Comparison of Operant Escape and Innate Reflex Responses to Nociceptive Skin Temperatures Produced by Heat and Cold Stimulation of Rats.

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Comparison of Operant Escape and Innate Reflex Responses to Nociceptive Skin Temperatures Produced by Heat and Cold Stimulation of Rats

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In behavioral tests, rats performed learned escape responses to thermal stimulation of the paws by 44.0, 47.0, or 0.3 °C. Licking, guarding, and jumping reflexes were evaluated at these temperatures. The frequency, latency, and duration of escape and reflex responses were compared and were related to hind-paw skin temperatures measured during stimulation of awake and anesthetized rats. The duration and latency of escape from heat were appropriately related to stimulus intensity. Escape occurred reliably for each intensity. Reflexes occurred unreliably and at long latency to 44.0 or 0.3 °C and were not appropriately related to heat intensity. The reflexes were relatively insensitive to thermal nociceptive stimulation other than heating of the skin at a high rate.

Behavioral methods for evaluating pain sensitivity in laboratory animals have almost exclusively involved observation of simple responses that can be elicited in decerebrate or spinal animals (Bennett, 2001; Woolf, 1984). These stereotyped responses can be described as innate reflexes even though they depend on and are modulated by connections between the spinal cord and brainstem. An important consideration for investigations intended to study pain is whether or not the reflex responses reveal the same effects of experimental manipulations as would a measure that requires cerebral processing of nociceptive input. That is, are circuits connecting nociceptive afferents to motoneurons through segmental spinal or spinal–brainstem–spinal connections affected by experimental treatments in exactly the same manner as long projections from the spinal cord to the thalamus, cerebral cortex, and limbic system? One way to evaluate this is to evoke segmental spinal responses (e.g., limb withdrawal and guarding), spinal–brainstem–spinal responses (e.g., licking and jumping), and operant escape responses under identical stimulus conditions and determine the extent to which the different responses covary. In this analysis, operant escape of nociceptive stimulation is regarded as the standard. Operant escape is a learned response that requires cerebral processing and recognition by the animal that a complex motor response in a task-specific environment will terminate nociceptive stimulation. The operant motor response is not stereotyped as are the reflex responses. The operant response cannot be evoked in decerebrate or spinal animals and is distinct from licking, guarding, and jumping, which can be evoked without task-specific learning and are not linked with a particular environment.

Evaluations of operant and reflex responses in the present study are presented in relation to previously proposed criteria for identifying behavioral responses that reveal the evocation of nociceptive sensations by laboratory animals (Vierck & Cooper, 1984). In general, presumed nociceptive responses by laboratory animals should be related to stimulus parameters, according to an extensive literature that has defined nociceptors as neural units selectively responsive to painful stimuli. In order to demonstrate these relationships, nociceptive testing procedures should satisfy the following criteria:

1. The intensities of stimulation should be known. For thermal stimulation, radiant heat is often utilized. An advantage of radiant heat is that it can be applied locally (e.g., to one paw). However, it is difficult to specify the stimulus intensity in terms of skin temperature at the time a response occurs. Also, nociceptive heat stimulation cannot be maintained at a given magnitude to test effects of prolonged stimulation without damaging the skin, and cold stimulation by radiant energy is not an option. For these reasons, thermal stimulation was delivered in the present study through a thermally regulated surface that could be maintained at...
any temperature from near freezing to levels (and durations) of heat that do not damage the skin. In order to document the effect of the applied thermal stimuli on cutaneous receptors, we measured surface and subcutaneous skin temperatures over time at each of the temperatures utilized for behavioral testing.

2. Behavioral responses should occur at latencies that correspond to the expected onset of pain sensations. For stimuli that reach nociceptive intensities abruptly (e.g., electrical stimulation), flexion and withdrawal reflexes are easily elicited but occur at latencies shorter than is required for conscious appreciation of sensory intensity. Withdrawal latencies are fixed at approximately 60 ms across a wide range of stimulus intensities, but operant escape latencies range from a minimal reaction time of approximately 200 ms to seconds, depending on the stimulus intensity and trial duration. For thermal stimulation, documentation of relationships between latencies of reflex and operant escape responses by laboratory animals is needed, particularly for cold and the low-to-moderate levels of nociceptive heat stimulation utilized in the present study.

3. Responses should occur preferentially and reliably to nociceptive stimulation. For electrical stimulation, flexion and withdrawal reflexes are elicited by stimulus intensities below those that elicit operant escape responses (Greenspan, Vierck, & Ritz, 1986; Le Bars, Gozauiu, & Cadden, 2001). The reliability of occurrence of operant escape and reflex responses to nociceptive intensities of thermal stimulation and resultant skin temperatures were evaluated in the present study.

4. Behavioral responses should be graded in proportion to stimulus intensities within the range of tolerable nociception. This is a critical consideration that is missing from investigations restricted to threshold assessment, as exemplified by measurement of reflex latencies to thermal stimulation. If varying the intensity of nociceptive stimulation does not produce a stimulus–response function consistent with expectations (e.g., derived from pain reports and/or physiologically demonstrated sensitivity of nociceptors), it is difficult to interpret changes in the response produced by an experimental manipulation. For example, determination of a stimulus–response function increases confidence that the lowest intensity that elicits the response represents a threshold for nociception (Greenspan et al., 1986; Vierck et al., 1995). Plotting a stimulus–response function provides the data necessary to fully describe a change in nociceptive sensitivity in terms of allodynia or hyperalgesia after a manipulation that increases sensitivity. Also, there are advantages to assessment of multiple response features in relation to stimulus intensity. For example, we monitored the latency, frequency, and duration of operant escape and innate reflex responses in the present study in order to determine the variable (or variables) that discriminate between nociceptive stimulus intensities. The relative sensitivities of operant and reflex measures for near-threshold (44.0 °C) and suprathreshold (47.0 °C) levels of nociceptive heat stimulation were evaluated.

**Method**

Lick–guard–jump (LGJ) tests and plantar skin temperature measurements from awake and Ketamine-anesthetized rats were conducted at Vanderbilt University. Operant escape, LGJ testing, and plantar skin temperature measurements from Nembutal-anesthetized rats were conducted at the University of Florida. The rats were housed 3 or 2 to a cage in a temperature-controlled environment, with a 12-hr light–dark cycle and free access to food and water. All procedures were approved by the Vanderbilt University or University of Florida chapters of the Institutional Animal Care and Use Committee and conformed to National Institutes of Health and American Pain Society guidelines for the ethical treatment of animals in research.

**Skin Temperature Measurements**

Progressive changes in skin temperature during application of heat or cold to the plantar surface of a hind paw were assessed by three methods:

1. Thermal stimulation was delivered to 4 anesthetized female Long–Evans rats by holding a preheated thermode on the plantar skin of one hind paw. Before thermal stimulation, the rats were administered 40 mg/kg Nembutal and were placed on a heating pad to maintain core temperature within normal limits (monitored with a rectal probe; YSI, Yellow Springs, OH). After a surgical level of anesthesia was established, a 25-ga needle with an indwelling thermistor (YSI Model 524) was inserted subcutaneously, within the plantar skin of one hind paw. The output of the thermistor was amplified and recorded continuously to establish the baseline (skin temperature and then determine responses to 10 min of stimulation at 44.0 °C and 47.0 °C (2 rats) or 0.3 °C (2 rats). Skin temperature was allowed to return to baseline after 44.0 °C stimulation, and then 10 min of 47.0 °C was applied. At the termination of recordings, the rats received an overdose of Nembutal (80 mg/kg ip).

2. Three female Long–Evans rats were anesthetized with Ketamine (50 mg/kg), and a 20-ga hypodermic needle with an indwelling thermistor (YSI Model 552) was inserted under the plantar surface of one hind paw. Each rat was then positioned on a thermally regulated plate so that the plantar surfaces of all four paws contacted the plate. Plantar skin temperature was sampled at 60-s intervals throughout 10 min of stimulation at 44.0 °C.

3. Skin surface temperatures were obtained from 6 awake female Long–Evans rats during 10-min trials on the thermally regulated plate without the operant escape option. The rats were unrestrained within an enclosure that surrounded the plate. At 1-min intervals, one hind paw was raised and inverted for 1–2 s by the experimenter, and plantar skin temperature was recorded by infrared reflectance (80T-IR infrared telethermometer and 187 Tru-Rms multimeter; Fluke Corporation, Everett, WA). The focal length during temperature measurement was approximately 0.5 cm. The rats were accustomed to handling and showed no aversive reaction to brief hind paw manipulations that were shorter than the duration of the average nocifensive response (e.g., lick or guarding). Skin temperatures were obtained in separate 44.0 °C sessions under three different conditions: (a) during 10-min sessions of 44.0 °C stimulation after preexposure to 38.0 °C in an identical apparatus situated adjacent to the test apparatus, (b) during 10-min sessions of 44.0 °C stimulation with no preexposure to 38.0 °C, and (c) during 10-min sessions begun 3 hr after bilateral plantar application of 0.9% capsaicin cream and immediately after a 38.0 °C pretest. The capsaicin cream was made by combining commercially available topical capsaicin cream (0.007%) with additional powdered capsaicin to achieve the desired concentration.

**Operant Escape and LGJ Testing**

The methods of behavioral testing of rats placed on a thermally regulated surface, with or without an escape option, have been described in detail elsewhere (Vierck, Acosta-Rua, Nelligan, Tester, & Mauderli, 2002). For both testing procedures, the rats were placed on a 15.2 × 22.9-cm aluminum plate with internal channels for circulation of heated or cooled water, supplied by a thermostatically controlled circulator (Neslab Model RET-111, Newington, NH; or Polyscience Model 9105, Niles, IL). The design of the channels ensured a uniform temperature over the entire surface. Plate surface temperatures of 44.0 °C, 47.0 °C, or 0.3 °C were maintained and
continuously monitored by means of YSI contact temperature probe input to a YSI Model 4900 thermometer. For LGJ testing, the plate was bounded by a clear Plexiglas enclosure with a ventilated, hinged roof at a height of 30.5 cm. For operant escape testing, the Plexiglas enclosure consisted of two compartments defined by a hanging septum with a 6.4 × 6.4-cm opening cut out of the bottom front corner, permitting the rats free access to either compartment. A 15.2 × 15.2-cm compartment at one end was brightly lit (268.6 cd/m²) by a 35-W cool halogen fixture (Tru Aim Model MR16/FMW; Sylvania, Minneapolis, MN) in the ventilated roof. The floor of the lit (escape) compartment was a thermally neutral platform that was tilted 12° toward the hanging septum. An adjacent 15.2 × 20.3-cm compartment on the side of the hanging partition opposite the escape platform was dimly lit (5.1 cd/m²), and the floor of this compartment was the thermally regulated plate. Thus, during operant escape testing, the rats could stand on the thermally regulated plate in relative darkness or escape from thermal stimulation to stand on the brightly lit, but thermally neutral, platform.

Training to escape thermal stimulation involved gradual increases in floor temperature across single daily trials. First, temperatures were increased from 39.0 °C to 47.0 °C in 2.0 °C increments in successive trials with the platform light off; then daily trials in the same sequence were conducted with the platform light on. Then, individual rats were tested with the platform light off; then daily trials in the same sequence were increased from 39.0 °C, or 0.3 °C, or 0.3 °C in successive trials. First, temperatures were increased three times before data acquisition, to acclimate the rats to the procedure. The LGJ trials were 10 min for 44.0 °C and 0.3 °C stimulation at 47.0 °C stimulation (35.5 s).

During LGJ testing (Figures 1B–1D), licking was the most prevalent innate reflex response to all temperatures presented, but the latencies of lick responses differed considerably from operant escape responses. For 44.0 °C and 0.3 °C, average first operant escape latencies were 74.8 s and 45.5 s, respectively, whereas first lick latencies for these temperatures were 295.9 and 263.3 s, respectively (Table 1). Three operant escape responses to 44.0 °C or 0.3 °C occurred before the first lick response to those temperatures. Lick latencies were similar to operant escape latencies for 47.0 °C stimulation (35.5 and 42.7 s, respectively), but stimulus–response functions could not be directly compared for operant escape and lick responses. Lick latencies for 44.0 °C and 47.0 °C could be compared only for trial durations of 2 min (required to avoid tissue damage during 47.0 °C LGJ trials), but no licking occurred in the first 2 min of 44.0 °C stimulation.

### Table 1

Mean (±SEM) Percentage of Trials With One or More Responses, First-Response Latencies (in Seconds), and First Interresponse Intervals (IRIs; in Seconds)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>n (escape)</th>
<th>Escape</th>
<th>n (LGJ)</th>
<th>Lick</th>
<th>Guard</th>
<th>Jump</th>
<th>Events*</th>
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<tr>
<td>Percentage of trials with one or more responses</td>
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</tr>
<tr>
<td>44.0</td>
<td>12</td>
<td>100.0 ± 0.0</td>
<td>16</td>
<td>78.8 ± 25.9</td>
<td>30.6 ± 32.6</td>
<td>57.7 ± 41.8</td>
<td>95.0 ± 8.0</td>
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<tr>
<td>47.0</td>
<td>17</td>
<td>100.0 ± 0.0</td>
<td>16</td>
<td>98.8 ± 5.0</td>
<td>33.8 ± 27.0</td>
<td>22.5 ± 34.2</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td>0.3</td>
<td>16</td>
<td>99.0 ± 4.2</td>
<td>20</td>
<td>65.0 ± 34.7</td>
<td>43.3 ± 26.0</td>
<td>57.2 ± 41.1</td>
<td>94.9 ± 8.8</td>
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<td>First response latencies</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44.0</td>
<td>12</td>
<td>74.8 ± 41.2</td>
<td>16</td>
<td>295.9 ± 70.1</td>
<td>235.8 ± 111.7</td>
<td>298.5 ± 110.3</td>
<td>218.3 ± 65.8</td>
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<tr>
<td>47.0</td>
<td>17</td>
<td>35.5 ± 20.4</td>
<td>16</td>
<td>42.7 ± 20.5</td>
<td>58.4 ± 27.6</td>
<td>64.4 ± 28.1</td>
<td>35.7 ± 17.9</td>
</tr>
<tr>
<td>0.3</td>
<td>16</td>
<td>45.5 ± 6.7</td>
<td>20</td>
<td>263.3 ± 100.5</td>
<td>336.8 ± 105.5</td>
<td>275.9 ± 139.5</td>
<td>198.8 ± 64.5</td>
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<td>First IRI</td>
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</tr>
<tr>
<td>44.0</td>
<td>12</td>
<td>45.6 ± 27.4</td>
<td>16</td>
<td>116.0 ± 45.3</td>
<td>135.0 ± 100.7</td>
<td>73.0 ± 49.8</td>
<td>76.0 ± 30.3</td>
</tr>
<tr>
<td>47.0</td>
<td>17</td>
<td>27.0 ± 25.4</td>
<td>16</td>
<td>20.0 ± 8.4</td>
<td>19.0 ± 13.0</td>
<td>21.0 ± 15.0</td>
<td>16.0 ± 6.6</td>
</tr>
<tr>
<td>0.3</td>
<td>16</td>
<td>13.8 ± 5.7</td>
<td>20</td>
<td>109.0 ± 92.0</td>
<td>69.0 ± 51.8</td>
<td>67.0 ± 43.6</td>
<td>66.0 ± 32.6</td>
</tr>
</tbody>
</table>

*Note.* Lick–guard–jump (LGJ) trials were 10 min at 44.0 and 0.3 °C, and 2 min at 47.0 °C.

*Any innate response, without regard to type.*
In contrast to licking, which occurred more reliably during 47.0 °C trials than during 44.0 °C trials, jumping was more prevalent at 44.0 °C than at 47.0 °C. Jumping occurred in bursts in certain sessions by some rats, and thus it was not consistently elicited by any stimulus condition. First-response latencies for LGJ responses to 47.0 °C were not far apart: 42.7, 58.4, and 64.4 s, respectively, in 120-s trials (Table 1); but the probabilities that at least one response would occur were 98.8%, 33.8%, and 22.5%, respectively (Table 1). The probabilities for guards and jumps are particularly surprising because 47.0 °C is clearly suprathreshold for activation of nociceptors. Thus, measurement of first latencies for innate responses to nociceptive stimulation can be misleading relative to response probability.

The distribution of LGJ responses can also be considered in terms of latencies and probabilities for any innate response, without regard for the type (labeled Events in Table 1). When this was done, a high probability of innate responses was observed for all temperatures presented (ranging from 94.9% to 100%). However, first-event latencies were 218.3 s and 198.8 s, respectively, for 44.0 °C and 0.3 °C (Table 1). Thus, latencies for the first instance of any reflex response were substantially greater than operant escape latencies for these temperatures (74.8 s and 45.5 s).

Paradigms that monitor only first-response latencies can be misleading because rats engage in competing activities such as exploration when first introduced into the testing environment. In addition, skin temperatures are unknown and variable at the beginning of a trial, before the rats have received thermal stimulation. One way to evaluate the effect of these factors is to compare first-response latencies with subsequent interresponse intervals (IRIs). The first IRI for operant escape was calculated by subtracting the latency and duration of the first escape response from the second escape latency. First IRIs for the LGJ task were calculated by subtracting the first-response latency from the latency to the second response of the same kind, regardless of side. The innate reflex responses were not sufficient in duration or frequent enough to bring skin temperature below a nociceptive level for either paw.

The first IRI for all response categories was shorter than the first latency for that response (Table 1). Table 1 also shows that the first

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Figure 1. The timing of sequential responses during trials of thermal stimulation (44.0, 47.0, or 0.3 °C) when the escape platform was available (A) or not available (B–D). The ordinates show the average percentage of the 1st through nth response for each category during 10-min trials. The abscissa shows the timing (latency) of the 1st through nth response. A: Operant escape responses occurred on nearly all trials at each temperature, numbered four or greater on more than 50% of the trials, and were distributed at regular intervals throughout trials. B–D: On most trials, only licks occurred in response to 47.0 °C. Guards and jumps occurred early but infrequently (on less than 50% of the trials) for 47.0 °C stimulation. All innate reflex responses occurred late on 44.0 °C and 0.3 °C trials, and the frequency of innate responses, particularly jumps and guards, was lower than that of escape.
IRI for LGJ events considerably exceeded the first IRI for operant escape. The IRI before the second escape from 44.0 °C was 45.6 s, and IRIs before the second LGJ event during 44.0 °C trials were a minimum of 73 s. Thus, LGJ responses consistently occurred after a longer exposure to 44.0 °C than operant escape responses, even though skin temperatures remained at nociceptive levels during LGJ testing but returned to non-nociceptive levels during escape responding.

**Skin Temperature Thresholds for Elicitation of Operant Escape and LGJ Responses**

Figure 2 shows plantar skin temperatures at 1-min intervals in response to 44.0 °C stimulation for three methods of measurement. Subcutaneous temperatures were nearly identical when recorded from thermisters placed intradermally in rats anesthetized with Nembutal or with Ketamine. The maximum subcutaneous temperature attained during 10 min of 44.0 °C stimulation was 42 °C. Surface skin temperatures measured with infrared reflectance from awake rats were only slightly lower than subcutaneous temperatures, despite opportunities for the awake rats to lick and guard the paw from which surface measurements were obtained.

For operant escape responding, important considerations are whether the rats consistently wait for thermal stimulation to reach nociceptive levels before climbing on the thermally neutral platform, and whether they remain on the platform long enough to bring skin temperatures out of the nociceptive range. To illustrate these relationships, Figure 3 shows high-resolution (1-s sampling rate) recordings of subcutaneous skin temperatures and the average timing of operant escape responses for each testing temperature. Escape responses to heat occurred after the initial high rate of skin temperature increase and after skin temperature reached a level that could excite nociceptors minimally (38.0 °C; Fleischer, Handwerker, & Joukhadar, 1983; Handwerker, Anton, & Reeh, 1987; Leem, Willis, & Chung, 1993; Lynn & Carpenter, 1982). Also, response durations showed that the rats remained on the thermally neutral platform long enough for skin temperatures to return to non-nociceptive levels. For 0.3 °C stimulation, operant escape latencies revealed sensitivity similar to human thresholds for cold pain (less than 25.0 °C), and the rats remained on the platform long enough to stabilize skin temperatures above 25.0 °C.

Subcutaneous recordings with 1-s resolution enabled the estimation of skin temperatures at the first latency of all responses.
elicited by heat or cold. Because skin temperature plateaus were reached before elicitation of operant escape or LGJ behaviors by heat, thresholds were similar (and varied little) for each response (see Table 2). On 44.0 °C trials, operant escape threshold was 40.5 °C, and LGJ thresholds ranged from 41.2 °C to 41.3 °C. On 47.0 °C trials, escape threshold was 42.4 °C, and LGJ thresholds ranged from 42.6 °C to 43.0 °C. In contrast, escape thresholds of 16.1 °C for cold stimulation differed considerably from LGJ thresholds, which ranged from 3.4 °C to 4.1 °C.

Another way of expressing thresholds is in terms of time an animal will tolerate thermal stimulation after skin temperature reaches a plateau at nociceptive temperatures. Table 2 shows initial response latencies minus the time required to reach 40.0, 41.0, or 20.0 °C for stimulation at 44.0, 47.0, or 0.3 °C, respectively. For 44.0 and 0.3 °C, there is a substantial disparity in adjusted initial latencies for operant escape (40 and 26 s for 44.0 °C and 0.3 °C) and for innate responses on the LGJ task (ranging from 206 to 305 s). For 47.0 °C stimulation, the adjusted escape latency was 25 s, compared with a range of 33–54 s for LGJ responses.

Response Frequency and Duration as Measures of Nociception

Response duration has been utilized infrequently as a measure of sensitivity to nociceptive thermal stimulation. However, this measure is relied on to reveal sensitivity to chemical stimulation of a paw, such as in the formalin test (Dubuisson & Dennis, 1977). A test of the validity of response duration as a measure of sensitivity to nociceptive thermal stimulation is available by comparison of response durations for different temperatures. Average operant escape durations for the first four responses of a trial were reliably greater for 47.0 °C than for 44.0 °C (see Table 3). Therefore, appropriate stimulus–response functions for operant escape are provided by both response latency and duration. In contrast, durations of innate reflex responses were not appropriately related to stimulus intensity. For example, average response durations for the first four lick and guard responses of a trial were shorter for 47.0 °C stimulation than for 44.0 °C stimulation (Table 3). Also, there was a disparity between operant escape and innate response durations for 0.3 °C stimulation, relative to responses to heat. Longer escape durations for 0.3 °C than for 47.0 °C indicate that cold stimulation is highly aversive. However, lick and guard response durations were shorter for 0.3 °C stimulation than for 44.0 °C stimulation.

In addition to the latency and duration of responses, the number of responses per trial could provide a measure of nociceptive intensity. Table 3 shows that the number of operant escape responses was not a good measure of the intensity of nociceptive thermal stimulation. Approximately equal numbers of escape responses occurred at 44.0 °C and 47.0 °C. The number of LGJ responses to 44.0 °C and 47.0 °C could not be compared directly, because trial durations were different, of necessity.

Delayed Effects of Capsaicin on Reflex and Operant Responses

Peripheral application of algesic chemicals before behavioral testing of nociceptive sensitivity is a valuable procedure for investigating therapeutic procedures intended to reduce sensitization (Vierck, Kline, & Wiley, 2003). In order to determine whether such procedure, cutaneous application of capsaicin, produces behavioral sensitization not accounted for by changes in skin temperature, we painted 0.9% capsaicin in emollient cream on the plantar surfaces of both hind paws 3 hr before operant escape or LGJ testing. Figure 4A shows that capsaicin did not affect skin temperatures during 10 min of 44.0 °C stimulation preceded by a 10-min pretest exposure to 38.0 °C. The pretest exposure was designed to stabilize baseline skin temperatures. However, lick–guard responding to 44.0 °C was significantly increased throughout 10-min trials preceded by the 38.0 °C pretest, F(1, 5) = 7.21, p = .02 (Figure 4B). Therefore, prior application of capsaicin increased lick–guard responsivity without affecting skin temperatures at the time of testing. This effect applied also to operant escape responding. The total duration of escape responding of 17 rats during 44.0 °C trials was greater 3 hr after the hind paws were painted with capsaicin (359.1 s), compared with trials preceded by application of hand cream to the paws (252.5 s), t(5) = 3.61, p = .001.

Discussion

A common strategy for investigation of nociceptive reflexes is to apply radiant heat that increases skin temperature rapidly or to place the animals on a surface maintained at a relatively high temperature (e.g., 50–55 °C). These methods have apparently evolved for several reasons: (a) Behavioral variability is minimized, (b) reflex responses are best elicited by abrupt-onset stimulation, and (c) data collection can be accomplished quickly with reflex assays. However, other desirable features of a nociceptive test are sacrificed by the use of high rates of increase in skin temperature. In particular, clinical pain can be associated with prolonged input from C nociceptors that are preferentially activated by low rates of heat transfer (Yeomans, Pirec, & Proudfit, 1996), as produced by temperatures from 43 °C to 45 °C (Vierck et al., 2002).

Prolonged stimulation at just-suprathreshold levels for nociception prevents tissue destruction and permits observation of the latencies and durations of multiple responses, supplementing the traditional measurement of only the first-response latency. These assessments of responsivity to prolonged stimulation provide opportunities to evaluate temporal summation of nociceptive inten-

Table 2

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Escape</th>
<th>Lick</th>
<th>Guard</th>
<th>Jump</th>
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First latency after 40 °C

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<th>Jump</th>
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</table>

p
Skin temperatures obtained at 1-min intervals from Figure 4. Measurement of skin temperature by intradermal thermistors or by infrared reflectance from the plantar skin surface revealed a thermoregulatory compensation for applied heat that can be attributed to sympathetic cutaneous vasoconstriction (Hirata, Nagasaka, Nunomura, & Cabanac, 1988; Janig, 1975; Nagasaka, 1987). Over 10 min of stimulation at 44.0 °C, the maximal skin temperature was 41.8 °C, and 47.0 °C stimulation raised skin temperature to 44.6 °C. The skin temperatures in response to 44.0 °C stimulation were similar for anesthetized rats and for awake rats in which locomotion, licking, and guarding provided intermittent pauses in stimulation. For 44.0 °C and 47.0 °C, skin temperature rose within approximately 30 s to 40.0 °C and 42.0 °C for 44.0 and 47.0 °C, respectively, and then progressed gradually to a maximum over the remainder of 10-min trials. In contrast to the rapid initial rise during application of heat, stimulation by 0.3 °C produced a gradual, negatively accelerating reduction in skin temperature that reached a minimum of 1.7 °C at the end of 10-min trials. This profile of skin temperature reduction is consistent with a passive storage of cold that is not substantially counteracted by thermoregulatory compensation (Mauderli, Vierck, Cannon, Rodrigues, & Shen, 2003).

Skincare temperatures at the first-response latency appear to provide estimates of thresholds for evocation of the different behavioral reactions, but this can be misleading. Skin temperatures at the first operant or innate reflex response to heat ranged from 40.5 °C to 41.3 °C for 44.0 °C stimulation and 42.4 °C to 43.0 °C for 47.0 °C stimulation. Thresholds for excitation of nociceptive afferents by heat are as low as 38.0 °C (Fleischer et al., 1983; Handwerker et al., 1987; Leem et al., 1993; Lynn & Carpenter, 1982). Therefore, all the observed responses can be regarded as nociceptive. However, the substantial plateau in temperatures produced by prolonged application of heat must be taken into account to appreciate the value of a threshold estimate.

For 44.0 °C stimulation, operant escape latencies averaged 74.8 s, which was 40 s after skin temperatures reached 40 °C, at the beginning of the plateau. In contrast, the first innate reflex (lick, sensitivity as a result of an experimental manipulation. Therefore, the present study compared operant and reflex responses to levels of thermal stimulation that activate nociceptors, are tolerable over a prolonged period of stimulation (10 min of 0.3 °C or 44.0 °C), and do not damage the skin (i.e., are not noxious).

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Table 3

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Escape</th>
<th>Lick</th>
<th>Guard</th>
<th>Jump</th>
<th>Eventsa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Response durationsb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44.0</td>
<td>53.9 ± 31.2</td>
<td>6.5 ± 3.2</td>
<td>3.5 ± 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47.0</td>
<td>82.8 ± 52.9</td>
<td>2.3 ± 0.7</td>
<td>1.4 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>111.9 ± 81.6</td>
<td>3.9 ± 3.1</td>
<td>0.8 ± 0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of responses per trial

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Lick</th>
<th>Guard</th>
<th>Jump</th>
<th>Eventsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.0</td>
<td>8.9 ± 3.0</td>
<td>5.9 ± 2.5</td>
<td>3.1 ± 3.3</td>
<td>18.5 ± 18.3</td>
</tr>
<tr>
<td>47.0</td>
<td>8.3 ± 2.0</td>
<td>7.9 ± 3.2</td>
<td>2.2 ± 1.5</td>
<td>1.4 ± 2.0</td>
</tr>
<tr>
<td>0.3</td>
<td>8.8 ± 3.0</td>
<td>5.9 ± 4.5</td>
<td>6.8 ± 6.0</td>
<td>8.5 ± 11.3</td>
</tr>
</tbody>
</table>

Note. Lick–guard–jump trials were 10 min at 44.0 and 0.3 °C, and 20 min at 47.0 °C. Any innate response, without regard to type. Average of the first four responses in a trial.
guard, or jump) occurred at 218 s—a minimum of 3 min after skin temperature reached 40 °C. The considerable discrepancy in operant and reflex latencies after excitation of cutaneous nociceptors shows that the neural circuits subserving the different responses are not comparably activated by low-level nociceptive stimulation. If nociceptive neurons at the spinal origin of operant and reflex circuits were activated similarly, it would be expected that operant escape latencies would be longer than innate reflex latencies because of longer conduction distances to the cerebrum, a delay imposed by decision making, and time required to locomote to and climb on the platform. However, for 44.0 °C stimulation, simple withdrawal (guarding), licking, and jumping latencies were, on average, 2.7, 3.6, and 3.6 min longer than escape latencies. Thus, comparison of operant escape and LGJ response latencies to skin temperatures reveals considerable disparities in the duration of nociceptor discharge required to elicit a response.

Not only were there long delays before occurrence of any reflex response to low-level nociceptive stimulation, but there was also a low probability that any of these responses occurred within the 10-min trials. The simplest segmental response (guarding) occurred on only 30.6% of 44.0 °C trials without the escape option, and licking was less consistently elicited (78.8%) than operant escape (95.8%). The late and unreliable occurrence of innate reflex responses to 44.0 °C stimulation is likely attributable to preferential activation of C nociceptors by slow rates of heat transfer to temperatures that do not stimulate A-delta nociceptors (Yeomans & Proudfit, 1996). Innate LGJ responses are most reliably elicited by intensities of thermal stimulation that impose high rates of heat transfer to the skin, preferentially activating A-delta nociceptors. Segmental flexion–withdrawal reflexes function to remove a limb quickly to prevent skin trauma and are elicited optimally by abrupt-onset stimulation at high temperatures. However, slow rates of discharge by C nociceptors likely occur under conditions of pathological pain, and therefore operant sensitivity to 44.0 °C may be an especially important test of clinically relevant nociception.

Stimulus–response functions provide more information than thresholds, which describe only one point along the continuum of nociceptive sensitivity. Operant escape responses were reliably elicited by 44.0 °C and 47.0 °C, and escape latencies and durations were appropriately related to these stimulus intensities. The criterion of a stimulus–response function was satisfied for escape latency and duration. However, LGJ responses were related to 44.0 °C and 47.0 °C stimulation in patterns that do not describe an appropriate stimulus–response function. Latencies of LGJ responses for 44.0 °C were disproportionately long, and lick and guard durations were actually greater for 44.0 °C stimulation than for 47.0 °C. Jumps occurred sporadically in bursts, and only by a few rats. In addition, LGJ frequencies across a full range of nociceptive heat intensities cannot be compared, because trial durations at 47.0 °C and above must be kept short (below LGJ latencies at 44.0 °C), to prevent skin damage.

Testing of responses to stimulation at 0.3 °C revealed disparities between operant escape and reflex sensitivity that were similar to those observed for heat stimulation. Escape responses were reliably elicited (on 100% of the 0.3 °C trials), but LGJ responses occurred on only 43.2–65.0% of the trials. First-escape latencies were 45.5 s, and LGJ latencies ranged from 263.3 to 336.8 s. Skin temperature threshold for escape was 16.1 °C, which is comparable to the human threshold for cold-induced pain (Harrison & Davis, 1999; Morin & Bushnell, 1998), but LGJ thresholds ranged from 4.1 °C to 3.4 °C. Therefore, reflex responses occurred too late to affect skin cooling through a considerable portion of the nociceptive range, and licking is not an adaptive response to cold. A notable feature of 0.3 °C escape responses was a long average duration (111.9 s) compared with escape from 44.0 °C (53.9 s) or 47.0 °C (82.8 s). These differences are consistent with a slower rate of recovery of skin temperature from cold stimulation relative to heat stimulation (Mauderli et al., 2003). For both heat and cold stimulation, the data show that duration of operant escape responses was highly regulated by the rats’ remaining on the escape platform until skin temperature returned to a non-nociceptive level.

In contrast, comparison of skin temperatures recorded from anesthetized rats and freely behaving rats showed that LGJ responses were ineffective in reducing skin temperatures during heat stimulation.

A distinct advantage of operant escape testing over evaluation of innate reflex responses is that long trial durations and a variety of temperatures can be utilized without imposing tissue damage. The animals escape before skin temperatures reach high nociceptive and noxious (skin damaging) levels, and they remain on the platform long enough for skin temperatures to return to non-nociceptive levels. This sequence is repeated multiple times throughout a trial, providing the opportunity to evaluate the reliability of latency and duration as measures of nociception. Such analyses of sequential responses showed substantial differences between the first escape response and subsequent responses. First-escape latencies were longer than subsequent IRIIs, and first-escape durations were shorter than subsequent response durations in a trial. These results suggest that it is not advisable to rely on first-response latency as a measure of nociception. When introduced into the test apparatus, the rats engage in exploratory activities, can be influenced by any stress resulting from handling, and have not fully sampled the temperature of the floor.

Consideration of skin temperature for algesiometric tests is particularly relevant for studies involving effects of nociceptor sensitization by algesic chemicals that induce a release of inflammatory agents and affect blood supply to the treated area. In the expectation that direct effects on skin temperature would abate over time, we conducted operant escape and LGJ tests 3 hr after applying capsaicin in emollient cream to the plantar surface of the hind paws. A 38.0 °C pretest was used to standardize foot temperatures. Skin temperature measurements during 44.0 °C trials after the pretest revealed no effect of capsaicin application. However, both LGJ and operant escape durations during 44.0 °C trials were significantly increased by prior application of capsaicin. Thus, pretreatment with capsaicin in emollient cream produced a sustained increase in thermal nociception that could be dissociated from direct effects on skin temperature.

In summary, we evaluated operant escape and LGJ responses to heat and cold to examine whether they were comparably and appropriately determined by the quality and intensity of nociceptive input. If response latencies, durations, and frequencies were similar across stimulus conditions, the strategy of observing innate reflex responses as a substitute for operant escape would be strengthened, on the basis that the spinal origins of neural circuits responsible for each behavior appeared to respond similarly to thermal nociceptive input. However, for near-threshold nociceptive heat and suprathreshold cold, there were few similarities.
between operant and reflex responsivity. For both these stimulus conditions, operant escape responses were reliably elicited, but LGJ responses were not. The latencies and durations of operant escape responses were appropriately related to skin temperatures, but the latencies of LGJ responses were extremely long, and LGJ durations did not increase with nociceptive heat intensity. Peripheral sensitization of nociceptors by prior cutaneous application of capsaicin increased operant and innate reflex sensitivity, but not skin temperatures, indicating that each behavior can be enhanced by increased C-nociceptor input, even though the reflexes are not reliably elicited by stimulus conditions that primarily activate C nociceptors.

References


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