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Behavioral characteristics of capsaicin mediated cutaneous, myogenic, and arthrogenic orofacial nociception in rats

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ABSTRACT

Objective: To assess changes in orofacial tactile sensitivity and gnawing related to capsaicin-mediated cutaneous, myogenic, and arthrogenic nociception in the rat.

Design: After recovery from anesthesia, orofacial tactile sensitivity and gnawing were assessed using operant testing methods following capsaicin application. Twenty female CD-Hairless rats were tested with bilateral capsaicin cream application to the cheek or with isoflurane anesthesia alone. Following several weeks of recovery, animals (n = 20) received either 10 µL unilateral masseter injections of vehicle, or phosphate buffered saline (PBS) to assess injection sensitization. After several weeks, masseter capsaicin (1.0%) injections (10 µL) were assessed compared to vehicle and PBS (n = 13). Weeks later capsaicin TMJ injections were evaluated. Animals (n = 11) received either 10 µL unilateral TMJ injections of capsaicin solution (1%) or vehicle.

Results: Capsaicin cream to the skin significantly altered gnawing activity (increased puncture time by 248 s ($p = 0.0002$)) and tactile sensitivity (decreased tolerated bottle distance by 0.980 cm compared to isoflurane only ($p = 0.0001$)). Similarly, capsaicin masseter injection increased puncture time (339.6 s, $p = 0.07$) and decreased tolerated bottle distance (1.04 cm, $p = 0.005$) compared to vehicle. However, intra-articular capsaicin in the TMJ only modified gnawing (increased puncture time by 133 s), with no changes found in tactile sensitivity compared to vehicle.

Conclusion: Application of capsaicin to the skin and masseter had similar behavioral effects; however, intra-articular injections to the TMJ only affected gnawing. These data indicate the behavioral changes in rodent models of myogenic and cutaneous pain may be markedly different than models of arthrogenic pain originating from the TMJ.

1. Introduction

Temporomandibular disorders (TMDs) are the second most commonly occurring musculoskeletal condition resulting in pain and disability, affecting 5–12% of the population (NIH, 2018). Patients with TMDs present a variety of symptoms, including irregular temporomandibular joint (TMJ) sounds, muscle and joint pain, and limited jaw movement (Peck et al., 2014; Velly et al., 2010). Providing new treatment options has proven difficult, exacerbated by the diversity of clinical causes and symptoms.

Behavioral testing is often used to investigate rodent models of pain and disability. For orofacial tactile sensitivity, current methods typically measure a withdrawal response using an investigator driven stimulus (Imamura, Kawamoto, & Nakanishi, 1997; Vos & Strassman, 1995). While semi-quantitative in nature, these particular tests can produce misrepresentative results due animal stress and experimenter bias, making comparisons across experimenters and labs challenging (Balcombe, Barnard, & Sandusky, 2004; Chesler, Wilson, Lariviere, Rodriguez-Zas, & Mogil, 2002; Imbe, Iwai-Liao, & Senba, 2006; Terman, Shavit, Lewis, Cannon, & Liebeskind, 1984). Operant-based behavioral tests allow the animal to make a participation decision, the results of which can be interpreted to assess pain and dysfunction. Moreover,

Abbreviations: TMJ, temporomandibular joint; TMD, temporomandibular disorder; IACUC, The Institutional Animal Care and Use Committee

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allowing the animal to make decisions factoring their individual pain or capabilities removes the experimenter from the assay, reducing both animal stress and experimenter bias. As such, operant assays of orofacial pain have potential to produce more consistent results across experimenters and labs (Cha, Kohan, Zuo, Ling, & Gu, 2012; Mauderli, Acosta-Rua, & Vierck, 2000; Neubert et al., 2005; Nolan, Hester, Bokrand-Donatelli, Caudle, & Neubert, 2011).

Capsaicin can be used to elicit hypersensitivity in tissues and provide a localized pain response. Capsaicin selectively binds to TRPV1 receptors (Rosenbaum & Simon, 2007) causing a release of neuropeptides from sensitized afferent terminals. Arteriolar vasodilation, plasma extravasation, and pain including hypersensitivity and allodynia are a result of application to tissues (Gazerani, Wang, Cairns, Svensson, & Arendt-Nielsen, 2006; Holzer, 1993, 1991; Jancsó, Jancsó-Gábor, & Szolcsányi, 1967; Jancsó, Jancsó-Gábor, & Szolcsányi, 1968; LaMotte, Lundberg, & Torebjörk, 1992; Lembeck & Holzer, 1979; Lin, Li, Xu, Zou, & Fang, 2007; Nilsson et al., 2014). Injected capsaicin produces thermal and mechanical hyperalgesia and edema at the injection site for over two hours in the hind paw, (Gilchrist, Allard, & Simone, 1996; Kinnman & Levine, 1995; Zhang, Wu, Fang, & Willis, 2003), masseter muscle (Ro, Lee, & Zhang, 2009; Ro, Lee, Capra, & Zhang, 2007) and TMJ of rats (Tang, Haas, & Hu, 2004). Application of capsaicin can also be used to model clinical pain related to TRPV1 activation.

In this paper, tactile hypersensitivity and gnawing are assessed in capsaicin-mediated models of cutaneous, myogenic, and arthrogenic pain using operant testing methods developed by our group (Rohrs et al., 2015). While capsaicin application does not directly mimic clinical etiology of TMD, the methods of evaluating rodent models of orofacial pain and TMJ dysfunction described here can be used to increase our understanding of clinical TMD etiology and pain. Etiologies of TMDs can have varying presentation of clinical symptoms including tactile hypersensitivity, masticatory muscle pain and joint pain. As such, future rodent models of TMD, that may more appropriately mimic clinical TMD etiology, can be appropriately evaluated using operant-based testing for orofacial hypersensitivity and TMJ dysfunction. Evaluating clinically relevant rodent models of TMD using operant-based methods will lead to more effective treatment options for TMD patients.

2. Methods

2.1. Animals

The testing procedures and general handling of animals described herein are in compliance with the ethical guidelines and standards established by the Institutional Animal Care & Use Committee (IACUC) at the University of Florida and the Guide for Care and Use of Laboratory Animals. All procedures were performed on IACUC approved research protocols (National Research Council (US), 2011).

2.2. Experimental design

Twenty, female CD-Hairless rats (3 month old, Charles River) underwent the testing procedures described in Fig. 1. To establish baseline behavior, animals were tested every 2–4 days until a consistent behavioral baseline was established (Baseline 1). Consistent baseline behavior was classified as 3 consecutive trials without significant change in mean result (bottle distance or puncture time, procedures detailed below). If animals did exhibit consistent baseline behavior or failed to participate in the operant based behavioral assays, the animal was excluded from all subsequent testing (3 animals removed).

Orofacial capsaicin cream application was first utilized to investigate behavioral changes associated with cutaneous hypersensitivity (procedures detailed below). For this experiment, animals were separated into two groups (n = 8, 9). Group 1 received capsaicin cream applied to the skin over the masseter under isoflurane anesthesia, while Group 2 animals received isoflurane only. The groups were then switched every 2 days and this process was repeated for three additional trials, resulting in four total capsaicin cream sensitivity trials. The first two trials were used to test orofacial tactile sensitivity, while the third and fourth trials were used to test gnawing. Using the crossed-group experimental design, all animals were tested under both treatment conditions for both behavioral tests.

Following cutaneous testing, animals were separated into three groups to examine the behavioral effects of masseter injections (non-noxious, procedures detailed below). The first set of experiments investigated the masseter injection alone, as muscle injection can incite hypersensitivity by themselves (Lund et al., 2010). Two groups received masseter injections of phosphate buffered saline (PBS, pH 7.4, n = 5) or vehicle (n = 6) under isoflurane anesthesia, while the third received isoflurane anesthesia only (n = 6). At 20 min after recovery from anesthesia, gnawing was tested for 10 min immediately followed by 10 min of orofacial tactile sensitivity testing.

To ensure baseline behavior remained consistent, remaining animals (n = 13) were tested at mid-point, with all animals assessed for tactile sensitivity and gnawing over three days with no treatments (Baseline).

Following the second baseline, the effects of capsaicin masseter injections were investigated. Here, animals were separated into three groups: injection of capsaicin (1%), vehicle, or PBS (n = 5, 4, 4). Again, at 20 min after recovery from anesthesia, gnawing was tested for 10 min immediately followed by 10 min of orofacial tactile sensitivity testing.

Finally, the remaining eleven CD-Hairless rats were used to evaluate the effects of intra-articular capsaicin (1%) injection to the rat TMJ. Here, animals were separated into two groups: injection of capsaicin or vehicle. As with cutaneous sensitivity testing, animal groups were switched three days later and re-assessed, resulting in n = 11 for both treatments. For behavioral testing on both testing days, animals recovered from anesthesia for 20 min; then, gnawing was tested for 10 min immediately followed by 10 min of orofacial tactile sensitivity testing. Following this experiment, all animals were humanely euthanized.

2.3. Capsaicin application to the skin

To assess the behavioral effects of capsaicin-mediated cutaneous hypersensitivity in the orofacial region of rats, animals were lightly anesthetized over 5 min using 3–4% isoflurane in an induction box, then transferred to 2% isoflurane via mask inhalation where capsaicin cream (0.1%, Capsazin-HP) was applied bilaterally to the skin over the masseter (Neubert, Rossi, Malphurs, Vierck, & Caudle, 2006; Rohrs et al., 2015). After 5 min, capsaicin cream was removed with a damp towel. For controls, animals were anesthetized for 10 min to match the total anesthesia time for capsaicin treatment, but did not receive any cream or solution on their skin. All animals were placed in a warm recovery box for 20 min prior to behavioral testing.

2.4. Masseter injection protocol

To assess the behavioral effects of capsaicin-mediated myogenic hypersensitivity in the orofacial region of rats, capsaicin solution was prepared by dissolving 10% capsaicin (Sigma-Aldrich) in ethanol, then mixing capsaicin in ethanol solution with Tween-80 (Sigma-Aldrich) and PBS in 1:1:8 ratio respectively. Vehicle solutions were prepared by mixing ethanol, Tween-80, and PBS (1:1:8 ratio). Animals were lightly anesthetized over 5 min using 3–4% isoflurane in an induction box, then transferred to 2% isoflurane via mask inhalation. The skin was prepared for injection using povidone-iodine and ethanol in triplicate, followed by a fourth application of povidone-iodine. For injections, all animals received a 10 μL injection to the right masseter using a 29 gauge insulin syringe (Ambalavanar et al., 2006; Niu, Saloman, Zhang, & Ro, 2011; Tang et al., 2004). Isoflurane only control group was