Tail-Flick Test: II. The Role of Supraspinal Systems and Avoidance Learning

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It is held that the tail-flick test of pain depends on a spinal reflex because a similar response is observed in spinaly transected rats. But when subjects were manually held and a cool heat setting was used, supraspinal systems facilitated the response (Experiment 1). This effect did not depend on the rate at which the tail was heated (Experiment 2) but rather on the co-occurrence of visual, auditory, and tactile cues that predict impending pain (Experiments 3 and 4). Subjects rapidly learned to exhibit a tail movement during these co-occurring cues, and this avoidance response was instrumental in nature (Experiment 5). Optimal learning was observed when the visual signal was presented 8–12 s before a heat-elicited response is normally observed (Experiment 6), and a low dose of morphine inhibited the performance of the instrumental response (Experiment 7).

Over the last 20 years, researchers have made considerable progress in understanding the neural mechanisms that modulate pain. This progress has hinged on the development and validity of some standard tests of pain reactivity. Some of the more popular tests include assessing the latency to respond to a thermal (e.g., the hot plate test; Eddy & Leimbach, 1953) or a chemical irritant (the formalin test; Dubuisson & Dennis, 1977) applied to the paw, vocalization to shock (Carroll & Lim, 1960), shock discrimination (Grilly & Genovese, 1979), and tail withdrawal from radiant heat (the tail-flick test; D’Amour & Smith, 1941). Although each of these tests has some unique advantages, many researchers have chosen to rely on the tail-flick test. Indeed, a literature search (BRS-Medline) of animal studies on pain measurement conducted between 1992 and 1995 revealed that the tail-flick test was used in approximately 40% of the articles.

Adoption of the tail-flick test has been encouraged by a number of factors. Chief among them is that tail-flick latencies to radiant heat are systematically affected by a variety of pharmacological manipulations known to alter pain in humans (Amit & Galina, 1986; Irwin, Houde, Bennett, Hendershot, & Seegers, 1951). Other important factors contributing to the popularity of this test include the following: The behavioral test is easy to perform and does not require any complicated equipment; relative to other tests, little variability is observed in baseline responding; the test can be conducted while subjects are loosely restrained in tubes, which minimizes the extent to which subjects are disturbed while testing (Grau, 1987); and, finally, evidence suggests that stimuli that induce changes in pain reactivity on this test do so without generally disrupting either sensory processing or the subject’s ability to execute the required motor response (Hayes, Bennett, Newlon, & Mayer, 1978; Illich & Grau, 1990).

Yet another advantage of the tail-flick test is that a great deal is known about the neural circuitry that underlies the reflex. Early studies revealed that the response is organized at the level of the spinal cord, for it can readily be elicited in spinalized subjects (Irwin et al., 1951). Further work has detailed the afferent neurons and motor output (Grossman, Basbaum, & Fields, 1982) and has shown how supraspinal systems can regulate this reflex by means of fibers that descend through the dorsolateral funiculus (Grossman et al., 1982; Necker & Hellung, 1978). Knowing how the response is produced and modulated yields a basic advantage—it helps researchers infer how an experimental manipulation affects the transmission of nociceptive information.

There is, however, one caveat to this simple story: Under some conditions the tail-flick response appears to depend on a different neural circuit that involves supraspinal systems (for a discussion of other problematic issues see Illich, King, & Grau, 1995; Kallina & Grau, 1995; King, Joynes, Meagher, & Grau, 1996; Prentice, Joynes, Meagher, & Grau, 1996). The clearest evidence for this comes from a study by Jensen and Yaks (1986) in which they tested spinalized and sham-operated rats on the tail-flick test under two heat intensities. When an intense heat source was used, the response appeared to depend on a spinal reflex, for both groups exhibited similar latencies. However, when the intensity of the heat source was decreased, only the sham-operated rats responded. This suggests that the response observed in sham-operated rats is not a spinal reflex but instead depends on supraspinal systems. This simple experiment has some very important implications, for it implies that different neural systems may mediate the tail-flick response, depending on the intensity of the test stimulus, a hypothesis that is now widely accepted (e.g., Blass, Cramer, & Fanselow, 1993; Cannon, Walsh, Henry, & Bolan, 1990;
Iwamoto & Marion, 1993; Suh, Fujimoto, & Tseng, 1992). Beyond this, the observation could help to explain some discrepancies in the literature (cf. Davis & Henderson, 1985; Illich & Grau, 1991) and provide a way to compare spinally and supraspinally mediated measures of pain reactivity by using the same type of eliciting stimulus (radiant heat) and the same nominal response (a tail movement).

In the present study we explored the variables that determine whether the response observed reflects a spinal reflex or a supraspinally organized behavior. Experiment 1 provided a replication of Jensen and Yaksh (1986). We showed that when rats are gently held during the test trial, a clear sham–spinal difference emerges when heat intensity is decreased. It is natural to assume that a change in the thermal characteristics (e.g., ramp speed) underlies the emergence of the spinal–sham difference (Blass et al., 1993; Cannon et al., 1990; Iwamoto & Marion, 1993; Suh et al., 1992). However, other cues coevolve with a change in heat intensity. For example, the onset of radiant heat often produces a change in illumination, yielding a visual signal that predicts impending pain. Decreasing heat intensity increases the duration of co-occurring visual cues and possibly their usefulness. With a longer cue, rats may rapidly learn to avoid the painful thermal stimulus by exhibiting a tail movement before the heat reaches their nociceptive threshold. To examine this possibility, we tested rats by using a procedure that allowed us to independently manipulate heat intensity and cue duration. When care was taken to minimize the impact of other cues, spinalized and sham-operated rats exhibited similar latencies across a wide range of heat intensities (Experiment 2). When heat intensity was kept constant and a visual cue was added, sham-operated rats quickly acquired an avoidance response (Experiment 3). Experiment 4 showed that tactile and auditory cues also support this learning, and Experiment 5 demonstrated that the response is instrumentally conditioned. In Experiment 6 we examined the optimal parameters for this learning. We conclude by showing that the avoidance response was highly sensitive to morphine (Experiment 7).

Experiment 1

Our first experiment was designed to replicate Jensen and Yaksh (1986). Tail-flick latencies were assessed in sham-operated and spinalized rats across four heat intensities.

Method

Subjects. The subjects were 16 male albino Sprague-Dawley (Rattus norvegicus) rats from Harlan (Houston, TX). They were 100–120 days old and weighed 450–500 g. The rats were individually housed, were maintained on a 12-h light–dark cycle, and were tested during the light phase. Rats had access to food and water ad libitum.

Apparatus. Subjects were held with the left hand just behind the forepaws, with the thumb under the right forepaw and the rest of the fingers wrapped around the back and under the left forepaw. The hindpaws were allowed to rest on a platform, located 1.0 cm below the top of the aluminum block on which the rat’s tail rested. The forepaws were held approximately 3.0 cm above the platform.

The same experimenter (R. Joynes) held the rats throughout the experiment. A second experimenter (T. King) placed the subject’s tail on the tail-flick device and immediately started the test. The tests were conducted in a room illuminated by light from an adjacent room and a 25-W red light. The room was maintained at 25 (±0.5) °C.

Tail-flick tests were conducted with a tail-flick device that used a 375-W movie light focused onto the rat’s tail by means of a condenser lens positioned 8 cm below the light source. The rat’s tail rested in a 0.5 cm-deep groove that was cut into an aluminum block that was positioned 4.7 cm below the condenser lens. A photocell, located under the groove, was used to automatically turn off the radiant heat if the rat moved its tail laterally 0.5 cm. The duration of the trial was timed to the nearest 0.01 s. The light source illuminated approximately 2.0 cm of the rat’s tail, and its intensity was modulated by an alternating current potentiometer (Leviton, #6681-W, Little Neck, NY). Four intensities were selected, the coolest of which generated tail-flick latencies 5–7 s longer than those Jensen and Yaksh (1986) obtained under their cool setting. The rate of increase for each intensity, as measured by a Fisher scientific thermometer (Model 14-983-10B, Pittsburgh, PA), is depicted in Figure 1. Cutoff times of 8, 10, 12, and 15 s, from the hottest intensity (A) to the coolest intensity (D), were used to prevent tissue damage.

Surgery. Surgeries were performed in the 1st or 2nd hr of the rats’ dark cycle. Subjects were anesthetized with pentobarbital (50 mg/kg), and the spinal cord was transected at the level of the second thoracic vertebrae (T2). After an anterior–posterior incision was made over the spinal cord, the tissue in the region of T2 was cleared. The spinalized subjects had their spinal cord transected by cauteryization, and the remaining gap was filled with Gelfoam.

Figure 1. The ramp speed, as measured by a thermometer, for four heat intensities: Intensity A (hottest) to Intensity D (coolest). In each case, the metal tip of the thermometer was warmed to 28 °C and placed directly under the heat source in the groove used to position the rat’s tail. The rate at which the temperature increased was then recorded over a 16-s period. The results confirm that test temperature varied in a systematic fashion across the four intensities used and provide the data needed to calibrate our equipment across experiments. However, we cannot directly infer tissue temperature as a function of time and intensity from these data, for the rate of heating will depend on the absorption characteristics of the skin and the depth of the nociceptors. For present purposes, though, we need only assume that an increase in physical temperature causes an increase in the rate at which the skin is heated.
(Upjohn, Kalamazoo, MI). The wound was then closed with michel clips (for further details see Meagher, Grau, & King, 1990). The sham operations were done in the same fashion, with the exception that the spinal cord was not transected. All animals were injected with saline solution and were allowed to recover for at least 18–20 hr. Although food and water were made available during the recovery period, both sham-operated and spinalized rats exhibited little consumption. To maintain hydration, supplemental injection of saline was given, and spinal rats had their bladders expressed at regular intervals.

**Procedure.** Subjects received either a sham operation (sham) or a spinalization (spinal) and were tested 18–20 hr later. Before testing, rats were brought into the testing room and restrained while their tails rested on the tail-flick device. The rats were acclimated in this fashion three times, with a 2-min intertrial interval (ITI), before the first test. The behavioral criterion for a response was a lateral movement of the tail. This response moved the tail out of the groove, activating the photocell and stopping the timer. All subjects received four blocks of training. Within each block, each subject was tested six times with one heat setting. Test trials were spaced 2 min apart and were counterbalanced across blocks of training. Subjects were placed into a holding bin located in a separate room during the ITI.

**Results**

The mean tail-flick latencies of sham and spinal subjects across all trials for heat intensities (A–D) are depicted in Figure 2A. When we tested the sham-operated and spinalized rats, using Intensities A and B, they exhibited similar latencies; but under the cooler settings, spinalized rats took far longer to respond. An analysis of variance (ANOVA) revealed a significant main effect of operation and heat intensity (both Fs > 40.61, ps < .05). Importantly, the Operation × Heat interaction was also significant, \( F(3, 42) = 10.49, p < .05 \), indicating that the spinal–sham difference depended on heat intensity.

Further analyses of the data revealed some unexpected outcomes. On the first trial of testing (see Figure 2B), sham-operated rats generally exhibited longer tail-flick latencies. An ANOVA confirmed that both operation, \( F(1, 8) = 6.73, p < .05 \), and test temperature, \( F(3, 8) = 31.06, p < .05 \), had a significant impact on these latencies. The Operation × Heat interaction was not significant, \( F(3, 8) < 1.00, p > .05 \). It seems, then, that sham-operated rats do not initially exhibit shorter latencies. Rather, this tendency must be acquired across trials. This is illustrated in Figure 2C, which depicts tail-flick latencies as a function of test trial collapsed across test temperatures. An ANOVA confirmed that there was a significant trials effect, \( F(5, 70) = 2.97, p < .05 \), and that the change observed across trials depended on operation \( F(5, 70) = 2.68, p < .05 \). The Blocks × Trials × Operation

**Figure 2.** The mean tail-flick latencies (A) and the latencies observed on the first trial (B) across four heat intensities (Intensities A, B, C, and D) for subjects that had received a spinal transection (SPINAL, open circles) or a sham operation (SHAM, filled circles). C: The change in tail-flick latencies (collapsed across test temperature) observed over the four blocks of six training trials for spinalized subjects (SPINAL, open circles) or for sham-operated subjects (SHAM, filled circles). Error bars represent the standard error.
interaction was also significant, $F(15, 210) = 1.74, p < .05$. No other significant differences were found (all $F$s < 1.02, $ps > .05$).

Discussion

As reported by Jensen and Yaksh (1986), sham-operated rats responded sooner than spinalized rats when tested by using a cool setting. But even under the coolest setting, spinal rats generally responded, albeit later than the sham-operated rats. In contrast, spinal rats did not respond under the coolest test temperature used by Jensen and Yaksh. Presumably, this reflects a difference in the terminal test temperature; before the cutoff used for the coolest settings, our device probably reaches a higher temperature. Indeed, this may be true simply because we used a longer cutoff (15 s vs. 10 s). None of our spinal rats responded before the 10-s cutoff used by Jensen and Yaksh; whereas only 1 of the 8 sham-operated rats exhibited latencies that exceeded this criterion. Thus, if a lower cutoff had been used, identical results would have been obtained. What is new and surprising is that spinal rats did not exhibit longer latencies at the start of testing. Rather, the spinal–sham difference emerged across test trials as an additional process came into play.

Experiment 2

As noted above, a change in heat intensity typically affects a variety of co-occurring cues (e.g., visual and tactile), not just tail temperature. In our next experiment we attempted to minimize the contribution of these cues by testing rats while they were restrained in covered Plexiglas tubes. If the thermal characteristics of the test stimulus underlie the emergence of a spinal–sham difference, then shielding rats from extraneous cues should have little effect on the results obtained.

Spinalized and sham-operated rats were tested using the same heat settings in Experiment 1. Because undergoing a sham operation may have some secondary consequences that could affect the tail-flick response (e.g., the stress of surgery may induce a residual hypoalgesia), we also tested a group of unoperated subjects (naive) under identical conditions.

Method

Subjects. Subjects were 24 rats of the same age, sex, and strain as those used in Experiment 1.

Apparatus. Subjects were restrained in Plexiglas tubes that were 22 cm in length and 6.8 cm in diameter. The front of each tube was closed with a Plexiglas sheet. A second sheet of Plexiglas formed a floor, 5.5 cm wide (lying 5.5 cm from the top of the tube) on which the rats could lie. The exterior surfaces of the tubes were painted black, and holes were drilled through the tops of the tubes to provide ventilation for the rats. The subject's tail protruded from the end of the tube, between the base and a band of tape, and could move freely in response to stimulation. The tail-flick device was the same as that used in Experiment 1, and the same four heat intensities were used.

Surgery. Surgeries were performed as described in Experiment 1.

Procedure. Subjects received a sham operation (sham), spinalization (spinal), or no operation (naive) and were tested 18–20 hr later. The rats were placed in the tubes and were allowed to acclimate for 15 min. Each subject was then tested under each intensity three times at 2-min intervals. Test order was counterbalanced across subjects by using a Latin square design.

Results

The results are depicted in Figure 3. It is clear that longer tail-flick latencies were obtained with the cooler test temperatures and that similar results were obtained across the three groups. An ANOVA confirmed that test temperatures had a significant impact, $F(3, 63) = 110.77, p < .001$. Neither the main effect of operation, $F(2, 21) = 1.05, p > .05$, nor its interaction with test temperature, $F(6, 63) < 1.00, p > .05$, approached statistical significance.

Further analyses were performed to evaluate whether a spinal–sham difference emerged across trials. Tail-flick latencies generally declined across test trials (approximately 2 s), but the magnitude of this effect did not vary across groups. An ANOVA showed that the trials effect was significant, $F(2, 42) = 9.20, p < .001$. Neither the main effect of test block nor its interaction with trials was significant (both $Fs < 2.12, ps < .05$). Most importantly, the main effect of operation, and all of its higher order interactions, did not approach statistical significance (all $Fs < 1.27, ps > .05$).

Discussion

When care was taken to minimize the contribution of other cues, a spinal–sham difference failed to emerge under
the cooler test temperatures. We have replicated this observation by using a different test apparatus and a much wider range of heat intensities (King, Payne, & Grau, 1993). In no case did sham rats exhibit shorter tail-flick latencies when they were shielded from co-occurring cues, nor was there any indication that a spinal–sham difference would emerge across trials. As is often the case, tail-flick latencies declined slightly across test trials, but a similar change was observed in all three groups. This decline presumably reflects a change in tail temperature. As we (Kallina & Grau, 1995) and others (Hole & Tjølsen, 1993) have shown, repeated testing generally produces an increase in tail temperature that causes tail-flick latencies to drift downward across trials.

**Experiment 3**

In Experiment 2 we minimized co-occurring cues and varied test temperature. In Experiment 3, co-occurring cues were manipulated while test temperature was kept constant. All rats were tested with the hottest heat setting (A) used in Experiments 1 and 2. A co-occurring visual cue was provided by illuminating the end of the restraining tube. Two cue durations were used: a long signal that preceded the onset of heat by 4 s and a brief signal that did not begin until heat onset. Control subjects experienced the signal during the ITI.

**Method**

**Subjects.** Subjects were 36 rats of the same age, sex, and strain as those used in Experiment 1.

**Apparatus.** Subjects were restrained in the tubes described in Experiment 2 and were tested by using the tail-flick device described in Experiment 1. A visual cue was provided by illuminating a 24-V light (GE 1820) that was positioned immediately in front of the tubes, between the clear Plexiglas front panel and the exterior covering.

**Procedure.** Subjects received either a sham operation or a spinal transection and were tested 18–20 hr later. The rats were placed in the tubes and were allowed to acclimate for 15 min. Tail-flick latencies were measured with the hottest temperature setting (A) that was used in Experiments 1–2. Twelve subjects (6 spinal and 6 sham rats) were exposed to a 4-s light cue before heat was applied to the tail (long signal). The light and the heat were turned off immediately after the rat flicked its tail from the groove. If the rat performed a tail flick during the 4-s light cue, the heat was never applied to the tail, and the light was turned off. Tail-flick latencies were recorded, by a stopwatch, from the time the light was turned on until the tail flick was performed. Twelve other subjects, prepared in the same fashion, experienced the visual cue alone during the interval between tail-flick tests (ITI signal). The duration of the cue was determined by yoking each of these rats to a similarly prepared subject in the long-signal condition. Rats in the ITI signal condition experienced the cue for the same duration as their long-signal partner but, the cue was unpaired with the tail-flick test that was performed (in the absence of a visual cue) 1 min after cue offset. Another 12 subjects (6 spinal and 6 sham) had the light and the heat presented at the same time, but without the 4-s warning period (short signal). Both the light and the heat were turned off as soon as the rats flicked their tails from the groove, and tail-flick latencies were recorded. Tail-flick trials were performed at 2-min intervals. After 20 training trials, tail-flick latencies were assessed in the absence of the visual cue (no signal), with a concurrent visual cue (short signal), and with the onset of the visual cue preceding the onset of radiant heat by 4 s (long signal). Each subject was tested twice under each condition. These tests were conducted in an ABCCBA order that was counterbalanced across subjects.

**Results**

The tail-flick latencies recorded over the 20 training trials for sham (Figure 4A) and spinal (Figure 4B) subjects are depicted in Figure 4. All groups, except for the sham subjects that had a 4-s warning signal (long signal), exhibited

![Figure 4](image-url)
similar tail-flick latencies across all 20 trials. An ANOVA revealed that the main effects of operation, signal condition, and the Operation × Signal interaction were all significant (all Fs > 9.29, ps < .05). There was also a significant trials effect, F(19, 570) = 9.03, p < .05. Both the Trials × Operation and the Trials × Signal Condition interactions were significant (all Fs > 1.45, ps < .05). Although the overall Trials × Operation × Signal Condition interaction was not statistically significant, F(38, 570) = 1.23, p > .05, trend analysis revealed a significant linear component, F(1, 570) = 7.05, p < .05. This indicated that the linear trend observed across trials depends on both operation and signal condition.

The results obtained when sham (Figure 5A) and spinal (Figure 5B) subjects were tested with a brief, long, or no signal are depicted in Figure 5. Again, subjects exhibited similar tail-flick latencies except for the sham-operated group that was trained with the long signal. When tested with the long signal, but not the brief signal, these subjects exhibited much shorter tail-flick latencies. Indeed, when the long signal was presented, these subjects generally responded approximately 1 s before the thermal stimulus was turned on. An ANOVA revealed that operation, F(1, 30) = 18.14, p < .05, training condition, F(2, 30) = 6.34, p < .05, and test condition, F(2, 60) = 8.31, p < .05, had a significant impact on tail-flick latencies. Each of the interaction terms was also significant (all Fs > 6.01, ps < .05), indicating that the impact of the test cue depended on both operation and training condition.

**Discussion**

Experiment 1 showed that under some test conditions, sham-operated rats exhibit shorter latencies, which suggests that supraspinal systems can facilitate the execution of the motor response. What was not clear is when this facilitatory process is engaged. Most have assumed that the critical variable is simply the rate at which the skin is heated. However, when this alone was manipulated in Experiment 2, no spinal–sham difference was observed. In contrast, when test temperature was kept constant, and a co-occurring cue (a light) was provided, sham-operated rats rapidly learned to exhibit a tail-flick response. Interestingly, whether or not this learning emerged depended on signal duration; intact rats only learned when a long signal was provided. This implies that decreasing test temperature in Experiment 1 brought supraspinal mechanisms into play because it increased the duration, and apparently the usefulness, of co-occurring cues.

In many situations, changes in tail-flick latencies are confounded by a change in tail temperature (Hole & Tjølsen, 1993). The difficulty is that an increase in tail temperature can cause a decrease in tail-flick latencies (Kallina & Grau, 1995). It is clear, however, that such an effect cannot account for the learning observed in sham subjects trained with a long signal. First, because these rats received less heat exposure, their tails should be cooler, which would act to lengthen, not shorten, tail-flick latencies. Indeed, this might help to explain why these subjects generally exhibited longer latencies when the cue was omitted during testing. Second, and more important, sham-operated rats tested with a long signal tended to respond before radiant heat was even applied. Clearly, it was the visual signal, not the thermal cue, that elicited the response. Given this, it is difficult to see how a change in tail temperature could account for our learning effect.

**Experiment 4**

Experiment 3 showed that when a long visual cue is provided, intact rats rapidly learn to exhibit a tail movement before the radiant heat becomes noxious. In the present
experiment we explored whether other cues (tactile and auditory) that often accompany the presentation of radiant heat with the tail-flick test can support this learning. As in Experiment 3, the hottest tail-flick setting was used, and the cues were provided 4 s before the onset of radiant heat.

Method

Subjects. Subjects were 6 rats of the same age, sex, and strain as those used in Experiment 1.

Apparatus. To expose rats to the tactile cue, the restraining tubes, which were described in Experiment 3, were shortened to 16.2 cm. The tail-flick device was set to the same intensity used in Experiment 3.

The visual cue was presented as described in Experiment 3. An auditory cue was generated by an Elgenco Gaussian noise generator (Model 602A, Santa Monica, CA) that was amplified by a Realistic SA-10 amplifier (Model 31-1982b, Fort Worth, TX) and presented through Realistic 3-in. surface mount speakers (Model 12-1852, Fort Worth, TX) mounted 14 cm above the restraining tubes. Noise intensity was adjusted to 80 dB as measured by a decibel meter placed at the open end of the restraining tube. The tactile cue was provided by gently holding the rat's hindquarters, just rostral to the tail, between two fingers and the thumb.

Procedure. A within-subject design was used to determine whether rats could learn to use other cues (tactile or auditory) to avoid the noxious heat. To stabilize nociceptive thresholds, a single unsignaled tail-flick test was administered. All subjects then received 10 blocks of training. Within each block, each subject was tested under four different cue conditions: visual, auditory, tactile, or no cue. The cues were presented in the same manner as the long signals used in Experiment 3, and the order was counterbalanced across subjects. Thus, the cue was turned on 4 s before heat was applied to the tail and remained on until a tail-flick response occurred. If a subject responded during the first 4 s of the cue, heat was not applied on that test trial. Tail-flick latencies were recorded by a stopwatch from the time the cue was turned on until the tail-flick occurred. Training trials were spaced 2 min apart.

Results and Discussion

The tail-flick latencies recorded over the 10 blocks of training trials are depicted in Figure 6. In the absence of a cue (no cue), tail-flick latencies remained stable across test trials. When a light, noise, or tactile cue was provided, subjects rapidly learned to exhibit a tail flick to avoid the noxious heat. An ANOVA confirmed that both the trials effect and the Trials × Signal interaction were significant (both $F$s > 2.77, $p$s < .05). The main effect of signal treatment was also significant, $F(3, 15) = 12.71, p < .05$. A Duncan's multiple-range test showed that, relative to the no-cue condition, subjects responded sooner when a signal was presented. No other differences were significant.

Further analyses were then performed to determine how quickly subjects acquired the avoidance response under each cue condition. A Bonferroni $t$ test was used to make these comparisons because it maintains the overall error rate at .05 for a family of contrasts. To obtain the appropriate mean square error for these comparisons, we performed three additional ANOVAs that compared the performance observed over trials under one cue condition with the latencies observed when no cue was provided. On a trial-by-trial basis, performance under each cue condition was then compared with the latencies recorded when no cue was provided. Using this test, we found that when a tactile or visual cue was provided, a significant difference emerged by the third test trial (Bonferroni $t = 2.97, p < .05$). In contrast, the auditory cue did not have a significant effect until the ninth test trial (Bonferroni $t = 3.47, p < .05$). Although the relative effectiveness of these cues appears to differ, it is clear that a variety of stimuli can support learning on the tail-flick test.

Experiment 5

It is clear that rats can learn to exhibit a tail movement to a signal that predicts nociceptive stimulation. It is natural to assume that this represents an instrumental avoidance response. From this perspective, the response-reinforcer contingency plays a critical role in maintaining the target response. Alternatively, the learning observed could reflect a form of Pavlovian conditioning. According to this view, the signal functions as a conditioned stimulus (CS) while the noxious heat provides an unconditioned stimulus (US). Pairing the US with the US may endow the signal with the ability to elicit a conditioned response (CR) similar to the unconditioned response (UR) elicited by the US. With this type of learning all that should matter is the number of CS–US pairings; the instrumental response-reinforcer contingency should be irrelevant.

In the present experiment we examined these alternatives by using a yoked-control design. One group of rats was trained with a long visual cue as described in Experiment 3. For these subjects, performing a response during the cue terminated, or prevented, the radiant heat. For rats in the yoked group, the response-reinforcer contingency was removed. Each of these rats was yoked to a rat that had control and experienced the same number of CS-US pairings, at the same intervals, and for the same stimulus durations. If the
signal-elicited response reflects a Pavlovian CR, then this experience should be sufficient to endow the signal with the ability to generate a conditioned tail movement.

Method

Subjects. Subjects were 30 rats of the same age, sex, and strain as those used in Experiment 1.

Apparatus. Subjects were restrained and tested with the same apparatus described in Experiment 3.

Procedure. Subjects were placed in the restraining tubes and were allowed to acclimate for 15 min. One group was trained with a long visual cue as described in Experiment 3. Subjects in this group received 20 training trials, at 2-min intervals, with a light cue provided 4 s before the onset of radiant heat. Both the light and the heat were terminated by a tail-flick response. If a response was made before the onset of heat, heat was never applied to the tail. Another group of subjects was yoked to rats in this group. For the experimenter to gain control over the application of radiant heat, rats in this group had their tail taped to a Plexiglas bar (5.0 × 1.3 cm) that extended from the base of the tube. The tip of the tail was taped to a second bar (4.5 × 1.4 cm) positioned on the distal side of the aluminum block that lies under the radiant heat source. Each yoked rat received the exact same exposure to the visual cue and heat as their trained partner. For comparison, an unsignaled group was also included. These subjects experienced the cue during the ITI (between unsignaled tail-flick tests) unpaired with the noxious heat. Cue duration was determined by a trained partner, and a tail-flick response terminated the thermal stimulus. Starting 2 min after the last training trial, all subjects were tested with and without the visual cue in an ABBA counterbalanced order.

Results

The tail-flick latencies observed during the 20 trials of training are depicted in Figure 7A. Relative to subjects that received the signal during the ITI (ITI signal), rats that received signaled controllable heat (trained) learned to exhibit a tail movement. An ANOVA revealed a significant effect of cue condition, $F(1, 18) = 10.18, p < .05$, and test trial, $F(19, 342) = 5.87, p < .05$. Importantly, the change observed across trials depended on cue condition, $F(19, 342) = 1.65, p < .05$.

The mean tail-flick latencies observed after training are depicted in Figure 7B. Rats trained with the cue exhibited shorter latencies when tested with the cue. The cue had no impact on performance in the other two groups. Both the main effects for training condition and test cue were significant (both $Fs > 3.88, ps < .05$). The training Condition × Cue interaction was also significant, $F(2, 27) = 8.39, p < .05$. To further analyze this interaction, we calculated the mean difference between cued and uncued test trials for subjects in each group. An ANOVA confirmed that the magnitude of the cue/no-cue difference varied across groups, $F(2, 27) = 8.03, p < .05$. A Duncan’s multiple-range post hoc test revealed that the cue/no-cue difference observed in the trained group was significantly greater than that observed in the other two groups. None of the other comparisons were significant.

![Figure 7](image-url)

Discussion

When a response–reinforcer contingency existed, rats rapidly learned to exhibit a tail movement during the cue. Rats that received the same cue–heat pairings, but lacked control, failed to learn. Thus, simply experiencing the cue–heat relation is not enough to generate and maintain a cue-elicited tail movement. This implies that the response observed during the cue does not reflect a Pavlovian CR. Instead, rats given cued tail-flick tests appear to acquire an instrumental response that depends on the response–reinforcer contingency.
Experiment 6

The preceding experiments demonstrated that rats can learn to flick to a visual warning cue to avoid a painful thermal stimulus. In our next experiment we explored how this learning varies as a function of cue duration. Cue duration was manipulated by varying the cue–heat interval; the cue preceded the onset of heat by 0, 2, 4, 8, or 16 s. For comparison, a sixth group was trained without a cue.

Method

Subjects. Subjects were 24 rats of the same age, sex, and strain as those used in Experiment 1.

Apparatus. Subjects were restrained and tested with the same apparatus described in Experiments 1–3.

Procedure. Subjects were placed in the restraining tubes and were allowed to acclimate for 15 min. All rats then received 10 tail-flick tests at 2-min intervals. One group of rats was tested without the visual cue. The other five groups had a cue presented, for the appropriate duration (0, 2, 4, 8, or 16 s) before heat was applied. A tail-flick response during the heat terminated both the visual cue and the thermal stimulus. For the four groups that received a cue before the onset of heat, a response during the cue alone terminated the cue and prevented the application of heat. Tail-flick latencies were recorded by a stopwatch from the time the cue was turned on until the response occurred.

Results

The mean tail-flick latencies for each cue duration are depicted in Figure 8. A comparison to the unsignaled group (no cue) revealed that the cue had no effect when its onset preceded heat by 2 s or less. Increasing cue duration up to 8 s led to shorter latencies while further increasing cue duration undermined performance. An ANOVA confirmed that the groups differed, $F(4, 15) = 3.87, p < .05$. To explore how performance varied as a function of cue duration, we used a trend analysis to compare the average latencies for the five groups that experienced the cue. This analysis revealed that both the linear, $F(1, 15) = 5.60, p < .05$, and the cubic, $F(1, 15) = 5.46, p < .05$, trends were significant. The linear component indicated that latencies generally decreased as the cue–heat interval was increased. The cubic trend showed that this relationship contained two significant inflections: one at 2 s and another at 8 s. Neither the quadratic nor the quartic components were significant (both $F$s < 3.60, $ps > .05$).

Discussion

As found in Experiment 3, subjects failed to learn when the cue and heat came on at the same time. Indeed, learning was not observed until the cue preceded the heat by 4 s. Further increasing cue duration to 8 s enhanced learning, but beyond this performance deteriorated. Of course, these values must depend on the relative intensity of the heat source, for what is surely critical is the cue to nociceptive threshold interval. Given that rats responded to our uncued thermal stimulus at approximately 4 s, it would appear that under the present experimental conditions, optimal learning is observed when the cue occurs 8–12 s before a heat-elicited response is normally observed.

Experiment 7

We have shown that rats can learn to exhibit an instrumental response to avoid a thermal nociceptive stimulus. We assume that this learning is motivated by the anticipation of pain. As such, the signaled version of the tail-flick test may provide a window into an aspect of pain processing that has received relatively little attention. But before we can argue that this supraspinally mediated response to thermal stimulation provides a new measure of pain, we must show that it is systematically affected by manipulations that are known to alter human pain. In the present experiment we began to address this issue by testing the impact of morphine on the acquisition and performance of the avoidance response.

Method

Subjects. Subjects were 24 rats of the same age, sex, and strain as those used in Experiment 1.

Apparatus. Subjects were restrained and tested with the same apparatus described in Experiments 1–3.

Procedure. On Day 1, half of the subjects were injected intraperitoneally with 1 mg/kg morphine. The remaining subjects were injected with an equivalent amount of saline. Fifteen minutes after the injection, subjects were placed in the restraining tubes and were allowed to acclimate for 15 min. All subjects then received 20 training trials at 2-min intervals. For each trial, the visual cue was presented for 8 s, then the heat was applied to the tail. A response terminated both the visual cue and the heat. If subjects responded before heat onset, the signal was terminated and heat was not applied.

On Day 2, half of the subjects in each condition were given morphine (1 mg/kg ip), whereas the remaining subjects received saline. Fifteen minutes later, they were placed in the restraining...
tubes and were allowed to acclimate for 15 min. The rats were then tested in the presence of the visual cue or with no cue in an ABBA counterbalanced order.

Results

Because tail-flick latencies on Day 1 did not depend on Day 2 group assignment (all F’s < 1.37, ps > .05), the data from Day 1 were collapsed across Day 2 drug condition.

Mean tail-flick latencies observed across trials on Day 1 in morphine- and saline-treated subjects are depicted in Figure 9. An ANOVA confirmed that drug treatment had a significant impact, F(19, 380) = 2.99, p < .05. Although the overall Trials X Drug interaction was not statistically significant, F(57, 380) = 1.14, p > .05, its cubic component was significant, F(1, 380) = 5.96, p < .05. This trend reflects the rapid acquisition observed in the saline-treated rats (Trials 1–5), which was followed by a period during which performance declined (Trials 6–8) and then recovered. This produced an acquisition curve with two inflections (a cubic trend). The linear, quadratic, and quartic components were not significant (all F’s < 1.15, ps > .05).

The results from Day 2 are depicted in Figure 10. In the absence of a cue (open bars), subjects that received morphine for the first time (saline–morphine) were hypoalgesic relative to the saline controls, whereas subjects that had previously experienced morphine exhibited little hypoalgesia to morphine on Day 2 (acute tolerance). A very different pattern was observed when subjects were tested in the presence of a cue (filled bars). Subjects given saline on Day 2 exhibited shorter tail-flick latencies irrespective of their drug treatment on Day 1, whereas rats given morphine on Day 2 uniformly exhibited longer tail-flick latencies. An ANOVA showed that both the main effect of drug, F(3, 20) = 10.36, p < .05, and the main effect of cue condition, F(1, 20) = 28.79, p < .05, were significant. Of importance, the Cue X Drug interaction was also significant, F(3, 20) = 6.76, p < .05. To further analyze this interaction, we calculated the mean difference between cued and uncued test trials for subjects in each group. An ANOVA confirmed that the magnitude of the cue/no-cue difference varied across groups, F(1, 20) = 28.78, p < .05. A Duncan’s multiple-range test revealed that the cue/no-cue difference was significantly greater in subjects given saline on Day 2 relative to the groups given morphine. No other comparisons were significant.

Discussion

These results have several important implications. First, the avoidance response is highly sensitive to morphine, which suggests that it is motivated by the pain-inducing characteristics of thermal stimulation. Second, the results indicate that morphine affects the performance of the avoidance response, not its acquisition. Subjects that received saline on Day 1, and had acquired the avoidance response, exhibited much longer latencies when given morphine on Day 2. Conversely, rats that received morphine on Day 1 performed as well on Day 2 as subjects that received saline on both days. It appears that morphine-treated rats learned about the relationship between the stimuli and responding on Day 1, even though performance
was degraded. Finally, it is apparent that our conclusions depend on how pain reactivity is assessed. If we focus on the uncued data from Day 2, it appears that prior exposure to morphine produced acute tolerance. In contrast, the data from the cued condition suggest that morphine retained its hypoalgesic potency.

It is clear from these results that morphine attenuates the performance of the avoidance response. Importantly, this decrement in response vigor cannot be attributed to state-dependent learning, for rats trained and tested in the presence of morphine failed to exhibit a response; whereas those trained under morphine, but tested in the presence of saline, responded as rapidly as rats that received saline before training and testing. What is less clear is how morphine undermines the performance of an aversively motivated response, both here and in other test paradigms (Gallagher, Kapp, McNall, & Pascoe, 1981; Hertz, 1960; Mauk et al., 1983; Mauk, Warren, & Thompson, 1982). One hypothesis is that morphine has this effect because it attenuates the induction of a central state (e.g., fear) that is necessary to the expression of learned behavior (Mauk et al., 1983).

General Discussion

At the most general level, in the present article we are concerned with the problem of pain measurement: How can researchers infer changes in pain and monitor the mechanisms that contribute to it? This is a critical issue, not only to those interested in the psychology of pain but also to those who hope to uncover the underlying neurobiological mechanisms.

As outlined by Chapman (1989) and Donaldson (1989), the way researchers choose to measure a hypothetical construct, such as pain, depends on the model of the underlying process. In the pain literature, most researchers have adopted, at least in a casual way, the model outlined by Basbaum and Fields (1984). This model assumes that neural mechanisms within the brainstem inhibit the flow of nociceptive information within the spinal cord. Because a critical step in pain modulation, the inhibition of the nociceptive signal, was thought to occur within the spinal cord, researchers have sought measures of this inhibitory process. At a behavioral level, this has fueled interest in spinal nociceptive reflexes, for it has been assumed that the descending inhibitory signal acts on the afferent input and, thus, inhibits both the ascending nociceptive message and the motor output.

Although a variety of spinal reflexes might be studied in rodents, most researchers have adopted the tail-flick test, for it does not require sophisticated equipment, is easy to perform, and is sensitive to manipulations known to reduce pain in humans (e.g., administration of morphine). On this last criterion, the tail-flick test and related spinal reflexes (e.g., paw withdrawal) have fared quite well. The only caveat is that the dose required to produce analgesia in rodents is often much higher than that needed to eliminate pain in humans (Hammond, 1989). Another factor contributed to the popularity of the tail-flick test is that it can be repeatedly applied. The benefits of repeated testing are obvious: (a) By pretesting subjects, baseline latencies can be established, which can be used to help to control for individual variability, and (b) it allows researchers to monitor the induction and time course of putative antinociceptive treatments. Of course, the latter issue can be addressed by using separate groups of subjects, but this greatly increases the numbers of subjects required and, hence, the cost.

The Problem of Avoidance Learning

At a recent conference on pain measurement, Hammond (1989) noted how typical assessment procedures could support learning and cautioned researchers that they may be underestimating its contribution. Interestingly, much of the subsequent discussion focused on the potential role of learning (Jensen, 1989). Motivated by the observations of Jensen and Yaksh (1986), the participants assumed that learning would depend on the “warmth” cues that precede nociceptive thresholds. Because very few tail fibers appear to signal warmth (Jensen, 1989), and because Jensen and Yaksh (1986) observed a spinal–sham difference only when a cooler test temperature was used, most concluded that learning contributed little to their results. By minimizing the presumed contribution of learning, researchers gain a greater confidence in the validity of the essential inference that underlies the test—that changes in tail-flick latencies reflect the induction of pain modulatory systems at the level of the spinal cord. It also provides a rationale for using more powerful within-subject designs that rely on repeated testing.

Our results suggest a very different perspective—that rats can rapidly learn to exhibit an avoidance response on the tail-flick test. Experiment 1 showed that when subjects were manually restrained, sham-operated rats responded sooner when tested with a cool setting. Interestingly, this effect was not observed at the start of training but instead emerged across test trials. Experiment 2 demonstrated that, when subjects are shielded from co-occurring cues, spinalized and sham-operated rats exhibit similar latencies across a wide range of test temperatures. The implication is that warmth cues provide little basis for learning on the tail-flick test, as others have suggested. However, this does not mean that learning cannot take place, for a variety of other cues may signal impending pain. Supporting this, Experiment 3 showed that when a visual cue was provided 4 s before the onset of heat (approximately 8 s before the thermal stimulus reached nociceptive threshold), rats rapidly learned to exhibit a tail movement. Experiment 4 demonstrated that tactile and auditory cues can also support learning, and Experiment 6 revealed that optimal learning occurs when the signal is presented 8–12 s before the radiant heat reaches nociceptive thresholds. Importantly, these observations were made using the hottest setting (Intensity A, Experiments 1 and 2).

Taken together, these results suggest a very different interpretation of the spinal–sham difference observed with the cool test setting in Experiment 1. They imply that the emergence of a spinal–sham difference depends on an increase in the duration of co-occurring cues rather than on
the decrease in test temperature. If a salient cue is provided 8–12 s before a heat-elicited response is normally observed, intact rats rapidly learn to exhibit an avoidance response, and this is true irrespective of the heat intensity used to elicit the response. The only reason that heat intensity appears to matter is that it generally covaries with other cues (e.g., visual and tactile) that can support learning only if they provide adequate warning; a long cue (8–12 s) supports learning, whereas a brief cue (4 s) does not. Interestingly, a similar observation has been made in studies of avoidance learning using shock; long (6 s) warning cues support avoidance learning, whereas brief (3 s) cues do not (Schwartz, 1958).

Underlying Mechanisms

Instrumental mediation. Theoretically, the learning observed on the tail-flick test could reflect either a Pavlovian CR or an instrumental avoidance response. In Experiment 5 we addressed this issue by using a yoked-control design. Rats that could control the termination of heat exhibited shorter latencies when tested on the cued version of the tail-flick test; whereas yoked rats, which had experienced the same signal–heat pairings, did not. This implies that the response observed is instrumental in form. Of course, Pavlovian conditioning may still contribute. Indeed, two-factor theories of avoidance learning assume a Pavlovian CR motivates acquisition of the avoidance response (Rescorla & Solomon, 1967). Moreover, the relative contribution of Pavlovian and instrumental processes may vary as a function of training; Pavlovian mechanisms may play a greater role early in training.

Motivated by pain. Finally, we assessed the impact of morphine. We found that a low dose of morphine disrupted the performance, but not the acquisition, of the instrumental response. The experiment also showed how cued and uncued versions of the tail-flick test can lead to very different conclusions; while a low dose remained effective on the cued version of the test, it was ineffective when the cue was omitted. Thus, although the uncued test may be insensitive to clinically effective doses of morphine (Hammond, 1989), the cued version appears highly sensitive. This raises the intriguing possibility that the cued version of the tail-flick test provides more information about the affective–evaluative component of pain and in this way could be more clinically relevant. Thus, rather than being viewed as yet another nuisance variable, avoidance learning may provide a window into an understudied component of pain processing—the affective–evaluative aspect of pain. Moreover, because trained rats can effectively be "switched" from the avoidance response to a spinal reflex by simply omitting the signal, information can be gained about both the regulation of nociceptive information within the spinal cord and its subsequent affective impact. Importantly, this is achieved by using the same noxious stimulus to motivate the response and the same response criterion.

Implications

At a practical level, our results have several important implications. One concerns the impact of baseline testing. During such tests, researchers typically observe a rapid decline in tail-flick latencies. Most have assumed that this shift reflects an increase in tail temperature. But our results suggest another possibility—that co-occurring cues reliably signal impending pain and, consequently, support the development of an avoidance response. It is important to realize that simply increasing test temperature does not preclude such learning. Indeed, learning was observed in Experiments 3–7 with our hottest tail-flick setting. What is critical is whether cues reliably predict impending pain during the optimal window for learning, which in our laboratory is 8–12 s before the thermal stimulus reaches nociceptive thresholds. Moreover, as Experiment 5 demonstrated, nearly any cue can support such learning. The implication is that, to minimize the possibility of learning, subjects should be shielded from co-occurring cues.

Our results also imply that decreasing test temperature will not necessarily alter the neural mechanism involved. As long as subjects are shielded from co-occurring cues, the tail-flick response appears to be spinally mediated (Experiment 2). This is important, for in some cases, it may be advantageous to test subjects with a cooler setting. For example, in studies of hyperalgesia, in which the experimental manipulation shortens tail-flick latencies, greater resolution may be obtained by lowering the test temperature. As long as subjects are shielded from the cues that predict impending pain, the response should reflect a spinal reflex. Of course, any strong claims regarding the spinal nature of the response should be verified by demonstrating that the response survives spinal transection.

These experiments also have implications for studies assessing the impact of manipulations known to influence instrumental learning. For example, it is well established that an extended exposure to inescapable shock produces a learning deficit (Maier & Seligman, 1976) and increases tail-flick latencies (Maier, Drugan, & Grau, 1982). We have assumed that this reflects a spinally mediated antinociception, but it is possible that longer latencies are observed, at least in part, because inescapable shock interferes with an instrumental component of the tail-flick test. Yet another example is provided by studies of drugs, such as scopolamine, that disrupt learning and memory. We have shown that scopolamine facilitates a spinal nociceptive reflex, causing rats to appear hyperalgesic relative to saline controls (Grau, Illich, Chen, & Meagher, 1991). But the opposite should be observed on a cued version of the test, for the drug should impair the acquisition of the avoidance response. As a consequence, relative to saline controls, scopolamine-treated rats would appear hypnalgesic.

A Pavlovian CS may also have very different effects on tail-flick latencies, depending on whether co-occurring cues are present. When subjects are shielded from such cues, a CS that has been paired with shock produces a conditioned antinociception, a CS-elicited increase in tail-flick latencies (Illich & Grau, 1991). In contrast, Davis and Henderson
(1985) found that a CS paired with shock produces enhanced pain reactivity, or conditioned hyperalgesia. Of interest, Davis and Henderson used a test paradigm that should encourage learning; a cool setting was used, rats were manually held, and testing occurred in a darkened room. Notice that these are the same conditions that yielded rapid learning in Experiment 1. Given this, it seems likely that avoidance learning contributed to the performance of the tail-flick response in their paradigm. Because a CS that has been paired with shock facilitates the performance of aver絲ously motivated avoidance responses (Rescorla & Solomon, 1967), the CS would appear to induce hyperalgesia on a cued version of the tail-flick test.

Conclusions

Over the last three decades, a great deal of progress has been made in researchers’ understanding of the physiological and psychological mechanisms involved in pain perception. But to continue making progress, researchers must have a clear picture of what can, and cannot, be inferred from their behavioral measures (Chapman, 1989). On the face of it, there was little a priori reason to believe that different mechanisms (spinal vs. supraspinal) would be engaged, depending on whether subjects were manually held (Experiment 1) or restrained in covered tubes (Experiment 2). In the absence of this knowledge, inconsistencies can emerge (cf. Illich & Grau, 1991; Davis & Henderson, 1985) and considerable effort can be expended by attempting to account for why a presumed common mechanism yields opposite results, when in fact, different mechanisms are being studied.

The tail-flick test has served researchers well over the last 25 years, and it clearly remains a very useful tool. But there is an increasing need to move beyond the pragmatic operationalism, adopted by most, to a classicist perspective that relies on multiple measures. Researchers cannot infer phenomena such as hypoalgesia and the induction of diffuse inhibition within the spinal cord using the tail-flick test alone. There is mounting evidence that pain, simple withdrawal reflexes, and the induction of diffuse noxious inhibitory controls sometimes move in opposite directions. Manipulations that generally inhibit nociceptive neurons within the cord sometimes facilitate spinal nociceptive reflexes (Morgan, Heinricher, & Fields, 1994). Conversely, longer tail-flick latencies do not necessarily imply diminished pain; the effect observed may be localized to the region where the noxious stimulus was applied (Prentice et al., 1996) and be accompanied by an increase in the affective–motivational component of pain (Illich et al., 1995; King et al., 1996).

The implication is that greater caution is warranted regarding the interpretation of data collected using simple spinal nociceptive reflexes. These tests can provide a window into the regulation of nociceptive information within the spinal cord, but they can also be misleading. To avoid this trap, researchers need to bolster their inferences with multiple measures of nociception and pain while being careful to select operations that rely on different stimuli, processing mechanisms, and behavioral outputs (Prentice et al., 1996).

References


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