NEUROBIOLOGY OF LEARNING AND MEMORY **67,** 64–68 (1997)
ARTICLE NO. NL963736

BRIEF REPORT

Evidence for Spinal Conditioning in Intact Rats

ROBIN L. JOYNES, PAUL A. ILLICH, AND JAMES W. GRAU[1]

Department of Psychology, Texas A&M University, College Station, Texas 77843

Prior work suggests that spinal systems are sensitive to the stimulus relationships that underlie Pavlovian conditioning. We studied this phenomenon in Sprague–Dawley rats by pairing a vibrotactile conditioned stimulus (CS) with a tailshock unconditioned stimulus (US). Experiment 1 showed that spinal rats exhibit differential conditioning, having longer tail-flick latencies on the tail-flick test during a CS that was paired with the US (conditioned antinociception). Experiment 2 showed that rats trained with the cord intact still exhibit differential conditioning after the cord is cut. This suggests that spinal learning contributes to behavioral plasticity in intact subjects. © 1997 Academic Press

Organisms learn about their world by encoding the spatial and temporal relationships that exist between environmental stimuli. This type of learning can be studied in animals using the procedure of Pavlov, in which a stimulus (the conditioned stimulus, or CS) is paired with a biologically meaningful event (the unconditioned stimulus, or US). As a result of this experience, the paired CS (the CS^+) elicits a response not observed to a CS presented in an explicitly unpaired fashion (the CS^-). Pavlov believed that this learning depended on the cerebral cortex (Pavlov, 1927). However, subsequent studies have demonstrated that lower level neural systems in the brainstem (Bromily, 1948; Norman, Buchwald, & Villablanca, 1977) and the spinal cord (Beggs, Steinmetz, & Patterson, 1985; Durkovic, 1975; Fitzgerald & Thompson, 1967; Grau, Salinas, Illich, & Meagher, 1990; Patterson, Cegavske, & Thompson, 1973) are sensitive to environmental relationships. Spinal learning is observed in a variety of species (Durkovic, 1975; Grau et al., 1990; Ince, Brucker, & Alba, 1978; Shurrager & Culler, 1940) and exhibits many of the basic characteristics identified by Pavlov [e.g., extinction, differential conditioning, overshadowing (Beggs et al., 1985; Grau et al., 1990; Illich, Salinas, & Grau, 1994)].

Research on spinal learning has been motivated by two basic issues. First, neuroscientists would like to develop model systems to study the biological mechanisms that underlie learning in vertebrates. Given that a great deal is known about spinal architecture (Price, 1988) and that spinal neurons can exhibit some interesting forms of plasticity (Coderre, Katz, Vaccarino, Melzack, 1993; Leahy & Durkovic, 1991; Wolpaw & Carp, 1990; Woolf & Thompson, 1991), spinal learning may provide an attractive model paradigm. Second, the study of spinal learning should help to elucidate the relationship between learning and neural complexity. Presumably, the forebrain enriches basic learning mechanisms in some important ways. A comparison of the rules that govern learning at different levels of the nervous system should facilitate the identification of properties that are unique to higher level systems.

To demonstrate spinal conditioning, researchers have routinely transected the cord prior to training. When learning is observed, it can be concluded that all of the mechanisms needed to encode the CS–US relation must exist within the cord. However, isolating the cord in this fashion may do more than prevent supraspinal mediation; it may effectively release spinal systems from tonic inhibition, allowing them to exhibit forms of plasticity that would not normally occur in intact subjects.

[1] This research was supported by a grant from the National Institute of Mental Health (MH48994) to J. W. Grau and M. W. Meagher. The authors thank Tamara King for her help and advice. Reprint requests and correspondence concerning this article should be addressed to Robin L. Joynes, Department of Psychology, Texas A&M University, College Station, TX 77843. E-mail: RLJ050A@ACS.TAMU.EDU.

1074-7427/97 $25.00
Copyright © 1997 by Academic Press
All rights of reproduction in any form reserved.

The present experiments address this issue by assessing whether spinal mechanisms encode the relationship between a cutaneous CS and US when the cord is intact. To do this, we describe a conditioning procedure that can be used to establish conditioned antinociception in spinal rats as well as in intact subjects. We then test whether the learning established with this paradigm survives a spinal transection.

In prior studies we routinely used a 1-mA hindleg shock as our CS (Grau et al., 1990; Illich et al., 1994). Although this stimulus elicits little movement or antinociception in spinal rats, it generates a great deal of struggling and a robust antinociception in intact subjects (Meagher, Grau, & King, 1990) and, as a consequence, could not be used. As an alternative we considered using vibrotactile stimuli. However, it was unclear whether these stimuli could support spinal learning. Given this, Experiment 1 was designed to test whether vibrotactile stimuli can support differential conditioning in spinal rats.

For Experiment 1 the subjects were 12 male Sprague–Dawley rats (400–480 g) obtained from Harlan (Houston, TX). Animals were individually housed and maintained on ad libitum food and water. A 12:12 hr light:dark cycle was maintained and subjects were tested during the second half of the light cycle.

Rats were anesthetized with 50 mg/kg pentobarbital (ip). 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. The cord was transected at T2 as follows: (a) after T2 was localized tactilely, an anterior–posterior incision was made; (b) the muscle tissue was cleared, and tissue retractors were used to expose the vertebral column; (c) a small rongeur was used to remove the T1 segment of bone; and (d) the exposed cord was transected by cauterization. The exposed spinal cord was covered with Oxycel (Parke-Davis), and the wound was closed with Michel clips. All subjects received ip saline (9%) to prevent dehydration during recovery. The rats were placed in a temperature-controlled environment (approximately 26.5°C) during recovery and their bladders were expressed manually as needed (approximately three times per 24-h period). All rats were trained/tested 18–24 h after surgery and were euthanized at the end of the test period with a lethal dose of pentobarbital (ip). Transections were confirmed by (1) inspecting the cord during the operations and (2) observing the behavior of the subjects after they recovered from the anesthesia to ensure that they exhibited paralysis below the level of the forepaws. In addition, the spinal cord was examined postmortum in randomly selected subjects to confirm that the transections were complete.

Subjects were loosely restrained in Plexiglas tubes (22 cm in length and 6.8 cm i.d.) during training and testing (Meagher, Chen, Salinas, & Grau, 1993). Vibrotactile stimulation was generated by passing a pulsating current (20 Hz) through miniature solenoids (Guardian, TP4X7) attached to the plantar surface of each hindpaw. These solenoids were capable of exerting a maximum force of 2.22 to 3.33 N. They were attached to a 2×4.5 cm aluminum plate that was taped to the base of one hindpaw in such a way that the plunger (1.2 mm in diameter) contacted the center portion of the paw. Extending from the side of this plate was a 2.5-cm-wide, 5.8-cm-long section of aluminum that was folded around the base of the solenoid to form a "U". The base of this "U" laid about 3 mm below the plunger and served to limit its range of movement and maintain it within its sleeve. A BRS/LVE shock generator was used to generate a constant current tailshock that served as the US. The shock was delivered through electrodes that were constructed from a modified fuse clip and coated with electrode paste. Nociceptive thresholds were tested with a radiant heat tail-flick device (for further details see Meagher et al., 1990).

After surgery, subjects were placed in the tubes and the vibrotactile devices were attached to the ventral surface of each hindpaw. The tail electrodes were taped to the rat's tail approximately 15 cm behind the rear of the tubes. The subjects then received differential conditioning during which vibrotactile stimulation to one hindleg (the CS^+) was paired with the tailshock US (2 s, 6 mA) while stimulation of the other paw (the CS^-) occurred during the intertrial interval. Whether left or right paw stimulation served as the CS^+ was counterbalanced across subjects. During training, the CS^+ and CS^- were presented 30 times each in a random order on a variable time schedule (mean interval between individual stimuli = 1 min; max = 90 s, min = 30 s; total training time = 1 h). The vibrotactile stimuli were presented for 10 s and the US occurred during the last 2 s of the CS^+. At the end of training, subjects were removed from the restraining tubes and returned to their home cages. A 1-h break was given to all subjects between training and testing. During testing, subjects were again placed in the tubes and the vibrotactile stimuli were reattached to their paws. Baseline nociceptive thresholds (latency to withdraw the tail from a radiant heat source) were then assessed three times at 2-min intervals with the tail-flick device. Tail-flick latencies during the CS^+ and

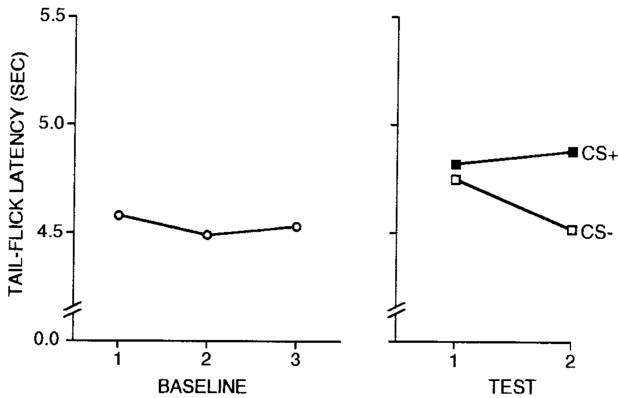

FIG. 1. The mean tail-flick latencies observed across the three baseline test trials (leftmost panel), during the CS^+ (rightmost panel, filled symbols), and during the CS^- (open symbols) in spinalized rats.

CS^- were then measured two times each in an ABBA order at 2-min intervals. Whether the CS^+ or CS^- was presented first was counterbalanced across subjects. The tail-flick test was initiated approximately 2 s after the onset of the vibrotactile stimulus. If a tail-flick response was not observed after a 6-s exposure to radiant heat, the trial was terminated to prevent tissue damage.

Baseline scores are depicted in the left panel of Fig. 1. An analysis of variance (ANOVA) revealed that baseline tail-flick latencies did not differ across trials, $F(2, 22) < 1.0$, $p > .05$. When tail-flick latencies were then tested during the CSs, longer latencies were observed during the CS^+, $F(1, 11) = 6.55$, $p < .05$. This effect accounted for 37% of the variance (η^2). Inspection of the data from individual subjects showed that only 1 of the 12 subjects exhibited longer latencies during the CS^-.

Experiment 2 looked at whether subjects trained with the cord intact would exhibit a CS^+/CS^- difference after the cord was transected. Twenty-four subjects received differential conditioning, using vibrotactile CSs and a tailshock US (2 s, 0.5 mA). The vibrotactile CS elicited leg movement, but few other signs of discomfort. The tailshock US elicited vigorous movement and some vocalization. Half of the subjects then received a spinal transection while the remaining subjects underwent a sham operation in which the cord was exposed but not transected. Twenty-four hours after training, tail-flick latencies were assessed during the CSs as described above.

Baseline tail-flick latencies are illustrated in the left panels of Fig. 2. Initially, sham-operated rats exhibited much longer tail-flick scores. This presumably reflects the induction of antinociception from reexposure to the shock context, an effect that appears to extinguish over the course of testing. As a consequence, similar tail-flick latencies were observed across the two groups on the last baseline test. An ANOVA confirmed that the groups differed overall, $F(1, 22) = 31.50$, $p < .001$, and that the magnitude of this difference varied across trials, $F(2, 44) = 9.84$, $p < .01$. The main effect of trials was also significant, $F(2, 44) = 7.07$, $p < .01$.

Tail-flick latencies during the CSs are depicted to the right of the baseline scores. As expected, sham-operated rats exhibited longer latencies during the CS^+. This difference grew slightly as testing was continued. On the first test trial, spinal rats exhibited a similar CS^+/CS^- difference, but it weakened over test trials. An ANOVA revealed a main effect of CS type, $F(1, 22) = 9.66$, $p < .01$. Neither the main effect of operation (sham/spinal) nor its interaction with CS type were significant, both Fs < 1.0, $p > .05$. An analysis of the trials effect revealed that the magnitude of the CS^+/CS^- difference depended upon both test trial and operation. None of the other trials effects were significant, all Fs $(1, 22) < 3.95$, $p > .05$.

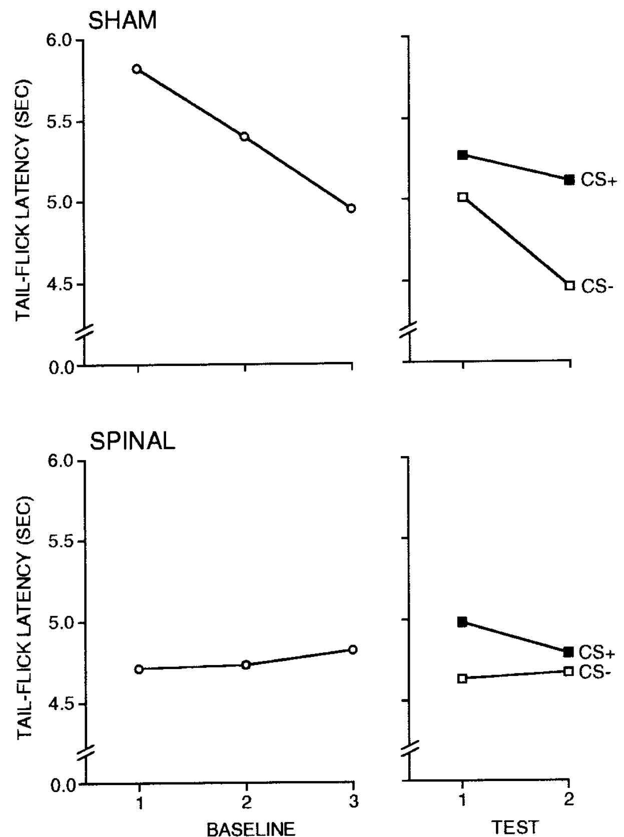

FIG. 2. The mean tail-flick latencies observed across the three baseline test trials (leftmost panels), during the CS^+ (rightmost panels, filled symbols), and during the CS^- (open symbols) in sham-operated (top panels) and spinalized (bottom panels) rats.

To further verify that the CS^+/CS^- difference emerged in both sham and spinalized rats, we conducted a separate means analysis for each condition. A separate ANOVA for sham-operated subjects verified that the CS^+/CS^- difference was significant, $F(1, 11) = 4.93$, $p < .05$. Comparing the latencies during the CS^+ and CS^- for each spinalized subject revealed that only 1 of the 12 subjects exhibited longer tail-flick latencies during the CS^-. A separate ANOVA confirmed that this CS^+/CS^- difference was significant, $F(1, 11) = 7.35$, $p < .05$. This difference accounted for 40% of the variance.

Experiment 1 showed that conditioned antinociception can be obtained in spinalized rats using vibrotactile CSs, rather than the shock CSs routinely used in prior studies. It is tempting to suggest that these results also demonstrate that *neutral* stimuli can support conditioning at the level of the spinal cord. At issue is whether the CSs produce a CR-like response, either before conditioning or after exposure to the US alone. If they do, which is clearly the case for the shock CSs used in earlier studies (Joynes & Grau, in press), the learning observed may be attributed to "alpha conditioning" rather than "true associative learning" (Kimble, 1961). Because our vibrotactile CSs are presumably less noxious, the fact that they support conditioning might be taken as evidence that neutral CSs can produce true associative learning at the level of the spinal cord. However, we are reluctant to make any strong claims based on this evidence, for it is possible that the vibrotactile stimuli per se do have some, albeit small, impact on spinal antinociceptive systems. Moreover, when placed against a finger, human observers report that the stimuli are less aversive than 1-mA shocks, but are nonetheless highly "annoying," a quality that might be enhanced by their repeated presentation or exposure to the US. Given this, it seems safer to assume that differential conditioning in spinal rats may require CSs that have some capacity to generate a CR-like response prior to training. Indeed, other observations suggest that this may be a necessary condition (Joynes & Grau, in press). In these studies, we explored three ways in which instituting a CS–US relationship can influence reactivity to a CS: protection from habituation, pairing-specific enhanced sensitization, and associative learning. Our results suggest that conditioned antinociception in spinalized rats reflects the simplest mechanism, protection from habituation. For this mechanism to generate a CS^+/CS^- difference, an untrained CS must have some capacity to produce a CR-like response (for a detailed discussion of this issue see Joynes & Grau, in press).

Experiment 2 revealed a number of interesting effects. First, sham-operated, but not spinalized rats, exhibited longer tail-flick latencies at the start of testing. This effect, which appeared to extinguish over the course of the baseline trials, most likely reflects the induction of antinociception from reexposure to the shock context (Chance, 1980; Fanselow & Baackes, 1982). Evidence suggests that this form of learning requires integration of noncutaneous sensory cues and relies on neural systems in the brain (Phillips & LeDoux, 1992). Transecting the cord prior to testing would prevent these brain systems from influencing tail-flick latencies and hence, eliminate the context-induced change in baseline latencies.

When the cutaneous CSs were presented during the testing session, 1 h after training, sham-operated rats exhibited longer tail-flick latencies during the CS^+. Subjects that were spinalized prior to testing also exhibited a significant conditioned antinociception. However, the antinociception appeared somewhat weaker. Perhaps context conditioning amplifies the impact of the CS^+ in intact rats. In addition, the CS–US relationship is surely encoded by neural systems in the brain as well, and this could enhance the antinociception elicited by the CS^+.

What is important about the present results is not that supraspinal systems amplify the CR, but rather, that any learning survives at all (also see Wolpaw & Carp, 1990). This suggests that spinal learning is not limited to the reduced, transected, preparations used in past studies—it also occurs in intact subjects. Interestingly, this learning was demonstrated using a milder US (a 0.5-mA 2-s tailshock) than that (a 3- to 6-mA 2-s tailshock) used in prior studies (Grau et al., 1990; Illich et al., 1994). We used a milder US because more severe tailshocks can have debilitating effects on intact rats (Campbell & Masterson, 1969) and because we sought to minimize the subject's pain and suffering. However, in spinal rats, our attempts to produce learning with such a weak US have routinely failed, and as a consequence, higher intensities (3–6 mA) are normally used. It is not clear why intact subjects exhibit spinal learning using a weak US that fails to establish a CR when subjects are transected prior to training. One possibility is that this difference in sensitivity to stimulation is an artifact of the radical nature of the surgical procedure, which may effectively dampen neural excitability within the spinal cord. Alternatively, supraspinal systems could, by means of descending fibers, increase the operational range of spinal mechanisms, allowing them to encode a wider range of relationships. In either case, it is clear

that spinal mechanisms are sensitive to environmental relationships and that these systems continue to function in intact subjects.

REFERENCES

Beggs, A. L., Steinmetz, J. E., & Patterson, M. M. (1985). Classical conditioning of a flexor nerve response in spinal cats: Effects of tibial nerve CS and a differential conditioning paradigm. *Behavioral Neuroscience,* **99,** 496–508.

Bromily, R. B. (1948). Conditioned responses in a dog after removal of neocortex. *Journal of Comparative and Physiological Psychology,* **41,** 102–110.

Campbell, B. A., & Masterson, F. A. (1969). Psychophysics of punishment. *In* B. A. Campbell & R. M. Church (Eds.), *Punishment and aversive behavior.* New York: Appleton–Century–Crofts.

Chance, W. T. (1980). Autoanalgesia: Opiate and non-opiate mechanisms. *Neuroscience and Biobehavioral Reviews,* **4,** 55–67.

Coderre, T. J., Katz, J., Vaccarino, A. L., & Melzack, R. (1993). Contribution of central neuroplasticity to pathological pain: Review of clinical and experimental evidence. *Pain,* **52,** 259–285.

Durkovic, R. G. (1975). Classical conditioning, sensitization, and habituation in the spinal cat. *Physiology and Behavior,* **14,** 297–304.

Fanselow, M. S., & Baackes, M. P. (1982). Conditioned fear-induced opiate analgesia on the formalin test: Evidence for two aversive motivational systems. *Learning and Motivation,* **13,** 200–221.

Fitzgerald, L. A., & Thompson, R. F. (1967). Classical conditioning of the hindlimb flexion reflex in the acute spinal cat. *Psychonomic Science,* **47,** 345–351.

Grau, J. W., Salinas, J. A., Illich, P. A., & Meagher, M. W. (1990). Associative learning and memory for an antinociceptive response in the spinal rat. *Behavioral Neuroscience,* **104,** 489–494.

Illich, P. A., Salinas, J. A., & Grau, J. W. (1994). Latent inhibition, overshadowing, and blocking of a conditioned antinociception response in spinalized rats. *Behavioral and Neural Biology,* **62,** 140–150.

Ince, L. P., Brucker, B. A., & Alba, A. (1978). Reflex conditioning in a spinal man. *Journal of Comparative and Physiological Psychology,* **92,** 796–802.

Joynes, R. L. & Grau, J. W. (in press). Mechanisms of Pavlovian conditioning: How does the spinal cord encode temporal relations? *Behavioral Neuroscience.*

Kimble, G. A. (1961). *Hilgard and Marquis' conditioning and learning.* New York: Appleton–Century–Crofts.

Leahy, J. C., & Durkovic, R. G. (1991). Differential synaptic effects on physiological flexor hindlimb motoneurons from cutaneous nerve inputs in spinal cat. *Journal of Neurophysiology,* **66,** 460–72.

Meagher, M. W., Grau, J. W., & King, R. A. (1990). Role of supraspinal systems in environmentally induced antinociception: Effect of spinalization and decerebration on brief shock-induced and long shock-induced antinociception. *Behavioral Neuroscience,* **104,** 328–338.

Meagher, M. W., Chen, P-S, Salinas, J. A., & Grau, J. W. (1993). Activation of the opioid and nonopioid hypoalgesic systems at the level of the brainstem and spinal cord: Does a coulemetric relation predict the emergence or form of environmentally-induced hypoalgesia? *Behavioral Neuroscience,* **107,** 493–505.

Norman, R. J., Buchwald, J. S., & Villablanca, J. R. (1977). Classical conditioning with auditory discrimination of the eye blink in decerebrate cats. *Science,* **196,** 551–553.

Patterson, M. M., Cegavske, C. F., & Thompson, R. F. (1973). Effects of a classical conditioning paradigm on hind-limb flexor nerve response in immobilized cat. *Journal of Comparative and Physiological Psychology,* **84,** 88–97.

Pavlov, I. P. (1927). *Conditioned reflexes* [G. V. Anrep, trans.]. London: Oxford Univ. Press.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience,* **106,** 274–285.

Price, D. D. (1988). *Psychological and neural mechanisms of pain.* New York: Raven Press.

Shurrager, P. S., & Culler, E. (1940). Conditioning in the spinal dog. *Journal of Experimental Psychology,* **44,** 252–262.

Wolpaw, J. R., & Carp, J. S. (1990). Memory traces in spinal cord. *Trends in Neuroscience,* **13,** 137–142.

Woolf, C. J., & Thompson, S. W. (1991). The induction and maintenance of central sensitization is dependent on *N*-methyl-D-aspartic acid receptor activation: Implications for the treatment of post-injury hypersensitivity states. *Pain,* **44,** 293–299.