Tail-Flick Test I: Impact of a Suprathreshold Exposure to Radiant Heat on Pain Reactivity in Rats

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KALLINA, C. F. AND J. W. GRAU. Tail-flick test I: Impact of a suprathreshold exposure to radiant heat on pain reactivity in rats. PHYSIOL BEHAV 58(1) 161–168, 1995.—Radiant heat applied to the tail elicits a vigorous tail-flick response in rats. This tail-flick reflex is frequently used to measure pain reactivity. Prior research has shown that a suprathreshold exposure to the radiant heat used to elicit this reflex causes a reduction in tail-flick latencies in pentobarbital anesthetized rats. Experiment 1 replicated this observation and showed that the effect is not due to a change in tail temperature. Experiment 2 showed that reduced latencies (hyperalgesia) are not observed if the spinal cord is transected prior to testing. Experiment 3 revealed that pentobarbital anesthesia is required to observe thermal-induced hyperalgesia, for this effect is not observed in awake subjects. Experiments 4 and 5 extended this observation by showing that awake rats also fail to exhibit hyperalgesia if other measures of pain reactivity are employed (shock-induced vocalization and motor reactivity). Implications of the results are discussed.

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WHETHER or not we perceive an aversive event as painful depends on a variety of social, cultural and experiential variables (13). At one extreme, people sometimes fail to notice strong noxious stimuli, stimuli which would normally induce a great deal of pain (4). At the other extreme are cases where people report intense pain in response to a mildly aversive stimulus, or even nonaversive stimulation (12). Over the last 25 yr, we have learned a great deal about how the body can reduce the level of pain experienced (1). Much less is known about the environmental events, psychological factors, and physiological systems that enhance pain reactivity. Surprisingly, this is true even though it has been known for many years that exposure to a variety of stimuli (e.g., horizontal oscillation, a novel context, being held by the nape of the neck, or a mild tail pinch) can enhance pain reactivity in rodents (7,15,16), and that this “hyperalgesia,” can be observed on both spinally mediated [e.g., tail withdrawal from radiant heat, the tail-flick test (9)] and supraspinally mediated (e.g., shock-induced vocalization) measures of pain reactivity (7, 15).

A particularly interesting example of environmentally induced hyperalgesia was recently presented by Baldwin, Weibrecht, O’Neil, and Cannon (3). These authors showed that rats appear hyperalgesic on the tail-flick test after they have experienced a single suprathreshold exposure to radiant heat, an exposure well below the cut-off normally used to prevent tissue damage. This finding is intriguing, not only because it provides a novel way to induce hyperalgesia, but also because it has implications for studies that use the tail-flick test to measure pain reactivity. For example, suppose an experimental manipulation produces a reduction in pain reactivity (hypalgesia) that causes subjects to experience a prolonged exposure to radiant heat on the tail-flick test. According to Baldwin et al. (2), the suprathreshold exposure to radiant heat will engage a hyperalgesic response that would counteract the hypalgesia elicited by the experimental manipulation. Consequently, subjects may subsequently exhibit normal levels of pain reactivity, not because the hypalgesia has dissipated, but rather because it is counter-acted by an opponent hyperalgesic response.

Serious questions remain, however, concerning the validity and generality of Baldwin’s basic effect. One problem is that a suprathreshold exposure to radiant heat may induce a local increase in tail temperature. Because tail-flick latencies tend to decrease as tail temperature increases (8), rats may exhibit faster tail-flick latencies after a suprathreshold exposure to radiant heat simply because their tails are warmer. Experiments 1 and 2 address this issue in 2 ways. First, we concurrently measure tail temperature and evaluate the contribution of this variable with statistical techniques. Secondly, we test whether a suprathreshold exposure to radiant heat decreases tail-flick latencies in spinalized rats; if the effect is simply due to an increase in tail tem-
perature, then spinal rats should also exhibit faster tail-flick latencies after a suprathreshold to radiant heat. Experiments 3 and 4 explore the generality of Baldwin’s effect and another problematic issue: that all prior demonstrations of this effect used pentobarbital anesthetized subjects. Given this, it is currently unclear whether a suprathreshold exposure to radiant heat affects tail-flick latencies in awake subjects. Experiment 3 addresses this issue by testing the impact of a suprathreshold exposure to radiant heat on tail-flick latencies in awake rats. Experiments 4 and 5 assess the impact of this manipulation on shock-induced vocalization and motor reactivity.

**GENERAL METHOD**

**Animals**

The subjects were male albino Sprague–Dawley rats obtained from Harlan (Houston, Texas). The rats were 100–120 days old during testing and weighed between 450–510 g. The animals were individually housed and maintained on a 12/12 h light/dark cycle. Subjects were tested during the last 4 h of the light cycle. Food and water were available ad lib.

**Apparatus**

Restraining tubes. During behavioral testing, rats were restrained in one of two 22 cm (length) × 6.8 cm (internal diameter) Plexiglas tubes. The front of each tube was closed with a Plexiglas sheet. A second sheet of Plexiglas formed a floor (5.5 cm wide, lying 5.3 cm from the top of the tube) on which the rats could lie. The tubes were covered with duct tape to minimize visual distractions. Twelve holes were drilled in the top of the tubes to provide ventilation. A band of porous tape was positioned over the rear of each tube to prevent the subjects from backing out of the tubes. The subject’s tail protruded from the end of the tube, between the flat base and the band of tape, and could move freely in response to stimulation.

Tail-flick device. To assess changes in nociceptive reactivity, and to provide a suprathreshold exposure to radiant heat, we used a radiant heat tail-flick device. The radiant heat source was a 375-W movie light (Sylvania, Type EBR), positioned 18 cm above the rat’s tail. A condenser lens was placed 8 cm below the light and served to focus the radiant heat onto the rats’ tails, producing an area of illumination of approximately 2 cm in diameter. The rat’s tail rested on an aluminum block which had an 0.8-cm-wide, 0.4-cm-deep groove cut into it. A lateral movement of the tail by at least 0.5 cm illuminated a photo cell positioned below the groove and terminated the trial. The duration of the trial was automatically timed to the nearest 0.01 s. Prior to Experiment 1, heat intensity was adjusted to obtain baseline tail-flick latencies in the 4–5 s range. The same heat intensity was then used in all of the experiments.

Thermometer. Tail temperature was monitored with an Archer Digital Thermometer (Model 277-0123) which was taped to the dorsal surface of the rat’s tail, 4 cm from the base of the tail.

Shock generator. Shock thresholds were measured using a BSR/LVE shock generator (SG-903). The shock source was connected to the rat’s tail 2 cm behind the thermometer with adhesive tape. The electrodes were constructed from modified fuse clips and were lightly coated with electrode paste before they were attached to the tail.

Test environment. The apparatus was located in an isolated room. A space heater maintained the room temperature at 25.0–26.0°C. This fan, together with the chamber fans, provided a background noise level of approximately 60 dB.

**Surgery**

Spinal transections were performed in the third or fourth hour of the animals’ dark cycle. The transection was made at the level of the second thoracic vertebra (T2) 15 min following an injection of sodium pentobarbital (40 mg/kg). A 4 cm anterior-posterior incision was made, beginning approximately 1 cm from the base of the skull, and the tissue in the region of T2 was cleared. T1 was detached from T2, creating an opening immediately caudal to the cord. The exposed cord was then transected by cautery. The remaining gap was filled with Gel-Foam, and the wound was closed with Michel clips. The transections were confirmed by (a) inspection of the cord during the operation; and (b) observing the behavior of the subjects after they recovered to ensure that they exhibited paralysis below the levels of the forepaws. Subjects were tested the next day.

**General Procedure**

**Pentobarbital anesthesia.** Subjects were removed from their homecages and injected (ip) with 40 mg/kg sodium pentobarbital (V-Pento; distributed by A-J Buck & Son) and returned to their homecages. This dosage is routinely used in our laboratory to prepare subjects for surgery. It produces a moderate to strong anesthesia with little or no respiratory dysfunction. Twenty-five minutes after the injection, subjects were checked to make sure they were flaccid, breathing normally, and could exhibit a tail-flick reflex. The subjects were then placed in the restraining tubes.

**Acclimation.** Immediately after subjects were placed in the restraining tubes, the thermometer was attached to the rat’s tail. Shock electrodes were also attached in Experiments 4 and 5. The subjects were then given 15 min to acclimate to the test environment.

**Tail-flick tests.** At the start of each tail-flick test, the subject’s tail was placed beneath the heat source and tail-temperature was recorded. Radiant heat was then applied, centered on a spot approximately 7 cm from the tip of the tail, and the latency to exhibit a tail-flick response was recorded.

**Shock reactivity.** Shock thresholds were determined by increasing tail shock in 0.05 mA increments at 3-s intervals and noting the intensity at which a motor response (Experiments 4 and 5 and a vocalization (Experiment 4) were first observed. The shock was then terminated.

**Suprathreshold exposure to radiant heat.** After baseline testing, half of the subjects in each experiment received a 6-s exposure to radiant heat. This was accomplished by manually restraining the rat’s tail on the tail-flick device at the same location used to establish baseline responding. Control subjects were treated the same with the exception that the thermal stimulus was not applied.

**Statistics**

An analysis of variance (ANOVA) was used to analyze the baseline nociceptive thresholds and temperatures. To control for differences in baseline thresholds, an analysis of covariance (ANCOVA) was used to analyze the data collected after subjects received a suprathreshold exposure to radiant heat. For each dependent variable, the subject’s baseline score served as the covariate. Finally, to address the possibility that the change in tail-flick latencies observed after heat were due to a change in tail temperature, the mean tail-flick latencies were reanalyzed with two covariates: baseline tail-flick latencies and the mean temperature recorded after heat exposure.
PAIN REACTIVITY

EXPERIMENT 1: REPLICATION AND ROLE OF TAIL TEMPERATURE

Experimental Design

Our first experiment was designed to replicate the finding that a suprathreshold exposure to radiant heat induces hyperalgesia on the tail-flick test in pentobarbital-anesthetized rats (3). In addition, we measured tail-temperatures to determine whether heat exposed rats exhibit shorter tail-flick latencies because their tails are warmer. Sixteen pentobarbital anesthetized rats were acclimated to the restraining tubes and given 5 tail-flick tests at 1 min intervals. Half the subjects then received a suprathreshold exposure to radiant heat. One min later, tail-flick latencies were recorded 10 more times at 1 min intervals. Tail-temperature was recorded immediately before each tail-flick test.

Results

Mean tail-flick latencies (a) and tail temperatures (b) are depicted in Fig. 1. Baseline tail-flick latencies and temperatures are depicted on the left side of each graph. It is clear that both groups exhibited similar latencies and temperatures prior to our experimental manipulation, both $F$'s $< 1.0, p > 0.05$.

After the suprathreshold exposure to radiant heat, heat exposed rats exhibited shorter tail-flick latencies and this was true across the entire 10 min of testing. An ANCOVA confirmed that there was a main effect of heat, $F(1, 13) = 7.04, p < 0.05$. Neither the trials effect, $F(1, 9) < 1.0, p > 0.05$, nor its interaction with the between subjects treatment, $F(1, 9) = 1.74, p > 0.05$, approached statistical significance.

Exposure to heat also appeared to produce a small increase in tail temperature. However, this effect did not reach statistical significance, $F(1, 13) = 1.46, p > 0.05$. There was a significant trials effect, $F(9, 117) = 2.62, p < 0.05$, but this effect did not interact with the effect of heat treatment, $F(9, 117) < 1.0, p > 0.05$.

Even though heat exposure did not have a significant effect on tail temperature, it is possible that the change in tail temperature could contribute to the effect of heat exposure on tail-flick latencies. To address this possibility, an additional ANCOVA was performed. Because the main effect of heat treatment did not interact with the trials effect, this analysis was performed on the mean tail-flick latencies obtained after heat exposure. In this analysis, both baseline tail-flick scores and the mean temperatures recorded after heat treatment, were used as covariates. The ANCOVA confirmed that a suprathreshold exposure to heat had a significant effect on tail-flick latencies that could not be accounted for in terms of a change in tail temperature, $F(1, 12) = 5.17, p < 0.05$.

EXPERIMENT 2: SPINAL TRANSECTION

Experimental Design

If a suprathreshold exposure to radiant heat lowers tail-flick latencies because it produces a local change, either at the site of stimulation or within the spinal cord, then it should also lower tail-flick latencies in spinalized rats. To address this possibility, 16 spinalized rats were anesthetized and tested as described in Experiment 1.

RESULTS

The mean tail-flick latencies (a) and tail temperatures (b) are depicted on Fig. 2. Baseline tail-flick latencies and temperatures are shown on the left side of each graph. Group differences, in either baseline tail-flick latencies, or baseline temperatures, did not approach statistical significance, all $F$'s $(1, 14) < 1.0, p > 0.05$.

After the suprathreshold exposure to radiant heat, spinalized rats did not exhibit hyperalgesia on the tail-flick test. An ANCOVA confirmed that the main effect of heat exposure was not significant, $F(1, 12) < 1.0, p > 0.05$. Neither the trials effect, nor its interactions with the between subjects variables, were statistically significant, all $F$'s $(9, 117) < 1.75, p > 0.05$.

In contrast to Experiment 1, heat exposure produced a clear increase in tail temperature. An ANOVA showed that both the main effect of heat, $F(1, 13) = 4.74, p < 0.05$, and its interaction with test trial, $F(9, 117) = 2.08, p < 0.05$, were significant. The main effect of test trial was not statistically significant, $F(9, 117) = 1.19, p > 0.05$.
One potential criticism of this experiment is that we administered pentobarbital. This step was taken because all previous demonstrations of the phenomenon used anesthetized subjects. It could be charged, however, that the pentobarbital anesthesia was unnecessary and that its presence somehow contaminated the results. To address this possibility, we replicated the experiment (N = 16) using awake spinal rats. Again, we found no evidence that a suprathreshold exposure to radiant heat affects tail-flick latencies in spinalized subjects. The mean tail-flick latencies (± SE) were 3.45 ± 0.21 and 3.85 ± 0.26 for the heat exposed and nonexposed groups, respectively. An ANCOVA confirmed that this difference was not statistically significant, F(1, 13) < 1.0, p > 0.05.

EXPERIMENT 3: NECESSITY OF PENTOBARBITAL ANESTHESIA

Experimental Design

In Experiments 1 and 2 of this report, and in Baldwin’s original demonstration of thermal-induced hyperalgesia (3), subjects were tested under pentobarbital anesthesia. Given this, it remains unclear whether a suprathreshold exposure to radiant heat affects pain reactivity in awake subjects. The present experiment addressed this issue by testing the effect of heat exposure on tail-flick latencies in pentobarbital anesthetized, saline injected and un.injected rats (N = 60).

RESULTS

Figure 3 depicts tail-flick latencies (a–c) and tail temperatures (d–f) in pentobarbital-treated (a,d), saline-treated (b,e), and uninjected subjects (c,f). Baseline tail-flick latencies and temperatures are depicted on the left side of each graph. It is clear that all three groups exhibited similar latencies prior to our experimental manipulation, all F’s < 1.0, p > 0.05. Within each pretreatment condition, there was little difference in temperature, both F’s < 1.0, p > 0.05. There was, however, an overall difference in temperature across the 3 pretreatment conditions, F(2, 54) = 44.04, p < .0001. Post hoc comparisons of the groups means with Duncans’ multiple range test revealed that the saline treated subjects exhibited higher temperatures than pentobarbital treated or uninjected rats. No other differences were significant.

After the suprathreshold exposure to radiant heat, heat exposed rats that had received pentobarbital exhibited shorter tail-flick latencies than the nonexposed controls, and this was true across the entire 10 min of testing. In contrast, heat exposure had virtually no effect on tail-flick latencies in either the saline injected or uninjected groups. An ANCOVA confirmed that the impact of heat depended on drug treatment, F(2, 53) = 3.23, p < 0.05. Neither the main effects of heat or drug treatment were statistically significant, both F’s (1, 53) < 2.70, p > 0.05. The trials effect, and its interaction with the between subjects variables, also did not reach statistical significance, all F’s < 1.32, p > 0.05. Post hoc comparisons of the group means with Duncans’ multiple range test confirmed that heat exposed rats pretreated with pentobarbital were hyperalgesic relative to their nonexposed controls and both of the saline injected groups (p < 0.05). No other group difference approached statistical significance (p > 0.05). Importantly, this analysis yielded the same results irrespective of whether it was performed on the adjusted or the unadjusted means.

Heat exposed rats also appeared to have higher tail temperatures. However, this effect did not reach statistical significance, F(1, 53) < 1.0, p > 0.05. Neither the main effect of drug treatment, nor its interactions with heat exposure, were statistically significant, both F’s(2, 53) < 1.0, p > 0.05. There was a significant trials effect, F(9, 477) = 14.86, p < 0.05, and a trials by drug interaction, F(18, 477) = 2.30, p < 0.005. However, the trials by heat exposure interaction did not approach statistical significance, F(9, 477) < 1.0, p > 0.05.

As in Experiment 1, it was possible that changes in tail temperature contributed to the hyperalgesia observed on the tail-flick test in anesthetized rats. To address this possibility, an additional ANCOVA was performed on the mean tail-flick scores using both baseline scores and the temperatures recorded after heat exposure as covariates. This analysis confirmed that the impact of heat exposure depended on drug treatment, and that this interaction could not be accounted for in terms of a change in tail temperature, F(2, 52) = 3.25, p < 0.05. Again, neither the main effect of heat, or drug, treatment reached statistical significance, both F’s < 2.76, p > 0.05.
PAIN REACTIVITY

FIG. 3. (a–c) Tail-flick latencies and (d–f) tail temperatures in (a,d) pentobarbital anesthetized, (b,e) saline injected, and (c,f) un.injected rats that received a suprathreshold exposure to radiant heat (●) or nothing (□). In each panel the baseline scores (BL) are depicted on the left side. The scores recorded over a 10 min period after heat exposure are depicted to the right of the baseline scores. The error bars indicate the SE.

Close inspection of the results from the un.injected rats suggest that heat exposure may have induced a transient change in pain reactivity, one that faded within a couple of minutes. To evaluate this possibility, an ANCOVA, covarying out baseline tail-flick scores and postheat temperatures, was performed on the data collected at the 1 min test point in un.injected subjects. Although it is difficult to justify this analysis, it did yield a statistically significant difference, $F(1, 18) = 6.70, p < 0.05$. However, even if this effect proves reliable, it is clear that a suprathreshold exposure to radiant heat has, at best, a transient effect on pain reactivity in awake subjects.

EXPERIMENT 4: IMPACT ON OTHER MEASURES OF PAIN REACTIVITY

Experimental Design

Experiment 3 revealed that a suprathreshold exposure to radiant heat has little impact on tail-flick latencies in awake rats. The present experiment evaluates the generality of this finding by testing awake rats in another way: by measuring their threshold to vocalize and/or exhibit a motor response. These tests were chosen because they have proven particularly sensitive to hyperalgesia in other situations (15,16). For purposes of comparison, tail-flick latencies and tail temperatures were also recorded. After un.injected rats ($N = 40$) were acclimated to the restraining tubes, baseline tail-flick latencies and shock thresholds were measured 2 times each. The tests were conducted at 2 min intervals in an ABBA order that was counterbalanced across subjects. Half of the subjects then received a suprathreshold exposure to radiant heat. One min later, tail-flick latencies and shock thresholds were assessed again 2 times each. These tests were conducted at 2 min intervals using the same ABBA order employed during baseline testing.

RESULTS

The tail-flick latencies (a) and temperatures (b) are depicted in the upper portion of Fig. 4. The vocalization (c) and motor response (d) data are presented below. The baseline scores are depicted on the left side of each graph. It is clear that the groups did not differ prior to heat exposure, all $F$'s$(1, 18) < 3.24, p > 0.05$.

The mean scores obtained after heat exposure are depicted to the right of the baseline data. As was found in Experiment 3, heat exposure did not have a significant effect on tail-flick latencies or temperature in awake rats, both $F$'s$(1, 17) < 1.30, p > 0.05$. Similarly, vocalization thresholds were unaffected by prior heat exposure, $F(1, 17) = 1.0, p > 0.05$. However, heat exposure did produce a small, but statistically significant, decrease in the intensity needed to produce a motor response, $F(1, 17) = 4.86, p < 0.05$.

EXPERIMENT 5: PENTOBARBITAL ANESTHESIA AND MOTOR REACTIVITY

Experimental Design

It is not clear why motor reactivity was the only measure to yield any evidence of thermal-induced hyperalgesia in awake rats. One possibility is that this measure, unlike tail-flick laten-
FIG. 4. (a) Tail-flick latencies, (b) tail temperatures, (c) vocalization thresholds, and (d) motor thresholds observed in awake rats that experienced a suprathermal exposure to radiant heat (filled bars) or nothing (open bars). The error bars indicate the SE.

cies, is unaffected by anesthesia; that similar levels of hyperalgesia would be observed using this measure irrespective of whether or not subjects received pentobarbital. Alternatively, the magnitude of thermal-induced hyperalgesia observed on our test of motor reactivity may, like the tail-flick test, be much larger in pentobarbital anesthetized rats. From this perspective, one would need to assume that motor reactivity yields a significant hyperalgesia in awake rats simply because it is a more sensitive measure. The present experiment evaluates these 2 alternatives by testing the impact of pentobarbital anesthesia on the hyperalgesia observed with the motor reactivity test. Rats (N = 24) received either saline or pentobarbital as described in Experiment 3. After subjects were acclimated to the tubes, motor reactivity was assessed 2 times at 2 min intervals. Half the rats then experienced a suprathermal exposure to radiant heat. One min later, motor thresholds were reassessed 4 times at 2 min intervals.

RESULTS

Motor thresholds are depicted in Fig. 5. As usual, there were no differences in baseline responsiveness, both F’s(1, 20) < 1.0, p > 0.05.

Exposure to heat induced a strong hyperalgesia in the pentobarbital anesthetized rats (a). In contrast to Experiment 4, little hyperalgesia was observed in the unanesthetized subjects. An ANCOVA confirmed that the impact of heat exposure depended on drug treatment, F(1, 19) = 10.52, p < 0.05. Neither the main effect of heat, or drug treatment, reached statistical significance, both F’s(1, 19) < 4.36, p > 0.05.

GENERAL DISCUSSION

Prior research had shown that a suprathermal exposure to radiant heat induces hyperalgesia on the tail-flick test. In Experiment 1, we attempted to replicate this basic effect and rule out an alternative explanation for the finding: that it reflects a heat-induced increase in tail temperature. As reported by Baldwin et al. (3), we found that heat exposed rats exhibit hyperalgesia on the tail-flick test. Importantly, we were able to show, using statistical techniques, that this hyperalgesia does not reflect a change in skin temperature. Experiment 2 explored whether supraspinal systems play an essential role in the production of thermal-induced hyperalgesia. We addressed this issue by testing whether the effect survived spinal transection. We found that it did not, which implies that thermal-induced hyperalgesia depends on su-
praspinal systems. Interestingly, heat exposure induced a significant rise in tail temperature in the pentobarbital anesthetized/spinalized subjects. Given this, if hyperalgesia simply reflects a change in skin temperature, this group should have exhibited a strong hyperalgesia. The fact that they did not provides further evidence that the hyperalgesia is unrelated to changes in skin temperature (11).

Experiment 3 addressed another troubling aspect of this phenomenon: that it has only been studied in pentobarbital anesthetized rats. Given this, the possibility remained that thermal-induced hyperalgesia would not be observed in awake rats. Supporting this, we found that a suprathreshold exposure to radiant heat induced hyperalgesia in pentobarbital anesthetized, but not awake subjects. Moreover, this was true irrespective of whether the awake controls were injected with saline or naïve. Experiment 4 extended these observations by assessing the impact of a suprathreshold exposure to radiant heat on other measures of pain reactivity, measures that have proven particularly sensitive to hyperalgesia in other domains (15,16): shock elicited vocalization and motor reactivity. Again, we failed to find hyperalgesia on the tail-flick test. Similarly, a suprathreshold exposure to radiant heat did not alter vocalization thresholds. Hyperalgesia was observed when motor thresholds were tested, but this effect was relatively small. Moreover, even with this measure, a much more robust hyperalgesia was observed when subjects were anesthetized with pentobarbital (Experiment 5). Thus, it is clear that a suprathreshold exposure to radiant heat has a much greater impact on pain reactivity in pentobarbital anesthetized rats.

It is not entirely clear why awake rats fail to exhibit hyperalgesia after a suprathreshold exposure to radiant heat. However, a potential explanation can be derived from recent work on the opposite phenomenon, environmentally induced hypoalgesia. This work has shown that exposure to a variety of aversive stimuli can elicit a hypoalgesia in rats that elevates tail-flick latencies (1). Interestingly, when a relatively mild aversive stimulus is used to induce the hypoalgesia, it is eliminated by pentobarbital anesthesia (5,6). Given these observations, it could be argued that a suprathreshold exposure to radiant heat induces a hypoalgesia in awake rats, a hypoalgesia that effectively counteracts the hyperalgesia. Because anesthesia eliminates this hypoalgesic response, a much stronger hyperalgesia would be observed in anesthetized rats. Alternatively, little hyperalgesia may be observed in awake rats because the mechanisms which produce this effect are tonically inhibited by higher psychological/neural mechanisms. From this perspective, it could be argued that the hyperalgesia is mediated at a relatively low level of the nervous system (e.g., brainstem), and that this mechanism is normally inhibited by higher brain processes. If pentobarbital differentially disrupted these higher brain systems, then it would effectively “release” the lower-level hyperalgesic mechanism from inhibition, allowing the hyperalgesia to be expressed. Clearly, further research is needed to evaluate these possibilities.

What is clear from our findings, and those reported by Baldwin et al. (2,3) is that a suprathreshold exposure to radiant heat has a reliable effect on tail-flick latencies, and motor thresholds, in pentobarbital anesthetized rats. This, in of itself, is an important observation for researchers in the pain literature often test their subjects while they are anesthetized. This step is sometimes taken for methodological convenience and sometimes in an effort to minimize the subject’s pain and suffering. In either case, any experimental manipulation that causes subjects to experience a suprathreshold exposure to radiant heat will engage a hyperalgesic response, a hyperalgesia that will complicate the interpretation of subsequent tail-flick latencies. This point is nicely illustrated by a recent experiment reported by Baldwin et al. (2).

Kaplan and Fields (10) had previously shown that rats given a single injection of morphine become hyperalgesic when administered an opioid antagonist. Kaplan and Fields (10) assumed that animals exhibited hyperalgesia because the antagonist had abruptly terminated the opioid’s action, producing an abstinence syndrome. However, there is another reason why their morphine treated rats may have subsequently exhibited hyperalgesia: because subjects were tail-flick tested during morphine treatment, these subjects experienced prolonged exposures to radiant heat. Naturally, because the saline controls exhibited normal tail-flick latencies, they received much less exposure to the radiant heat during this pretesting. When the opioid’s action was then terminated by the administration of the antagonist, subjects pre-treated with morphine might have appeared hyperalgesic simply because they had previously experienced prolonged exposures to radiant heat. If this is true, then omitting these prolonged exposures to radiant heat should eliminate the subsequent hyperalgesia. Supporting this, Baldwin et al. (2) showed morphine
treated rats, given an opioid antagonist, only exhibit hyperalgesia if they were previously tested during the period when they were hypoalgesic. What this finding calls into question is the cause of the hyperalgesia, suggesting that it is due to the prolonged exposures to radiant heat rather than opioid abstinence. It is important to note that this does not in any way undermine other claims made by Kaplan and Fields (10). For example, they also presented evidence that neurons within the rostral ventromedial medulla play a critical role in the production of hyperalgesia. This finding still stands. The only point in contention is what triggered the hyperalgesia—morphine abstinence or differences in exposure to radiant heat. At present, evidence appears to favor the second alternative.

It is also interesting to note that both Baldwin et al. (2) and Kaplan and Fields (10) tested their subjects while they were anesthetized with pentobarbital. Our results suggest that this may be a necessary condition for observing thermal-induced hyperalgesia; that suprathreshold exposure to radiant heat has little impact on pain reactivity in awake rats (Experiments 3-5). Given this, if the hyperalgesia studied by Kaplan and Fields (10) is due to their morphine treated rats having experienced prolonged exposures to radiant heat, then we would predict that little hyperalgesia would have been observed if subjects were tested while they were awake.

Researchers have now shown that a variety of stimuli can induce hyperalgesia, including vibration, novelty, tail-pinching and illness (15–17). It is unclear how thermal-induced hyperalgesia is related to these phenomena. However, one characteristic that these events do appear to share is that, compared to the kinds of stimuli that are normally used to induce hypoalgesia, the stimuli used to induce hyperalgesia are relatively mild. This observation has led some authors to suggest that the impact of an aversive event may depend on its severity: Very mild stimuli may elicit hyperalgesia while more severe stimuli produce hypoalgesia (14). What appears different about thermal-induced hyperalgesia is that it is most robust in pentobarbital anesthetized rats. However, it is difficult to draw firm conclusions on this issue since others have not investigated whether the magnitude of hyperalgesia observed in other situations is affected by pentobarbital anesthesia. Given this, the possibility exists that all of these manipulations induce hyperalgesia through similar mechanisms, and that in all cases, the effects would be even stronger in the presence of pentobarbital anesthesia. Again, further work is needed to explore these issues.

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