PAVLOVIAN CONDITIONING IN AWAKE PENTOBARBITAL ANESTHETIZED RATS

A Senior Thesis

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Abstract

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Prior research shows that very intense noises can produce antinociception in awake rats. Experiment 1 examined the antinociceptive effects of a tone and a noise at various intensity levels on awake and pentobarbital anesthetized rats. Both the tone and noise reduced reactivity to a noxious thermal stimulus applied to the tail in both awake and anesthetized subjects. Experiment 2 examined whether Pavlovian conditioning can be established in pentobarbital anesthetized rats. Half of the subjects experienced an auditory cue (the conditioned stimulus, or CS) paired with an aversive tailshock (the conditioned stimulus, or US). The remaining subjects experienced the CS and US in an unpaired fashion. US intensity was set to a value known to induce a strong antinociception in both awake and anesthetized rats. CS intensity was set, based on the results from Experiment 1, to a level that generated a weak antinociception. Rats trained while awake exhibited longer tail-flick responses in the presence of the context, but they did not appear to exhibit any conditioning to the auditory cues. Rats trained under anesthesia did not exhibit conditioning to the context, but did display reduced tail-flick latencies (conditioned hyperalgesia) to the paired auditory cues. Implications of the results are discussed.

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Pavlovian Conditioning in Awake and Pentobarbital Anesthetized Rats

Organisms learn about their environment by encoding the relationships between stimuli. One example of this is Pavlovian conditioning (Pavlov, 1927). In this procedure, a neutral stimulus, such as a contextual or discrete cue (the conditioned stimulus, or CS) is paired with a biologically meaningful event, such as a shock (the unconditioned stimulus, or US). After repeated pairings, the neutral stimulus comes to elicit a new response, known as the conditioned response (CR).

Pavlovian conditioning is frequently studied in rats by comparing a discrete CS (e.g. a light or tone) with an aversive shock (US). After a few CS-US pairings, rats exhibit a variety of defensive responses, including freezing and diminished pain reactivity (conditioned analgesia). Similar effects are observed when the context (e.g. shock chamber) is paired with the shock (Fanselow, 1986).

It has been generally assumed that Pavlovian conditioning depends on conscious processes. However, recent reports suggest that unconscious subjects can exhibit some forms of learning. For example, researchers claim to have observed learning in anesthetized humans (Ghonheim & Block, 1992). A large part of the literature is devoted to clinical studies on human patients. These clinical studies have looked at verbal information processing during surgery. Their findings suggest that patients exhibit implicit memory

for surgical events which occurred under general anesthesia (Kihlstrom & Couture, 1992). For example, anesthetized patients have been shown to answer more questions about "common facts" correctly after having been presented with statements about them during surgery (Jelicic, De Roode, Bovill, & Bonke, 1992). However, it unclear what type of mechanism underlies this learning (single stimulus, Pavlovian or operant conditioning), and some have questioned the validity of these studies (Shanks & St. John, 1994).

In the present study we examine Pavlovian conditioning in sodium pentobarbital treated rats. Sodium pentobarbital is a commonly used anesthesia in animal research, and is thought to disrupt forebrain activity, which many believe to be responsible for associative learning. Experiment 1 is a parametric study of the antinociceptive effects of tones and noises of various intensities on saline and pentobarbital treated rats. The results of this experiment were then used to select the CS values for Experiment 2. In Experiment 2 we attempt to establish a conditioned antinociceptive response in saline and pentobarbital treated rats by pairing auditory stimuli with tailshock.

Experiment 1

Anesthesia could disrupt learning simply because it prevents the CS or US from being detected or processed. To avoid this problem we sought stimulus values that had similar behavioral consequences in both awake and

anesthetized subjects. Previous studies have revealed that a 1.5-s 1-mA tailshock US produces about the same level of antinociception (reduced pain reactivity) in awake and anesthetized subjects (Meagher, Chen, Salinas, & Grau, 1993). However there was little data to guide the selection of the CS parameters. Consequently, Experiment 1 explores the behavioral effects of two auditory cues (a 1000-Hz tone and a static-like noise) on pain reactivity in awake and pentobarbital anesthetized rats.

Method

Subjects. The subjects were 24 male Sprague-Dawley rats from Harlan (Houston, TX). Each rat weighed between 300 and 400 grams and was 120-150 days old. The rats were individually caged and kept on a 12-hr light/dark cycle. All testing was performed during dark cycle. The rats had continual access to food and water.

Apparatus. The rats were placed in blackened Plexiglas tubes for testing. The tubes were 19 cm long, 6.9 cm in internal diameter. Inside each tube was a flat base (5.7 cm wide, 5.6 cm from the top of the tube) on which the rats were placed. The front of the tube was blocked off by blackened Plexiglas. Ventilation holes were drilled in the top of the tube. The rats were kept in the tube by adhesive tape placed above and below the tail at the rear of the tube, allowing the tail to protrude and move freely. Each tube was placed in an open testing chamber during testing. Testing took place in an isolated room maintained at about 24 (C.

The 1000-Hz tone and white noise stimuli were delivered by a Realistic 3-in. surface mount speaker (Model 12-1852) placed 13.5 cm above the top of the tube. The tones were generated by a Heathkit model IG5282 audio generator. The pulsing white noise stimuli were generated by an Elgenco model 602A Gaussian noise generator coupled to a pulse former which turned the noise output on and off every 10 ms. Both the tone and noise were amplified using an Optimus 50-W amplifier (Model 12-1970A) and passed through a Radio Shack SSM-50 stereo sound mixer to control the stimulus intensity. The tone and noise intensities were calibrated using a Columbia model SPL-204 sound pressure level meter placed inside the tube. Ventilation fans provided a background noise of about 45-dB.

A radiant heat tail-flick device was used to assess pain reactivity. The radiant-heat source was a 375-W reflector movie light (General Electric) positioned 18 cm above the base of the device. A condenser lens located 11 cm below the light focused the radiant heat on the tail. The aluminum base had a V-shaped groove cut into it (0.7 cm wide, 0.3 cm deep), in which the rats' tails were placed. Lateral movement (0.5 cm minimum) of the tail was detected by a photocell placed beneath the groove, which automatically terminated the trials. A timer automatically measured how long each trial lasted to the nearest 0.01 sec. To prevent tissue damage, trials were manually terminated if no response occurred after 8 sec.

Procedure. The rats were removed from their cages and half were injected (i.p.) with sodium pentobarbital (Sigma Chemical Co.) at a dose (48 mg/kg) designed to induce a plane of anesthesia in which subjects were flaccid, while spinal reflexes remained intact. The other half were injected (i.p.) with an equal volume of 0.9-percent saline. Approximately ten minutes later, the rats were placed in their tubes and allowed to acclimate for 15 min.

Tail-flick latencies were tested during 0, 95, 105, and 115-dB 1000-Hz tone stimuli and during 0, 85, 95 and 105-dB white noise stimuli. Thirty-two tail-flick tests were performed at 1-min intervals. Twenty-two seconds prior to every fourth test a 30-sec tone or noise stimuli was presented. Thus after three baseline trials, a 30-sec tone or noise stimulus preceded and overlapped every fourth trial. Each subject was presented with each of the four tone intensities followed by each of the four noise intensities or vice versa, counterbalanced using a latin square design.

Results

The impact of the tone on tail-flick latencies of saline and pentobarbital treated rats is depicted in the bottom panel of Figure 1. Both groups exhibited longer tail-flick latencies when tone intensity was increased. An analysis of variance (ANOVA) revealed that neither the main effect of drug treatment, nor its interaction with tone intensity, approached statistical significance, both Fs < 1.0, both $p > 0.05$ Although the main effect of tone intensity was not significant, $F(3, 66) = 2.35$, $p > 0.05$, trend analysis revealed a significant linear

component, $F(1, 66) = 6.76$, $p < 0.05$. Neither the quadratic nor the cubic component approached significance, both Fs < 1.0, $p > 0.05$.

The impact of the noise on tail-flick latencies of saline and pentobarbital treated rats is shown in the top panel of Figure 1. At lower intensities, greater antinociception was observed in saline treated rats. More intense noise stimuli elicited a similar level of antinociception in both groups. An ANOVA revealed that noise intensity had a significant impact, $F(3, 66) = 6.08$, $p < 0.001$. While the main effect of treatment drug was not significant, $F(1, 22) < 1.0$, $p > 0.05$, the overall interaction with noise intensity was marginally significant, $F(3, 66) = 2.66$, $p < 0.06$. Further analysis revealed a significant quadratic component $F(1, 66) = 7.70$, $p < 0.001$. Neither the linear nor the cubic components were significant, both Fs < 1.0, $p > 0.05$.

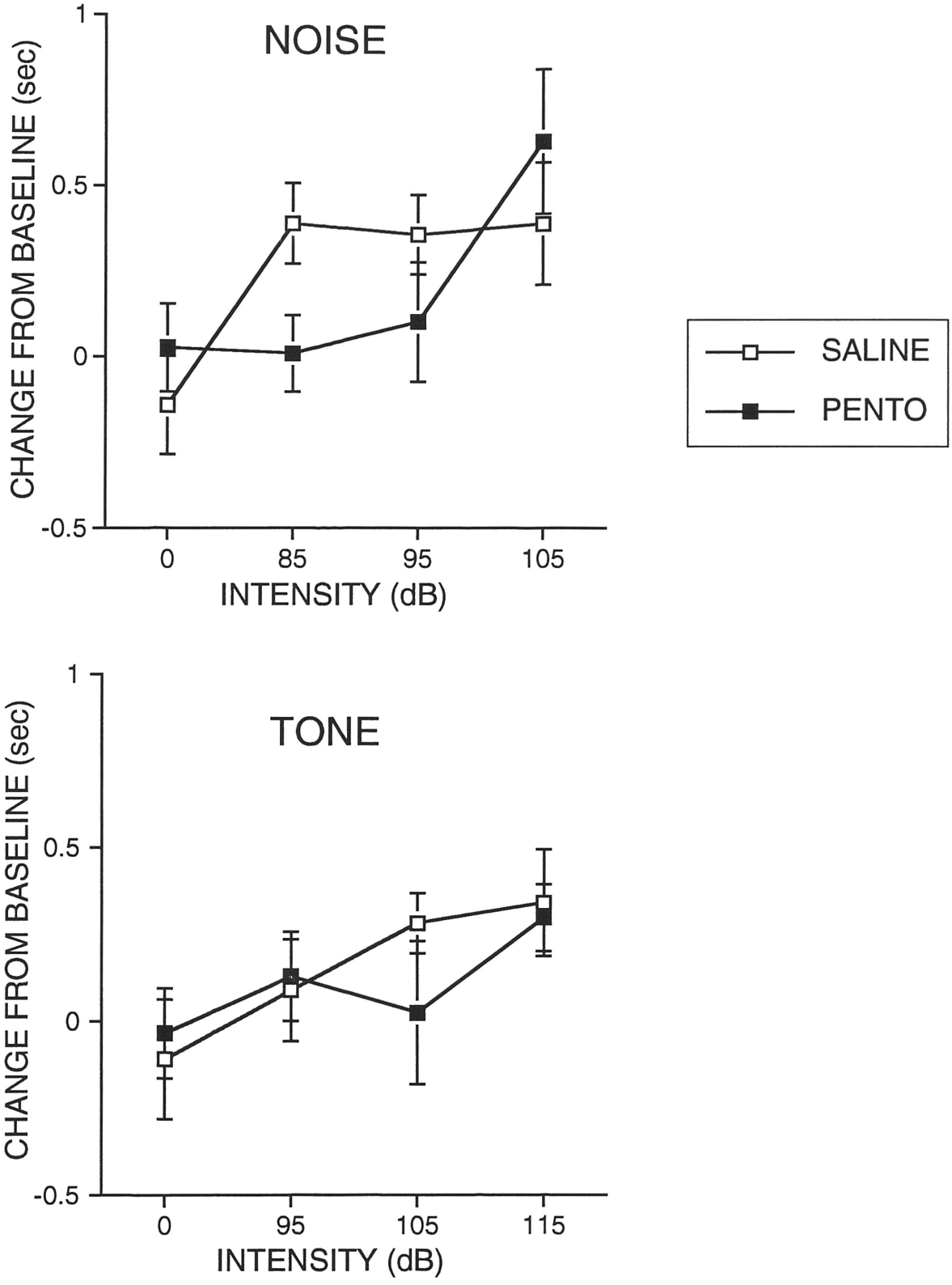

Figure 1. The impact of the noise (top panel) and 1000-Hz tone (bottom panel) on tail-flick latencies of awake (saline, open box) and anesthetized (pentobarbital, filled box) rats. The error bars indicate the standard error of the mean (S.E.M.).

Discussion

Both the tone and the noise produced a significant antinociception as measured by change from baseline on tail-flick latencies. The noise generated antinociception at lower intensity values than the tone. While pentobarbital treated rats appeared somewhat less sensitive to the noise stimulus, it produced comparable levels of antinociception in both groups at an intensity of 100-dB. Drug treatment did not appear to affect responsiveness to the tones, which did not elicit a strong antinociception until the intensity was increased to 115 dB.

Experiment 2

Experiment 2 examines whether Pavlovian conditioning is observed in pentobarbital anesthetized rats. Based on results from Experiment 1, CS values were selected that produced a similar level of antinociception during both the tone and the noise, and across both the saline and pentobarbital treated groups. Inspection of Figure 1 shows that a noise stimulus of 100-dB produced a change from baseline on the tail-flick test of about 0.35-s for both saline and pentobarbital treated rats. Figure 1 also shows that a 115-dB tone produced a change from baseline of about 0.30-s for both the saline and pentobarbital treated rats. Consequently, we used a 100-dB noise and a 115-dB tone for our CSs. The US was a 1.5-s 1-mA tailshock that has been shown to generate a strong antinociception on the tail-flick test in both awake and anesthetized rats (Meagher, Chen, Salinas, & Grau, 1993).

Method

Subjects. The subjects were 32 rats of the same sex, strain, weight and as in Experiment 1. All training and testing was performed during the dark cycle.

Apparatus. The tubes, chambers and noise presentation were the same as described in Experiment 1.

Constant current, 60-Hz AC, 1.5-sec, 1-mA tailshocks were generated by 660-V transformers. Shock was applied through electrodes constructed out of modified fuse clips mounted 1.7 cm apart. The electrode plates were lightly coated with electrode paste and taped to the rat's tail about 5 cm from the tip.

Procedure. The rats were removed from their cages and half were injected (i.p.) with a doses of 48 mg/kg sodium pentobarbital as described in Experiment 1. The other half were injected (i.p.) with an equal volume of 0.9-percent saline. The rats were placed in the tubes, restrained with tape, attached to the electrodes, and closed up in the chambers. Half the subjects (paired) in each drug condition then received 10 CS-US pairings on a variable interval schedule with an average of 156-s

between CS-US pairs. CS duration was 30-s and the US was presented during the last 1.5-s of the CS. The remaining subjects (unpaired) experienced the CS and US in an unpaired fashion, on a variable interval with a mean of 78-s. For half the paired and unpaired rats the tone served as the CS, while the

remaining subjects experienced the noise as the CS. After training, the subjects were returned to their cages.

Testing occurred 24 hours after training. Each rat was tested in the same tube and chamber in which it was trained. Immediately after being placed in the tube, each rat was given three tail-flick tests at 2 minute intervals. Each rat was then closed up in the chamber for 26 minutes. Testing resumed immediately upon opening the chambers. Sixteen tail-flick tests were then given at 2-min intervals. Twenty-two seconds prior to every fourth test a 30-sec tone or noise stimuli was presented. Thus after three tail-flick trials, a 30-sec tone or noise stimulus preceded and overlapped every fourth trial. The third tail-flick test in each set was used as the baseline. Each subject was presented with two 30-sec 1000-Hz tone stimuli and two 30-sec white noise stimuli, in counterbalanced ABBA order.

Results

The baseline tail-flick latencies observed across five test trials are depicted in Figure 2. At the start of testing, subjects trained under pentobarbital exhibited lower tail-flick latencies. This difference disappeared over the course of testing. An ANOVA confirmed that drug treatment had a significant impact, $F(1,28) = 5.00$, $p < 0.05$. Both the main effect of trials and its interaction with drug treatment were statistically significant, both Fs$(1,24) > 4.34$, $p < 0.01$. Neither the main effect of training condition, nor any of the

other interaction terms, approached statistical significance, all $Fs < 1.0$, $p > 0.05$.

Tail-flick latencies during the pre-exposed CS are depicted in Figure 3, and the latencies observed during the novel CS are depicted in Figure 4. In saline treated rats (upper panel, Figure 3), similar latencies were observed irrespective of whether the CS was paired or unpaired with the US, and this was true for both stimuli. In contrast, the paired CS elicited shorter latencies (conditioned hyperalgesia) in subjects trained under pentobarbital (lower panel, Figure 3). This learning effect did not generalize to the novel stimulus (lower panel, Figure 4). Oddly, saline treated rats did respond differently to the novel CS depending on both their training history and novel CS type (noise vs. tone); while a novel tone elicited antinociception regardless of training history, a novel noise only elicited a strong antinociception in unpaired subjects (upper panel, Figure 4). An ANOVA revealed that the impact of the test CS (noise vs. tone) depended on training condition, $F(1, 24) = 4.97$, $p < 0.05$. Whether the test CS elicited a significant antinociception also depended on its training history (whether or not the CS was pre-exposed), drug treatment during training, and the training condition (paired vs. unpaired), $F(1, 24) = 4.28$, $p < 0.05$. No other main effects or interactions reached statistical significance, all $Fs < 3.32$, all $p > 0.05$.

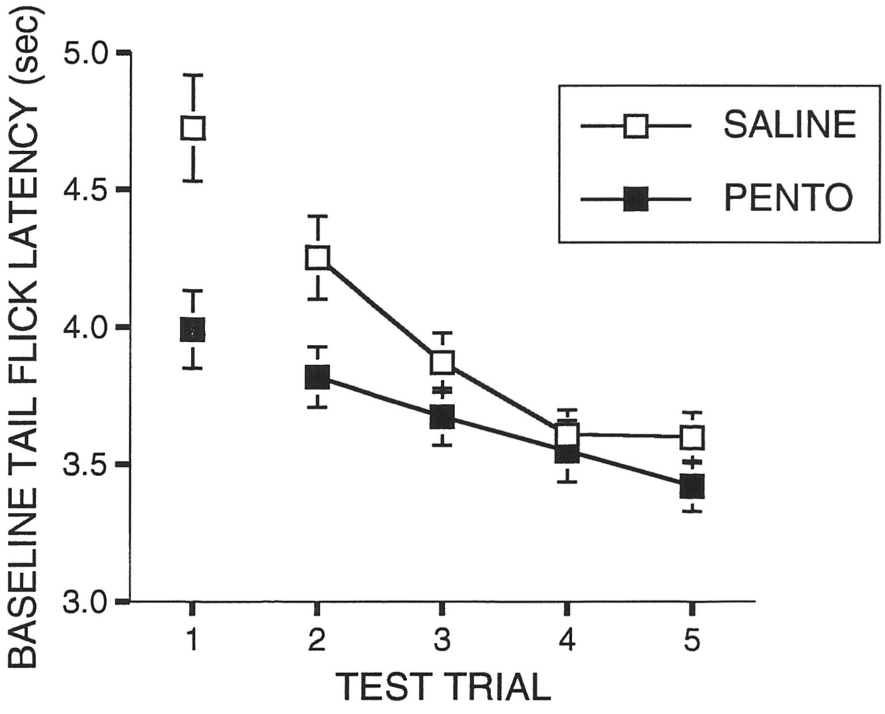

Figure 2. Baseline tail-flick latencies observed over the five test trials for rats that were awake (saline, open box) or anesthetized (pentobarbital, filled box) during training. There was a 26 minute resting period between test trials 1 and 2. The error bars indicate standard error of the mean (S.E.M.).

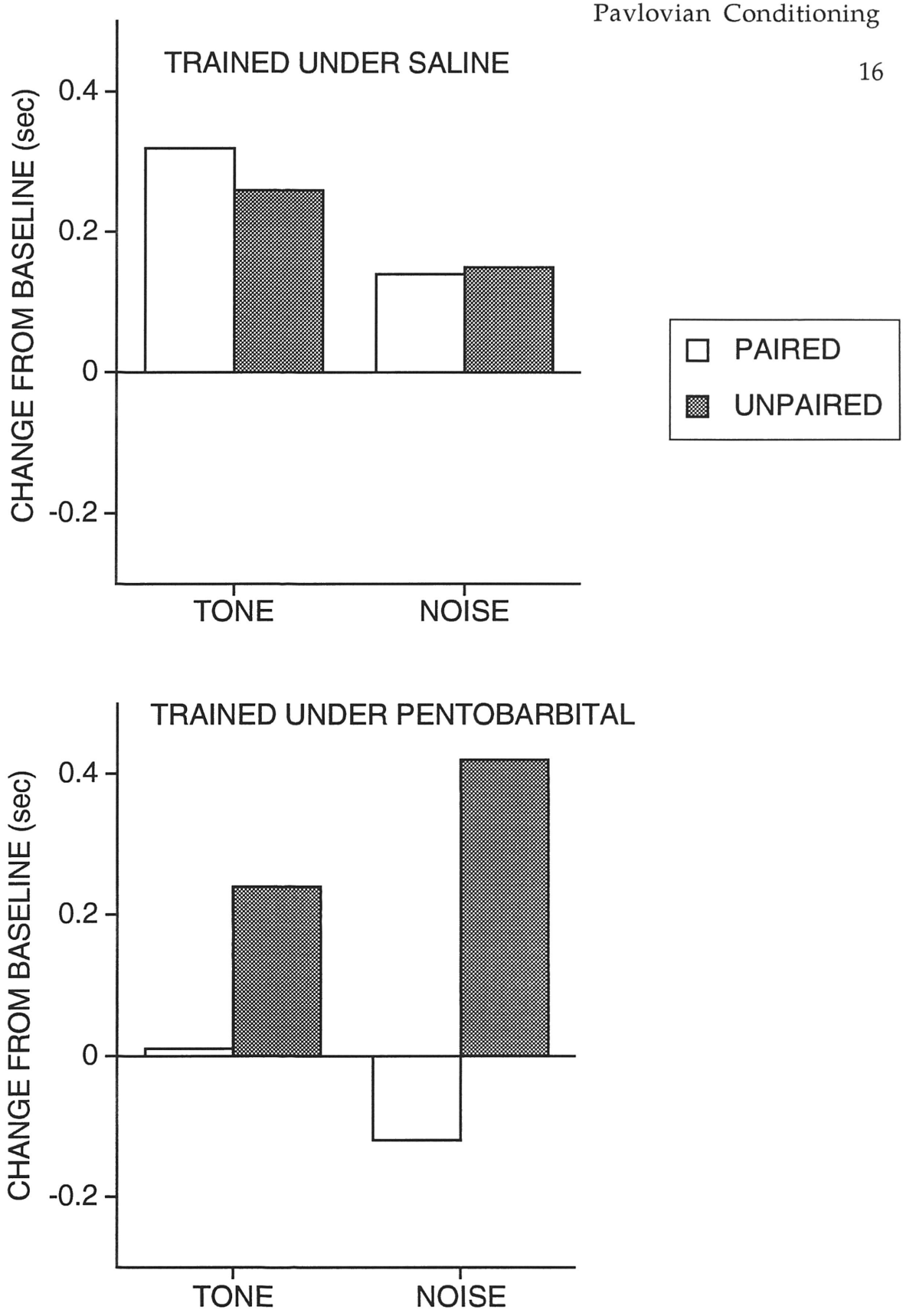

Figure 3. Tail-flick latencies to the pre-exposed CS (tone or noise) for rats that were awake (top panel) or anesthetized (bottom panel) during either paired (open box) or unpaired (shaded box) training.

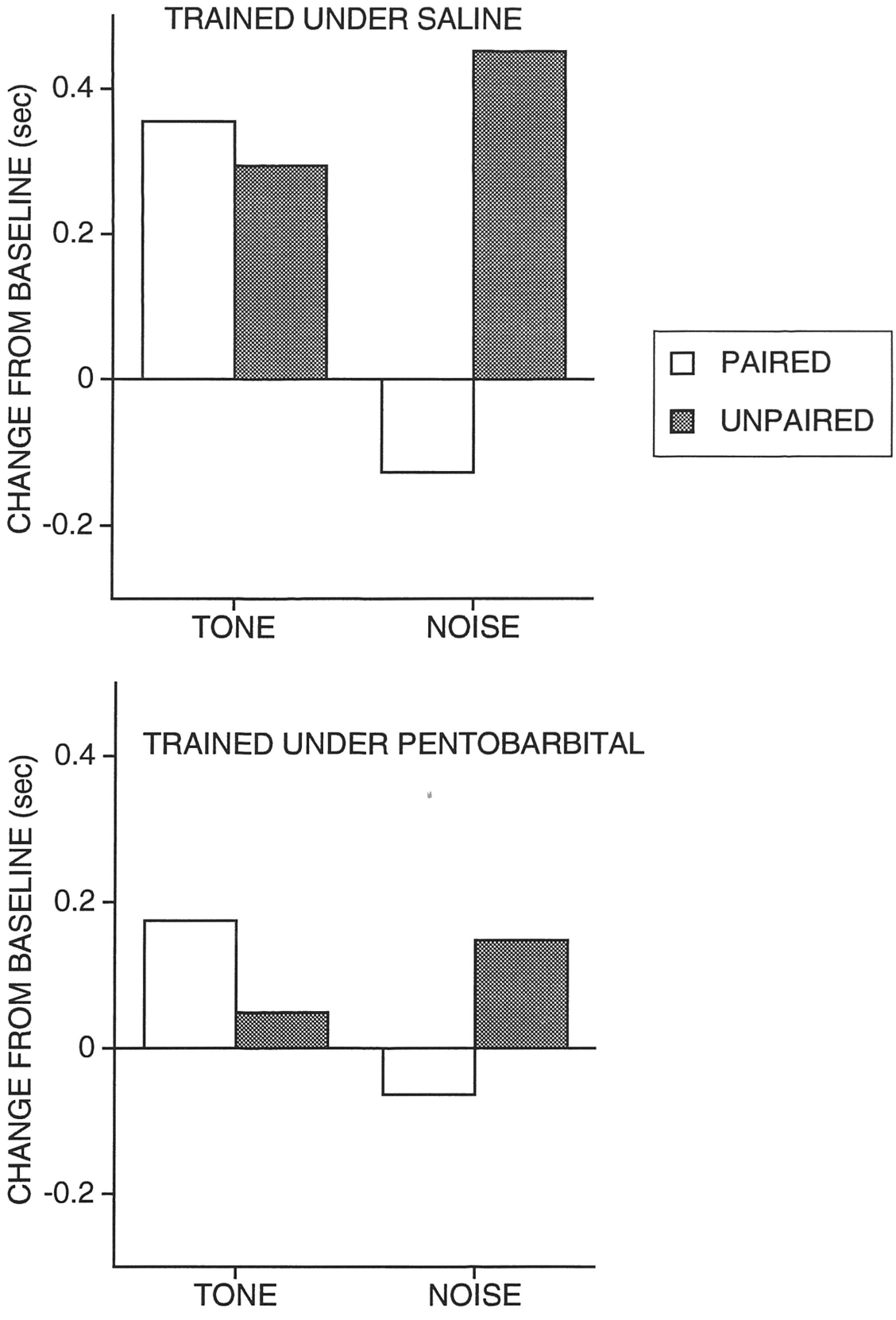

Figure 4. Tail-flick latencies to the novel CS (tone or noise) for rats that were awake (top panel) or anesthetized (bottom panel) during either paired (open box) or unpaired (shaded box) training.

Discussion

Whether or not subjects learned a context-shock association appeared to depend on whether they were anesthetized during training. Subjects treated with pentobarbital during training did not show a conditioned antinociception to the context, while saline treated subjects did. In the absence of further shock, the antinociception observed in the saline treated rats extinguished over the course of testing.

Oddly, only anesthetized rats appeared to learn about the discrete CS. Specifically, shorter tail-flick latencies were observed during the paired CS in rats given pentobarbital prior to training, but not in the saline treated controls.

The results from the novel CS were more difficult to interpret. It is clear that the conditioned hyperalgesia observed in pentobarbital treated subjects did not generalize to the novel CS. What is less clear is why subjects trained under saline responded differently to the novel noise depending on their training history.

General Discussion

The present experiments looked at whether Pavlovian conditioning can occur in anesthetized subjects. To address this issue, we sought CS and US values that produced similar behavioral effects in both awake and unconscious subjects. Experiment 1 was designed to establish the CS parameters by comparing the antinociceptive consequences of tone and noise

stimuli over a range of intensities in awake and anesthetized rats. A 100-dB noise stimulus and 115-dB tone stimulus were found to produce similar levels of antinociception to each other, and across saline and pentobarbital treated groups. The fact that noise stimuli can induce antinociception in awake rats replicated an earlier study by Helmstetter and Bellgowan (1994). These authors attributed the noise-induced antinociception to the induction of fear. Because unconscious subjects presumably experience little fear, their account suggests that anesthesia should eliminate the noise-induced antinociception. Indeed, this may help to explain why 85-dB noise stimuli induce antinociception in awake, but not unconscious, rats. What is problematic is that at higher intensities antinociception was observed irrespective of whether rats were awake or unconscious. This suggests that auditory stimuli can activate the antinociceptive systems at a relatively low level of the nervous system (e.g., brainstem), and, thus, this antinociception is not mediated by psychological processes.

Using the 100-dB noise and 115-dB tone CSs derived from Experiment 1, Pavlovian conditioning was demonstrated in both awake and pentobarbital anesthetized rats (Experiment 2). Awake rats exhibited conditioned antinociception to the context, which was paired with shock during training, but did not appear to show any learning to the discrete cues. The anesthetized rats exhibited conditioned hyperalgesia to the discrete auditory CS, which had

been paired with shock during training, but showed no learning effects to the context.

Pentobarbital is thought to eliminate consciousness, which many argue is required to make the associations necessary for Pavlovian conditioning. The differences in conditioning between saline and pentobarbital treated subjects in this study are consistent with the idea that different types of learning occur depending upon whether conscious processes are available.

Pavlovian conditioning has been observed in a number of other paradigms that disrupt higher psychological functions. For example, Pavlovian conditioning has been obtained in decerebrate subjects (Mauk & Thompson, 1987), and spinalized rats have been shown to exhibit a form of Pavlovian conditioning for an antinociceptive response (Grau, Salinas, Illich, & Meagher, 1990). This learning may depend on simpler, nonassociative mechanisms. Supporting this, Joynes and Grau (1996) have shown that a protection from habituation mechanism underlies Pavlovian conditioning in spinalized rats.

The results of Experiment 2 suggest that pentobarbital treated subjects are capable of exhibiting Pavlovian conditioning. However, the pentobarbital treated subjects showed a different form of learning than the saline treated subjects. Prior work suggests that learning about contextual stimuli requires higher neural functions (e.g., hippocampus) while simple mechanisms may mediate leaning about discrete cues (Sutherland & Rudy, 1989). This could

help explain the results of this study. Awake rats could learn about the shock context and this learning may have overshadowed learning about the discrete auditory cue. Because pentobarbital disrupts higher brain functioning, it disrupted learning about the contextual stimuli. Learning about the discrete cue was, however, spared.

In some ways it is surprising that the saline treated rats failed to learn about the discrete CS. However, it is not clear whether their failure to exhibit conditioned hypoalgesia reflects a failure to learn or the development of an opposite process, a conditioned analgesia, that counteracts the conditioned hyperalgesia observed in the pentobarbital treated rats.

One problem with using anesthesia is that there is no way to precisely determine the level of consciousness of the subjects. Under anesthesia the level of consciousness may vary between subjects and within subjects over time. This makes it difficult to make strong conclusions about the conscious state of anesthetized subjects. In this study the subjects were given a dose that made them flaccid, but left their spinal reflexes intact. This was necessary to be able to test their behavioral responses. Our subjects may be said to have been only lightly anesthetized, but a difference was observed between the responses of pentobarbital and saline treated subjects. The pentobarbital was observed to have an effect on how the subjects learned, without eliminating learning altogether.

Ordinarily, comparison of the paired and unpaired groups' responses to the novel CS helps distinguish the mechanism which underlies the conditioning. However, in this study, the training history of the subjects affected the way they responded to the novel CS. Given this, it is not entirely clear that the subjects were able to discriminate the novel CS from the CS experienced during training.

Further research is needed to better clarify the mechanisms involved. Weaker auditory stimuli may be unconsciously detected or processed in such a way that they do not produce a response on their own. Could anesthetized subjects learn about such stimuli? Further work is also needed to evaluate the impact of CS exposure in the absence of shock. Does repeated presentation of an auditory cue cause habituation, and does this learning depend on conscious processes? Further studies are also needed to isolate the neural mechanisms involved. For example, would a similar pattern of results be obtained on decerebrate subjects?

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