Failure to Observe Spinal Antinociception

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Abstract

In this research, it was attempted to elicit the phenomenon of spinally activated antinociception. Surprisingly, the effect failed to be observed. We then attempted to determine whether the effect was due to a difference in surgical or testing procedures. Since it was due to testing, we then atempted to determine if the loss of antinociception was due to pre- or post shock testing procedures. It was determined that it was probably due to an unknown sensory cue present in the post shock procedures.

Failure to Observe Spinally Mediated Antinociception

A vast amount of research has shown that multiple pain control mechanisms exist within the body. These endogenous systems can be activated by a variety of aversive stimuli, including electric shock (Akil, Madden, Patrick, & Barchas, 1976; Watkins, Cobelli, & Mayer, 1982), cold water (Bodnar, Kelly, Brutus, & Glusman, 1980), and chemical burns (Davis, Meyer, Turnquist, Pappagallo, Filloon, & Campbell, 1992). It has been repeatedly observed that exposure to an aversive stimulus can produce a profound decrease in pain reactivity, or hypoalgesia (e.g., Akil, et al., 1976; Grau, Hyson, Maier, Madden, & Barchas, 1981; Meagher, Grau, & King, 1990). For example, exposure to electric shock has been shown to suppress spinally mediated withdrawl reflexes to a heat stimulus (Grau, et al., 1981).

It has been shown that this hypoalgesia is at least partially due to the suppression of pain signals at the level of the spinal cord (Basbaum & Fields, 1984). Prior research has tended to focus on how neural pathways, originating in the brainstem and descending through the dorsal lateral funiculus, control the activation of these spinal hypoalgesic systems (Basbaum, Marley, O'Keefe, & Clanton, 1977). It has been repeatedly observed, however, that transecting the spinal cord ("spinalization") does not always eliminate the hypoalgesia observed after the presentation of an aversive stimulus (Watkins, Cobelli, & Mayer, 1982; Meagher, 1990). Thus, it appears that the spinal cord can directly activate its own endogenous

hypoalgesic systems, rather than requiring activation by descending brainstem systems. For example, Meagher (1990) found that spinalization eliminated the hypoalgesia produced by a series of three mild (0.75-sec, 3.0 mA) to moderate (25-sec, 1.0 mA) shocks, but did not eliminate the hypoalgesia produced by a series of three severe (25-sec, 3.0 mA) shocks. In this case, the severity of the noxious stimulus determined whether hypoalgesic systems in the spinal cord could be directly activated, rather than requiring activation by the brainstem.

Much of the research on intrinsic pain control systems has focused on the neurochemical mechanisms which are involved in decreasing pain reactivity. In the 1970's, researchers discovered endogenous opioids (Hughes, Smith, Kosterlitz, Fothergill, Morgan, & Morris, 1975), peptides manufactured by the body that can bond with receptors on nociceptive neurons, which are nerve cells that transmit pain signals. Bonding with these receptors leads, through cAMP second messenger systems, to changes in the cell membrane's permeability to ions, such as potassium and calcium, resulting in decreased ability for the neuron to release neurotransmitter. The transmission of pain signals is, in turn, inhibited.

In behavioral research on pain control systems, the terms opioid and non-opioid are often used to describe and explain different types of hypoalgasia. An opioid hypoalgesia is one that can be blocked by opioid antagonist drugs, such as naloxone (e.g., Akil, et al., 1976) and that exhibits a cross tolerance to the narcotic morphine (Mayer &

Hayes, 1975). A non-opioid hypoalgesia displays neither of these characteristics, and may involve other neurochemical mechanisms.

It has been shown in previous work that both opioid and non-opioid systems may be involved in directly activated spinal antinociception (a more specific term referring to a diminished pain reactivity in response to a stimulus that can potentially damage tissue). Meagher, Chen, Salinas, & Grau (1993) administered naltrexone, an opioid antagonist drug, to spinalized rats, which then received a series of three tailshocks. It was found that naltrexone blocked the antinociception produced by mild (2-sec, 3.0 mA) shock but not by severe shock (75-sec, 3.0 mA). One interpretation of these results is that an opioid system is involved in the antinociception produced by mild shock, while a non-opioid system is involved in the antinociception produced by severe shock.

One problem with this interpretation is that only a single dose of naltrexone was used . This dose may not have been sufficient to block the antinociception produced by a severe shock schedule if the shock caused the release of a high concentration of opioids. Thus, the antinociception produced by severe shock may have actually been opioid in nature. This was the basic issue that this project was intended to address. By administering naltrexone across a range of doses, we could have determined whether higher doses would block the antinociception produced by severe shock.

The first experiment involved administering a severe tailshock to spinalized rats in order to replicate the basic effect of spinal

antinociception. Surprisingly, this typically reliable effect was not observed. The next experiment tested whether antinociception failed to be observed due to differences in surgical technique or in behavioral testing procedures. The third experiment was designed to determine if antinociception was attenuated due to sensory cue present during either the preparation phase or the post-shock phase of behavioral testing.

Method

Subjects

The subjects were 76 male Sprague-Dawley rats obtained from Harlan (Houston). The rats weighed between 350-400 grams. They were maintained on a 12:12-hr light/dark cycle. The subjects were individually housed and maintained on ad libitum food and water.

Apparatus

Pain reactivity was measured using the "tail-flick" test, the most common measure of pain reactivity. This test measures the latency at which a rat withdraws its tail away from a radiant heat source. The radiant heat source was a 375-Watt movie light bulb (type EBR) that was positioned 18 cm above the platform on which the tail rested during testing. A condenser lens was located 8 cm below the light bulb to focus the radiant heat on the rat's tail. Each trial was terminated by a lateral movement of the tail (0.5 cm minimum) which was detected by a photocell over which the tail had been positioned. In the event that the rat did not flick its tail within 8 seconds after the tail flick apparatus had been turned on, the trial was terminated manually in order to prevent tissue damage. Tail-

flick latencies were automatically recorded to the nearest 0.01 second by a timer.

During behavioral testing, the rats were restrained in Plexiglass tubes. To minimize the possibility of visual stimuli altering the subjects' behavior, the tubes were wrapped in duct tape. Ventilation holes were drilled in the tops of the tubes. A band of adhesive tape, stretched across the rear of the tube, kept the rats confined while allowing the tail to move freely.

Tailshock was provided by a 660-V transformer that produced a constant current 3.0-mA shock. The electrode was lightly coated with electrode paste and was taped to the rat's tail approximately 15 cm behind the rear of the tube.

All testing was conducted in the same room. A space heater maintained the room temperature at approximately 26.5 degrees Celsius and provided a background noise level of about 60 dB.

Surgery

Surgery was conducted while the rats were anesthetized with thiopental. To stabilize and position the rat's body for surgery, its head was held in a stereotaxic instrument and a small "pillow" was placed under its chest. After locating T2 tactilely, a 3 cm midline incision was made. The muscle tissue around the spinal column was then separated and drawn apart by a retractor, thereby exposing the column. The transection was performed immediately caudal to T2 using a heat cautery unit to minimize blood loss. Transections were confirmed by: (a) visually and tactilely inspecting the cord during surgery; and (b) observing the behavior of the subjects after they had regained consciousness to ensure that they exhibited paralysis

below the level of the forepaws and did not vocalize to the tailshock used as a nociceptive stimulus. Immediately following behavioral testing, the rats were euthanized using an overdose of pentobarbital anesthesia.

Experiment 1: The Effect of Long Shocks on Pain Reactivity

In this experiment, I attempted to replicate the spinally activated antinociceptive effect of the long shock (75-sec, 3.0 mA) parameter previously employed by Meagher, et al., (1993). This was done in order to assure that spinal antinocoception would be reliably elicited before attempting to block the effect with naltrexone.

Method

Subjects. The subjects were 12 rats, as described in the general method.

Apparatus. The apparatus was the same as described in the general method.

Procedure. Eight to 10 hours after the rats had been spinalized, they were placed in the restraining tubes and allowed to acclimate for 15 min. All of the subjects then received three tail-flicks at 2-min intervals to determine baseline levels of pain reactivity. The last two tail-flicks were averaged to provide a measure of the rats' baseline tail-flick latency. Immediately after baseline testing, the shock electrodes were attached to the rats' tails with adhesive tape.

The experiment involved a single factor (shock condition) design with repeated measures. Six of the subjects received a series of three 75-sec, 3.0 mA tailshocks. The remaining six subjects served as unshocked controls and were restrained for the same duration of

time as the shocked rats. Following the period of shock or restraint, the shock electrodes were removed and five tail-flicks were administered at 2-min intervals.

Results

The results are depicted in Figure 1. The baseline levels of pain reactivity are shown on the left side of the panel. An ANOVA indicated that tail-flick latencies did not differ between between groups prior to shock treatment, $F = .06$, $p > .05$.

The mean tail-flick latencies observed after shock are shown to the right of the baseline data in the panel. It is apparent that the 75-sec shock condition elicited a weak antinociceptive effect, compared to the unshocked condition. The between subjects term of an ANOVA confirmed a significant main effect of shock condition, $F(1, 10) = 6.29$, $p < .05$. The within subjects term of the ANOVA showed that neither the trials effect, nor its interaction with shock condition approached statistical significance, all F's < 1.15, $p > .05$.

Discussion

Although a significant spinal antinociception was observed after the presentation of shock, the effect was very weak compared to the results of previous research. Importantly, this weak effect was observed after the presentation of a severe shock schedule. Because this was a cause for concern, a second experiment was designed to determine if methodological differences in surgical or testing procedures were involved in attenuating the antinociception.

Experiment 2: Are Surgical or Testing Procedures Involved in the Attenuation of Spinal Analgesia?

Because the presentation of electric shock failed to elicit the typically robust and reliable phenomenon of spinal antinociception, the purpose of the next experiment was to begin to determine which variable or variables were causing the failure to observe this behavior. More specifically, it was thought that a difference in the experimenter's surgical technique or testing procedures might have prevented antinociception from being elicited. In Experiment 2, the surgical techniques and testing procedures of two different experimenters were compared to determine their impact on spinal antinociception.

Method

Subjects. The subjects were 32 rats, as described above.

Apparatus. The apparatus was the same as described above.

Procedure. The spinalization surgery was the same as described above. However, half of the rats were spinalized by Thomas Prentice, while the other half were spinalized by Dr. James Grau. Approximately 24 hours after surgery, subjects underwent behavioral testing. Half the rats were tested by Prentice and the other half were tested by Dr. Grau. The behavioral testing procedures were the same as previously described. Half of the rats in each condition received a series of three 2-sec, 3.0 mA shocks, while the other half served as unshocked controls and were restrained for the same length of time. The complete experiment involved a 2 (surgeon) x 2 (tester) x 2 (shock condition) factorial design.

Results

The results are depicted in Figure 2. The baseline levels of pain reactivity are depicted on the left side of each panel. An ANOVA indicated a main effect of surgeon on baseline levels of pain reactivity, $F(1, 24) = 4.68$, $p < .05$. No other effects were observed.

The mean post-shock tail-flick latencies are shown to the right of the baseline data in each panel. It is apparent that shocked groups tested by Dr. Grau showed a strong antinociceptive response compared to all other groups. Furthermore, this effect was present regardless of which surgeon performed the spinalization. Shock did not, however, elicit antinociception in groups prepared by Prentice, regardless of which experimenter performed the surgery.

The between subjects terms of an ANOVA confirmed a significant main effect of shock condition, $F(1,24) = 37.58$, $p < .001$, and a marginally significant main effect of tester, $F(1,24) = 4.004$, $p > .05$. More importantly, the interaction between tester and shock condition was significant, $F(1, 24) = 6.82$, $p < .05$. Neither the main effect of surgeon, nor its interaction with shock or tester approached significance, all F's $< .909$, $p > .05$. The interaction between surgeon, tester, and shock was also non-significant, $F(1, 24) = .89$, $p > .05$.

The within subjects terms showed that the trials effect, $F(4, 96) = 4.93$, $p < .005$, as well as its interaction with tester , $F(4, 96) = 3.01$, $p < .05$, were significant. None of the other within subjects terms approached statistical significance, all F's < 1.59, $p < .05$.

Discussion

This experiment showed that the absence of spinal antinociception following the presentation of shock was determined by the person who tested the subjects, rather than the person who performed the surgeries. Thus, the effect is apparantly not sensitive to slight variations in surgical technique. Spinal antinociception was, however, attenuated by an unknown variable or variables present when Prentice tested the rats.

Experiment 3: The Effect of the Experimenter on Spinal Antinociception During Behavioral Testing

Because the previous experiment showed that the absence of spinal antinociception was due to variables present when the subjects were tested, the next step was to attempt to determine at which point during testing these variables had their effect. The behavioral testing procedure was broken down into a preparatory phase (pre-shock) and a testing (post-shock) phase. The purpose of this experiment was to discern whether the unknown variable was present during preparation, during testing, or both.

Method

Subjects The subjects were 32 rats, as described above.

Apparatus The apparatus was the same as described above.

Procedure In this experiment, all of the spinalizations were performed by Prentice, since Experiment 2 showed that antinociception was not affected by the person performing surgery. Approximately 24 hours after spinalization, the rats were tested using the tail-flick procedure discussed earlier. Half of the rats in the experiment received a series of three 2-sec, 3.0 mA tail-shocks while the other half served as unshocked controls.

The preparation phase of the experiment involved: a) placing the rats in the tubes and allowing them to acclimate; b) taking baseline tail-flicks; c) coating the shock electrodes with electrode paste; d) taping the tails to the electrodes; and e) beginning the administration of shock or restraint. Half of the rats were prepared by Dr. Grau while the other half were prepared by Prentice.

Immediately after the administration of the shock condition, the testing phase of the experiment began. The testing phase of the experiment involved: a) untaping the rats tails from the electrodes; and b) administering post-shock tail-flicks. The complete experiment involved a 2 (preparation) x 2 (testing) x 2 (shock condition) design with repeated measures.

Results

The results are depicted in Figure 3. The baseline levels of pain reactivity are depicted on the left side of each panel. An ANOVA indicated that tail-flick latencies did not differ between groups prior to all shock treatments, all $F's < 2.56$, $p > .05$.

The mean tail-flick latencies observed after shock are shown to the right of the baseline data in each panel. It is apparent that shocked groups tested by Dr. Grau showed a strong antinociceptive

response compared to all other groups. Furthermore, this effect was present regardless of which experimenter prepared the rats for testing. In contrast, shock did not elicit antinociception in groups tested by Prentice, regardless of which experiimenter prepared the rats for testing.

The between subjects terms of an ANOVA confirmed a significant main effect of tester, $F(1, 24) = 19.19$, $p < .001$, and a significant main effect of shock, $F(1, 24) = 40.89$, $p < .001$. Most important, the interaction between shock and tester was significant, $F(1, 24)$, $p < .001$. Neither the main effect of preparation, nor its interaction with shock or tester approached statistical significance, all F's $< .596$, $p > .05$. Furthermore, the interaction between preparation, tester, and shock was nonsignificant, $F(1, 24) = .847$, $p > .05$.

The within subjects terms showed that both the trials effect, $F(4,96) = 7.95$, $p < .001$, and its interaction with tester, $F(4,96) = 3.25$, $p < .05$, were significant. The three-way interaction between test trial, tester, and shock condition, $F(4,96) = 3.30$, $p < .05$ was also significant. None of the other within subjects terms approached statistical significance, all F's $< .867$, $p > .05$.

Post hoc comparisons with Duncan's multiple range test confirmed that shocked groups tested by Dr. Grau exhibited a significant antinociception relative to all other groups. No other differences were statistically significant.

Discussion

This experiment showed that the attenuation of spinal antinociception did not depend on which experimenter prepared the subjects for testing. The absence of the effect did, however, depend

on which person tested the rats, and apparently entered the experiment after shock had been presented. Importantly, this indicates that the phenomenon is not highly sensitive to slight differences in preparing rats for testing, such as different styles of taping the tails to the electrodes.

General Discussion

In this research, I attempted to elicit the phenomenon of spinally activated antinociception using tailshock as a nociceptive stimulus. This effect had been reliably observed in previous work. Antinociception did not occur, however, and it was not clear what variable could be causing the phenomenon to not appear. Two attempts to experimentally determine when this variable was entering into the research were then undertaken. The first of these experiments showed that the absence of spinal antinociception depended on which experimenter carried out the behavioral testing, and did not depend on which person performed the spinalization. In the next experiment, it was determined that the unknown variable had its effect after shock had been presented and did not result from preparing the rats for testing.

It should be emphasized that these non-typical results could not stem from any possible differences in the rats which were used in these experiments. This is apparent because subjects which were tested by Dr.Grau exhibited displayed the same pattern of results as subjects in previous work. Thus, the failure for Prentice to observe antinociception in shocked groups must result from a cue in the rats' environment which has its effect through one or more of the sensory modalities.

Three of the sensory modalities can probably be eliminated from consideration. First, it is unlikely that a visual stimulus could be involved because during testing the rats are restrained in tubes which they cannot see through. Second, a taste cue is unlikely because there should be nothing different for the rats to taste when Prentice tests than when Grau tests. Finally, an auditory stimulus is probably not involved because the noise level of the room is the same regardless of who tests the rats.

It is more probable that an olfactory or tactile cue caused the attenuation of spinal antinociception. An olfactory cue is possible because the rats are not isolated from their olfactory environment while they are in the testing tubes. It is also likely that their olfactory environment is not constant when two different experimenters test the rats. It is also possible that a tactile cue could have caused Prentice-tested rats to fail to exhibit spinal antinociception. A tactile stimulus could only have been presented while untaping the rats' tails from the shock electrodes or while placing the tail on the tail-flick apparatus, since the subjects are not touched at any other time during testing.

Experimentally, one could determine if an olfactory or tactile stimulus was involved by testing whether Prentice's mere presence in the testing room could attenuate antinociception while another experimenter conducted behavioral testing. If antinociception was attenuated, then this would indicate an apparent olfactory cue, since Prentice would have never handled the rats. However, if antinociception was not attenuated, this would seem to indicate that a tactile cue caused Prentice to fail to observe spinal

antinociception. Another manipulation could then be undertaken to determine if the tactile cue occurred while untaping the rats' tails from the electrodes or while placing the tails on the tail-flick apparatus.

Another way to approach the issue of determining why antinociception was attenuated would be to test the effect of morphine on pain reactivity in Prentice-tested rats. This experiment could be conducted in non-spinalized subjects so that we might determine if the sensory cue has its effect on an intact central nervous system. In addition to possibly answering this question, there are several practical advantages to using this method.

One advantage is that this method is methodologically simple. Since the experiment could be carried out in intact subjects, surgery, as well as tailshock, would not be necessary. There would be no need to shock the tails to stimulate the release of endogenous opioids because an exogenous opioid, morphine, would serve in their place. Since it would be unnecessary to use tailshock in this experiment, it would also be unnecessary to tape the tails to electrodes, thus eliminating the possibility of a tactile cue while unfastening the tails. The rats would simply undergo baseline tail-flick testing, followed by a subcutaneous injection of morphine, then tested for post-injection pain reactivity.

A third advantage of testing the effect of morphine is that we might determine part of the physiological mechanism that is causing the attenuation of antinociception, regardless of whether the sensory cue is olfactory or tactile. For example, if morphine failed to produce antinociception, this would seem to indicate that the

stimulus triggers a mechanism that has its effect after opioids are already present in the system, such as an antagonist or a metabolizing agent. However, the results of this experiment could not be entirely conclusive, since the mechanism might attenuate a shock-induced antinociception, but not a morphine-induced antinociception.

Although these experiments have shown that the phenomenon of spinal antinociception is more sensitive to variations in the testing environment than it was once thought, it is not yet known what environmental cue or cues are responsible for attenuating the effect. Perhaps even more complex is the issue of the physiological mechanism that may be involved in this phenomenon. The answers to these questions are not yet apparent at this time, and can only be addressed with more research.

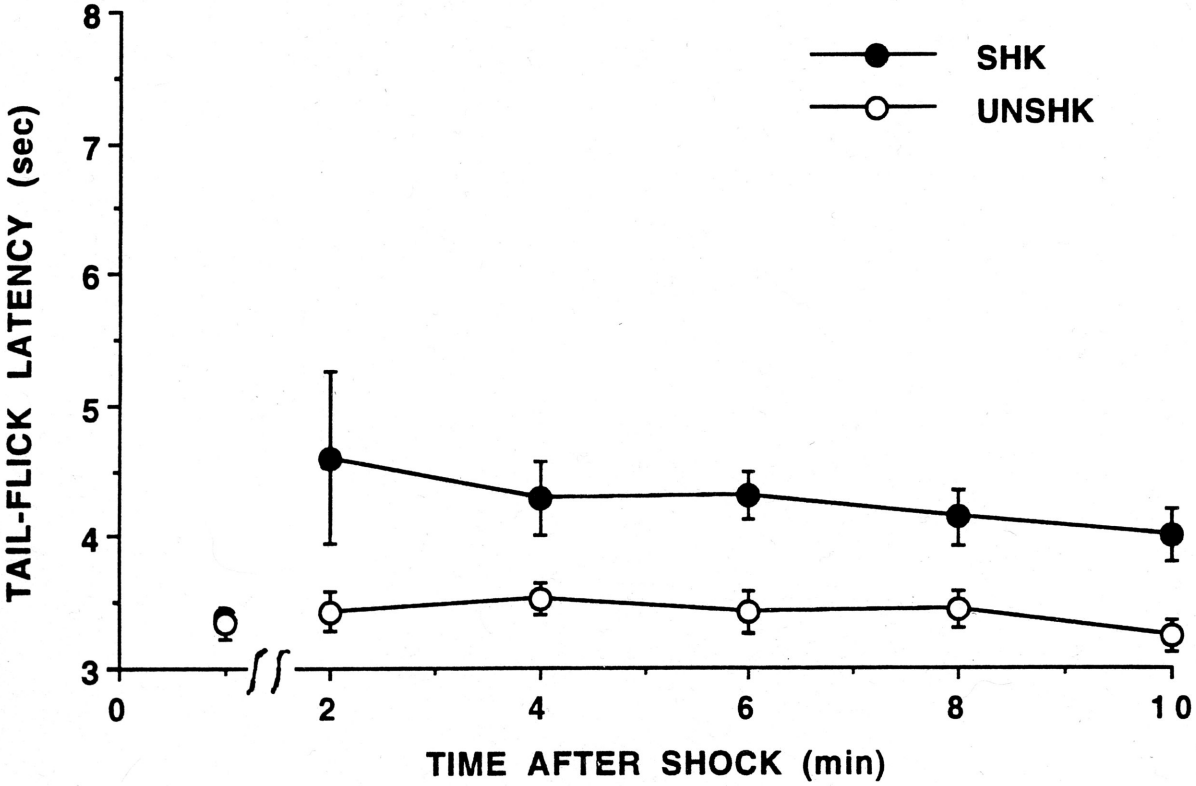
SHK
UNSHK
TAIL-FLICK LATENCY (sec)
8
7
6
5
4
3
0
2
4
6
8
10
TIME AFTER SHOCK (min)

Figure 1

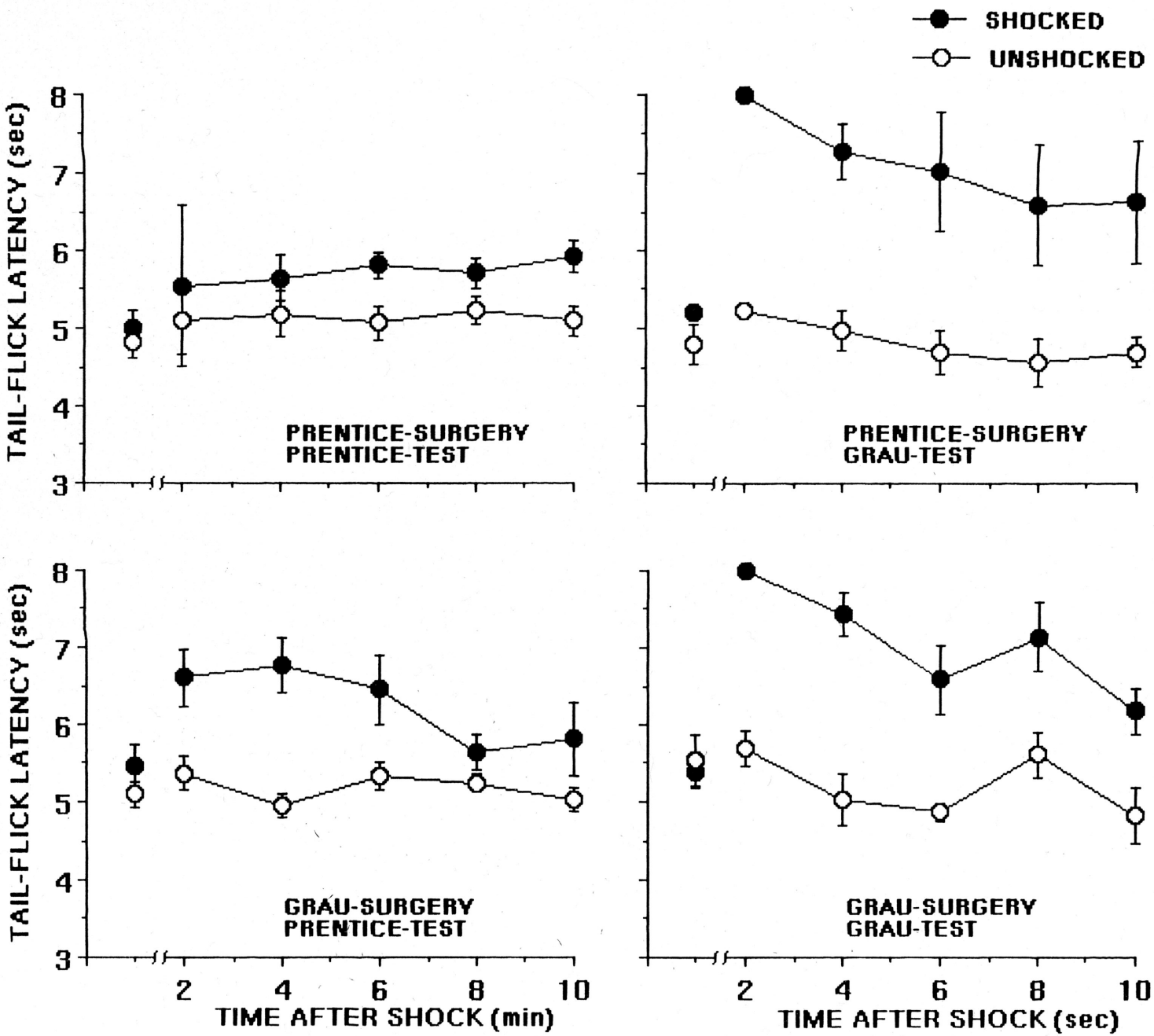
SHOCKED
UNSHOCKED
TAIL-FLICK LATENCY (sec)
8
7
6
5
4
3
PRENTICE-SURGERY
PRENTICE-TEST
PRENTICE-SURGERY
GRAU-TEST
GRAU-SURGERY
PRENTICE-TEST
GRAU-SURGERY
GRAU-TEST
2
4
6
8
10
TIME AFTER SHOCK (min)
TIME AFTER SHOCK (sec)

Figure 2

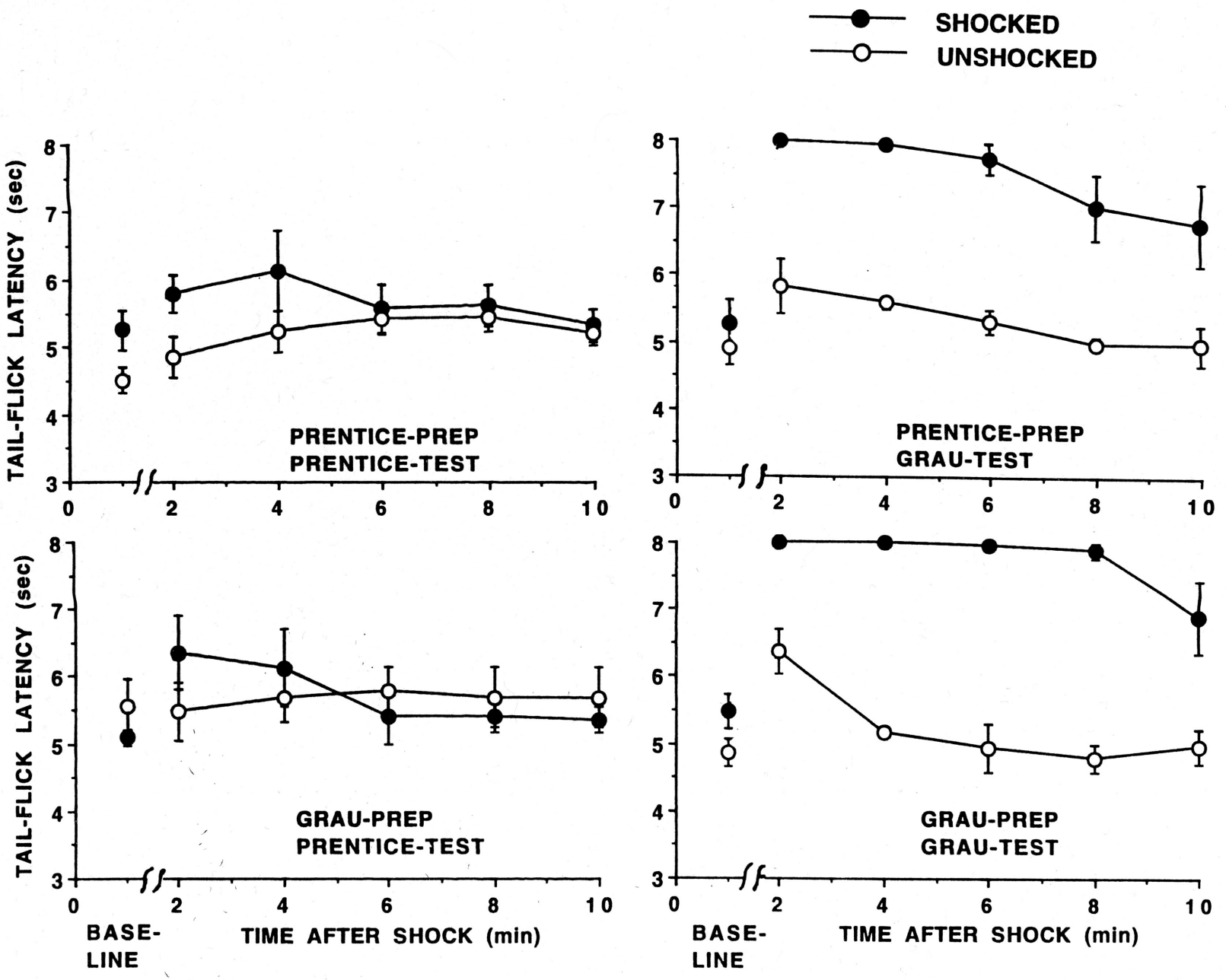
SHOCKED
UNSHOCKED
TAIL-FLICK LATENCY (sec)
PRENTICE-PREP
PRENTICE-TEST
PRENTICE-PREP
GRAU-TEST
GRAU-PREP
PRENTICE-TEST
GRAU-PREP
GRAU-TEST
BASE-
LINE
TIME AFTER SHOCK (min)
BASE-
LINE
TIME AFTER SHOCK (min)

Figure 3

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