The Variables which Determine whether a Conditioned Stimulus Elicits an Opioid or Nonopioid Analgesia in Rats

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Abstract

Previous studies suggest that a stimulus that has been paired with an aversive event may elicit a decrease in pain reactivity that is mediated by either an opioid or nonopioid system. The present studies examine the variables which determine the form of the conditioned response (the CR). In all experiments, one stimulus (CS+) was paired with shock whereas another (CS-) was presented alone. Pain reactivity was assessed by measuring the latency of tail withdrawal from radiant heat (the tail-flick test). Experiment 1 tested whether the intensity of the unconditioned stimulus (the US) is a critical determinant of the form. Results indicate that US severity does play a role in determining the nature of the CR. Specifically, a more severe US (0.5 s, 1.0 mA) induces a nonopioid-mediated CR while a mild US (0.5 s, 0.3mA) produces an opioid hypoalgesic CR. Experiment 2 evaluated the impact of the CS duration on the form of conditioned hypoalgesia. It was found that subjects exhibit a nonopioid CR regardless of the CS duration (either 60 s or 300 s). The results suggest that US severity, not CS duration, determines the form of the hypoalgesic CR on the tail-flick test.

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Introduction

Considerable evidence suggests that pairing a neutral stimulus (the "CS") with an aversive event (the "US") endows the CS with the capacity to elicit a strong decrease in pain reactivity, or "hypoalgesia" (Chance, White, Krynock, & Rosecrans, 1977; Fanselow & Baakes, 1982; Hayes, Bennett, Newlong, & Mayer, 1978; Watkins, Cobelli, & Mayer, 1982, Watkins, & Mayer, 1986). This change in pain reactivity reflects a "conditioned hypoalgesia" which is due to the formation of an association between the CS and the US. Like other classically conditioned responses, it exhibits extinction, blocking, second-order conditioning, and is strongest when forward conditioning procedures are employed (Ross 1985, 1986; Ross & Randich, 1985; Watkins & Mayer, 1982).

The discovery of conditioned hypoalgesia provoked researchers to speculate about the neurochemcial basis of the conditioned response (the "CR") based on evidence indicating that unconditioned hypoalgesia (i.e., a decrease in pain reactivity caused by direct exposure to an aversive event) has two forms of mediation (Akil, Madden, Patrick & Barchas, 1976; Lewis, Cannon, & Liebeskind, 1980; Hayes et al.,1978, Lewis et al., 1980). One type of intrinsic pain modulation exhibits morphine-like qualities and is referred to as the "endogenous opioid system". Endogenous

opioids are blocked by opiate antagonists (e.g., naloxone and naltrexone) and exhibits cross-tolerance to morphine (Akil et al., 1976, Lewis et al., 1980). The second form of unconditioned hypoalgesia is not sensitive to these manipulations and is therefore referred to as the "nonopioid system" (Grau, 1987; Lewis, et al., 1980). Prior research indicated that a variety of variables (e.g., US intensity and CS duration) were critical in determining the form of the unconditioned hypoalgesic response. Specifically, a mild representation of the US activated the opioid system whereas a more severe US representation induced hypoalgesia mediated by the nonopioid system (Grau 1987; Fanselow, 1984; Terman, Shavit, Lewis, Cannon, & Liebeskind, 1984). Based on this evidence, researchers began searching for the form of the hypoalgesic CR.

Initial research suggested that the CR was mediated by the nonopioid system since it was insensitive to the administration of naloxone (Chance & Rosecrans, 1979; Hayes et al.,1978). However, subsequent work from a number of laboratories (in particular, Watkins, Cobelli, & Mayer, 1982) indicated that conditioned hypoalgesia can be blocked by an opiate antagonist, indicating involvement of the opioid system. These conflicting results were due to the fact that researchers (Chance & Rosecrans, 1979; Hayes et al.,1978) observing a nonopioid CR administered the opiate antagonist after exposing their subjects to stimuli associated with shock (CS+), whereas Watkins et al. (1982) administered the drug prior to the presentation of any CS+. This indicated that a moderate dose of naloxone can prevent, but not reverse, the

hypoalgesic CR (Watkins et al., 1982). Since this initial controversy, opioid conditioned hypoalgesia has been observed across a wide range of test conditions and on a variety of pain reactivity tests (e.g., tail-flick test, formalin test, hot plate test, and cold swim) (Fanselow & Baakes, 1982; Levine, Feldmesser, Tecott, Lane & Gordon, 1984; Matzel & Miller, 1987; Oliverio & Castellano, 1982). This led to the generally accepted view that conditioned hypoalgesia is mediated by the opioid system.

Contrary to this established view, recent evidence from the laboratory of Ross and Randich (1985) suggests that under some circumstances, conditioned hypoalgesia is mediated by the nonopioid system. These researchers found that pairing a 30 s light with shock elicits a strong hypoalgesia on the hot plate test. More importantly, administration of naloxone prior to the CS+ did not attenuate the conditioned hypoalgesia, unlike the effects reported by Watkins et al (1982). This, along with other evidence (Chance & Rosecrans, 1979 b: Fanselow, 1984), suggests that sometimes the nonopioid system mediates the hypoalgesic CR. At present, it is not clear what variables determine whether conditioned hypoalgesia is mediated by the opioid or nonopioid system.

A comparison of studies suggests that two variables seem to be important determinants of the form of conditioned hypoalgesia: the US intensity and the CS duration (Chance et al, 1977; Fanselow & Baakes, 1982; Hayes et al.,1978; Ross & Randich, 1985; Watkins et al., 1982). Researchers found that using a relatively mild US intensity resulted in activation of the opioid system whereas a

more severe US produced a nonopioid CR (Fanselow & Baakes, 1982; Fanselow, 1984). These results, however, may not represent a pure conditioned effect, but may be confounded by other unconditional effects. Thus, it is not known if the intensity of the US is critical in purely conditioned hypoalgesia. In addition, casual comparison across studies indicates that a long duration CS produses an opioid conditioned hypoalgesia (Fanselow & Baakes, 1982; Matzel & Miller, 1987), whereas a relatively short CS results in non-opioid mediation (Chance, 1979; Ross & Randich, 1985). However, no one has yet systematically manipulated only the CS duration to determine its effects on the form of the CR. The purpose of the present paper is to examine whether these two variables influence the form of the conditioned response. Experiment 1 tests the impact of US severity and Experiment 2 tests the impact of CS duration.

Experiment 1

The purpose of this experiment is to explore the impact of manipulating the US intensity on the form of the conditioned hypoalgesic response. Prior work by Watkins and Mayer (1982) suggested that conditioned hypoalgesia is always opioid mediated, which was supported by evidence from a number of other laboratories (Fanselow & Baakes, 1982; Levine et al., 1984; Matzel & Miller, 1987; Oliverio & Castellano, 1982). This general view

was incorporated into many current theories of conditioned hypoalgesia.

Evidence contrary to the accepted idea of opioid-mediated conditioned hypoalgesia was provided by Fanselow (1984), who reported that US severity may play a role in determining the form of the conditioned response. After receiving either a mild (0.75 s, 1.0 mA) or severe (3 s, 4.0 mA) shock, subjects in both shock conditions showed a decrease in pain reactivity, as evidenced on the formalin test. Most importantly, Fanselow observed that naloxone blocked the hypoalgesia observed after mild shock, but had little impact on the hypoalgesia observed after severe shock. On the basis of this work, Fanselow suggested that shock severity may be critical in determining the form of the conditioned response: mild stimuli may establish an opioid hypoalgesic CR, whereas severe shocks may induce a nonopioid CR.

However, the validity of these results can be questioned. First, the hypoalgesia Fanselow observed does not clearly represent a conditioned effect. He did demonstrate that testing the subjects outside the shock context attenuates the hypoalgesia, which suggests that it was, at least in part, a conditioned effect. However, a considerable hypoalgesia existed after this manipulation, indicating that unconditioned processes may also have played a role. This is especially true in the severe shock condition, which has led researchers to speculate that the nonopioid hypoalgesia observed by Fanselow may reflect an unconditioned response or "pseudoconditioning" (Maier, 1989). Secondly, Grau and associates (1981) reported that increasing

shock severity can have the opposite impact. Here too, it is unclear whether a conditioned or unconditioned hypoalgesic response is elicited.

The present experiment circumvents this problem of pseudoconditioning by using a differential conditioning paradigm to assess the impact of shock severity. In this procedure, one auditory stimulus (the CS-) is presented alone while the other (the CS+) is paired with the unconditioned stimulus, shock. This procedure allows one to test for associative learning by comparing, within subject, the observed magnitude of the response to the CS+ to that observed to the CS-. In addition, because the critical comparison is within subject, any difference between the CS+ and CS- cannot be attributed to exposure to the CS or US alone, controlling for sensitization and pseudoconditioning, respectively.

Subjects.

Thirty-two male Sprague-Dawley rats obtained from Harlan (Houston, Texas) were the subjects. The animals were between 100 and 120 days old and weighed in the range of 420 to 490 grams. The subjects were individually housed and fed ad libitum food and water. They were maintained on a continuous light-dark cycle of 12 hours in each condition. The experiment was conducted during the final fourth of the light phase and the first fourth of the dark phase.

Apparatus.

Each subject was restrained in a Plexiglas tube (22 cm long, and 6.8 cm in diameter) during training and testing. A 5.5 cm wide Plexiglas sheet extended across the base of each tube, 5.3 cm from the top, to form a stable platform on which the rat rested. A clear Plexiglas sheet covered the front of each tube. The external surfaces of each tube was covered with duct tape to prevent the rats from being distracted by extraneous visual stimuli. Thirteen ventilation holes (0.9 cm in diameter) were drilled through the midsection of each tube. A band of adhesive tape was used to seal the rear of each tube in order to secure the position of the rat. The subject's tail protruded from the rear of the tube between the band of adhesive tape and the top of the tube. Tail-shock was provided by a constant current 1.0 mA shock produced by a 660 volt transformer. A modified fuse clip was used as the shock electrode, and was lightly coated with electrode paste. The electrode was secured to the rat's tail with adhesive tape approximately 15 cm from the base of the tail. Tail flick latencies were assessed with a radiant heat tail-flick device. This device utilized a 375 watt movie light positioned 18 cm above the base of the apparatus. A condenser lens was positioned 8 cm below the light and served to focus the light on the rat's tail. The tail was positioned over a photo cell which automatically terminated the trial when the rat moved its tail laterally 0.5 cm. The auditory stimuli were a pure tone (1000 Hz) and a train of clicks (7 Hz). A Heathkit Audio Generator (Model IG-5282) generated the tone. The click was formed by running the

output from an Elgenco Gaussian Noise Generator (Model 602A) through a pulse former which turned the ouput on and off every 10 msec. A Realistic SA-10 Amplifier (Model 31-1982b) was used to amplify the stimuli. The stimuli were presented through Realistic 3 Inch Surface Mount Speakers (Model 12-1852) mounted 14 cm above the restraining tubes. Presentation of the stimuli were controlled by a Radio Shack Model IV computer.

The apparatus was located in an isolated room maintained at a temperature of approximately 25 ^{o}C. Ventilation fans provided a background noise of about 60 dB and the stimuli were presented at an intensity about 20 dB above this background noise level.

Procedure

A differential conditioning paradigm was employed. Each subject was weighed prior to being placed in the restraining tubes. The tone served as the CS+ and the train of clicks served as the CS- for one half of the subjects, and this relation was reversed for the other half. During each training session, the subjects received 6 presentations: three CS+ and 3 CS-. The conditioned stimuli were presented for 60 s in duration. A 0.5 sec, 1 mA shock served as the unconditioned stimulus (US) for 16 subjects and a 0.5 s, 0.3 mA was the US for the other 16 subjects. The US was presented during the last 0.5 s of the CS+. The stimuli were presented on a variable time interval, averaging 15 min (10 to 20 min) between CSs. The stimuli were presented in a random order as determined individually and online by the computer

before each training period. Each training period lasted about 90 min and each subject received training for two consecutive days.

Twenty-four hours after the second day of training, half of the subjects received an intraperitoneal (IP) injection of saline (14 mg/kg) and the other half received an IP injection of naltrexone (14 mg/kg). The subjects were then placed in the restraining tubes for a 15 min acclimation period. Four tail-flick tests were then given at 2 min intervals with an 8 sec cutoff limit to prevent tissue damage. The last three trials were averaged to provide a measure of the rat's baseline tail-flick latency. One min and fifteen sec after the last tail-flick test, one half of the subjects received the CS+ while the other half received the CS-. Each CS was 60 s in duration. One tail-flick test was conducted approximately 45 s after CS onset. Pain reactivity was tested again at 2, 4, 6, 8, 10 and 12 min after the test during the CS presentation. The final three of these six tests were averaged to provide the rat's baseline tail-flick latency for the presentation of the other CS. The subjects that received the CS+ then received the 60 s duration CS- 1 min 15 sec after the final tail-flick test. This order of CS presentation is reversed when the CS- is presented first. Pain reactivity was again measured 45 s after CS onset. Three tail-flick tests were then given at 2 min intervals. Each rat was tested for two consecutive days. The second day differed such that the subjects that received saline were administered naltrexone, and those that were given naltrexone received saline.

Results

Baseline levels of pain reactivity are depicted in Figure 1 on the following page. It is apparent that tail-flick latencies generally decreased, both across the three trials prior to each CS, and across blocks (i.e., group of trials before each CS presentation). The decrease observed across trials is generally larger on the first block than on the second block of testing. Apparently, naltrexone produced a slight decrease in tail-flick latencies. These impressions were confirmed by an analysis of variance (ANOVA) which revealed that the main effects of test block and trial, and the block by trial interaction were all statistically significant, all Fs > 6.19, $p < 0.01$. Also, a main effect of drug administration was evident, $F(1,28) = 8.52$, $p < .01$. No other differences were significant, all Fs < 3.78, $p > .05$.

The results for the 1.0 mA condition are depicted in the top two panels of Figure 2 and 0.3 mA intensity results appear in the bottom two panels of Figure 2. In saline treated subjects, 1.0 mA US established a strong conditioned hypoalgesia that was maintained across the two days of testing. Inspection of the data obtained from rats trained with the 1.0 mA US, but tested under naltrexone, reveals that the drug did not attenuate the conditioned hypoalgesia. In fact, if anything, it augmented the hypoalgesia elicited by the CS+. This effect was especially apparent on day 2 of testing. When subjects trained with 0.3 mA shocks were tested under saline, a strong conditioned hypoalgesia was observed on day 1, but not on day 2. This suggests that the

Figure Captions

Figure 1

Both panels represent the average tail-flick latencies in sec versus the trial number. The upper panel corresponds to training with 1.0 mA, and the lower panel corresponds to training with 0.3 mA. A block represents the set of 3 trials just prior to the presentation of the CS. Open circles represent saline controls and filled circles represent naltrexone treated subjects. Results from Day 1 and Day 2 testing are displayed.

Figure 2

The top two panels represent the change from baseline in sec versus the trial number for the saline controls (upper left panel) and the naltrexone treated subjects (upper right panel) for the 1.0 mA condition. The lower two panels correspond to the upper two with the exception that subjects were trained with 0.3 mA shocks. Open circles for all four panels represent presentation of the CS+, and filled circles represent CS- presentation. Results from Day 1 and Day 2 testing are displayed.

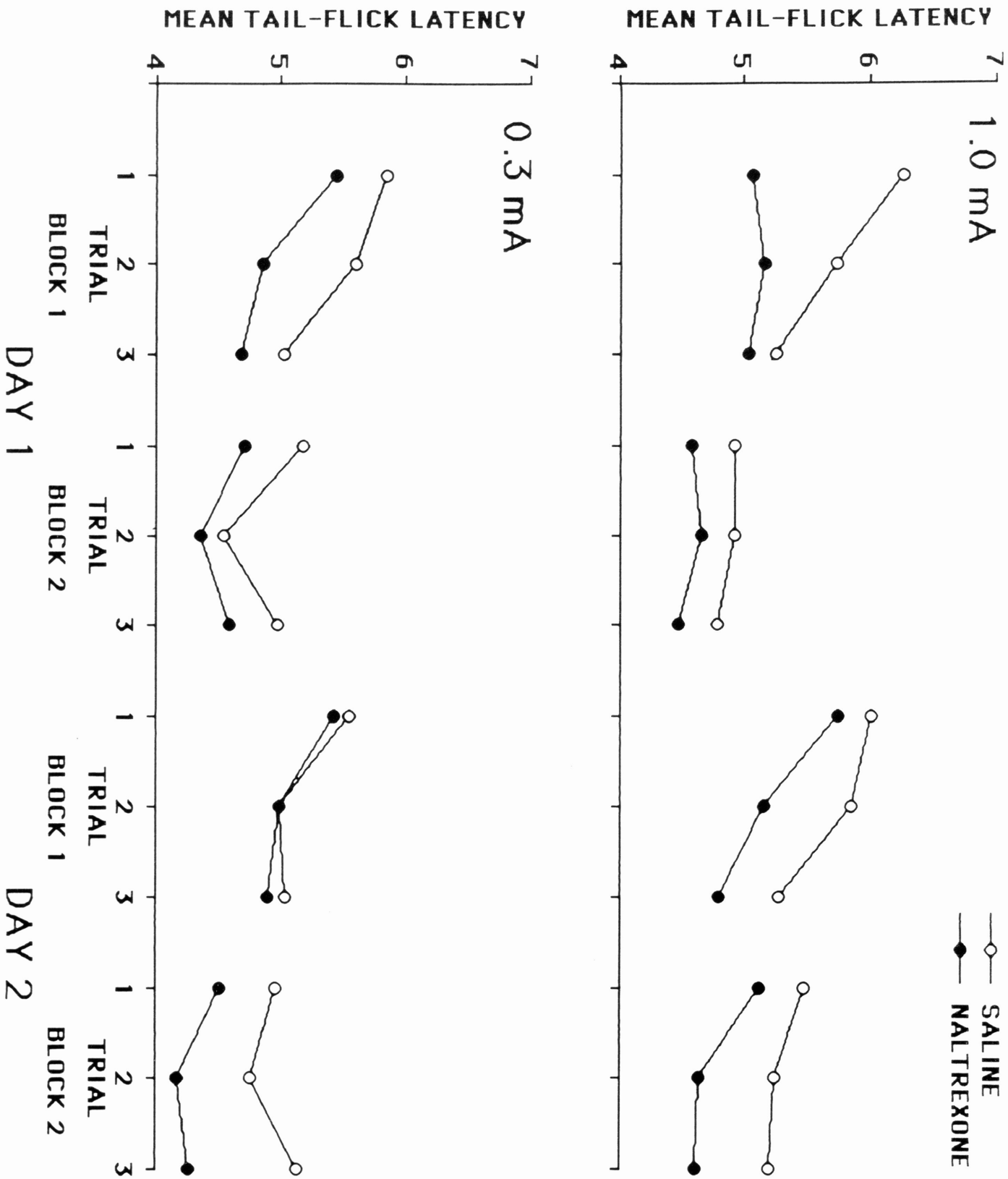

MEAN TAIL-FLICK LATENCY
1.0 mA
0.3 mA
SALINE
NALTREXONE
TRIAL
BLOCK 1
BLOCK 2
DAY 1
DAY 2

Figure 1

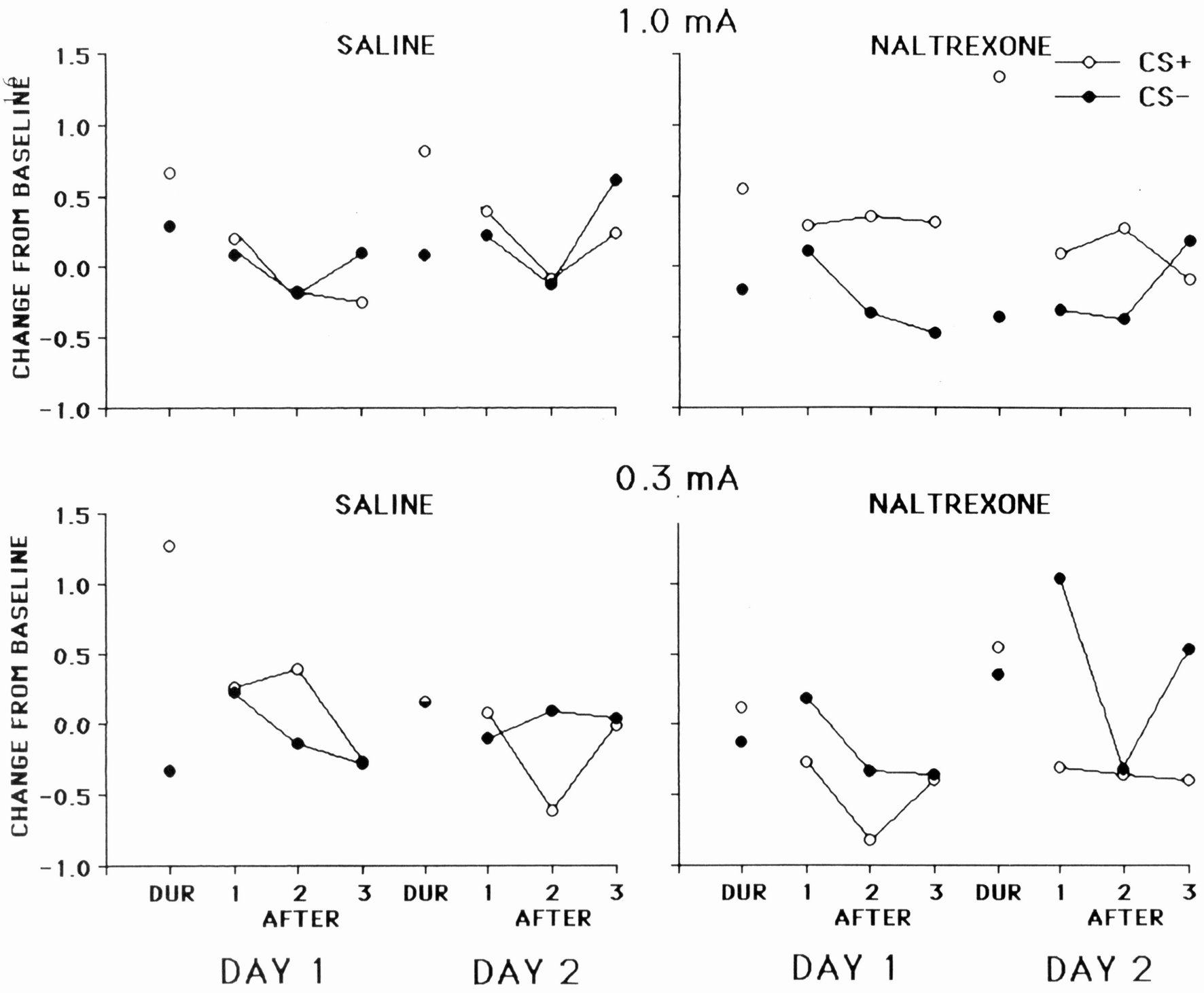

1.0 mA
SALINE
NALTREXONE
CS+
CS-
CHANGE FROM BASELINE
1.5
1.0
0.5
0.0
-0.5
-1.0
0.3 mA
SALINE
NALTREXONE
CHANGE FROM BASELINE
DUR
1
2
3
AFTER
DAY 1
DAY 2

Figure 2

presentation of the stimuli on the first day of testing caused extinction of the hypoalgesia. Most importantly, the conditioned hypoalgesia observed on day 1 was eliminated by naltrexone. In addition, little conditioned hypoalgesia was observed in naltrexone treated subjects on the second day of testing.

An ANOVA confirmed that the main effect of CS type was significant, $F(1,28) = 18.97$, $p < .001$. The higher-order interaction terms reveal that whether a difference was observed between the CS+ and CS- depended on both the combined impact of US intensity and day of testing, as well as US intensity and drug treatment, both Fs > 4.43, $p < .05$. A significant interaction between the impact of the drug treatment and day of testing was evident, $F(1,28) = 5.29$, $p < .05$. No other differences were significant, all Fs < 3.50, $p > .05$.

Results for tail-flick latencies after presentation of the CSs are depicted in Figure 2. After the CS presentations, a complex pattern of results was observed. In saline treated subjects, little difference was observed between the CS+ and CS-, and this was true irrespective of whether subjects were trained with the 1.0 or 0.3 mA US intensity. In naltrexone treated subjects, a strong conditioned hypoalgesia was observed in the subjects trained with 1.0 mA shocks, but not in subjects trained with 0.3 mA shocks. In fact, if anything, subjects trained with the 0.3 mA shocks exhibited the opposite pattern of results. In both cases, the magnitude of the difference between the CS+ and CS- depended on both the day of testing and the time following CS offset.

An ANOVA revealed that there was a significant change in tail-flick latencies across trials, and that the magnitude of the change observed depended on day of testing, both Fs > 4.87, p < .05. More importantly, a significant interaction between CS type and US intensity existed, $F(1,28) = 3.87$, $p < .06$. A marginally significant interaction between US intensity, test trial and drug treatment also existed, $F(2,56) = 3.03$, $p < .06$. Finally, a significant interaction between drug treatment, test trial, day of testing and CS type existed, $F(2,56) = 3.63$, $p < .05$.

Discussion

Results reveal that a decrease in tail-flick latencies occurs across trials and across blocks. Previous evidence indicates that this is generally the case in experiments utilizing radiant heat to measure pain reactivity. As a result of each tail-flick test, the temperature of the subject's tail increases slightly, lowering the subject's reactive threshold. In addition, naltrexone caused a slight decrease in baseline tail-flick latencies. One of two reasons can account for this effect. One possibility is that the period of restraint in the apparatus elicits an opioid analgesia. This is plausible since previous work has shown that restraint stress induces an analgesia which is blocked by naltrexone (Amir, Brown, & Amit, 1980). Alternatively, the training context itself may act as a CS+, causing a CS+(context)-US association which is opioid mediated.

The results of saline treated subjects tested under the 1.0 mA condition show that a strong conditioned hypoalgesia existed on

both days of testing, as expected. Subjects receiving naltrexone under the 1.0 mA US exhibited an enhanced conditioned hypoalgesia, suggesting that a severe conditioned stimulus activates the nonopioid system. This enhancement of the magnitude of the conditioned hypoalgesia may be due to naltrexone's ability to facilitate the acquisition and retention of associative learning in aversively motivated situations (Gallagher, 1982). This enhancement effect is especially apparent during day 2 of testing in the 1.0 mA condition.

Saline-treated subjects trained with 0.3 mA shocks exhibited a strong conditioned hypoalgesia on the first day of testing, but not on the second day, suggesting that the nonreinforced presentation of the stimuli on the first test day caused extinction of the hypoalgesia. Extinction occurred in the 0.3 mA condition and not the 1.0 mA condition because the association made between the CS+ and US was relatively weak in the former case, as compared to the latter. Therefore, a single nonreinforced presentation of the CS+ to the 0.3 mA rats was capable of inducing extinction of the hypoalgesia. More importantly, the conditioned hypoalgesia observed on day 1 in the subjects in the 0.3 mA condition was eliminated by naltrexone, indicating that a less severe US elicits an opioid mediated CR.

After the CS presentation in saline treated rats, little difference in tail-flick latencies was observed between the CS+ and CS- irrespective of the US intensity. In naltrexone treated subjects, a strong conditioned hypoalgesia was observed in the subjects receiving 1.0 mA, but not those receiving 0.3 mA shocks

after CS+ presentation. The effect seen in the 1.0 mA condition is due to the previously mentioned enhancemenent of learning and memory of the CS-US association by naltrexone. This effect was not seen in the 0.3 mA condition because the association between the stimulus and shock was relatively weak.

The primary aim of this experiment was to test the impact that US intensity has on determining the nature of the CR. Comparison of the form of the CR in the two shock conditions clearly shows that intensity does play a critical role in determining whether the opioid or nonopioid system is activated: relatively mild stimuli elicit an opioid mediated hypoalgesic CR, whereas a more intense shock elicits a nonopioid CR. These results agree with those found by Fanselow (1984). Here, however, it is clearly evident that the results are due to a conditioned response.

Experiment 2

Results from Experiment 1 indicate that the form of the hypoalgesic CR depends on the severity of the US. Specifically, a weak shock elicits an opioid mediated CR whereas a more intense shock activates the nonopioid system. A number of alternative accounts of this finding exist, one being that the form of the CR depends simply on the nature of the US (Fanselow, 1984). This predicts that the form of conditioned hypoalgesia should not vary as a function of the number of CS-US pairings or the duration of

the CS. However, a comparison across laboratories suggests that these variables are critical. For example, many studies reporting that the CR is opioid in nature (Fanselow & Baakes, 1982; Matzel & Miller, 1987) have used relatively long CS (30 s or more), whereas studies reporting that the CR is nonopioid in nature (Chance, 1979, Ross & Randich, 1985) have generally used briefer CS durations (30 s or less).

This observation led Fanselow to suggest that the critical factor may be the level of conditioned fear elicited by the CS+ (Lichtman & Fanselow, in press). This hypothesis suggests that increasing the level of fear elicited by the CS+ would change the form of the CR from opioid to nonopioid. Furthermore, this hypothesis suggests that the form of the CR depends on both the number of CS-US pairings and the CS+ duration. Specifically, a brief amount of training would activate the opioid system because the weak association established by such training would elicit only a low level of conditioned fear. Increasing the number of CS-US pairings should increase the strength of the CS-US association, and hence, the level of conditioned fear elicited by the CS+. Supporting this hypothesis, Lichtman and Fanselow (in press) recently reported that the form of the CR does depend on the number of CS-US pairings . Their results indicate that an extended training procedure produces a CR that is insensitive to naltrexone (i.e. nonopioid), whereas a briefer period of training establishes a CR that was attenuated by naltrexone.

As mentioned previously, Fanselow's hypothesis (i.e., the level of conditioned fear elicited by the CS is critical in the

form of the CR) also accommodates the observation that the form of the CR varies with CS+ duration. An opioid CR is observed when a long CS+ is employed because such a CS+ should elicit a relatively low level of conditioned fear. In addition, decreasing the duration of the CS+ would increase the expectation of the US, and consequently, the amount of fear elicited by the CS+. This, in turn, should act to change the form of the CR from opioid to nonopioid.

Fanselow and Baackes (1982) tested this hypothesis using a differential conditioning procedure, reporting that a 5 min CS+ elicits a strong opioid hypoalgesia on the formalin test. More importantly, this opioid CR was observed even though a relatively large number of CS-US pairings (8) were employed. One criticism of this finding is that the researchers did not test whether the form of the CR varies as the CS+ duration changes.

The present experiment tests whether the form of the CR varies as a function of the duration of the CS+. As previously noted, a number of laboratories have shown that this variable is important. However, a variety of procedural differences (e.g., method of pain assessment, modality of CS, and amount of training) could account for the differences observed across laboratories. To alleviate this problem, the present experiment directly tests the impact of changing the CS duration.

The results obtained from Experiment 1 clearly show that a 0.5 s, 1.0 mA shock establishes a nonopioid CR when the CS+ duration is 1 min. The present experiment directly tests which pain modulating system a CS+ 5 min in duration activates. This

CS+ length was chosen based on the previously mentioned experiment of Fanselow and Baackes.

As in Experiment 1, tail-flick latencies were assessed at 2 min intervals beginning 45 s after the CS onset. This procedure allows the testing of pain reactivity 3 times during the long CS (at 45 s, 165 s and 285 s). According to Fanselow's hypothesis, the level of conditioned fear should be lowest soon after the CS+ onset, and greatest near CS+ offset (when the US is always experienced). Thus, one might anticipate that the form of the CR will depend on the time during the CS+ that the pain reactivity is tested. Specifically, the CR may appear naltrexone reversible soon after CS+ onset when the level of conditioned fear should be relatively low, and become progressively naltrexone insensitive as CS termination is approached.

Subjects and Apparatus

The subjects were 32 rats of the same age, sex and strain as used in Experiment 1. The apparatus was the same as described in Experiment 1.

Procedure

One group (16 rats) received conditioning with a brief CS (60 s as used in Experiment 1) and the other group received conditioning with a long CS (300 s). A 0.5 s, 1.0 mA shock served as the US for both groups. Testing for the brief CS group is the same as reported in Experiment 1. During testing, the long CS subjects received four tail-flick tests, the last three of which

were averaged to obtain the base-line tail-flick latency. One min and 15 sec after the fourth tail-flick test, the CS was started, and tail-flick latency was measured 45 sec later, followed by two tail-flick tests at two minute intervals during the CS presentation. One tail-flick test was taken 45 sec after the conclusion of the CS presentation, serving as the after CS baseline. Three tail-flick tests were conducted at two min intervals, serving as the baseline for the other CS, and the procedure repeats as explained above. All other details of testing remain the same.

Results

Results from the baseline tail-flick latencies are depicted in Figure 3 on the following page. Analysis of the results obtained in this experiment reveal that the main effect of day of testing, and its interaction with other variables, did not have a significant effect on tail-flick latencies, all Fs < 3.55, p > .05. Consequently, the data in this experiment were collapsed across test days.

As in Experiment 1, baseline tail-flick latencies varied across test trials, and the magnitude of the change observed depended on test block. In addition, naltrexone treatment had a consistent impact of lowering baseline scores. An ANOVA confirmed that both the main effect of trials and test block, as well as their interaction, were significant, all Fs > 6.58, p < .05. The main effect of drug treatment was marginally significant, $F(1,30)$

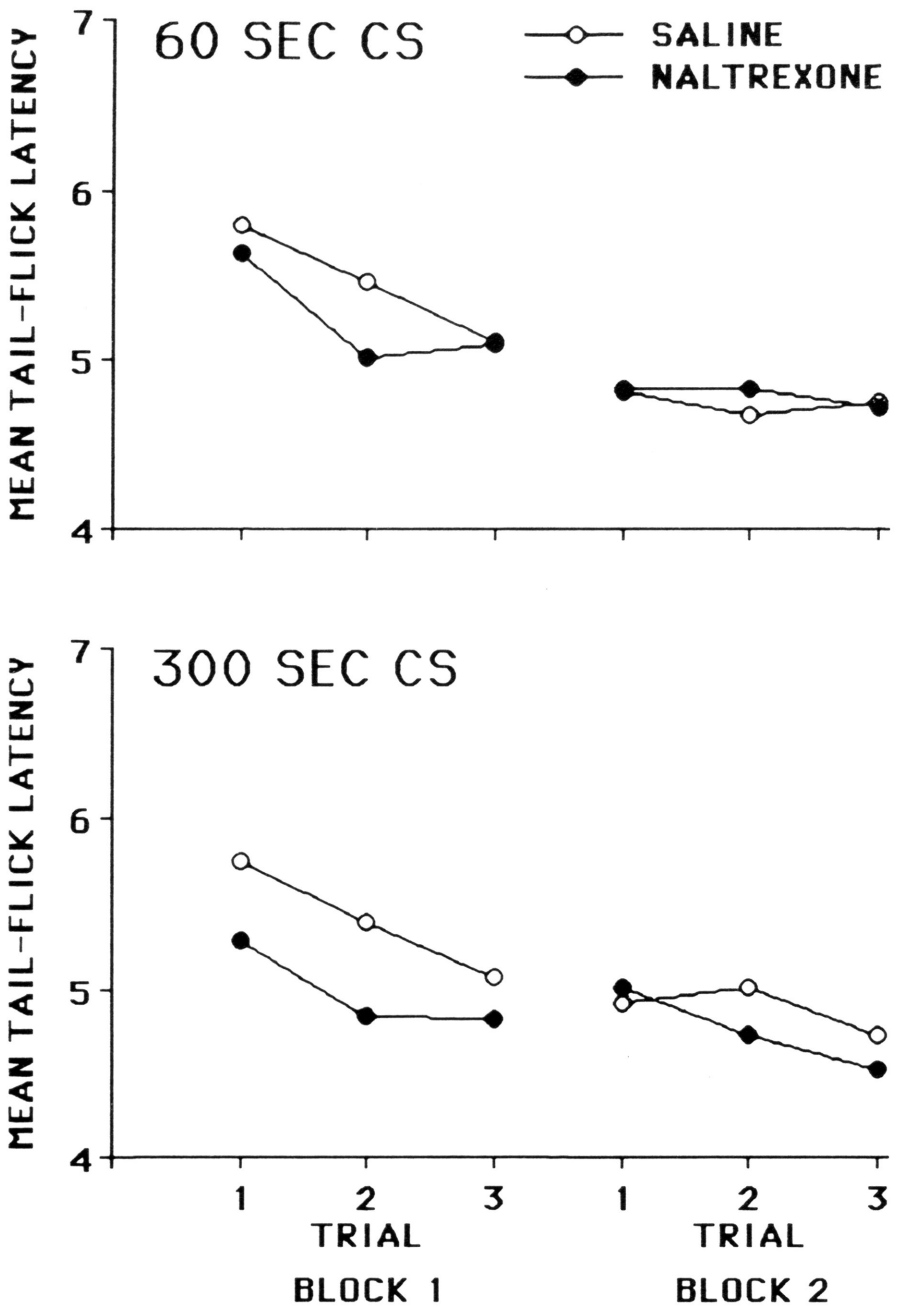
Figure Captions

Figure 3

Both panels demonstrate mean tail-flick latencies (sec) versus the trial number, with a block corresponding to the three latencies measured just prior to the CS presentation. The upper panel corresponds to a CS duration of 60 s, and the lower panel represents CS duration of 300 s. Open circles are the saline controls, and filled circles represent naltrexone treated subjects.

Figure 4

All four panels represent the change from baseline in sec versus the trial number. Open circles represent presentation of the CS+ and filled circles represent CS- presentation. The upper two panels correspond to subjects trained and tested with a CS 60 s in duration, and the lower two panels represent those in the 300 s CS duration condition. The upper and lower left panels represent results of the saline controls, and the two right panels are results for the subjects receiving naltrexone.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SALINE
NALTREXONE
MEAN TAIL-FLICK LATENCY
7
6
5
4
300 SEC CS
MEAN TAIL-FLICK LATENCY
7
6
5
4
1
2
3
1
2
3
TRIAL
TRIAL
BLOCK 1
BLOCK 2

Figure 3

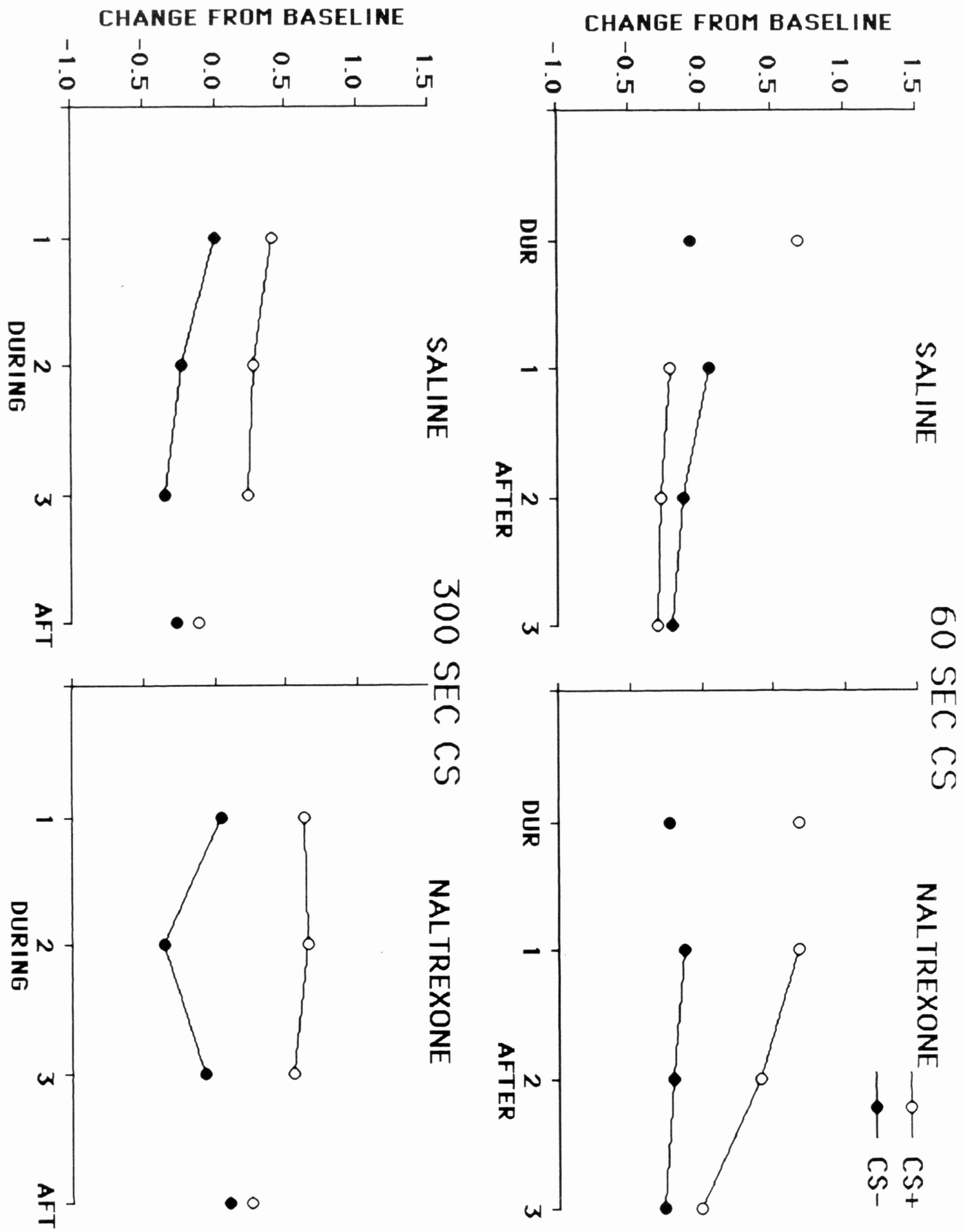
CHANGE FROM BASELINE
1.5
1.0
0.5
0.0
-0.5
-1.0
SALINE
60 SEC CS
NALTREXONE
CS+
CS-
DUR
1
2
3
AFTER
DURING
AFT

Figure 4

= 3.57, $p < .068$. No other differences were statistically significant, all Fs < 3.09, $p > .05$.

Analysis of the data obtained during the CS was begun by focusing on the results for subjects trained and tested with the long CS (depicted in the lower panels of Figure 4). Inspection of this data reveals that the long CS+ elicited a small hypoalgesia that was not attenuated by naltrexone, and that this was observed irrespective of whether pain reactivity was tested 45 s, 165 s, or 285 s after CS+ onset (test trials 1, 2 and 3, respectively). An ANOVA confirmed that there was a main effect of CS type, $F(1,15) = 8.29$, $p < .05$. Neither the main effects of drug treatment and test trial, nor any of the higher-order interactions, approached statistical significance, all Fs < 2.30, $p > .10$. Because the results obtained from the subjects trained and tested with the long CS did not vary as a function of test trial, the data across the three test trials were collapsed in the subsequent analyses.

Shifting attention to the data obtained from the subjects trained with the brief CS, we found that the CS+ elicited a strong conditioned hypoalgesia that was not attenuated by naltrexone as seen in the top panels of Figure 4. An ANOVA performed on the during CS scores observed across the two CS durations confirmed that the effect of CS type was significant, $F(1,30) = 27.1$, $p < .001$. Neither the main effects of stimulus duration and drug treatment, nor any of the higher-order interactions, approached statistical significance, all Fs < 0.72, $P > .10$.

After the long CS, the only effect which is apparent is a slight increase in tail-flick latencies in the naltrexone treated subjects. An ANOVA confirmed that there was a main effect of drug treatment, $F(1,15) = 7.77$, $p < .05$. Neither the main effect of CS type, nor its interaction with drug treatment, were significant, both Fs < 0.54, $p > .05$. After the brief CS, it appears that some conditioned hypoalgesia was observed in naltrexone treated subjects, but not the saline controls. Although ANOVA revealed a significant effect of test trial, $F(2,30) = 4.20$, $p < .05$, the interaction between drug treatment and CS type was not significant, $F(1,15) = 2.78$, $p > .05$. No other terms approached statistical significance, all Fs < 1.81, $p > .05$.

Discussion

As in Experiment 1, baseline tail-flick latencies varied across test trials and the magnitude of change depended on test block. This effect is due to the previously mentioned decrease in the subject's pain threshold due to an increased tail temperature. Also in accordance with Experiment 1, naltrexone caused a decrease in the baseline tail-flick latencies, which is due to opioid analgesia induced by either restraint or a context-US association.

Results for the subjects trained and tested with the 300 s CS+ revealed that the long CS+ elicited a small nonopioid hypoalgesia irrespective of the time at which pain reactivity was tested. Similarly, subjects exposed to a 60 s CS+ exhibited a strong nonopioid conditioned hypoalgesia.

Tail-flick latencies after the long or brief CS+ presentation increased slightly in the naltrexone, but not the saline, treated subjects. This may be due to naltrexone's ability to enhance the learning and memory of the of CS-US association (Gallagher, 1982).

The primary purpose of this experiment was to test the impact that the CS duration has on the form of the hypoalgesic CR. As previously mentioned, all subjects exhibited hypoalgesia regardless of the duration of the CS or the drug administered. This suggests that CS duration does not play a critical role in determining the form of conditioned hypoalgesia.

According to Fanselow (1984), a long CS duration should elicit a relatively low level of conditioned fear, and therefore induce an opioid CR, whereas a decrease in the duration of the CS+ would increase the conditioned fear level, thus activating the nonopioid system (Fanselow, 1984; Gibbon & Balsam, 1981). Findings of the present experiment are inconsistent with this hypothesis. Regardless of duration, these results show that conditioned hypoalgesia is mediated by the nonopioid system.

In agreement with Fanselow's hypothesis, however, it is possible that a much longer CS duration may elicit an opioid CR. In fact, one interpretation of naltrexone's attenuation of our baseline tail-flick latencies is consistent with this notion. Specifically, it is possible that the subjects acquired a context-shock association and the opioid conditioned hypoalgesia results from this. Because the context is the longest duration CS present, this extended duration CS may activate the opioid system. On the other hand, it is also possible that the unconditioned

effect of restraint stress alone elicits an opioid hypoalgesia. This interpretation is supported by previous studies which have found that restraint alone can induce a hypoalgesia attenuated by naltrexone (Amit, Brown, & Amir, 1980). Clearly, further work is required to resolve this issue.

General Discussion

The first experiment demonstrates that the intensity of the US plays a critical role in determining the form of the conditioned hypoalgesia. A mild US activates the opioid system whereas a more severe stimulus elicits nonopioid analgesia. This agrees with the results of Fanselow (1984), but the procedure used in the present experiment clearly indicates that the results are not due to pseudoconditioning, but are attributable to conditioned effects.

In Experiment 2, results show that CS duration is not a critical determinant of the form of the hypoalgesic CR. Regardless of CS+ duration, conditioned hypoalgesia was mediated by the nonopioid system. However, Fanselow (1984) found that a 300 s duration CS+ elicited an opioid CR on the formalin test with 8 CS-US pairings. At this point, the reasons for this inconsistency across laboratories is unclear. One explanation is simply a difference in procedures. For example, the method of assessing pain reactivity differs across studies (i.e., Fanselow employed the formalin test whereas we used the tail-flick test).

Alternatively, it is possible that CS duration was not a critical determinant of the form of the CR in Fanselow's study. Rather, the number of CS-US pairings alone may determine whether the opioid or nonopioid system is activated. Subsequent experiments could involve use of the formalin test to evaluate pain reactivity or manipulation of the number of CS-US pairings.

In both experiments, naltrexone attenuated the baseline tail-flick latencies. This opioid analgesia maybe due to either restraint stress or a conditioned association between the context and the shock. In the first case, the restraint stress itself leads to an unconditioned activation of the opioid system, which is blocked by naltrexone. In the second case, the subjects associate the experimental context (i.e., the Plexiglas tube, odor, etc.) with the shock, forming a CS-US association. If so, then the context may function as a long duration CS which elicits an opioid CR. From this perspective, it is possible that a 300 s duration CS was not long enough to activate the opioid system. Examining this issue may show that an extended CS+, such as context, is a critical determinant of the form of the CR. On the other hand, restraint may be the cause of the opioid analgesia.observed during baseline measurements. Further research is needed to elucidate the cause of this effect.

Understanding the variables which determine the form of conditioned hypoalgesia could have ramifications in the management of chronic pain. Knowledge in this may eventually result in the use of conditioning techniques to elicit intrinsic pain modulation that is not susceptible to tolerance.

References

Akil, H., Madden, J., Patrick, R. L., Barchas, J. D. (1976). Stress-induced increase in endogenous opiate peptides: Concurrent anlgesia and its partial reversal by naloxone. In Kosterlitz, H. (Ed.), Opiates and Endogenous Opioid Peptides. Amsterdam: Elsevier.

Amir, S., Brown, Z. W., & Amit, Z. (1980). The role of endorphins in stress: Evidence and speculations. Neuroscience and Biobehavioral Reviews, 4, 77-86.

Bolles, R. C., & Fanselow, M. S. (1980). A perceptual-defensive-recuperative model of fear and pain. Behavioral and Brain Sciences, 3, 291-323.

Cannon, J. T., Terman, G. W., Lewis, J. W., & Liebeskind, J. C. (1984). Body region shocked need not critically define the neurochemical basis of stress analgesia. Brain Research, 323, 316-319.

Chance, W. T. (1979). Autoanalgesia: Opiate and non-opiate mechanisms. Neuroscience & Biobehavioral Reviews, 4, 55-67.

Chance, W. T., White, A. C., Krynock, G. M., & Rosecrans, J. A. (1977). Autoanalgesia: Behaviorally activated antinociception. European Journal of Pharmacology, 44, 283-284.

Chance, W. T., Krynok, G. M., & Rosecrans, J. A. (1978). Antinociception following lesion-induced hypermotionality and conditioned fear. Pain, 4, 243-252.

Chance, W. T., & Rosecrans, J. A. (1979 a). Lack of effect of naloxone on autoanalgesia. Pharmacology, Biochemistry & Behavior, 11, 643-646.

Chance, W. T., & Rosecrans, J. A. (1979 b). Lack of cross-tolerance between morphine and autoanalgesia. Pharmacology, Biochemistry & Behavior, 11, 639-642.

Fanselow, M. S. (1984). Shock-induced analgesia on the formalin test: Effects of shock severity, naloxone, hypophysectomy, and associative variables. Behavioral Neuroscience, 98, 79-95.

Fanselow, M. S., & Baakes, M. P. (1982). Conditioned fear-induced opiate analgesia on the formalin test: Evidence for two aversive motivational systems. Learning and Motivation, 13, 200-201.

Gallagher, M. (1982). Naloxone enhancement of memory processes: effects of other opiate antagonists. Behavioral and Neural Biology, 35, 375-382.

Gibbon, J., & Balsam, P. D. (1981). The spread of association in time. In Locurto, C. M., Terrace, H. S., & Gibbon, J. G. (Eds.), Autoshaping and Conditioning Theory. New York: Academic Press.

Grau, J. W. (1987 a). The central representation of an aversive event maintains the opioid and nonopioid forms of analgesia. Behavioral Neuroscience, 101, 272-288.

Grau, J. W., Hyson, R. L., Maier, S. F., Madden, J., & Barchas, J. D. (1981). Long-term stress-induced analgesia and activation of the opiate system. Science, 213, 1409-1411

Hayes, R. L., Bennett, G. J., Newlong, P. G., & Mayer, D. J. (1978). Behavioral and physiological studies of non-narcotic analgesia in the rat elicited by certain environmental stimuli. Brain Research, 155, 69-90.

Levine, J. D., Feldmesser, M., Tecott, L., Lane, S., & Gordon, N. C. (1984). The role of stimulus intensity and stress in opioid-mediated analgesia. Brain Research, 304, 265-269.

Lewis, J. W., Cannon, J. T., & Liebeskind, J. C. (1980). Opioid and nonopioid mechanisms of stress analgesia. Science, 208, 623-625

Lichtman, A. H., Fanselow, M. S. (in press). Opioid and nonopioid mechanisms of conditional analgesia. Behavioral Neuroscience.

Maier, S. F. (1989). Determinants of the nature of environmentally induced hypoalgesia. Behavioral Neuroscience, 103, 131-143.

Matzel, L. D., & Miller, R. R. (1987). Recruitment time of conditioned opioid analgesia. Physiology & Behavior, 39, 135-140.

Oliverio, A., Castellano, C. (1982). Classical conditioning of stress-induced analgesia in the mouse: strain comparisons. Physiology & Behavior, 25, 171-172.

Ross, R. T. (1985). Blocking and unblocking of conditioned analgesia. Learning and Motivation, 16, 173-189.

Ross, R. T. (1986). Pavlovian second-order conditioned analgesia. Journal of Experimental Psychology: Animal Behavior Processes, 12, 32-39.

Ross, R. T., & Randich, A. (1985). Associative aspects of conditioned analgesia evoked by a discrete CS. Animal Learning and Behavior, 4, 419-431.

Terman, G. W., Shavit, Y., Lewis, J. W., Cannon, J. T., & Liebeskind, J. C. (1984). Intrinsic mechanisms of pain inhibition: Activation by stress. Science, 226, 1270-1277.

Watkins, L. R., & Mayer, D. J. (1982). Organization of endogenous opiate and nonopiate pain control systems. Science, 216, 1185-1192.

Watkins, L. R. & Mayer, D. J. (1986). Multiple endogenous opiate and nonopiate analgesia systems: Evidence of their existence and their clinical implications. Annals of the New York Academy of Sciences, 467, 273-299.

Watkins, L. R., Cobelli, D. A., & Mayer, D. J. (1982). Classical conditioning of front and hind paw footshock-induced analgesia (FSIA): naloxone reversibility and descending pathways. Brain Research, 243, 119-132.