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Research report

Effects of hot and cold stimulus combinations on the thermal preference of rats

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ABSTRACT

Traditional evaluation of pain in animals has primarily used reflexive withdrawal or nocifensive response from singly presented stimulation. However, daily experience of thermal sensation involves situations in which rapid temperature changes from cold to hot can occur. Therefore, in order to better understand integration of competing stimuli and their role in the motivational character of pain perception, behavioral tasks have been adapted to evaluate treatment-driven changes in hindpaws when exposed to two or more stimuli. However, such assessments of craniofacial sensitivity are lacking. In this study, we sought to characterize thermal preference for facial stimulation when rats are given the option of experiencing a hot or cold stimulus to obtain a milk reward, or abstaining from stimulation. We found that when both cold and hot stimuli were either non-noxious or where both stimuli were noxious the hot stimulus was preferred. When the hot stimulus was noxious, non-noxious cold was preferred. Unstimulated time was dependent on the combined aversiveness of the two stimuli, such that unstimulated time was the greatest with a highly aversive stimulus pair (−4 and 48 °C). We also found that pairing stimuli modulated successful task completion for each stimulus, but for nociceptive heat, this was not solely a consequence of thermal preference. Finally, we found that previous preference could both induce and abolish subsequent thermode preference independent of stimulus cues. The findings in this study will allow us to evaluate experimental pain states and analgesic treatments in a manner more relatable to the experience of the patient.

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1. Introduction

Traditional behavioral testing methods for evaluating thermal processing in animals have primarily used reflexive withdrawal from a single hot or cold stimulus. However, in our daily experience we encounter a mix of thermal stimuli that produce complex sensations. Even in a non-pathological state, the summation of disparate stimuli can produce unpleasant or painful sensations that are greater than might be produced by an individual stimulus, as occurs in the thermal grill illusion [9]. Recent molecular and electrophysiological studies indicate that this phenomenon may be due in part to the co-expression of molecular mediators of hot and cold thermal transduction within a single nociceptor, such as overlapping expression among transient receptor potential channels vanilloid 1 (TRPV1), melastatin 8 (TRPM8), and ankyrin 1 (TRPA1) [15,36]. Populations of heat- and/or cold-sensing nociceptors can also provide convergent input to second order neurons that transmit noxious signals to higher centers [10,37,38]. The central integration of these stimuli, as well as the context in which they are presented, influences pain perception and motivation.

Thus it is important to accompany molecular and electrophysiological studies with behavioral assays that evaluate pain-related decision making in the presence of differing stimuli. Some groups have used the thermal preference task to evaluate sensory integration of stimuli detected in the hindpaws of rodents either with two stimulating options [18,31,32,34] or a gradient of stimuli [18]. While this is sufficient to evaluate pain states targeting the hindpaw, no such method exists to evaluate pain within the head and face. It is important to evaluate facial pain specifically because there are forms of chronic pain unique to the trigeminal system, such as headaches and trigeminal neuralgia. Some differences have also been noted between the trigeminal and sciatic nervous systems, including differences in basal expression of the TRPM8 important for cold perception [15], expression of the inflammatory mediator interleukin-6 following chronic constriction injury [17], and the efficacy of serotonin antagonists [13]. These differences could have implications for how pain is processed in the craniofacial region.

We previously characterized an operant assay that assesses the ability of rats to obtain a milk reward while self-stimulating the face with a single stimulus [23,24]. We have also presented thermal preference for a hot stimulus with a combination of −4 and

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48 °C [24], which are both noxious, i.e. capable of causing tissue damage with sufficient duration of contact. Our goal in this study was to expand on these findings and characterize thermal preference with various hot and cold stimuli, including those that range from unpleasant, but not painful (e.g. 24 °C) to potentially painful, but non-noxious (e.g. 10 and 45 °C).

In humans, cold pain thresholds are reported at about 18 °C [35], but unpleasantness thresholds have been reported within +6 °C from pain thresholds [11]. Reflex and nocifensive tests of rats often identify statistically significant responses lower, near ∼5 °C [2], but this may reflect the threshold for pain intolerance occurring just prior to noxious stimulation. Or findings and an operant escape assay stimulating the hindpaw indicate that innocuous cold stimuli can modestly reduce operant outcome measures, suggesting aversion to these stimuli relative to equivalent warmth [20,24]. Heat pain thresholds in humans are reported at about 43 °C [35] and unpleasantness is more tightly coupled with pain, occurring within ∼1 °C of the pain threshold [11]. Our work in rats similarly indicates a threshold of response around 45 °C, with sharp decline in reward attainment as temperature increases [23]. Based on these data, we paired cold stimuli (24, 18, and 10 °C) with hot stimuli ranging from 42 to 52 °C.

We also examined the effect of previous experience on modulating subsequent preference and these findings suggest previous experience can serve to condition an aversion or preference, which could have implications for evaluating affect of pain. This assay could therefore provide a useful tool for evaluating perceptual and motivational aspects of pain states and analgesic treatments in a manner more relatable to the experience of the patient.

2. Materials and methods

2.1. Animals

Male hairless Sprague–Dawley rats (seven weeks old, Charles River, Raleigh, NC) were housed in groups of five in enriched housing (see ref for description) and were maintained in a standard 12-h light/dark cycle and were allowed access to food (Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet, Harlan Labs, Tampa, FL) and water *ad libitum* when not being tested. Rats' weights were recorded every week to monitor general health. Animal testing procedures and general handling complied with the ethical guidelines and standards established by the Institutional Animal Care & Use Committee at the University of Florida, and all procedures complied with the Guide for Care and Use of Laboratory Animals (1996).

2.2. Thermal preference testing of the face

Facial testing was completed using a reward-conflict operant testing paradigm as described previously [23,24]. Briefly, the rats were trained to drink sweetened condensed milk while making facial contact with a thermode. During the training period (approximately 2 weeks) their baseline intake was recorded, and the rats were considered ready for experimental testing once their average baseline intake was 10 g or greater at 37 °C. Training was performed in the single stimulus condition for all rats. Once trained, the facial testing region for each animal was depilitated under light isoflurane anesthesia (inhalation, 2.5%) once a week to maximize thermal stimulus contact. The rats were fasted over night (13–15 h) prior to each recorded session.

The thermal preference of the rats was recorded as previously described by Rossi et al. [24]. Rats were trained in the single task condition and initially placed in the thermal preference apparatus with both thermodes set at 37 °C to allow them to become accustomed to this new task. A second such session was recorded to ensure rats did not demonstrate a side preference. Rats were able to move freely from one side of the compartment to the other and explore both thermodes at will. Subsequently, rats (n = 5–10) were tested on separate days at the following temperature combinations: 10/42, 10/10, 10/45, 18/45, 18/52, 18/48, 10/48, 24/45 and 24/48 °C. Additional animals were also tested at 10/45 °C (n = 5), and 10/48 °C (n = 15), and found to exhibit the same pattern of preferences as the first group, thus their data were pooled. Cold and hot thermodes were alternated across testing sessions to prevent a learned side preference. Two days or more separated the introduction of a new stimulus pair, except for the conditioned aversion/preference experiments described in Section 3.3, where 10/42 °C, was followed one day later by 10/10 °C, and 52/18 °C was followed by 18/48 °C. We also present data previously published (thermal preference for −4 and 48 °C, n = 7 rats) for comparison with the other stimulus combinations and to illustrate the percentage of unstimulated time not reported in the previous publication [24].

The number of licking contacts with each thermode, were calculated for and totaled to determine the percentage of licks obtained on each side and at each temperature. The duration of time spent on each thermode was used to determine the percentage of time spent stimulating with the hot or cold stimulus, as well as the time spent not performing the reward task (i.e. unstimulated). An assessment of successful task completion for each stimulus was made by dividing the number of licks (rewarded attempts) by the number of stimulus contacts (the number of total contacts or attempts) for each stimulus. In order to evaluate the effect of temperature combination on the success ratios, the single stimulus ratios were compared with the ratios calculated for the same stimulus when paired with another. Single stimulus data were collected in a separate group of rats.

2.3. Statistical analysis

All statistical analyses were performed using SPSS (v. 16.0, SPSS, Inc.). Rats were excluded from consideration if they failed to switch sides. For within stimulus pair comparisons, paired t-tests were used to determine the difference between the percentage of licks on the cold and hot thermodes (or right and left for neutral conditions) and repeated measures analysis of variance (ANOVA) was used to determine significant differences in the percentage of time spent on either thermode or unstimulated. For cold stimuli paired with 45 °C stimulation, One-way ANOVAs were used to compare licks and time across the different stimulus pairs on the cold stimuli, on the 45 °C stimulus, or off the stimuli. This was also true for cold stimuli paired with 48 °C stimulation. For the hot stimuli, 45 and 48 °C, one-way ANOVAs with post hoc Tukey's test were used to compare success ratios for the single stimulus condition with success ratios for that stimulus when paired with various cold stimuli (i.e. success at 45 °C alone versus success at 45 °C when paired with 10 °C, with 18 °C, etc.). For the cold stimuli, t-tests were used to compare success ratios for the stimulus presented alone versus success ratios at that cold stimulus when paired with a hot stimulus (e.g. success at 10 °C alone versus success at 10 °C when paired with 45 °C, etc.). Repeated measures ANOVA was used to assess the effect of previous experience on duration spent on the thermode (left or right) targeted by the previous stimulating conditions, as well as unstimulated time. When significant effects were found with one-way ANOVA, post hoc comparisons were made using Tukey's test and for the repeated measures ANOVA, with least squared difference test (LSD). Statistical significant was set to $p < 0.05$.

3. Results

3.1. Thermal preference and unstimulated time

Although individuals may exhibit a side preference when exposed to a pair of neutral temperatures, this preference was not consistent across testing sessions. When all rats percentage of licks and time spent on each thermode were averaged no side bias was observed, as we have previously reported [17]. With few exceptions, individual rats exhibit a temperature preference each time they are exposed to hot and cold pair of stimuli, and most individuals exhibit the same preference, predictive of the group mean (Table 1). We tested a range of cold stimuli paired either with 45 or 48 °C. For all pairs including 45 °C as the hot stimulus, 45 °C is strongly preferred, with approximately 40% of testing time and >80% of total licks spent in contact with this stimulus (Fig. 1A and B, see Table 2 for within-pair statistics). There was no significant difference in

Table 1
Number of rats exhibiting a cold, hot, or no preference at the stimulus combinations tested, as determined by the percentage of licks; individuals in agreement with the group preference are denoted in bold.

Temperature pair (°C)	Cold preference	Hot preference	No preference[a]	Total n
−4 & 48	2	**11**	1	14
10 & 48	**37**	15	2	54
18 & 48	**8**	1	0	9
24 & 48	**7**	2	0	9
10 & 45	2	**12**	0	14
18 & 45	0	**9**	0	9
24 & 45	2	**10**	0	12
37 & 37[b]	11	14	2	27

[a] "No preference" is defined as 50 ± 5% of licks spent in contact with either stimulus.
[b] For 37 & 37 °C, the count in the "cold preference" column reflects number of rats with a left thermode preference.

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