHTS

- Confirmation of a painful experience to the thermal grill illusion (TGI) in humans.
- Neither rodents nor cats showed behavioural pain correlates when exposed to TGI.
- Hence, the relevance of TGI mechanisms derived from animal models is unclear.
- The results reinforce the value of behavioural measures in preclinical pain research.

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ABSTRACT

Simultaneous presentation of alternating innocuous warm and cold stimuli induces in most humans a painful sensation called thermal grill illusion (TGI). Here, pain is elicited although nociceptors are not activated. Upon back-translation of behavioural correlates from humans to animals, we found that neither cats nor rodents show adverse reactions when exposed to TGI stimulation. These results question that a TGI observed as a pain-related change in behaviour can be elicited in animals. While distinct neuronal patterns as previously reported may be measurable in animals upon TGI stimulation, their translational meaning towards the sensation elicited in humans is unclear.

In normal tissue pain is usually elicited when nociceptors are excited by noxious stimuli. However, the interlacing of innocuous cold and warm stimuli to the skin can cause the thermal grill illusion (TGI), a peculiar painful sensation without activation of nociceptors. Thus, the TGI has been discussed as a potentially valuable model for chronic pain states without nociceptor input, e.g. pain that outlasts tissue damage, pain in association with psychiatric disorders [1,2], or central neuropathic pain [3]. Most studies to date have been performed in humans, characterizing spatial and temporal TGI parameters [4], qualities and intensities of the perceived sensations [5,6], the influence of different diseases [1,2,7] and brain areas involved [5,8,9]. However, one study in anesthetized cats utilized electrophysiological recordings from the spinal cord and provided evidence that the TGI may result from altered spinal nociceptive processing [10]. In this study, the simultaneous presentation of innocuous 20 °C and 40 °C stimuli led to decreased responses of cells specific for innocuous cold stimuli, while the responses of multimodal heat, pinch and cold (HPC) cells remained unaltered, thereby causing a relative overweight of the latter. Such balance shifts have been described for noxious cold and moderate heat stimuli. In our study, we aimed at back-translating the sensations evoked in humans to animals in order to further characterize the underlying neuronal mechanisms in an animal model. For this reason, results in humans were confirmed and thermal grill devices for mice, rats and cats were designed and manufactured, and validated with respect to behavioural responses.

For the studies in healthy volunteers, a device was used which we have previously described and validated [11]. In brief, the device

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consisted of 15 aluminum tubes with 16 cm length and 12 mm diameter and a distance between tubes of 2.5 mm (see Fig. 1A). Heating and cooling of these tubes was accomplished by perfusion with either warm or cold water which was regulated to the respective temperatures using thermostats (cold: Huber UCO15, 25 L/min, Germany; warm: Julabo ED5, 15 L/min, Germany). Effective temperatures at the tubes were controlled using a digital thermometer. Nineteen participants (age 26.4 ± 4.1 years, 10 male, 9 female) were recruited by flyer advertisement and email requests. All participants were right-handed, according to the German version of the Edinburgh Handedness Inventory [12]. Subjects who presented with one of the following conditions were excluded: any organic origin of pain complaints, chronic pain, any neurological or psychiatric signs or symptoms as assessed by a standardized interview and a clinical examination, current use of analgesic or antidepressant medication, alcohol or substance dependency and use of alcohol within 12 h before the experiment. For inclusion, all subjects were required to give written informed consent to a protocol approved by the Ethics Committee of the Medical Faculty of the Friedrich-Schiller-University, Jena.

At first, cold and heat pain thresholds (CPT and HPT) of both palms were determined consecutively by an ascending method of limits as described previously [13]. In brief, a 9 cm^2 contact thermal stimulator (TSA-2001; Medoc, Israel) was used with a temperature decrease or increase of 0.5 °C/s (baseline temperature: 32.0 °C; minimal temperature: 0 °C, maximal temperature: 53.0 °C). To determine thermal pain thresholds, participants were asked to follow the written instruction: "When thermal perception becomes painful, press the stop button immediately." The investigation started with three learning trials and continued with five consecutive tests. The mean of the last five trials was calculated and used as the CPT or HPT for further experiments. Subjects were further asked to rate cold and heat stimuli regarding pain intensity for thresholds and all grill experiments (see below). For this assessment, a visual analogue scale (VAS) of 100 mm length was used on which participants indicated pain intensity. Here, the very left end of VAS indicated no pain (0 mm), while the very right end represented worst imaginable pain (100 mm). Based on thermal pain thresholds, the following combinations of cold and warm bars of the grill were chosen as test conditions: T_{cold} = CPT + 14 °C and T_{warm} = HPT–14 °C. Similarly, T_{cold} and T_{warm} of bars were set to 12 °C, 10 °C, 8 °C, 6 °C, 4 °C or 2 °C above or below CPT or HPT, respectively. Furthermore, the combination of the determined CPT and HPT was applied, similar to previously used protocols [1,2,11]. After setting the calculated temperatures as controlled by the attached digital thermometer, participants were asked to place their right or left hand in randomized order on the device for 30 s. After removing the hand participants had to indicate pain on the VAS. From the VAS pain ratings, the percentage of so-called responders was obtained, that is, of those participants who experienced the presented temperature differential to be painful. Considering the variability of VAS pain tests, statistical analysis revealed that VAS values greater than 6/100 indicate a painful sensation, such that participants were considered responders when values of 7/100 and higher were measured on the VAS scale, as applied previously [1,2]. Intervals between measurements were at least 3 min. Since data from both hands were comparable, only data from the right hands of the participants were included in the analyses.

For the studies in mice and rats, a new device was designed and built, aiming at a down-scaling according to the size of the hind paws of these animals (Fig. 1D–F). The main challenge was to present stable warm and cold stimuli, which was solved by choosing specific materials, distances between tubes and elaborate thermostat technology. The miniaturized device consisted of 21 stainless steel tubes of 9 cm length and 1.2 mm diameter and a distance between tubes of 1.8 mm to ensure proper insulation between temperatures (see Fig. 1C,F). Again, tubes were perfused by water of different temperature, yet in order to reach the desired temperatures more quickly, one water bath contained water of 1.0 °C (which was the lowest temperature that could be used, resulting in effective lowest device temperatures of 1.5 °C) and the other water of approx. 60 °C. These were mixed using electronically regulated proportional valves (ASCO Joucomatic, Germany) to reach the intended temperatures (also see Supplementary Fig. S1A in the online version, at doi:10.1016/j.bbr.2016.07.020). Again, effective temperatures at the tubes were calibrated using a digital thermometer. In cats, the device which was also employed in humans was used, yet with an additional box to contain the animal during the experiment and with sufficient space to place the paws next to the cold and warm bars, thereby allowing the cats to escape the stimulus once it became painful (see Supplementary Fig. S1C, D in the online version, at doi:10.1016/j.bbr.2016.07.020). In order to verify that the chosen temperatures were effective at the different tubes and to exclude any mixing phenomena, both devices were examined using an infrared camera (Variocam head, Jenoptik, Jena, Germany) which showed that the alternating bars indeed had the temperature set at the thermostats (see examples for a combination of 10 °C and 40 °C in Fig. 1C, F for the mouse/rat and the cat/human device, respectively). Animal experiments were performed according to local regulations with the approval of the local authorities (Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz, TLLV, Ref. 02-041/08 for mice and rats,

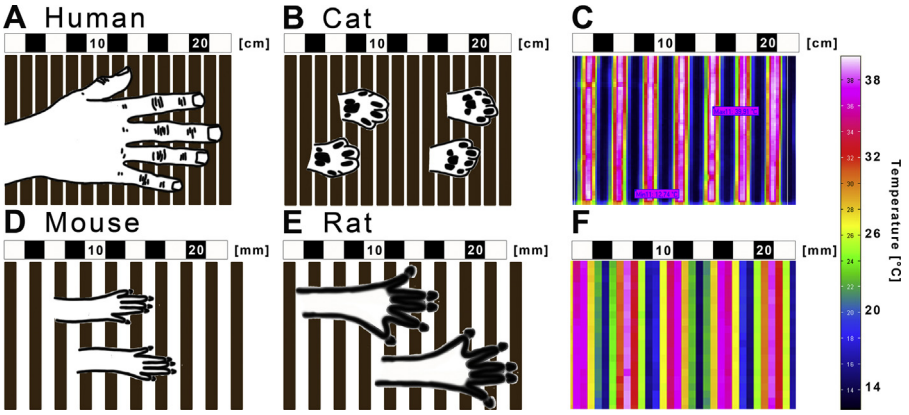

Fig. 1. Device scaling and temperature distribution. Human hand (A) and cat front and hind paws (B) in relation to the larger thermal grill device. Mouse (D) and rat hind paws (E) in relation to the miniaturized thermal grill device. Infrared camera pictures verifying clear distinction between warm and cold bars for the large (C) and the small device (F).