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Characterization of variables defining hindpaw withdrawal latency evoked by radiant thermal stimuli

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Abstract

We have examined the stability and sources of variation within the nociceptive model of rat hind paw withdrawal from an under-glass radiant stimulus (Hargreaves et al., 1988) using a system where stimulus intensity and floor temperature can be controlled and reproducibly changed. The current study demonstrates that: (i) increased stimulus intensity with a fixed surface temperature is associated with a monotonic decrease in mean response latency and its variance; (ii) for a fixed stimulus intensity, the mean paw withdrawal latency and variance increased as the glass floor temperature is lowered from 30°C to room temperature (25°C). Using subcutaneously-implanted thermocouples and a 30°C glass surface, the subcutaneous paw temperature observed at an interval corresponding to the time at which the animal displayed a paw withdrawal did not differ across multiple heating rates (41–42.5°C). This finding is in agreement with human studies of pain thresholds and C-fiber activity. These studies emphasize the importance of maintaining a fixed surface temperature to reduce experimental variability and the utility of this apparatus across multiple stimulus intensities to define agonist efficacy. © 1997 Elsevier Science B.V.

Keywords: Paw temperature; Heating rate; Thermal nociception; Radiant thermal stimulus; Hindpaw withdrawal

1. Introduction

The acute application of a sufficiently intense thermal stimulus to the body surface of the unanesthetized animal will evoke an organized escape response targeted at removing the stimulated site from the heat source. The adequate stimulus is believed to reflect the activation of small high threshold thermoreceptors and polymodal C-fibers, with the frequency of firing being proportional to stimulus intensity (Beitel et al., 1977; Konietzny, 1984). The response to the afferent input may be organized at the spinal level (e.g. a nociceptive reflex in the Sherringtonian sense) or the supraspinal level, reflecting a complex coordinated behavioral adjustment to the stimulus environment.

Behavioral models in the rodent have been developed which are believed to reflect these underlying physiological substrates (Yaksh, 1997). For assessment of spinally mediated nociceptive reflexes, the rat tail displays a brisk withdrawal consequent to being placed in a heated water bath (Janssen et al., 1963) or the application of a radiant stimulus (D'Amour and Smith, 1941). For supraspinally organized nociceptive reflexes, a common paradigm has been the withdrawal or licking of the hind paw or the jumping response evoked by placing the animal on a hot surface (Woolfe and MacDonald, 1944). The use of models based on spinal versus supraspinal organization is predicated on specific questions being addressed. However, it is certain that an essential element of nociception is the supraspinal processing of the input generated by the noxious stimulus and emphasizes the importance of assessing the supraspinal component of the afferent-evoked response.

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An important variant on the paw withdrawal model is the application of a radiant thermal stimulus to the plantar surface of the hindpaw of a rodent standing on a glass surface (Hargreaves et al., 1988). This model has gained favor as an analgesic drug bioassay for several technical reasons: (i) it permits independent testing of either hind paw; (ii) testing can be undertaken with minimum handling (i.e. the animal can be placed in the chamber before the stimulus is initiated); (iii) the end point (paw withdrawal) can be automatically detected in un-anesthetized, un-restrained animals; and (iv) stimulus intensity is easily and reproducibly changed. Utilization of this model as a drug bioassay merits consideration of model properties that contribute to response variability and sensitivity. Reduced variability permits smaller groups to be employed to define statistical differences, but if reduced variability also significantly reduces sensitivity, model utility in defining analgesic drug action will be lost. We believe that two variables merit specific consideration: stimulus intensity and glass surface temperature.

With respect to intensity, increasing thermal stimulus intensity in a variety of models will result in a reduced response latency (Carstens et al., 1979; Carstens and Wilson, 1993; Dirig and Yaksh, 1996). We sought to determine the relationship between stimulus intensity, mean response latency, and group variation. With the radiant stimulus model, increasing lamp current (amperage) results in an increased peak temperature as well an increased rate of rise. One question addressed in the current study was whether the temperature associated with the withdrawal response was independent of the rate of rise.

With respect to glass temperature, the glass surface upon which the rodent is placed acts as a heat sink or source, depending upon the relative temperature gradient (Hirata et al., 1990). Glass temperature can thus affect paw skin temperature. Changes in basal glass temperature could potentially alter withdrawal latency. This has been demonstrated in the tail flick test where decreases in basal tail skin temperature increased tail flick latencies (Berge et al., 1988). Accordingly, failure to control glass temperature could lead to sources of random variation. The current study thus incorporates the radiant thermal stimulus described above to examine the relationship between thermal intensity, glass surface temperature, tissue temperature, and withdrawal latency.

2. Methods

2.1. Animals

Male Sprague Dawley rats (300–325 g) were housed pair-wise in cages and maintained on a 12 h light/dark cycle with free access to food and water at all times. All studies were carried out under protocols approved by the Institutional Animal Care and Use Committee at the University of California, San Diego.

2.2. Thermal testing device

To assess thermal nociceptive responses, a commercially available device modeled conceptually after that described by Hargreaves et al. (1988) was employed (UARDG, Department of Anesthesiology, University of California, San Diego; La Jolla, CA, 92103-0818, attn. George Ozaki). As shown in Fig. 1, this device consists of a glass surface upon which the rats are placed individually in Plexiglas cubicles ($9 \times 22 \times 25$ cm). The glass surface temperature is maintained at either 30 ± 0.1°C or 25 ± 0.1°C by a feedback-controlled, under-glass, forced-air heating system.

The heating system is driven by a thermocouple attached to the bottom surface of the glass plate. The thermal nociceptive stimulus originates from a focused projection bulb mounted in a stimulus tower that is manually manipulated in a two-dimensional axis on ball bearing slides to permit the stimulus to be delivered separately to either hind paw of each test subject. This stimulus is positioned under the foot pad with the aid of an angled mirror mounted on the stimulus source which permits an exact visual targeting of the stimulation site prior to stimulus initiation. A timer is automatically actuated with the light source, and response latency is defined as the time required for the paw to show an abrupt withdrawal. Paw withdrawal is detected by an array of photodiode motion sensors mounted on the stimulus tower that stops the timer and terminates the stimulus. Stimulus current from a regulated source is monitored continuously to determine the amperage delivered to the light source and, thereby, the magnitude of the radiant stimulus to which the paw is subjected. In all cases, a cut-off of 20 s is employed to avoid tissue injury.

2.3. System calibration

2.3.1. Temperature-time profile of glass heating

To define stability of basal glass temperature over time in the absence of stimulation as well as changes in recorded temperature on the inner and outer glass surface with thermal stimulation, a thermocouple (36Ga, type T, Omega instruments) was attached to the inner and outer surface of the glass plate. Thermocouple signal was amplified and plotted on a Hewlett Packard 7754b four channel recorder system.

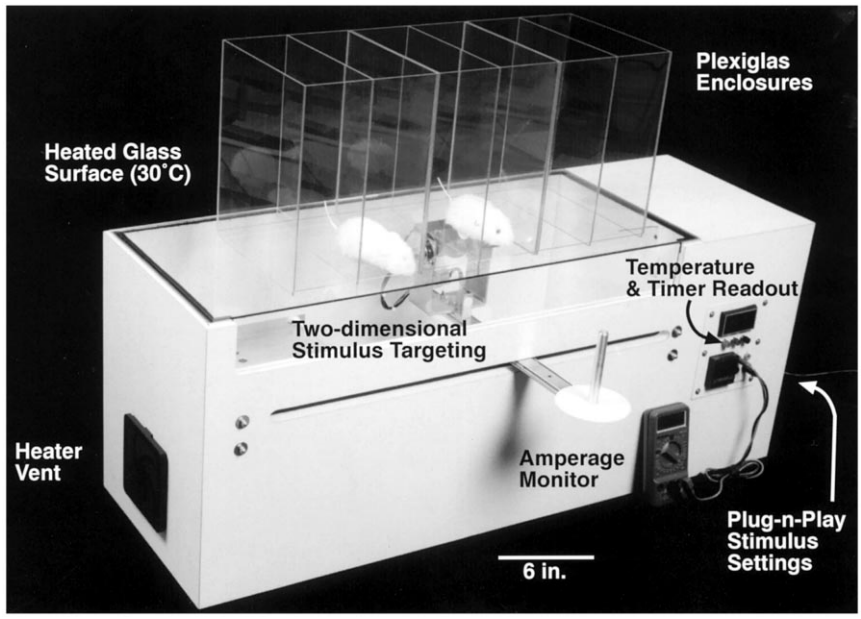

Fig. 1. Pictured above is the thermal stimulus device described in Section 2.2. Note the angled mirror-light assembly to facilitate stimulus site targeting and front electronics panel for monitoring of glass temperature and response latency as well as the 'plug-and-play' easy selection of three user-defined, pre-set stimulus currents.

2.4. Animal testing protocols

Unless otherwise noted, rats were acclimated to the test chamber for 20–30 min prior to testing. At each test exposure, the left and right paws were tested 1 min apart in random order. The response latency of the rat for a given test represents the average of the time to paw withdrawal for the left and right paw.

2.4.1. Response reliability

To assess the reliability of the testing paradigm, naive animals with no prior handling other than that involved in routine cage maintenance were handled for 2–3 min, placed in the testing apparatus, and tested every 20 min for 2 h at one of four stimulus intensities (stimulus currents: 5.0, 5.25, 5.5, and 6.0 A).

To establish population data, in a separate study, basal latencies were collected in groups of rats which had undergone implantation with intrathecal catheters (Yaksh and Rudy, 1976) and allowed at least 3 days of post-surgical recovery before inclusion in any study. Basal latencies (prior to any intrathecal injection) were collected from a total of 550 rats by four different trained observers on 13 different radiant thermal testing apparatuses.

2.4.2. Subcutaneous temperature profile

To define the relationship between the time dependent change in subcutaneous paw temperature, response latency, and stimulus intensity, a separate set of animals was used. Paw withdrawal latencies were assessed in triplicate with 4.9, 5.3, and 6.0 A stimuli at 25 and 30°C glass temperature settings ($n = 4$ at each glass temperature). Under halothane anesthesia (2% air-O_2), a T type thermocouple was implanted subcutaneously into the plantar surface of the right hindpaw such that the active tip of the probe was positioned within the subcutaneous tissue of the hindpaw pad in the same position as the light beam was focused. After implantation, thermal stimuli at the three intensities were applied for 20 s while the animals remained under anesthesia. Five minutes was allowed between stimuli, which were applied in an increasing order of intensity, to allow the paw tissue to return to baseline temperature. Thermocouple signal was amplified and written directly to a computer text file using Macintosh Labview v2.2 and an NB-DMA-8-G data transfer interface (National Instruments). The thermocouple was validated for conversion of mV signal to degrees Celsius using water at temperatures ranging from 23 to 65°C. The thermocouple signal was linear over this range ($r^2 = 0.99$, data not shown). In this manner, thermal paw withdrawal latencies in unanesthetized animals could be compared with the subcutaneous paw temperature assessed in the same animals under halothane anesthesia; this study design was applied using both 25 and 30°C glass temperatures.

2.5. Statistical analysis

Variations in paw withdrawal latency over time were explored using one way and two-way repeated measures ANOVA, with Scheffe's post hoc correction for multiple comparisons (GB-Stat, Dynamic Microsystems).

Withdrawal temperatures interpolated from paw heating curves were compared using one-way ANOVA with the post-hoc correction described above. Relationships between latency, lamp amperage, and latency standard deviation were explored using linear regression, and the minimum *a priori* criteria for statistical significance in all tests was $p < 0.05$.

3. Results

3.1. Stimulus characteristics

Glass surface temperature in five separate boxes was examined. Over repeated 24 h day night cycles, the mean glass temperature when set at 30°C was 30.0 ± 0.1°C, and the 25°C setting was 25 ± 0.1°C.

With increasing lamp amperage, the rate of rise and the maximum glass temperature measured by the thermocouple increased with the inner-glass temperature being greater than outer-glass. The outer glass temperature was consistently greater after a 20 s stimulus course when measured on 30°C glass as compared with 25°C. As shown in Table 1, there was a single exception to this that occurred at the 4.5 A intensity, but this stimulus intensity did not evoke a consistent, brisk paw withdrawal (data not shown) and was considered a non-noxious thermal stimulus. At all stimulus intensities that evoked a brisk paw withdrawal from the thermal stimulus, the outer glass temperature was greater after 20 s for the 30°C glass temperature setting than the 25°C setting. The outer-glass heating rates over the first 10 s at lamp settings of 5.0, 5.25, 5.5, and 6.0 A were 2.6, 3.1, 4.2, and 5.2°C/s, respectively. As shown in Fig. 2, increasing glass temperature to 30°C increased the magnitude of the heating curves at all intensities tested. Outer-glass temperatures did not converge until 15 s after stimulus initiation at the highest intensities.

Table 1
Effect of glass temperature and stimulus intensity on heat sink capacity of glass surface

Amperage	25°C Glass		30°C Glass	
	Outer glass	Inner glass	Outer glass	Inner glass
4	38.9 (0.1)	41.1 (0.1)	41.5 (0.2)	48.4 (0.1)
4.5	48.7 (0.2)	53.2 (0.1)	45.4 (0.3)	57.6 (0.1)
5	57.4 (0.3)	63.7 (0.1)	62.6 (0.2)	68.9 (0.1)
5.25	62.6 (0.3)	76.4 (0.1)	67.9 (0.5)	80.6 (0.1)
5.5	80.5 (0.4)	79.5 (0.1)	79.6 (0.4)	84.2 (0.1)
6.0	88.7 (0.6)	96.7 (0.1)	88.9 (0.5)	98.7 (0.3)

Glass temperature was maintained at 25 or 30°C. Lamp amperage was tested at the intensities below, and inner and outer glass temperature at the 20 s cutoff times shown. S.E.M. is indicated in parentheses.

To assess the dependability of the stimulus apparatus, 925 consecutive 20 s glass heating cycles were recorded at 1 min intervals. No differences in peak temperature or heating profile were observed (data not shown).

3.2. Paw withdrawal latencies

3.2.1. Naive rats

Basal withdrawal latencies at the four stimulus currents listed above (5.0, 5.25, 5.5, and 6.0 A) were examined at 30°C glass temperature. These basal withdrawal latencies were elevated immediately after animal placement in the apparatus, stabilized by 40 min, and were stable throughout the remainder of the 2 h timecourse (see Fig. 3). The mean latencies after 2 h of testing were 13.6 ± 1.1, 9.3 ± 0.8, 7.9 ± 0.5, and 5.4 ± 0.4 s for the 5.0, 5.25, 5.5, and 6.0 A stimuli, respectively.

3.2.2. Population data: handled groups

Pooled basal (pre-drug) response latency data from 79 separate experimental groups ($n = 550$ rats) examined by four trained observers on 13 different radiant thermal testing apparatuses were analyzed for relationships between paw withdrawal latency, latency variance, and stimulus intensity. Glass surface temperatures were maintained at 30 ± 0.1°C. As shown in Fig. 4A, there was a linear relationship between paw withdrawal latency and amperage, demonstrating that paw withdrawal latency and current amperage (as a measure of stimulus intensity) vary inversely ($r^2 = 0.72$). With increased latency (decreased intensity), there was an increase in the variance of withdrawal latency as shown in Fig. 4B ($r^2 = 0.37$), such that there was a decreased variance at lower latencies (higher intensities). In both cases, linear regression illustrated a relationship between the two variables with a slope that was significantly different from zero ($p < 0.0001$).

3.3. Glass surface temperature and response latency

As indicated in Table 2, response latencies for a given stimulus intensity were uniformly greater when assessed at a 25°C than with a 30°C glass surface. Thus at 5.3 A, the response latencies at 25 and 30°C were 7.0 ± 0.4 and 5.3 ± 0.1 s, respectively.

3.4. Tissue temperature

Using a 30°C glass setting, subcutaneous paw temperatures, as measured by implanted thermocouples, were maintained at 38°C, whereas 25°C glass decreased paw temperature to 33–34°C (See Fig. 5).

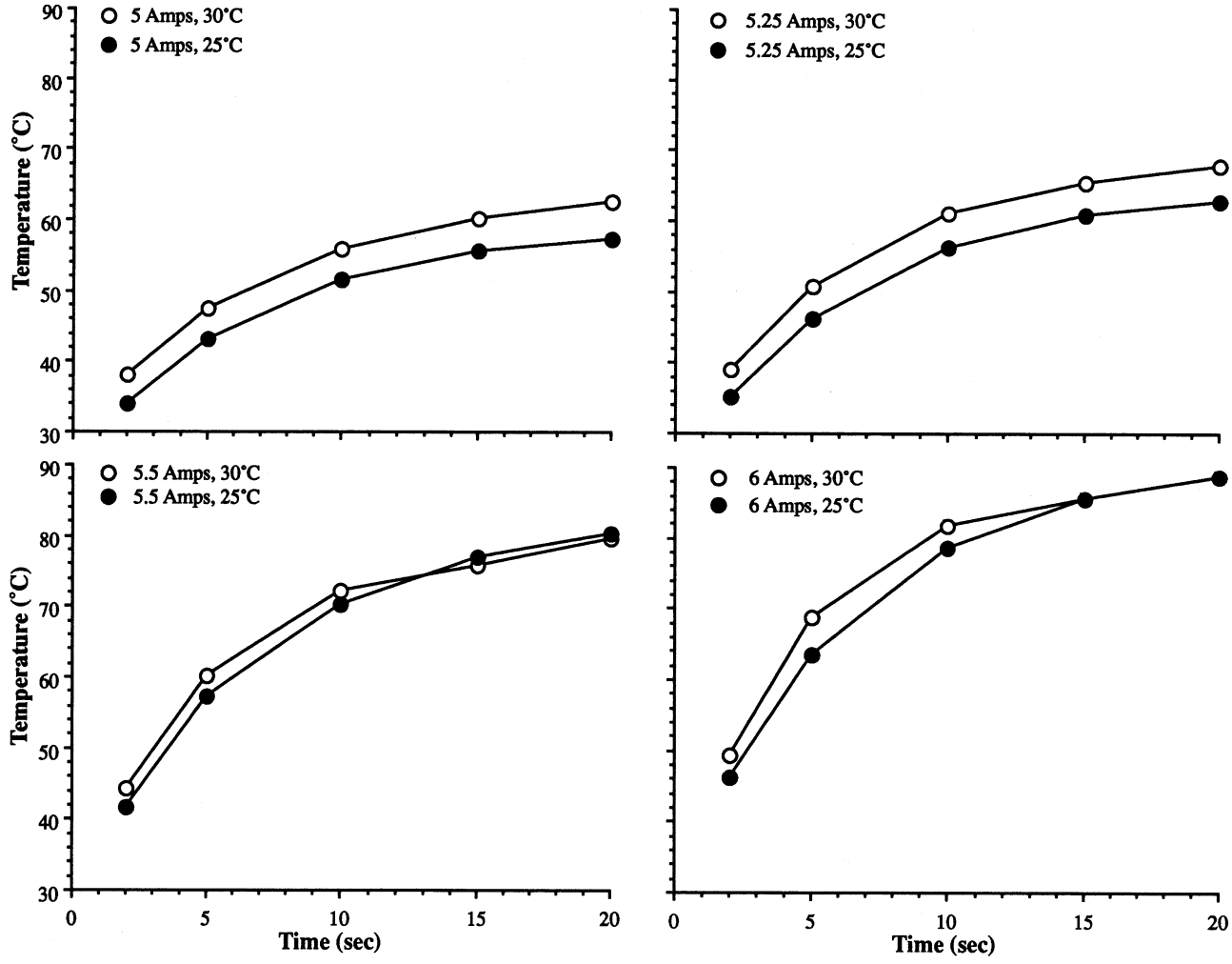

Fig. 2. Basal glass temperature determines peak temperature after stimulus. Heating curves are presented for the outer glass temperature during 20 s of heating by the radiant thermal source set at one of four lamp intensities (5, 5.25, 5.5, and 6.0 A). Glass temperature was monitored for separate stimulus time courses with the apparatus glass maintained at 25°C (●) or 30°C (○), with each tracing representing the mean of ten trials ± S.E.M.

After 20 s of heating at three different intensities (4.9, 5.3, and 6.0 A), paw temperatures did not return to baseline until 5 min after stimulus actuation. This was true for both 25 and 30°C glass settings. After 20 s of heating, the peak paw temperatures were higher for the high intensity stimulus and the 30°C glass setting subsets (See Table 2). Since each animals' withdrawal latency was assessed before thermocouple implantation, paw temperatures at the time of withdrawal (withdrawal temperature) for each animal were interpolated from the heating curves. Withdrawal temperature at each stimulus intensity were not different across stimulus intensity for the 30°C glass settings (41–42°C), but were different ($p < 0.05$) for the 25°C glass setting (37–41°C, see Table 2). Thus, at the 25°C glass temperature, higher intensity heating curves resulted in withdrawal at lower skin temperatures.

4. Discussion

While the more traditional testing paradigms such as the tail flick test have been rigorously studied (see Kawakita and Funakoshi, 1987), there have been few studies validating paw withdrawal from a radiant thermal stimulus (Hargreaves et al., 1988; Galbraith et al., 1993; Yeomans and Proudfit, 1994). The goals of the present study were (i) to examine the consistency of paw withdrawal latencies over time, (ii) determine the relationship between stimulus intensity, latency, and latency variance, and most importantly, (iii) investigate the effects of basal glass temperature on withdrawal latency and tissue temperature.

4.1. Model characteristics

As compared with traditional nociceptive tests such

as the hot plate or tail flick test, the current design introduces glass temperature as an intervening variable. The glass surface that the animals are placed on for testing will alter the heating of the hindpaw due to its capacity as a heat sink (Hirata et al., 1990). As described in Table 1, there was difference between inner and outer glass surface temperatures, with the inner surface being warmer than the outer at all temperatures and stimulus intensities. Due to this heat sink effect, all subsequent glass heating curves are presented as the outer surface glass temperature, as this was more representative of the stimulus applied to the hindpaw. An additional concern with respect to the heat sink capacity of the paw is its positioning on the glass. The degree of paw contact with the glass has a direct impact on thermal conduction, and thereby the efficiency of stimulus delivery. In all cases described herein, the thermal stimulus was delivered to the same area of the paw in animals in similar ambulatory positions. Thus, the latency differences observed at 25 and 30°C glass temperatures cannot be explained in terms of differences in hindpaw contact with the glass.

Current delivered to the projection bulb is defined as monotonically related to the stimulus intensity. Increasing bulb amperage provided an increased rate of rise and increased the outer glass temperature reached at 20 s. Measurements of outer glass surface temperature demonstrated that maintenance at 25°C decreased the overall magnitude of the heating curve at all points except the latest time points of the highest intensity stimuli. Thus, decreasing glass temperature to room temperature decreased the effective magnitude of the stimulus presented. In the present study, maximum glass temperature was set at 30°C in the apparatus since the rat hindpaw skin temperature is typically 30–31°C (Galbraith et al., 1993). This may seem a contradiction in light of the data presented for 30°C glass in Fig. 5, where subcutaneous paw temperatures were maintained at a normothermic 38°C. It is important to note that the results of Galbraith et al. (1993) were obtained using surface contact thermocouples, whereas the current study involved an invasive procedure to measure internal paw temperature. The subcutaneous probes would record in vivo temperature, whereas the surface probes would record external skin temperature, which

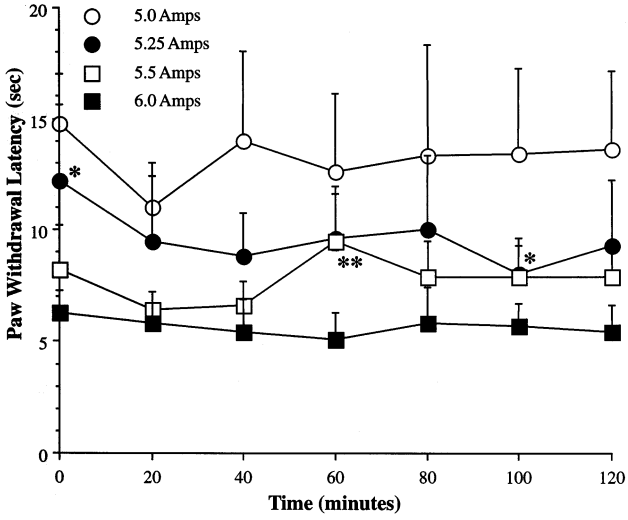

Fig. 3. Paw withdrawal latencies are stable over time. Basal hindpaw withdrawal latencies are presented over a 2 h timecourse tested at lamp amperages of 5.0 (○), 5.25 (●), 5.5 (□), and 6.0 (■). Each tracing depicts the averaged left and right hind paw withdrawal latencies from seven to 14 rats ±S.E.M. over the 120 min time course. There was no interaction between intensity and time (2-way repeated measures), and statistically significant variations in latency at each intensity (one way repeated measures) are indicated as $*p < 0.05$ or $**p < 0.01$.

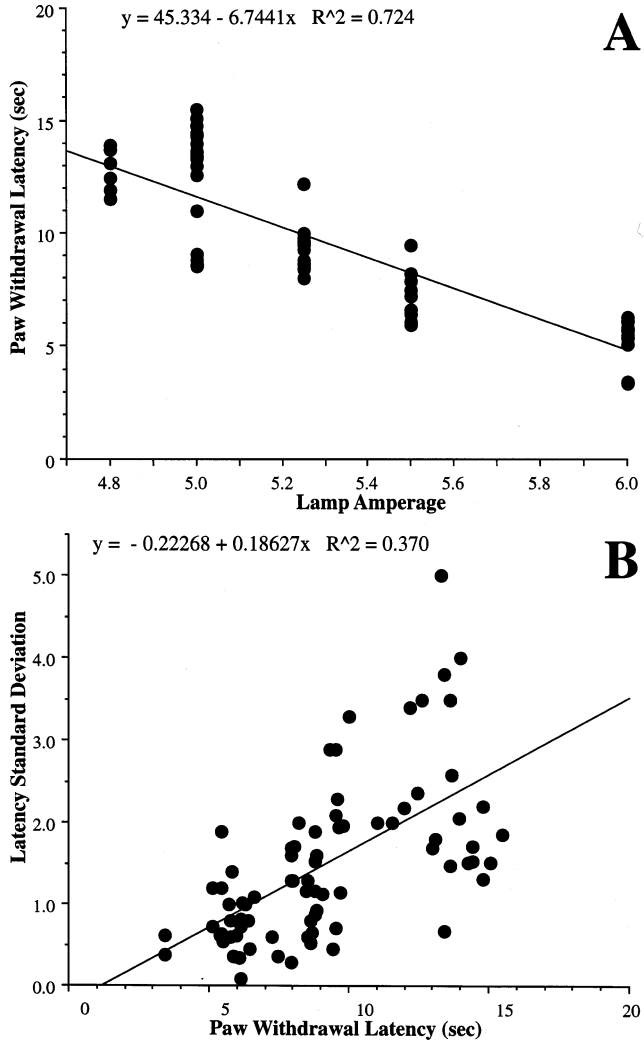

Fig. 4. Relationship between latency and intensity. Top panel (A) demonstrates the linear relationship between withdrawal latency and lamp amperage, supporting the use of lamp amperage as a measure and control of stimulus intensity. The bottom panel (B) demonstrates co-variance of paw withdrawal latency and the standard deviation from the same 79 separate groups of animals as depicted in Fig. 4A. In both panels, linear relationships shown had slopes significantly different from zero ($p < 0.0001$).

Table 2
Withdrawal latencies and subcutaneous withdrawal tissue temperatures were assessed at three different stimulus intensities

Amperage	25°C Glass			30°C Glass		
	Latency	Withdrawal temperature (°C)	Peak paw temperature (°C)	Latency	Withdrawal temperature (°C)	Peak paw temperature (°C)
4.9	14.3 (0.8)	41.1 (0.5)	42.4 (0.5)	9.1 (0.5)	42.4 (0.4)	45.2 (1.0)
5.3	7.0 (0.4)	38.5 (0.5)	44.5 (0.6)	5.3 (0.1)	41.6 (0.2)	47.4 (0.9)
6	3.7 (0.1)	37.0 (0.3)*	48.0 (0.9)	3.2 (0.1)	41.1 (0.1)	50.6 (1.2)

Note that the 30°C glass sets show no difference in withdrawal temperature across intensities, whereas the 25°C glass groups steadily decline. Significant differences are indicated by $p<0.05$ (*) as determined by one way ANOVA/Scheffe's post-hoc test; standard error of means are indicated in parentheses. While not shown for the sake of clarity, latencies within a glass temperature subset were significantly different from each other, and decreasing glass temperature to 25°C increased withdrawal latencies at all three stimulus intensities

is lower due to convection and evaporative cooling. This lower external temperature is more important in terms of creating a neutral, but clamped, glass/paw interface temperature. That this is the optimum temperature to clamp the glass is further substantiated by the observation that paw temperature remained normothermic when using the 30°C glass, whereas the 25°C glass trials were accompanied by paw cooling over the time course of the experiments described in Fig. 5.

4.2. Paw withdrawal latencies

In the present model, when animals were tested either acutely (at 20 min intervals) for periods of up to 2 h or over days, there was a remarkable stability in the withdrawal latency.

We believe this response stability arises from control of the several variables that can alter the stimulus condition, including control of glass surface temperature, monitoring and control of bulb current, and the ability of the stimulus lamp to deliver a repeatable stimulus despite multiple stimulus cycles.

Several investigators have reported a hyperalgesic response to repeated testing and suggested a peripheral sensitization of thermal nociceptors (Iwata et al., 1994; Brennan et al., 1995). Thermal stimuli that yield peripheral injury (e.g. as evidenced by inflammation) will yield an increased responsiveness. However, such sensitization in the face of rapid stimulus repetition may also result from the inability of the paw to shed heat associated with the stimulus. This is suggested in Fig. 5, where after 20 s of heating, paw temperature did not return to a normothermic baseline at 4.9 and 5.3 A stimuli until five min after stimulus initiation. At the highest intensity stimulus, paw temperature still had not returned to baseline after 5 min. Thus, continual testing of the paw at 5 min intervals or less under these conditions could produce a summation of tissue temperature and thereby increased thermal nociceptor activity. This would be interpreted as a facilitation phenomenon.

4.3. Thermal stimulation and central activation

Increasing bulb current results in an increase in the caloric output of the bulb. The detection system (whether thermocouple or subcutaneous thermoreceptor) will report an increase in temperature, the rate of rise, and the maximum temperature which is proportional to bulb current. As will be considered below, it is assumed that the nociceptive response to the stimulus will reflect two components: the absolute temperature and the rate of rise. Single unit recording from slowly conducting afferent populations (C-fibers) in humans have shown a discharge threshold in the range of 41–42°C (Yarnitsky et al., 1992). At temperatures above this threshold, there is a rapid increase in the discharge frequency of these axons. Moreover, for a given thermal stimulus, primary afferent discharge rates increase with increased rate of change (Beitel et al., 1977; Konietzny, 1984), and psychophysical studies in humans have also demonstrated that pain magnitude estimates and C-fiber primary afferent firing rates increase with increased rate of temperature change (Kenshalo et al., 1968; Yarnitsky et al., 1992).

In the present studies, measurement of subcutaneous temperatures under several stimulus conditions that match those employed in the behaving rat revealed that when the withdrawal latency of each animal was interpolated back to tissue temperature at the time of paw withdrawal, there was no difference in withdrawal temperature across stimulus intensities (30°C glass). Although withdrawal latency decreased threefold from 9.1 to 3.2 s with increasing stimulus intensity, the tissue temperature at withdrawal was not different (41.5°C). These behavioral observations are in close accord with the thresholds for activation of C-fiber populations described above (Yarnitsky et al., 1992), and suggest that the reaction (paw withdrawal) mechanism was dependent upon tissue temperature but not rate of rise.

It is interesting to note however the variance from this observation noted when a low intensity stimulus was employed with the 25°C glass surface. Here, the

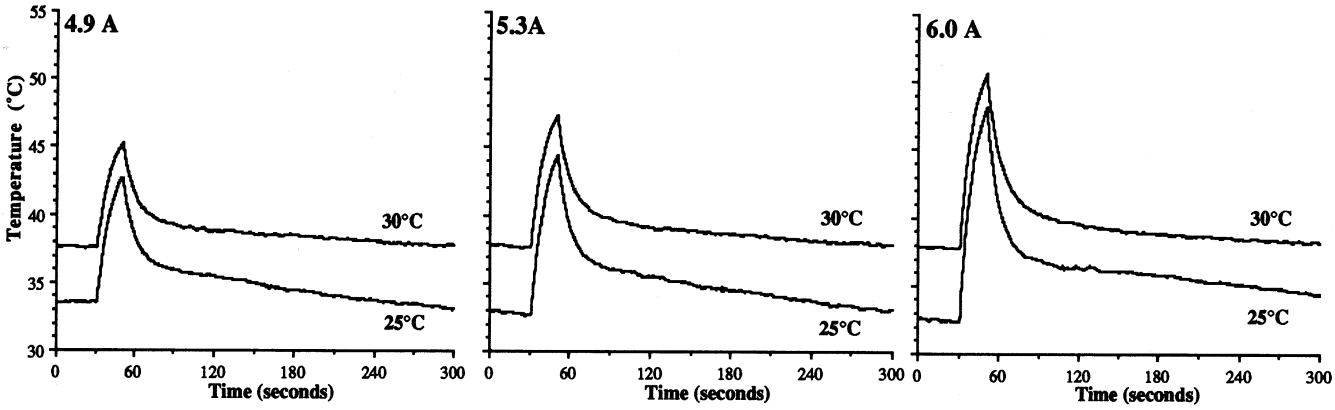

Fig. 5. Room temperature glass decreases paw temperature. Heating curves for subcutaneous paw temperature mirrored glass heating curves, such that basal and peak temperatures were depressed when room temperature glass was used, and peak paw temperatures co-varied with amperage (also see Table 2).

temperature corresponding to escape was less than that required to evoke escape at the 30°C glass setting. This anomaly may reflect upon the psychophysical observation that there is a facilitation of the response to even mild heat when the skin has been subjected to a cool/cold conditioning stimulus. While it seems irregular to evoke nocifensive responses at such low temperatures, cold/cool pain studies have been reported using temperature probes at temperatures up to 20–25°C as cold noxious stimuli (LaMotte and Thalhammer, 1982; Wahren et al., 1989; Yarnitsky and Ochoa, 1990). This phenomena has been shown to have a central correlate in the firing of dorsal horn marginal cells (Craig and Bushnell, 1994).

4.4. Practical significance of stimulus control

These observations emphasize two points. First, random variations in surface temperature arising from variations in room temperature can produce significant changes in response. Informal measurement of unregulated surface temperature in an air conditioned room range from 20 to 26°C. This range would clearly increase the variation in the response latency independent of other treatments. In addition, it has been shown that skin temperature can vary significantly in quiescent unrestrained rats, and systematic changes in skin temperature have been shown to alter response latencies (Hole and Tjolsen, 1993). The ability of the glass surface to clamp paw skin temperature may thus serve as an important virtue in reducing the test-to-test or rat-to-rat variability that may arise with varying glass and skin temperature. As shown in Fig. 4B, variance increased with increasing latency. If the variance estimate of a population is known, the number of subjects required to detect a given difference can be predicted (Samuels, 1989). Given the current study, it is possible to estimate the minimum number of animals required to detect a 25% difference using a stimulus intensity of 4.9 A with a 30°C glass surface (see Table 2). After setting α and β criteria at 0.90 and 0.05, respectively, detecting a 25% change from baseline at 25°C would require 23 animals. Conversely, using the same criteria at 30°C would require 16 animals, a 45% reduction in animals required per testing group.

In conclusion, the current study demonstrates that increased stimulus intensity is associated with a decreased mean withdrawal latency and variance. For a fixed stimulus intensity, the mean withdrawal latency and variance increased when the glass floor was maintained at room temperature (25°C). These studies emphasize the importance of maintaining a fixed 30°C surface temperature to reduce experimental variability and the utility of this apparatus across multiple stimulus intensities to define relative efficacy for families of antinociceptive agents.

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References

Beitel RE, Dubner R, Harris R, Sumino R. Role of thermoreceptive afferents in behavioral reaction times to warming temperature shifts applied to the monkeys face. Brain Res 1977;138:329–46.

Berge OG, Garcia-Cabrera I, Hole K. Response latencies in the tail-flick test depend on tail skin temperature. Neurosci Lett 1988;86:284–8.

Brennan RJ, O'Brien JP, Demarco PJ, Baldwin AE, Cannon JT. Tail flick: test interval can modify the development of both sensitization and morphine analgesia, Soc. Neurosci, 25th Annual Meeting, 457.4, 1995.

Carstens E, Wilson C. Rat tail flick reflex: magnitude measurement of stimulus-response function, suppression by morphine and habituation. J Neurophysiol 1993;70:630–9.

Carstens E, Yokota T, Zimmermann M. Inhibition of spinal neuronal responses to noxious skin heating by stimulation of the mesencephalic periaqueductal gray in the cat. J Neurophysiol 1979;42:558–68.

Craig AD, Bushnell MC. The thermal grill illusion: unmasking the burn of cold pain. Science 1994;265:252–5.

D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther 1941;72:74–9.

Dirig DM, Yaksh TL. Reduced thermal escape latencies after spinal Substance P receptor activation vary inversely with stimulus intensity, but remain a fixed fraction of basal latencies. Neurosci Lett 1996;220:93–6.

Galbraith JA, Mrosko BJ, Myers RR. A system to measure thermal nociception. J Neurosci Methods 1993;49:63–8.

Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988;32:77–88.

Hirata H, Pataky A, Kajander K, LaMotte RH, Collins JG. A model of peripheral mononeuropathy in the rat [letter; comment] [see comments]. Pain 1990;42:253–5.

Hole K, Tjolsen A. The tail-flick and formalin tests in rodents: changes in skin temperature as a confounding factor. Pain 1993;53:247–54.

Iwata K, Tsuboi Y, Yagi J, Toda K, Furukawa T, Yoshimoto A, Sumino R. Effect of interstimulus interval on perceived sensation and intradental nerve activity during thermal tooth stimulation in man. Brain Res 1994;635:211–6.

Janssen PAG, Niemegeers CJE, Dony JGH. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittel-Forschung 1963;13:502–7.

Kawakita K, Funakoshi M. A quantitative study on the tail flick test in the rat. Physiol Behav 1987;39:235–40.

Kenshalo DR, Holmes CE, Wood PB. Warm and cold thresholds as a function of rate of stimulus temperature change. Percept Psychophys 1968;3:81–4.

Konietzny F. Peripheral neural correlates of temperature sensations in man. Hum Neurobiol 1984;3:21–32.

LaMotte RH, Thalhammer JG. Response properties of high-threshold cutaneous cold receptors in the primate. Brain Res 1982;244:279–87.

Samuels ML. Statistics for the Life Sciences. San Fransico: Macmillan, 1989.

Wahren LK, Torebjork E, Jorum E. Central suppression of cold-induced C-fibre pain by myelinated fibre input. Pain 1989;38:313–9.

Woolfe G, MacDonald A. The evaluation of the analgesic action of pethidine hydrochloride (demerol). J Pharmacol Exp Ther 1944;80:300–7.

Yaksh TL. Pre-clinical models of nociception. In: Biebuyck JF, Lynch C, Maze M, Saidman LJ, Yaksh TL, Zapol W, editors, Anesthesia: Biologic Foundations, Philadelphia: Lippincott-Raven, Chapter 13, 1997 (in press).

Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6.

Yarnitsky D, Ochoa JL. Release of cold-induced burning pain by block of cold-specific afferent input. Brain 1990;113:893–902.

Yarnitsky D, Simone DA, Dotson RM, Cline MA, Ochoa JL. Single C nociceptor responses and psychophysical parameters of evoked pain: effect of rate of rise of heat stimuli in humans. J Physiol 1992;450:581–92.

Yeomans DC, Proudfit HK. Characterization of the foot withdrawal response to noxious radiant heat in the rat. Pain 1994;59:85–94.