EFFECT OF CONDITIONED FEAR ON RESPONSIVENESS TO HYPERALGESIA IN RATS

by
Shuh-Ren Jin

Abstract

A number of recent research studies have suggested that the response of pain to electric shock can be conditioned. However, mixed results stemmed from these studies producing either conditioned analgesia (insensitivity to pain) or hyperalgesia when triggered by various conditioned stimuli. The present study hypothesized that psychogenic hyperalgesia can be triggered by conditioned fear as measured by tail-flick latencies. In the present experiment, rats (N=22) were exposed to shock an equal number of times in conditioning sessions. Separate groups were then tested either with or without a conditioned fear stimulus (CS) in a tail-flick test. The hypothesis was supported. It was found that a fear CS appeared to produce significantly (p<.01) decreased tail-flick latencies indicating a hyperalgesic response. The nature of the tail-flick reflex and the procedures used by studies producing the various findings were discussed.
Effect of Conditioned Fear on Responsiveness to Hyperalgesia in Rats

There is much evidence indicating the existence of a system in the rat's brain that is biochemically and functionally related to narcotic analgesics, and is capable of inhibiting pain (Madden, Akil, Patrick & Barchas, 1977; Chance, White, Krynack & Rosecrans, 1978). It is reported that opioid peptides act as chemical mediators in this endogenous system of pain inhibition. The recognition that this system may contain mechanisms for the ongoing regulation of nociception poses the additional question of what environmental contingencies normally invoke these processes. Some of these environmental contingencies include many stimuli ranging from electrical shock (Madden, Akily, Patrick & Barchas, 1977) and cold water immersion (Bondnar, Kelly, Spiaggia, Ehrenberg & Glusman, 1978a) to food deprivation (Bodnar, Kelly, Spiaggia & Glusman, 1978b). A variety of recent research has focused on testing whether a stimulus that is not itself painful but that predicts a painful event is also capable of triggering the endogenous analgesia system (Chance, White, Krynock & Rosecrans, 1978; Davis & Hendersen, 1985; Hayes, Bennett, Newlon & Mayer, 1978; Fanselow & Bolles, 1979). The fact that Pavlovian conditioned fear stimuli can produce analgesia plays a prominent role in several theories of aversive learning and motivation (see Bolles & Fanselow, 1982). Several experiments designed to demonstrate that the analgesia can be classically conditioned to cues that proceed or accompany the aversive event have increased the potential implications of stress-induced analgesic reactions for behavioral and pharmacological phenomena. Chance et al. (1977) first reported that exposure to environmental stimuli previously associated with shock could produce analgesia on the tail-flick test. Rats were shocked in a distinctive context and then given a tail-flick test in that context.
Unfortunately, the only control group used was one in which no shock at all was delivered. Thus it is possible that the decreased responsiveness to radiant heat on the tail-flick test represented an unconditioned effect of shock rather than a classically conditioned antinociceptive response. In a second experiment, Chance et al. (1978) reported that exposure to a fear conditioning situation increased endogenous opiate activity in the brain as well as producing analgesia on the tail-flick test. However, their lack of conditioning control leaves open the possibility that these effects were due simply to the unconditioned effects of the shock (unconditioned stimulus, UCS) and were not a conditioned triggering of the endogenous analgesic system by the CS. Subsequently, MacLennan et al. (1980) improved some control procedure: each rat was exposed an equal number of times to two distinct environmental contexts, but shock was only received in one of them. It was found that rats that were reexposed to the context in which they had been shocked were significantly more analgesic than rats in the other two groups (which did not differ).

These results confirmed that it is possible to condition shock-induced analgesia in the rat. However, an unexpected result was found by Davis and Henderson (1985) in which the fear CS produced hyperalgesia or increased sensitivity to pain, instead of analgesia.

Davis and Henderson divided rats into two groups. One group received forward fear conditioning for two hours, with a variable inter-trial interval whose mean was 240 seconds. The CS was a 30-second, blinking light and tone compound stimulus, followed immediately by a 1 second, 2 milliampers (mA), scrambled shock UCS. The second group received the same CS exposure as the fear conditioning group, but the UCS was absent. In tail-flick testing, all animals were tested immediately after the 30-second CS.
Since other researchers have reported analgesia triggered by conditioned fear, the results of the preceding experiment were so unexpected. Furthermore, the conditioning of stress-induced analgesic reactions is a phenomenon of some potential significance (MacLennan et al., 1980). It is worthwhile to compare these two sets of findings in terms of experimental results and procedures. Therefore, the purpose of the present study is (i) to examine the effect of CS fear on the responsiveness of pain as measured by tail-flick latencies to verify that the psychogenic hyperalgesia can be triggered by conditioned fear; and (ii) to examine the differences between the two sets of results and experimental procedures if (i) were to be verified.
Experiment

Davis and Henderson's (1985) experiment III did not include a control for sensitization effects that a series of shocks may have on tail-flick latencies. Thus, the effect they obtained may not have required the association between CS and shock, representing instead a sensitization of the tail-flick reflex by the shocks administered in the conditioning phase. The present experiment attempted, therefore, to use a procedure that equalizes the treatment between experimental and control subjects; all animals were given equivalent fear conditioning (and, accordingly, equivalent shock experience). Separate groups were then tested either with or without CS.

Method

Subjects

The subjects were 22 naive, female, hooded rats of the Long-Evans strain. The animals were 70-90 days old at the start of the experiment. They were maintained on ad libitum food and water, and on a 12-hour light-dark cycle throughout the experiment.

Apparatus

Fear conditioning was conducted in four identical chambers. The sides of each conditioning chamber were aluminum, with front back, and top of clear Plexiglas. The chambers were 30 cm wide, 20 cm deep, and 21.5 cm in height. The grid floor of the chambers consisted of stainless steel rods .45 cm in diameter, and spaced 1.9 cm from center to center. The
conditioning chambers were housed in sound attenuating chambers, with a background noise level of 70 dB. In tail-flick testing, the rat's tail was positioned on a wooden board with two raised wooden guides one-quarter inch apart at the end of the board near the rat's body, tapering to a "v" eight inches away. A 600-watt photographer's lamp was located six inches above the board. A photocell located beneath a point two inches from the end of the rat's tail was activated by movement of the rat's tail, and automatically terminated the trial.

Procedure

The experiment consisted of three phases: handling, fear conditioning, and testing. Animals received four days of handling at the start of the experiment. The animals were handled by the experimenter for two sessions of two minutes each, separated by an intertrial interval of between 10 and 15 minutes that the animal spent in its home cage. On the third and fourth days of handling, the animals were grasped from behind, and placed in a position to receive an intraperitoneal (i. p.) injection, although no injection was delivered. On the fifth day of the experiment, all animals received 15 pairings of tone and shock. A thirty second tone from a Mallory Sonalert was followed immediately by a 1 mA, .75 seconds scrambled shock. Conditioning trials were delivered on a 280 seconds variable time schedule. On the sixth day of the experiment, the animals received an injection of saline. The drug injection here was employed to compare the saline group with the naloxone group in terms of the responsiveness to pain, but it was not the main concern of this paper. Actually, saline injection has no physiological
effect at all. Twelve minutes after drug injection, the animals received three tail-flick trials. Half the animals randomly chosen received the thirty seconds CS (group CS) while they were being held in the tail-flick apparatus, while the other animals experienced thirty seconds of silence (group NOCS). The stimulus exposure preceded all three tail-flick trials. There was a 20 seconds inter-trial interval. If the latency on a trial was less than 1 second, that trial was discarded, and another trial added. If a tail-flick had not occurred within 14.5 seconds, a trial was automatically terminated to reduce the possibility of tissue damage.

Results

The experimental results were analyzed by ANOVE with repeated measure (Kirk, 1982, P. 237). The results, consistent with expectations, indicated that the tail-flick latencies were significantly lower in the group that received fear conditioning (see Figure 1).
Figure 1: Effect of a fear CS on tail-flick latencies

The mean tail-flick latency for group SALINE + NOCS was 8.03 seconds, while for group SALINE + CS it was 5.51 seconds (see Table 1).

Table 1

Mean Scores (in seconds) of the Effect of a fear CS in Three Tail-flick Trials

<table>
<thead>
<tr>
<th>Trials</th>
<th>Groups</th>
<th>N</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NOCS</td>
<td>11</td>
<td>9.04</td>
<td>6.62</td>
<td>8.09</td>
<td>8.03</td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td>11</td>
<td>6.14</td>
<td>5.56</td>
<td>4.83</td>
<td>5.51</td>
</tr>
</tbody>
</table>
This difference was significant \( F(1,20)=8.225, P<.01 \). There was no significant decrease in latencies across trials \( F(2,40)=1.5636, p>.05 \). No significant interaction between trials and groups was present (see Table 2).

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**Table 2**

*Summary Table of ANOVA for the Effect of a Fear CS in Three Tail-flick Trials*

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>between subjects</td>
<td>360.240</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (Treatment)</td>
<td>161.983</td>
<td>1</td>
<td>104.983</td>
<td>8.225**</td>
</tr>
<tr>
<td>sub.w.groups</td>
<td>255.257</td>
<td>20</td>
<td>12.762</td>
<td></td>
</tr>
<tr>
<td>within subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (Trial)</td>
<td>35.007</td>
<td>2</td>
<td>17.503</td>
<td>1.564n.s</td>
</tr>
<tr>
<td>A x B (Interaction)</td>
<td>17.535</td>
<td>2</td>
<td>8.768</td>
<td>0.763n.s</td>
</tr>
<tr>
<td>B x Subj.w. groups</td>
<td>447.775</td>
<td>40</td>
<td>11.194</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>850.558</td>
<td>66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**F. 01;1,20=8.10**
Discussion

The results of this experiment confirm the study conducted by Davis and Henderson (1985). This study employed a tail-flick test of pain reactivity, gave each group an equivalent shock experience, and still found evidence for a conditioned nociceptive reaction. That is, a fear CS produce significantly decreased tail-flick latencies.

This conclusion is in sharp contrast to the way the inescapable shock treatment affects the same dependent measure, producing longer latencies (Lewis, Cannon, and Liebeskind, 1980; MacLennan, Jackson, and Maier, 1980). Previous authors offered the plausible hypothesis that tail-flick latencies lasted longer because the shock was perceived as less painful, due to an opioid-mediated analgesia process. Obviously, findings obtained in this experiment directly contradict the precious hypothesis. Why and how these contradictory results emerged is a question.

We will focus on examining the discrepancies between these two sets of results and those of the experimental procedures.

First of all, how can these two sets of results be explained?

Is it a Function of the Release of Different Hormones?

Just a few years ago, Pert and Snyder (1973) reported that certain areas of the brain are peculiarly sensitive to opiates (e.g., morphine) and opiate antagonists (e.g., naloxone). The critical areas were soon implicated in a variety of pain mechanism (see Liebeskin and Paul, 1977).
Hughes and his associates (Hughes et al., 1975) isolated and identified these opiate-like substances in brain tissue. Among them, opposite effects were found in adrenocorticotropic hormone (ACTH) and Beta-endorphine. Both ACTH and Beta-endorphine have been found in minute amounts in the rain (and in larger amounts in the pituitary), although their exact physiological functions are still the subject of speculation. However, when nonphysiological, pharmacological doses of ACTH or Beta-endorphin were injected into the periaqueductal gray of rats, opposite behavioral effects were observed (Jacque, 1978; Jacque & Wolf, 1981). Following ACTH, rats became hyperalgesic, agitated, and hypermobile (i.e. leaping and running frantically about the cage), while following Beta-endorphine, rats became analgesic, sedated, and catatonic (i.e. capable of being molded into awkward postures which they would then hold for long periods). Furthermore, it also suggested that analgesia could be mediated by stereospecific, naloxone-reversible receptor (of which the endogenous ligand was suggested to be Beta-endorphine), while the hyperalgesia (fearful hyperactivity and explosive motor behavior) could be mediated by a nonstereospecific, nonnaloxone-reversible receptor (of which the endogenous ligand was suggested to be ACTH) (Jacquet, 1978). Research findings (Lewis et al., 1980) consistent with the hypothesis that pituitary hormones, presumably Beta-endorphine, mediate at least certain forms of stress analgesia. However, can we infer that it is ACTH which mediates the fear-induced hyperalgesia in this experiment?

The answer is probably no. According to Jacquet's assumption, excitatory action or hyperexcitability is mediated by the receptor that is naloxone-insensitive. Nevertheless, the hyperalgesic reaction triggered by the conditioned fear has been blocked by naloxone in a concomitant experiment held with the same conditioning procedures (Davis and Henderson, 1985).
Then, how can this experiment explain the nature of decreased latencies of tail-flick reflex in this experiment?

**Arousal, Anxiety and Avoidance Behavior**

**Arousal and anxiety.** Conditioned fear has arousal properties (Miller, 1951). When a rat is brought onto the tail-flick table and threatened with electric shock, it will possibly be led to increased arousal. Arousal is somewhat related to anxiety. According to McReynolds' (1976) assumption, the relationship between anxiety and arousal is complex: the arousal system responds positively to increments in anxiety; the more sudden the increment the greater the response, but once the level of anxiety has reached and equilibrium, even though it is still high, the level of arousal tends to return to normal. It was also found that high arousal does lead to heightened sensitivity to pain (Handback, 1976). In this experiment, the appearance of CS might increase the rats' arousal level as well as anxiety level, once the radiant heat followed immediately, the decreased latencies of tail-flick reflex served as an indication of lower pain threshold. We expect that if the trial was repeated a few times, even though the anxiety of being shocked was still high, the level of arousal should decrease, and so should the increase in pain threshold, as McReynolds indicated.

**Avoidance behavior.** We assume that the tail-flick reflex is a kind of avoidance response. The Expectancy X Value theory implies the performance of the so-called avoidance response is sped up because other initially more dominant response tendencies have been weakened by punishment (Atkinson, 1983, p. 178). By using tail-flick test, the rat is physically constrained and it is only the movement of tail that can take the rat out of a threatening situation (e.g. terminate the radiant heat). From the viewpoint of
Expectancy X Value theory, the initial response followed by the CS is strong and rapid on the ground that the animal is expected to be punished by aversive stimulus (which is a negative incentive value); then the so-called avoidance responses which got the animal out of the threatening place should finally extinguish on subsequent trials because the animal is no longer being shocked (no shock followed and adjusted to radiant heat).

By now, we have discussed the behavioral and physiological justification as to the differences between the two sets of results (i.e., Lewis et al. 1980 vs. the present study). Next, the focus will be shifted to the experimental procedures. Three topics concerning the causes of different results will be discussed as follows: (i) the distribution and amount of shock intensities; (ii) contextual and discrete cues; and (iii) the time courses of tail-flick measurement.

(I) The Distribution and Amount of Shock Intensities

Chance et al. (1978) demonstrated that when subjects of experimental group were shocked (0.8 mA) for 15 sec/day for 8 days, and they exhibited longer tail-flick latencies than the control subjects. Madden et al. (1977) reported that extremely intense (3.0 mA) and prolonged (30 min) discontinuous shock caused an increase in the level of endogenous opioids, and that it produced analgesia on the tail-flick test. However, this effect dropped out over the course of 13 shock sessions, as if the animals were becoming tolerant to their own endorphines. They also become hyperalgesic. In this experiment, a 1 mA, .75 S scrambled shock was administered, and conditioning trials were delivered on a 280 seconds variable time schedule. The various distributions and amount of shock intensities were
employing and the variant results were stemmed. Accordingly, to what extent the various distribution and amount of shock intensities disturbs the body's natural biochemical balance to produce a sensitization to pain and an adaptation of the normal pain-inhibiting mechanism still remains a question.

(II) Contextual and Discrete Cues

Chancé et al. (1978) first reported that exposure to environmental stimuli previously associated with shock could produce analgesia on the tail-flick test. Subsequently, MacLennan et al. (1980) used a differential fear-conditioning procedure-equivalent exposure was given in two contexts, but shock was only received in one of them and found that exposure to the shock-associated apparatus caused analgesia on the tail-flick tests. These two experiments conducted the measure of pain sensitivity which was identical to the method and time course employed in this experiment produced analgesia. Perhaps the most startling of these is that this study used a discrete, fixed duration fear CS, while experiment yielding analgesia used a contextual fear cue (Davis and Henderson, 1985). It is possible the contextual and discrete cues have very different effects on responsiveness to pain.

(III) The Time-course of Tail-flick Measurement

The tail-flick testing conducted by Lewis and his group (Lewis et al., 1980) resumed 1 minute after the stress or control procedure, continuing at 1-minute intervals for 9 minutes and subsequently at 2-minute intervals until 15 minutes had elapsed since the procedure. Oliverio and Castellano (1982) verified the stress-induced analgesia. The rate were subjected to 60 tone presentation in absence of shock, à
session consisting of 60 5-sec tone spaced by one minute. In this experiment, tail-flick latencies was measured 3 trials right after CS appeared, and there was only a 20-second inter-trial interval. The longer tail-flick latencies were found in the first two experiments, while the shorter latencies were found in the last one. This suggests that the effect of the CS is somewhat different at immediate stage and later stage.

The phenomenon of two-stage time course can likewise be found in the measure of freezing. It was reported that all animals were active during the first 2 minutes and freezing occurred on only 2% of the behavior sample during this period (Fanselow and Bolles, 1979); the initial response to shock is jumping and movement (Fanselow, 1982). Thus, the latencies of tail flick or the pattern of activity that an animal shows after a shock or a fear CS is administered may reflect two different effects. Furthermore, the two-stage time course of responsiveness appears fairly consistent with the previous justifications in terms of arousal, anxiety and avoidance behavior.

Other Thoughts on the Procedure of This Experiment

The influence of other unconditioned stimulus. In stress situations, rat's cortisol levels increase as with fear; persistent immobilization for the rat is very aversive and stressful, inducing transient hypertension, adrenal enzyme synthesis, and increased shock-elicited fighting (Lamprecht et al., 1972; Kvetnansky et al., 1970). In addition to handling, radiant heat, and the novel situation (tail-flick table) could also be perceived as a joint unconditioned stimulus (aversive stimulus, with negative incentive value), so that the reported effects could be attributed to unconditioned reactions to UCS with the combination of conditioned fear to CS.
Hence, we can not tell the nature of tail-flick responses in this experiment: Is it a UR (response to tone, handling heat, and/or novel table) or a CR (response to CS)? In other words, the tail-flick reflexes could be a responsiveness to pain due to the conditioned fear, or just an unconditioned escape behavior (flight, startle, etc.) evoked by some situation-bound aversive stimulus.

Form UCS-UR to CS-CR. Some thoughts related to those given above are questions with regard to the strength of the connection between CS (tone) and UCS (shock). The UCS was supposed to be replaced by the CS at the end of the conditioning procedure. However, this study did not measure the UR or the CR in conditioning sessions, then how can we assume that the UCS has been substituted by CS and the UR has been replaced by the CR?
Summary

In summary, this experiment confirms that the decreased latency of tail-flick is triggered by fear CS in rats, indicating that a conditioned fear does not always produce psychogenic analgesia. In other words, conditioned fear is capable of exerting diverse and qualitatively different effects on the perception of pain. The mechanism behind this physiological phenomenon could be autonomous arousal, anxiety, or an avoidance response. In addition, there are many procedural differences between this experiment and those of Chance et al. (1977, 1978) and Fanselow & Bolles (1979), who obtained contradictory findings. Further experiments testing the effects of various fear CS on conditioned fear responses are needed.


制約恐懼對老鼠痛覺過敏反應的影響

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本實驗研究之目的在於驗證制約恐懼（conditioned fear）對老鼠痛覺過敏（hyperalgesia）反應的影響。過去的研究發現，老鼠在制約恐懼情境下的痛覺反應並不一致，有趨向兩極的痛覺反應傾向：其一是「痛覺喪失」（analgesia），其二是「痛覺過敏」。本實驗研究中以老鼠尾部反彈反射（tail-flick reflexes）的潛伏期（latencies）設定為測量痛覺反應之指標，假設經由制約恐懼的引發，會產生心因性的痛覺過敏反應，而非痛覺喪失。實驗分成三個階段進行：持握（handling）、恐懼制約（fear conditioning）與測試處理（testing）。持握階段係將老鼠把持於手掌，期使熟悉人手之持握。在恐懼制約階段，實驗組（N=11）與控制組（N=11）均接受等量的電擊處置。尾部反彈反射之實驗處理階段，實驗組接受制約恐懼的實驗處理，控制組則無。結果顯示實驗組較控制組有較短的尾部反彈潛伏期（P<.01），顯示出痛覺過敏的反應。對於本研究與其他實驗在研究方法、過程與結果的差異，均予檢驗與討論。