Pain as a stressor: Effects of prior nociceptive stimulation on escape responding of rats to thermal stimulation

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A B S T R A C T
In our previous studies, psychological stress was shown to enhance operant escape responding of male and female rats. The stressors that produced hyperalgesia were physical restraint and social defeat. Nociceptive input also elicits stress reactions, generating the prediction that pain would facilitate pain under certain circumstances. For example, the usual method of evaluating stress in laboratory animals is to test for effects after termination of the stressor. Accordingly, operant escape performance of male and female rats was evaluated during two successive trials involving nociceptive thermal stimulation. The intent was to determine whether nociceptive sensitivity differed on first trials and during pain-induced stress on second trials. Compared to a first trial of 44.5 °C stimulation, escape responding increased during a second trial of 44.5 °C stimulation (preceded by an escape trial of 10 °C). Similarly, escape from cold (10 °C) was enhanced when preceded by escapable 44.5 °C stimulation. Thus, prior nociceptive stimulation enhanced escape from aversive thermal stimulation. Facilitation of pain by a preceding pain experience is consistent with stress-induced hyperalgesia and contrasts with other models of pain inhibition by concurrent nociceptive stimulation.

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1. Introduction
A variety of stressors activate the limbic system, the hypothalamic-pituitary-adrenal axis (HPA) and the brain stem (Herman et al., 1996), resulting in descending modulation of nociceptive reflexes (Amit and Galina, 1986) and generating the impression that stress reduces pain sensitivity (Ford and Finn, 2008; Millan, 2002). However, descending modulation can influence motor output (Holmqvist and Lundberg, 1959; Lai et al., 1989; Taylor et al., 1997; Zemlan et al., 1983). Therefore, reflex suppression following stress may represent suppression of motor output rather than attenuation of pain sensations. Also, cerebral effects of stress must be considered. For example, ascending influences from the brain stem can alter sensory transmission (e.g., Svensson, 1987). The importance of stress influences on nociceptive transmission throughout the neuraxis is revealed when pain modulation is evaluated with procedures which reveal cerebral encoding of pain. This has been documented with restraint and social defeat stress which enhance subsequent escape from nociceptive thermal stimulation (King et al., 2003, 2007; Marcinkiewicz et al., 2009). The combination of reflex suppression but increased pain sensitivity is consistent with the clinical profile of stress effects on reflexes and pain (Ford and Finn, 2008; Nilsen et al., 2007; Temml et al., 2007; Vierck, 2006; Zautra et al., 2007).

Pain is stressful and can be expected to produce effects observed for other stressors. Generally, simultaneous stimulation at different, often remote sites has been utilized to evaluate influences of nociceptive input on nociceptive responses. For example, reflex responses of a hindlimb can be attenuated by concurrent nociceptive stimulation within cervical dermatomes (Villanueva and Le Bars, 1995). This is referred to as diffuse noxious inhibitory control (DNIC), suggesting that pain is inhibitory to pain. However, this inference requires demonstrations of mutual inhibition at both sites. Instead, DNIC may represent a contrast effect with unidirectional suppression at one site from another, depending on the relative intensity of stimulation at the two sites or according to a cervical to lumbar hierarchy. Supposition that DNIC represents a generalized inhibition of pain by pain requires documentation of sensation magnitudes elicited by different intensities of stimulation at disparate sites, singly and simultaneously (Lautenbacher et al., 2007). In addition, simultaneous stimulation at two sites requires controls for divided attention (Staud et al., 2003), and non-reflexive tests of pain processing are needed for laboratory animal studies.

If pain produces stress and stress increases pain, then sensitivity to nociceptive stimulation should increase over the duration of a stress response elicited by pain. Such a positive feedback loop
would not be evident with concurrent stimulation paradigms that do not evaluate changes over time. Because the stress response develops slowly, the testing paradigm for the present study involved two sequential periods (trials) of thermal stimulation spanning 30 min. Nociceptive sensitivity during the first trial, which is likely to initiate a stress reaction, was compared with sensitivity during the second trial, when stress should be well developed. Experience with other methods of stress induction predicted that escape responding would be enhanced during second trials, relative to first trials.

2. Methods

2.1. Subjects

Three groups of Long-Evans hooded rats were behaviorally trained and then tested. Escape from thermal stimulation was evaluated with 18 females and 14 males. All rats were maintained on a standard 12-h light/dark cycle and had access to food and water ad libitum. All experimental procedures complied with ethical guidelines and standards established by the Institutional Animal Care & Use Committee at the University of Florida and conformed to National Institutes of Health guidelines for care and use of experimental animals.

2.2. Apparatus

The testing apparatus for escape consisted of a dark (0.5 foot candles) compartment with a thermally regulated floor plate (6 in wide, 8 in long) and a brightly lit (3200 foot candles) compartment (6 in wide, 6 in long) with a thermally neutral platform. The two compartments were separated by a partition with a 2.5 by 2.5 in. opening on one side. The animals could ambulate freely between compartments, choosing between thermal stimulation in the dark plate compartment and an aversive level of bright light in the platform compartment. The apparatus was ventilated with forced room air.

2.3. Procedure

After a period of acclimatization to the testing apparatus in room lighting and without thermal stimulation, rats were trained to escape from nociceptive thermal stimulation. The training procedure involved two series of sessions with a gradual increase in magnitude of thermal stimulation. The first series was conducted without bright light over the platform, and heat and cold stimulation progressed on alternate days from 36 °C to 44 °C in 2 °C increments and from 36 °C to 20 °C, 15 °C, 10 °C and then 5 °C. The temperature sequences were then presented with the light on in the platform compartment.

Following completion of training, sessions of escape testing were conducted Monday – Friday. Each day, the animals were placed sequentially into two escape apparatuses situated side by side in a sound-isolated, dark room. A 15 min. first trial began when an animal was placed on the thermal plate in apparatus #1 and a 15 min. second trial began with placement on the thermal plate in apparatus #2. Occupancy of each escape platform was detected by electronic proximity detectors and was timed by proprietary software. Sequences of trials within days were 44.5 °C then 10 °C and 10 °C then 44.5 °C. Escape behavior was recorded during both daily trials, permitting comparisons of performance in response to 10 °C as a first trial and as a second trial after 44.5 °C and in response to 44.5 °C as a first trial and as a second trial after 10 °C. The sequences of cold then hot or hot then cold trials of nociceptive stimulation were used as a conservative approach to the possibility that thermoregulatory mechanisms would determine responses on the second trial. For example, if skin temperatures were the primary determinant of escape, a first trial of cold stimulation would decrease responding to a second trial of heat stimulation.

2.4. Data analysis

Our previous experiences with operant escape testing of rats have shown that first response latencies and the number of responses during a trial are poor indicators of nociceptive stimulus intensity. In contrast, the duration of platform occupancy (escape from thermal stimulation) reliably discriminates between thermal stimulus intensities and a variety of experimental manipulations presumed to affect pain intensity (Vierck et al., 2002, 2004, 2005, 2008a,b). In addition, the durations of successive occupancies of the plate and platform compartments provide important measures of changing sensitivity as a trial progresses. Therefore, as depicted in Figs. 1 and 2, escape responding was broken down into individual occupancies of the plate and platform compartments. These discrete events occur in an alternating sequence as pairs of plate and platform durations (events).

An experimental manipulation that produced hyperalgesia could decrease the duration of successive thermal stimuli endured (plate durations) or increase the duration of each escape response (platform durations). Because the number of these events can differ between animals in a time-limited session, durations were accumulated across 15 pairs of plate and platform occupancies. Summation of successive event durations for individual animals avoids problems associated with averaging across different numbers of observations late in a session. For a given test condition (e.g., first trials of 44.5 °C stimulation) the accumulated durations of successive plate durations were subtracted from the accumulated durations of paired platform durations to give the relative preference for the escape platform or the thermal plate as a trial progressed (see Fig. 1).

Increasingly positive platform durations minus plate durations indicate increasing pain sensitivity, and negative platform durations minus plate durations reveal relative insensitivity to nociceptive thermal stimulation. These difference scores were compared for first trials and second trials to indicate whether prior nociceptive stimulation increased or decreased pain sensitivity. Graphical displays of preference for the escape platform on first and second trials were used to determine the response number with the maximum difference in pain sensitivity (Fig. 2). T-tests for dependent groups were then utilized to evaluate the significance of the maximal difference in pain sensitivity on first and second trials.

3. Results

Panels A and B of Fig. 1 depict average durations of successive platform and plate occupancies of male rats during first and second trials of 44.5 °C escape testing. Both panels reveal that the durations of thermal stimulation (on the plate) and escape (on the platform) changed over 15 pairs of these events. Early in first trials at 44.5 °C (panel 1A), there was no preference for the thermal plate or escape platform (events 1–3), and the average occupancy within each compartment was short (10–20 s). However, as trials progressed, plate times diminished slightly and platform times increased (responses 4–10). Average platform times eventually decreased, because the trial timed out for most of the animals (e.g. an animal with a maximum of 10 rather than 15 platform pairs). Platform times increased earlier and to a greater extent during second trials of 44.5 °C that were preceded by a trial of 10 °C (panel 1B).
Panels 1C and 1D accumulate successive platform and plate times for first and second trials respectively, depicting a progressive increase in difference between platform and plate durations. Also, second trial platform times (1D) increased faster than first trial platform times (1C), reflecting a greater sensitivity to thermal stimulation on second trials. Escape performance is further transformed into one curve showing cumulative differences (platform–plate times) for stimulation during first trials (1E) and second trials (1F). This expression of event-by-event differences in platform and plate times is useful for comparing thermal pain sensitivity during first and second trials (e.g., Fig. 2B).

Fig. 2 compares pain sensitivity for males and females responding to 10 °C and 44.5 °C during first and second trials. For each panel, event-by-event estimates of pain sensitivity (platform–plate durations) during first trials were subtracted from the corresponding calculations for second trials. Because of slightly reduced plate durations and substantially increased platform durations during second trials of 10 °C and 44.5 °C stimulation, pain sensitivity was greater for second trials compared to first trials (panels 2A–D). The uniformly positive values in Fig. 2 show that pain sensitivity was increased throughout second trials of 10 °C and 44.5 °C stimulation for males and females. Statistical comparisons of maximal differences in pain sensitivity (Table 1) utilized t-tests for dependent samples of first and second trial performance for the event pairs shaded in Fig. 2. Comparisons of pain sensitivity (platform minus plate durations) on first and second trials were significant for males and females at 10 °C and 44.5 °C. The differences in pain sensitivity for these event pairs were determined by a significant increase in platform duration on second trials (increased latency to return to thermal stimulation). In contrast, latencies of
escape (plate times) from cold or heat stimulation were not significantly reduced by prior nociceptive stimulation.

4. Discussion

Painful experiences set in motion interrelated autonomic, emotional, motivational, sensory and motor effects that are recognized as components of a psychological stress response (Chapman et al., 2008; Craig, 2003). Stress can, in turn, contribute to development of chronic pain and/or exacerbate it, once established (Davis et al., 2001; Vierck, 2006). Results consistent with human clinical reports of mutually facilitatory relationships between pain and stress were produced in the present study, using an operant escape test of sensitivity to sequential trials of nociceptive thermal stimulation. For both male and female rats, escape from aversive cold or heat stimulation was enhanced for second trials relative to first trials that are presumed to have initiated a stress response. These results contrast with numerous preclinical studies showing suppression of nociceptive reflexes during concomitant nociceptive stimulation or previous stress (Amit and Galina, 1986; Ford and Finn, 2008; Villanueva and Le Bars, 1995).

Effects of pain and stress on reflex responses to nociceptive stimulation are usually attributed to modulation from the brain stem onto spinal nociceptive transmission. In the case of pain-induced stress, nociceptive input to any spinal level activates cells with projections to the periaqueductal gray and other nuclei of the brain stem (Craig, 2003) that project back to the spinal cord and modulate nociceptive reflexes (Basbaum and Fields, 1979). In addition, nocireceptive spinal cells project to the limbic system (e.g., the amygdala) and the hypothalamus – important components of central stress circuits that act via brain stem nuclei to modulate reflex responses (Chapman et al., 2008). An assumption implicit in the use of reflex measures of nociception is that descending modulation from the brain stem is globally expressed at spinal levels, similarly affecting reflex circuits and sensory transmission to the brain. This is highly unlikely, judging from demonstrations of different effects of a variety of experimental manipulations on reflex and operant tests of nociceptive sensitivity (Vierck and Light, 1999; Vierck et al., 2002, 2003, 2004, 2005, 2008a,b; Wiley et al., 2007), including comparison of the present study with others showing stress-induced hyporeflexia. In addition, effects of pain and stress on cerebral processing of pain must be considered, which requires a non-reflexive test of pain sensitivity.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Ave. Diff.</th>
<th>t</th>
<th>P</th>
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<tr>
<td>Platform–plate</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Males 10°C</td>
<td>236.0</td>
<td>4.47</td>
<td>&lt;0.001</td>
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<tr>
<td>Females 10°C</td>
<td>366.6</td>
<td>6.15</td>
<td>&lt;0.001</td>
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<td>297.2</td>
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<tr>
<td>Females 44.5°C</td>
<td>404.5</td>
<td>4.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platform</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Males 10°C</td>
<td>239.2</td>
<td>4.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Females 10°C</td>
<td>326.8</td>
<td>5.81</td>
<td>&lt;0.001</td>
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<tr>
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<td>409.1</td>
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</tr>
<tr>
<td>Plate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.81</td>
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</tr>
<tr>
<td>Females 44.5°C</td>
<td>1.02</td>
<td>0.32</td>
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Statistical comparisons of escape behaviors on first and second trials for males and females during 10°C and 44.5°C stimulation. T-tests for dependent samples compared three measures of responsivity for the event pair with the maximal difference in pain sensitivity on first and second trials (see Fig. 2). For males and females responding to 10°C and 44.5°C, platform durations were significantly increased during second trials, as were platform–plate durations. Plate durations on first and second trials were not significantly different. For maximal differences between trials 1 and 2 are indicated by * above the peak difference scores (shaded bars).

Fig. 2. Relative event-by-event performance on trials 1 and 2 is shown by subtracting the estimates of pain sensitivity in trial 1 (cumulative platform – plate durations, as in Fig. 1E) from the estimates of pain sensitivity in trial 2 (as in Fig. 1F). Positive values indicate that pain sensitivity was enhanced during second trials. Panels A (10°C) and B (44.5°C) show positive values throughout 15 event pairs for males. Panels C (10°C) and D (44.5°C) show positive values throughout 15 event pairs for females. T-tests for maximal differences between trials 1 and 2 are indicated by * above the peak difference scores (shaded bars).
An important advantage of operant tests of pain sensitivity is that measures of sensitivity other than response latency or threshold are obtained. Also, in contrast to reflex paradigms that predestine the duration and rate of stimulation used to assess nociception, during escape testing the animals regulate the timing of stimulation according to its intensity and their sensitivity. Their behavior provides measures of time on the thermal plate (escape latency) and time off the plate (escape duration). Plate durations represent the time that expires between the onset of each thermal stimulus and escape (an estimate of threshold). The measure of escape latency is often utilized in human studies of pain sensitivity, particularly involving long-duration stimulation which increases sensation intensity gradually over time before reaching a painful level (e.g., in cold pressor or tourniquet pain studies). This applies to the present study, where low levels of aversive thermal stimulation were utilized, producing baseline escape latencies of approximately 15 s. However, first trial noxious stimulation did not significantly affect escape latencies (plate durations) on second trials, indicating that thermal pain thresholds were not increased. That is, escape occurred in response to similar skin temperatures and levels of nociceptor activation on both first and second trials.

Platform durations (escape durations) can be regarded as latencies of escape from bright light, but they differ greatly with variations in plate temperature (Vierck et al., 2002) while light intensity is a constant. Platform durations are determined primarily by the animals’ reactions to the plate temperature that drove them to the platform. Therefore, platform durations appear to represent a measure of affective responses of the animals following termination of each noxious stimulus on the plate. Reluctance to return to the plate is proportional to the perceived intensity of the most recent exposure to noxious heat or cold stimulation. In the present study, platform durations increased within trials, revealing enhanced affective reactivity with stimulus repetition, in contrast to plate durations which were relatively stable and decreased slightly within trials. In addition, platform duration was significantly influenced by a prior trial of noxious stimulation. Platform durations for second trials of 44.5 °C and 10 °C were substantially increased by first trials of noxious stimulation thus. In addition to short term mechanisms for central sensitization that enhance pain during repetitive activation of C nociceptors within trials (Vierck et al., 1997), pain-induced stress extends the time-course of pain facilitation by the pain. The test–retest strategy provides a means for studying mechanisms of increased pain sensitivity and pain affect in experimental animal models of acute and chronic pain.

There is little doubt that pain is stressful. Accordingly, pain-induced stress enhanced escape from thermal stimulation within the same time frame as observed for physical restraint or social defeat experiences that are regarded as stressful (King et al., 2003, 2007; Marcinkiewcz et al., 2009). These interactions between stress and pain coding likely occur within cerebral structures and pathways that are distinct from brain stem and spinal reflex circuits. For example, anterior cingulate and prefrontal cortical regions have been implicated in affective processing of pain (Derbyshire and Jones, 1998; Craig, 2003; Johansen et al., 2001; Qiu et al., 2006) and evocation of stress reactions (Rauch et al., 2003; Radley et al., 2004). The increased affective reactions of rats to noxious stimulation on second escape trials likely resulted from interactions within these structures and related cerebral systems for stress and nociceptive processing.

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References


