Instrumental Learning Within the Spinal Cord: IV. Induction and Retention of the Behavioral Deficit Observed After Noncontingent Shock

Eric D. Crown and Adam R. Ferguson
Texas A&M University

Robin L. Joynes
Kent State University

James W. Grau
Texas A&M University

Spinalized rats given shock whenever 1 hind leg is extended learn to maintain that leg in a flexed position, a simple form of instrumental learning. Rats given shock independent of leg position do not exhibit an increase in flexion duration. Experiment 1 showed that 6 min of intermittent leg shock can produce this deficit. Intermittent tailshock undermines learning (Experiments 2–3), and this effect lasts at least 2 days (Experiment 4). Exposure to continuous shock did not induce a deficit (Experiment 5) but did induce antinociception (Experiment 6). Intermittent shock did not induce antinociception (Experiment 6). Experiment 7 addressed an alternative interpretation of the results, and Experiment 8 showed that presenting a continuous tailshock while intermittent legshock is applied can prevent the deficit.

Prior studies have shown that neurons within the spinal cord are surprisingly plastic and can support a wide range of behavioral phenomena, including habituation, sensitization, and Pavlovian conditioning (Coderre, Katz, Vacarrino, & Melzack, 1993; Durkovic, 1975; Durkovic & Dumanopoulos, 1986; Grau, Salinas, Illich, & Meagher, 1990; Groves & Thompson, 1970; Joyce & Grau, 1996; Patterson, Cegavske, & Thompson, 1973; Willis, Sluka, Rees, & Westlund, 1996). Spinal neurons also appear sensitive to response–reinforcer relations, a form of learning known as instrumental conditioning (Chopin & Buergel, 1976; Crown, Ferguson, Joyner, & Grau, in press; Crown & Grau, 2001; Grau, Barsow, & Joyner, 1998; Grau, Joyner, & Penland, 1996; Segal & Wolf, 1994).

Instrumental learning is typically studied by using a variation of Horridge’s master–yoke paradigm (Horridge, 1962). To isolate the lower spinal cord from the brain, the spinal cord is transected at the thoracic level. The next day, shock electrodes are attached to one hind leg at a location that elicits an upward movement of the foot (a decrease in ankle joint angle). Foot position is monitored with a contact electrode that is electrically insulated from the subject and taped to the foot. At rest, the uninsulated tip of this electrode contacts a underlying salt solution, completing a circuit that is monitored by a computer. By means of this apparatus, one group (master) is given legshock whenever the contact electrode touches the solution (response-contingent shock). Another group (yoked) is experimentally coupled to the master group. Each yoked rat receives shock at the same time and for the same duration as its master partner, but for the yoked rat, shock occurs independent of foot position (noncontingent shock). Under these conditions, master rats exhibit a progressive increase in flexion duration that minimizes contact with the underlying solution and thereby reduces net shock exposure (Grau et al., 1998). Even though yoked rats receive the same amount of shock, they do not exhibit a change in response duration. Further evidence for instrumental learning has been provided by experimentally manipulating the contiguity between the response (foot position) and the reinforcer (shock). Delaying shock onset by just 100 ms eliminates learning, suggesting that this instrumental relation is critical (Grau et al., 1998).

To further explore the behavioral consequences of contingent versus noncontingent shock; we tested master and yoked rats under common conditions with response-contingent shock applied to either the same (ipsilateral) or opposite (contralateral) leg (Grau et al., 1998; Joynes, Ferguson, Crown, Patton, & Grau, in press). Under these conditions, subjects that have previously experienced contingent shock learn more rapidly (positive transfer). Yoked rats that previously received noncontingent shock fail to learn (negative transfer), a behavioral deficit reminiscent of learned helplessness (Maier & Seligman, 1976; Overmier & Seligman, 1967; Seligman & Maier, 1967). It is important that both of these effects are observed on the contralateral leg, which implies that a common system (within the spinal cord) mediates the consequences of earlier shock treatment (Joyner et al., in press). It is as if contingent...
shock enables spinal cord plasticity, whereas noncontingent shock has a disabling effect.

The disabling of behavioral potential within the spinal cord may have important consequences for the recovery of function after spinal cord injury. For example, if neural tissue within the lumbar-sacral spinal cord is preserved, treadmill training can help restore some locomotive capacity after a spinal cord injury (Wernig, Muller, Nanassy, & Catrol, 1995; Wernig, Nanassy, & Muller, 1998). Other studies have shown that neural bridges can be used to span an injury, helping to restore some communication with the brain (Cheng, Cao, & Olson, 1996). Both of these therapies depend on the maintenance of behavioral potential in neural tissue below the injury site. Our work suggests that afferent noncontingent nociceptive stimuli can engage a process that renews behavioral potential within surviving spinal cord tissue and thereby hastens recovery. Given this, it is important to delineate the circumstances under which this behavioral deficit emerges and its long-term consequences. The present experiments address these issues by exploring the shock conditions that undermine behavioral potential (Experiments 1–3, 5), how long the effect lasts (Experiment 4), and the relation of this effect to other unconditioned consequences of noxious stimulation (Experiments 6–8).

**General Method**

**Subjects**

The subjects were male Sprague-Dawley rats obtained from Harlan (Houston, TX). The rats were approximately 100–120 days old (400–480 g) and were individually housed, with food and water continuously available. Rats were maintained on a 12-hr light–dark cycle and were generally tested during the last 6 hr of the light cycle. Experiments 1, 5, and 6–8 used 6 subjects per cell. Experiments 2–4 used 8 subjects per cell.

**Surgery**

In all but Experiment 4, the spinal cord was transected at the second thoracic vertebra (T2) as described in Grazu et al. (1998). Rats were anesthetized with pentobarbital (50 mg/kg). The tissue over T2 was cleared away, and the cord was transected by cautery. The exposed cord was then covered with Oxycel (Parke-Davis, Morris Plains, NJ), and the wound was closed with Michel clips. Rats were maintained in a temperature-controlled environment (approximately 25.5 °C) during recovery and testing. During recovery, the rat’s rear legs were maintained in a normal flexed position by a piece of porous tape that was gently wrapped once around the rat’s body. While recovering from anesthesia, hydration was maintained with supplemental injections of saline. Once awake, rats had water and food available ad libitum. Bladders were expressed at least twice per day and immediately before any behavioral procedures were performed. At the end of testing, subjects were euthanized with pentobarbital (100 mg/kg).

Experiment 4 required maintaining spinalized rats for a period that was 3 times longer than normal (9 versus 3 days). Because rats transected at T2 can be difficult to maintain over such an extended time frame, a lower level transection was performed. An anterior–posterior incision was made directly above the eighth thoracic vertebra (T8). The tissue was cleared, and a pair of fine scissors was used to sever the spinal cord at T8. Subjects were allowed to recover and were returned to their home cages, where they were closely monitored for the duration of the experiment.

Transsections were confirmed by (a) inspecting the cord during the operation, (b) observing the behavior of the subjects after they recovered to ensure that they exhibited paralysis below the level of the forepaws and did not vocalize to legshock or tailshock, and (c) examining the spinal cord postmortem in a randomly selected subset of the subjects.

**Apparatus**

Instrumental training was conducted while spinal rats were loosely restrained in tubes that allowed both hind legs to hang freely (see Grazu et al., 1998, Figure 1). Legshock was applied by attaching one lead from a BRS/LVE shock generator (Model SG-903, Laurel, MD) to a stainless steel wire inserted through the skin over the tibia, 1.5 cm from the tarsals. The other lead was attached to a stainless steel pin that was inserted 0.4 cm into the tibialis anterior muscle, 1.7 cm above the other electrode.

Foot position was monitored with a contact electrode constructed from a stainless steel rod that was positioned directly in front of the plantar protubrance and taped to the plantar surface of the rat’s foot. The lead 2.5 cm of the electrode was insulated from the foot with heat shrink tubing. A fine wire was attached to the end of the rod extending from the rear of the foot and was connected to a digital input monitored by a Macintosh computer. A plastic rectangular dish containing a salt solution was placed approximately 7.5 cm below the restraining tube. A drop of detergent was added to the solution to reduce surface tension. A ground wire was connected to a 1-mm stainless steel rod that was placed in the solution. When the contact electrode attached to the rat’s paw touched the solution, it completed the circuit monitored by the computer. The state of this circuit was sampled at a rate of 30 Hz.

To quantify the intensity of shock needed to induce an appropriate flexion at the ankle joint, we measured flexion force by attaching a monofilament plastic line to the rat’s foot immediately behind the plantar protubrance. The 40-cm length of line was passed through an eyelet attached to the apparatus directly under the paw, 16 cm beneath the base of the tube. The end of the line was attached to a strain gauge that was fastened to a ringstand. After the line was connected to the rat’s paw, the ringstand was positioned so that the line was taut, just barely engaging the gauge. The strain gauge was previously calibrated by determining the relationship between voltage and force in newtons. These data revealed a linear relation that allowed us to convert voltage to force.

A 660-V transformer was used to generate the tailshocks. These AC shocks were administered through electrodes constructed from a modified fuse clip that was covered with electrode paste and taped to the rat’s tail, approximately 7.5 cm from the tip. A computer was used to control the onset and offset of tailshock. The AC shocks were 80 ms in duration and occurred on a variable time schedule with a mean of 2 s (range = 0.2–3.8 s). For all experiments, a volt–ohm meter was used to verify the intensity of the tail- and legshocks.

For Experiment 6, tail-flick thresholds to radiant heat were measured with an automated tail-flick device. Heat was provided by a 375-W movie light that was focused onto the rat’s tail by means of a condenser lens positioned 8 cm below the light source. The light source illuminated approximately 2 cm of the rat’s tail. Light intensity was controlled by an AC potentiometer (#6681-W, Leviton, Little Neck, NY). The rat’s tail rested in a 0.5-cm-deep groove that was cut into an aluminum block positioned 4.7 cm below the condenser lens. If a subject failed to respond, the test trial was automatically terminated after 8 s of heat exposure to avoid tissue damage.

**Behavioral Procedures**

The behavioral procedures were initiated approximately 24 hr after surgery, with the exception of Experiment 4, in which this interval differed according to the rat’s condition. Before the application of legshock and instrumental training, the rat’s rear legs were shaved and marked for placement of the shock leads. The wire electrode was then inserted over the tibia at the distal mark, and the rats were placed in the restraining tubes. Next, the contact electrode used to monitor foot position was taped to the
paw. To minimize lateral movements of the tibia and fibula, we wrapped a 20-cm piece of porous tape around the ankle and taped it to a bar extending across the apparatus directly under the front panel of the restraining tube. Next, one lead from the shock generator was attached to the stainless steel wire inserted over the tibia. The shock generator was set to deliver a 0.1-mA shock, and the region over the second mark was probed to find a site that elicited a vigorous flexion response. The pin was then inserted perpendicular to the body, into the tibialis anterior muscle. After the line connected to the strain gauge was placed over the rat's paw, we verified that a single intense (1.6 mA) test shock (0.3 s) elicited a flexion response of at least 0.8 N. Shock intensity was then adjusted so that a 0.3-s shock produced a flexion force of 0.4 N, and the plastic line was removed. Finally, the level of the salt solution was adjusted by applying three 0.15-s legshocks to establish the resting position of the contact electrode. The height of the solution was then adjusted so that the tip of the electrode lay 4 mm below the surface.

Behavioral Measures

Three behavioral measures were used to monitor instrumental performance: time in solution, response number, and response duration (see Grau et al., 1998, Figure 2). The computer recorded when the contact electrode touched the underlying solution (time in solution). Whenever the electrode left the solution, the number of responses was increased by 1 (response number). To obtain a measure of performance over time, we divided the session into thirty 1-min time bins. We have previously shown how instrumental learning can be distinguished from a reactive system that is insensitive to the response-reinforcer relation (Grau et al., 1998). A key difference concerns response duration; only the instrumental account anticipates that contingent shock will produce a progressive increase in response duration. Response duration was derived from time in solution and response number by means of the following equation: Response duration = (60 s × time in solution) − (Response number + 1), where i was the current time bin. Because response duration is a simple measure of instrumental performance and helps to discount some alternative interpretations of our results (e.g., Church & Lerner, 1976), we use it as our primary measure of learning (see Grau et al., 1998, for further details). To address the possibility that behavioral differences reflect a loss of responding in the previously shocked rats, we also present response number.

Statistics

All results were analyzed with analysis of variance (ANOVA). Post hoc comparisons were made with Duncan's new multiple range test, except in Experiment 4, in which Bonferroni t tests were performed to evaluate specific group differences. In all cases, a criterion of p < .05 was used to judge statistical significance.

Experiment 1

Using the master–yoke paradigm, we have shown that exposure to noncontingent, but not contingent, shock induces a behavioral deficit (Grau et al., 1998; Joyner et al., in press). Although this paradigm is useful for demonstrating that the response–reinforcer relation matters, it can complicate further analysis of the behavioral deficit. One obvious limitation is that it requires a master group that we have already demonstrated does not exhibit a deficit. A second problem is that, because master rats learn at different rates, each yoked subject receives a different number, amount, and distribution of shock. This variability in shock exposure can introduce variability in the development and retention of the behavioral deficit and undermine our capacity to resolve group differences.

To address these problems, we sought a paradigm that would allow us to standardize the presentation of response-independent shock. This was achieved by developing a computer program that emulated the shock schedule generated by a typical master subject. From past studies, we determined that yoked rats typically receive shocks that are 80 ms in duration. It was less clear, however, how many shocks would be needed and whether the magnitude of the deficit depends on shock spacing. We addressed the latter issue by varying the average interval between shocks. For half of the subjects, the shocks occurred at a high frequency (2.5 Hz), whereas the remaining subjects received shock at an intermediate frequency (0.5 Hz). Net exposure to shock was manipulated by varying the period over which intermittent shock was applied; separate groups received 0, 72, 360, or 1,800 s of intermittent legshock.

We expected that increasing net shock exposure would produce a more robust behavioral deficit. But it was more difficult to predict how manipulating shock spacing would affect the development of the deficit. On the one hand, some unconditioned effects develop more rapidly with massed exposure (e.g., Joyner & Grau, 1996), which suggests that the higher frequency shock would produce the most robust deficit. On the other hand, in a variety of learning paradigms, increasing the interval between trials (spaced practice) produces a stronger effect (when the total number of stimuli received is equated; e.g., Gibbon & Balsam, 1981; Miller & Matzel, 1989). A third possibility is suggested by studies of wind-up. Wind-up refers to the increase in excitability observed in spinal cord neurons after repetitive C-fiber stimulation (for a review, see Herrero, Laird, & Lopez-Garcia, 2000). This phenomenon exhibits a nonmonotonic stimulus–response function, with the most robust wind-up being observed at intermediate frequencies (e.g., between 0.5 and 3.0 Hz). If a similar function governs the development of the behavioral deficit, it too may exhibit a nonmonotonic relation that peaks at an intermediate shock frequency. To help evaluate this possibility, an additional group was included that received 180 shocks at an even lower frequency (0.1 Hz). We hoped that a comparison of this group to those that received a comparable number of shocks (0.5 Hz for 360 s and 2.5 Hz for 72 s) would help to clarify the relation between shock frequency and the induction of the behavioral deficit.

Method

Fifty-four spinal rats were used in this experiment. Approximately 24 hr after spinal transection, rats were placed in the tubes. The shock electrodes were attached to their legs, and the shock intensity necessary to induce a 0.4 N change in flexion force was established. Subjects (24) assigned to the high frequency condition (2.5 Hz) were given a series of 80 ms legshocks (AC) spaced an average of 0.4 s apart (range = 0.04–0.76 s). Subjects (24) in the intermediate frequency condition (0.5 Hz) received shocks spaced approximately 2 s apart (range = 0.2–3.8 s). Subjects received shocks for a period of 0, 72, 360 or 1,800 s at either a high or intermediate frequency, yielding a 4 (duration of shock exposure) × 2 (shock frequency) experimental design. All groups (n = 6) were restrained for the same period (1,800 s). For groups that received less than 1,800 s of shock, the shocks were presented during the last portion of the restraint period. This equated the interval between shock termination and behavioral testing. An additional group of 6 subjects received 180 shocks at a low shock frequency (0.1 Hz) distributed over the 1,800-s session.
Immediately after shock treatment, the shock leads were removed and attached to the contralateral leg. Next, shock intensity was adjusted to a level that produced a flexion response of 0.4 N. To determine the impact of the prior shock treatment on instrumental behavior, subjects were tested with response-contingent shock for 30 min. During this test period, shock was applied to the contralateral leg whenever the contact electrode attached to that leg touched the underlying salt solution.

Results and Discussion

Impact of varying the duration and frequency of exposure. We first analyzed the results from the factorial design comparing the net duration of shock exposure (0, 72, 360, or 1,800 s) and shock frequency (0.5 or 2.5 Hz). Prior shock exposure had little effect on the shock intensity required to elicit a flexion response of 0.4 N on the contralateral leg (see Figure 1, top panel) or initial response duration (bottom panel). Independent ANOVAs confirmed that the group means for shock intensity and response duration did not differ as a function of net shock exposure or density (Fs < 2.60, p > .05).

The impact of prior shock treatment on test performance is illustrated in Figure 2. The top panels depict the change in response duration over time for subjects that received shock at an intermediate (left) or high frequency (center). The group means (±SEM) are depicted in the top right panel. Unshocked subjects exhibited a progressive increase in response duration that reached asymptote after approximately 10 min of training, whereas prior exposure to 360 or 1,800 s of shock to the contralateral leg disrupted learning.

An ANOVA confirmed that there was a main effect of training duration, F(3, 40) = 17.02, p < .01. Neither the main effect of shock frequency nor its interaction with training duration were significant (Fs < 2.84, p > .05). Post hoc comparisons of the group means showed that the 360- and 1,800-s shocked rats had significantly shorter response durations than the 0- and 72-s shocked rats (p < .05). The within-subjects terms confirmed that response duration changed as a function of training time. F(29, 1160) = 5.99, p < .01. The nature of this change depended on the duration of prior shock exposure, F(87, 1160) = 1.30, p < .05. Although the two-way interaction between time and shock frequency was not significant, F(29, 1160) < 1, p > .05, there was a significant three-way interaction between time, duration, and frequency, F(87, 1160) = 1.40, p < .05. This three-way interaction indicates that the change in response duration observed over time depends on both the duration of prior shock exposure and shock frequency.

Spinalized rats that previously received 360 or 1,800 s of noncontingent legshock generally exhibited a greater number of flexion responses (see Figure 2, bottom left and middle panels). The group means for response number are shown in the bottom right panel of Figure 2. An ANOVA confirmed that shock treatment had a significant effect, F(3, 40) = 7.31, p < .01. Neither the main effect of shock frequency nor its interaction with the duration of shock exposure approached significance (Fs < 3.56, p > .05). The within-subjects terms revealed that the number of responses exhibited varied across the test session, F(29, 1160) = 1.71, p < .01. The magnitude of this effect did not vary in a systematic way with either the duration or frequency of prior shock exposure (Fs < 1, p > .05).

Additional analyses of the frequency effect. Our basic factorial design demonstrated that shock delivered by means of a computer program designed to emulate the shock schedule produced by a master subject generates a robust behavioral deficit. As expected, increasing the net duration of shock exposure increased the magnitude of this effect. With both high (2.5 Hz)- and intermediate (0.5 Hz)-frequency shock, a robust deficit was observed after just 6 min of intermittent shock. Rats given 72 s of high-frequency shock received approximately the same number of shocks (180) as those that received 360 s of shock given at an intermediate frequency (0.5 Hz). With shock number held constant and set to the minimum effective value (180), only the intermediate frequency produced a deficit. Would further decreasing shock frequency augment the behavioral deficit (producing a monotonic function), or would a wider spacing undermine its development (a nonmonotonic function)? These issues were addressed by comparing the groups that received 180 shocks at the high or intermediate frequency to those that received the same number of shocks distributed over 1,800 s (frequency = 0.1 Hz). The mean response durations are presented in Figure 3. For comparison, we also present the pooled data for subjects that never received shock (unshocked). It is clear that a nonmonotonic relation exists and that the most robust deficit was observed when shocks were presented at an intermediate rate. An ANOVA confirmed that the groups differed, F(3, 25) = 5.72, p < .01. Trend analysis demonstrated that the quadratic component

![Figure 1](image-url) Mean (±SEM) shock intensity (top panel) needed to induce a 0.4-N change in flexion force on the testing limb and duration of the first flexion response (bottom panel) for unshocked and 72, 360, and 1,800 s shocked subjects from Experiment 1.
(one inflection) accounted for most of the variance (72.5%). $F(1, 26) = 12.45$, $p < .01$. Neither the linear (no inflection) nor cubic (two inflections) components approached significance ($F_s < 3.59$, $p > .05$). Post hoc comparisons showed that rats given 180 shocks over 360 s differed from the other three groups ($p < .05$). No other differences were significant ($p > .05$).

Thus, when the number of shocks given was held constant, their impact depended on trial spacing and was greatest at an intermediate frequency (0.5 Hz). This stimulus–response function resembles the one observed in studies of wind-up, in which the strongest effect is observed between 0.5 and 3 Hz (Herrero et al., 2000; Schouenborg, 1984).

**Experiment 2**

Using parameters designed to emulate the shock schedule produced by a master subject, we showed in Experiment 1 that 6–30 min of intermittent legshock induces a robust behavioral deficit in spinalized rats. As mentioned earlier, this behavioral deficit has features that resemble the phenomenon of learned helplessness (Maier & Seligman, 1976; Overmier & Seligman, 1967; Seligman & Maier, 1967). Theorists have argued that the consequences of uncontrollable intermittent shock do not reflect a simple unconditioned (unlearned) response (Maier & Seligman, 1976). Instead, it has been suggested that the deficit only occurs after the organism has learned that the shock occurs independently of its behavior. Experiment 1 allowed for such learning by repeatedly presenting

---

**Figure 2.** Performance over time during testing in Experiment 1. The change in response duration (top panels) and response number (bottom panels) observed across the testing phase for rats that were previously unshocked or experienced either intermediate or high frequency shock for 72, 360, or 1,800 s. The right-hand panels display the group mean (±SEM) response duration (top) and response number (bottom) during the testing phase.

**Figure 3.** Mean (±SEM) response duration for unshocked controls and subjects that received a similar number of shocks (180) at 0.1, 0.5, or 2.5 Hz.
shock independent of foot position. This learning could then interfere with the system's capacity to respond appropriately when a contingency is introduced between foot position and shock. Alternatively, within the spinal cord, a simpler rule might apply, one that does not require the abstraction of response–shock independence. At this level of the nervous system, the behavioral deficit could reflect a simple unconditioned response, an effect that passively develops irrespective of how shock is applied. If so, then it should not matter whether the inducing event elicits a flexion response. Shock to the tail, which we know from prior studies elicits very limited movement of the leg or tail, could be equally effective. The present experiment evaluates this possibility by exposing spinalized rats to 1,800 s of 3 mA tailshock presented through cutaneous tail electrodes at a frequency of 0.5 Hz.

Method

Sixteen rats were used in this experiment. Twenty-four hours after spinal transection, rats were placed in the tubes and shock electrodes were attached to their tails. Once in place, rats (n = 8) received either a series of 30-s, 3 mA (AC) tailshocks spaced approximately 2 s apart (0.5 Hz) over a period of 1,800 s, or an equivalent period of restraint. The tail electrodes were then removed, shock electrodes were attached to the test leg, and initial flexion force was equated across subjects. Finally, each subject was tested for 1,800 s with response-contingent shock.

Results and Discussion

Tailshock had no effect on either the shock intensity needed to elicit a 0.4 N flexion force or initial response duration. The mean (±SEM) shock intensity ranged from 0.45 ± 0.05 mA to 0.49 ± 0.004 mA, and the duration of the first response ranged from 0.23 ± 0.17 s to 0.25 ± 0.12 s. Independent ANOVAs of the group means confirmed these small differences did not approach significance (Fs < 1, p > .05).

The impact of shock exposure on response duration is illustrated in the top panel of Figure 4. As usual, the unshocked consorts exhibited an increase in response duration over the 30-min test period. Rats that had received tailshock (shocked) failed to learn. An ANOVA revealed a significant main effect of pretreatment condition, F(1, 14) = 505.48, p < .01. In addition, the within-subjects terms showed that response duration varied over time and that the magnitude of this effect depended on whether the subjects had previously received tailshock (Fs > 4.93, p < .01).

Rats that received noncontingent tailshock also made more contacts with the salt solution than unshocked rats (see Figure 4, bottom panel). An ANOVA revealed a significant main effect of shock condition, F(1, 14) = 16.31, p < .05. Neither the main effect of time nor the Shock Condition × Time interaction were significant (Fs < 1.02, p > .05).

Like noncontingent shock, tailshock produced a behavioral deficit. This implies that the induction of the behavioral deficit does not depend on the elicitation of a flexion response. Further, the deficit can be induced when shock is applied to a different location, suggesting that the development of the deficit does not, in any obvious way, depend on direct stimulation of the leg muscles. The simplest interpretation of these results is that the behavioral deficit reflects an unconditioned response that passively develops as a function of shock exposure.

Experiment 3

We have previously shown that 1.5-mA AC tailshocks can induce a strong antinociception in spinalized rats that decreases behavioral reactivity to radiant heat applied to the tail (the tail-flick test; Meagher, Chen, Salinas, & Grau, 1993). Perhaps the behavioral deficit observed after intermittent shock is mediated by this antinociceptive system, which could generally undermine the capacity of noxious stimuli to support learning. Consistent with this hypothesis, we have previously shown that both the behavioral deficit and the antinociception can be reversed by the opioid antagonist naltrindone (Crown & Grau, 2001; Joynes & Grau, in press; Meagher et al., 1993).

If these two effects of shock exposure are mediated by a common system, then a common set of parameters should predict their emergence. We previously established that spinally mediated antinociception does not emerge until an intensity of approximately 1.5 mA (Meagher et al., 1993). Is the same true for the behavioral deficit? To evaluate this possibility, we gave rats 1,800 s of tailshock at 0.0, 1.0, 1.5, or 3.0 mA prior to testing in our instrumental paradigm. We also examined the development of the behavioral deficit as a function of shock exposure. From the results of Experiment 1, we expected that 360 s of intermittent tailshock would produce a behavioral deficit.

Method

Sixty-four rats were used in this experiment. Twenty-four h after spinal transection, rats were prepared for tailshock as described in Experiment 2. Four groups of rats (n = 8) received a series of AC tailshocks (80 ms) at 0.0, 1.0, 1.5, or 3.0 mA, presented an average of 2 s apart (range = 0.2-
3.8 s) over a period of 1,800 s. Another four groups of rats (n = 8) were given 3-mA, 80-ms tailshocks at the same frequency for 0, 72, 360, or 1,800 s. As in Experiment 1, subjects were restrained for a period of 30 min. To equate the interval between the last shock and the start of testing, rats given less than 1,800 s of shock received the stimuli at the end of the pretreatment phase. After tailshock, rats were tested with contingent leg-shock as detailed in Experiments 1 and 2.

Results

The mean (±SEM) shock intensity required to elicit a 0.4 N flexion response before testing ranged from 0.40 ± 0.03 mA to 0.48 ± 0.08 mA. Initial response durations ranged from 0.13 ± 0.02 s to 0.22 ± 0.10 s. Independent ANOVAs of the group means confirmed that these differences did not approach statistical significance (Fs < 1.90, p > .05).

Shock intensity. The effect of varying pretreatment shock intensity on test performance is illustrated in Figure 5. Rats that were previously unshocked exhibited a progressive increase in response duration over the course of testing (top panels). Although 1.0-mA tailshocks had relatively little effect, prior exposure to more intense tailshock interfered with learning. An ANOVA revealed a significant main effect of shock condition (unshocked, 1.0, 1.5, or 3.0 mA), time, and a significant Shock Condition × Time interaction (Fs > 1.86, p < .01). Post hoc comparisons of the group means showed that the 1.5 and 3.0 mA shocked rats displayed significantly shorter response durations than unshocked and 1 mA shocked rats (ps < .05). No other differences were statistically significant (p > .05).

Subjects that received 1.5–3.0-mA tailshocks also made more contacts with the salt solution (see Figure 5, bottom panels). An ANOVA yielded a significant main effect of shock condition and time (Fs > 2.90, p < .01). The Shock Condition × Time interaction, however, was not significant, F(87, 812) < 1, p > .05. Post hoc comparisons of the group means showed that the 1.5 and 3.0 mA shocked rats made significantly more contacts with the salt solution than did the unshocked and 1 mA shocked rats (ps < .05). No other differences reached statistical significance (p > .05).

Duration of exposure. The impact of varying the duration of tailshock exposure is illustrated in Figure 6. Inspection of the response durations (top panels) suggests that just 360 s of intermittent tailshock disrupted instrumental behavior. An ANOVA yielded a significant main effect of shock condition (unshocked, 72, 360, or 1,800 s) and time, as well as a Shock Condition × Time interaction (Fs > 2.00, p < .01). Post hoc tests

![Figure 5](image-url)  
**Figure 5.** Performance over time during testing in Experiment 3. The change in response duration (top panels) and response number (bottom panels) observed across the testing phase for unshocked, 1 mA, 1.5 mA, and 3 mA shocked subjects. The right panels display the group means (±SEM).
showed that rats receiving 360 s or more of shock generally exhibited shorter response durations than rats that received 72 s of shock or no shock (ps < .05). In addition, there was a significant difference between the groups that received 360 versus 1,800 s of shock. No other differences were statistically significant (p > .05).

Subjects that received 3-mA intermittent tailshock for 360 s or more also made a greater number of contacts with the salt solution (see Figure 6, bottom panels). An ANOVA revealed a significant main effect of shock condition and a significant Shock Condition × Time interaction (Fs > 1.48, p < .01). The main effect of time was not significant, F(29, 812) < 1, p > .05. Post hoc comparisons of the group means showed that rats given 360 s of shock or more differed from both the 72-s shocked group and the unshocked controls. Rats given 1,800 s of tailshock generally exhibited more responses than those that received just 360 s of shock (ps < .05). No other differences approached significance (p > .05).

Discussion

When the duration of intermittent shock exposure was held constant at 1,800 s, we found that a behavioral deficit emerged at a shock intensity of 1.5 mA, the same intensity required to produce antinociception (Meagher et al., 1993). When shock intensity was held constant and the duration of intermittent shock exposure was varied, we found that at least 360 s of shock was required, the same value obtained in Experiment 1.

In intact subjects, constant current AC shocks, applied using the same apparatus, elicit a tail movement at an intensity of approximately 0.2 mA and a vocalization at 0.4 mA (Meagher et al.,
Although both behavioral antinociception and its presumed physiological correlate, diffuse noxious inhibitory controls (DNIC), can be elicited in intact animals with shocks near the vocalization threshold, shocks at this level generate little antinociception or DNIC after the spinal cord has been transected (Grau et al., 1996; LeBars, Dickenson, & Besson, 1979; Meagher et al., 1993). To directly engage antinociceptive systems within the spinal cord, through intraspinal connections, requires much more intense shocks (e.g., 1.5 mA AC). At the neural level, increasing shock intensity alters both the number and kind of fibers engaged: weak shocks activate non-nociceptive myelinated fibers (Aα and Aβ), moderate shocks also engage myelinated nociceptive afferent neurons (Aδ), and further increasing shock intensity engages C-fibers (Wall & Wools, 1984). Given this intensity function, and the fact only very intense shocks induced the behavioral deficit, it is tempting to speculate that C-fiber input may be necessary to engage the behavioral deficit. Of course, intermittent shock would also engage myelinated fibers (e.g., Aβ and Aδ). From the present results, we cannot determine whether this co-occurring activity is necessary. It appears, though, that Aβ and Aδ activation is not sufficient to induce the behavioral deficit. If it was sufficient, the deficit should have emerged at a much lower shock intensity. Physiological studies are being conducted to explore these implications.

**Experiment 4**

Elsewhere (Joynes & Grau, in press), we have shown that the behavioral deficit observed after intermittent shock lasts 20–24 hr. Does it last longer? Is the potential for learning permanently ruined? The present experiment explored these issues by evaluating the duration of the behavioral deficit produced by intermittent tailshock (360 or 1,800 s) over a period that ranged from 1 to 8 days.

**Method**

Seventy-two rats were used in this experiment. Four groups of spinalized rats (n = 8) were exposed to 360 s of intermittent tailshock as described in Experiment 2. Another 4 groups received 1,800 s of intermittent tailshock. Shock intensity was set to a value of 1.5 mA, an intensity that we knew from Experiment 3 was sufficient to produce a robust behavioral deficit. A final group of rats (n = 8) remained unshocked. To equate the interval between surgery and testing, subjects were tested 9 days after surgery. Rats received tailshock 1, 2, 4, or 8 days prior to testing. The unshocked controls received an equivalent period of restraint 8 days before testing. As always, whether testing occurred on the left or right hind leg was counterbalanced across groups.

**Results and Discussion**

Before testing, the mean (±SEM) shock intensity required to elicit a flexion force of 0.4 N ranged from 0.75 ± 0.05 mA to 0.78 ± 0.07 mA. Initial response durations ranged from 0.11 ± 0.01 s to 0.18 ± 0.05 s. In neither case did the group differences approach statistical significance (Fs < 1.34, p > 0.05).

The impact of prior shock exposure on response duration is illustrated in the top panels of Figure 7. Instrumental learning was blocked by 360 or 1,800 s of intermittent shock when rats were tested 24–48 hr after shock exposure. At 96 hrs, only 1,800 s of intermittent shock undermined learning, and at 192 hr, it appears that shock treatment had relatively little effect. An ANOVA comparing the effect of shock treatment (360 or 1,800 s) as a function of retention interval (24, 48, 96, or 192 hr) revealed significant main effects of both retention interval and shock duration (Fs > 6.54, p < 0.05). The interaction between retention interval and shock duration approached significance, F(3, 56) = 2.48, p = 0.07. The within-subjects components revealed a significant main effect of time, a Time × Retention interval interaction, a Time × Shock Duration interaction, and a three-way interaction between time, retention interval, and shock duration (Fs > 1.28, p < 0.05). Post hoc comparisons of the group means showed that rats given 360 or 1,800 s of shock 24 hr before testing exhibited shorter response durations than all other groups (ps < 0.05). Rats tested 48 hr after 1,800 s of intermittent shock had significantly shorter response durations than rats tested at 48 hr that received 360 s of intermittent shock and both groups that were tested 24 hr after shock exposure (ps < 0.05). Rats tested 96 hr after 1,800 s of intermittent shock exhibited shorter response durations relative to the 1,800-s shocked group tested at 192 hr and the 360-s shocked groups tested at 96 and 192 hr. No other differences were significant (p > 0.05).

The impact of shock exposure on response number is illustrated in the bottom panels of Figure 7. Groups that did not exhibit an increase in response duration tended to respond more frequently. An ANOVA revealed a significant main effect of shock duration or a Retention Interval × Shock Duration interaction (Fs > 1.63, p < 0.05). This ANOVA also revealed a significant main effect of time as well as a significant Time × Shock Duration interaction (Fs > 1.59, p < 0.05). The Time × Retention Interval interaction and the three-way Time × Retention Interval × Shock Duration interaction were not significant (Fs < 1.16, p < 0.05). Post hoc comparisons indicated that over the course of testing rats in the 24 hr shocked conditions (24 hr–360-s shocked, 24 hr–1,800-s shocked) made more contacts with the salt solution than did rats in the 96-hr and 192-hr conditions (96 hr–360-s shocked, 96 hr–1,800-s shocked, 192 hr–360-s shocked, 192 hr–1,800-s shocked; ps < 0.05). No other differences were significant (p > 0.05).

To assess whether the recovery observed at 192 hr was complete, we compared the two shock groups tested at this interval to the unshocked controls. For these three conditions, the differences in the shock intensity necessary to elicit a 0.4 N flexion force and initial flexion durations did not differ (Fs < 1, p > 0.05). An ANOVA examining response duration over the 1,800-s test period did not reveal any overall group differences, F(2, 21) < 1, p > 0.05. There was, however, a significant main effect of time as well as a significant Time × Shock Duration interaction (Fs > 1.59, p < 0.01). Closer inspection of the results (see Figure 8) suggests that this interaction was due to differences in the rate of learning during the early phase of training (first 5 min). Independent ANOVAs showed that there was a significant Time × Shock Duration interaction for Minutes 1–5, F(8, 84) = 2.17, p < 0.05, but not for Minutes 6–30, F(24, 504) = 2.27, p > 0.05. Comparisons of the shocked groups to each other, and to the unshocked controls, confirmed that the groups did not differ at the start of testing (Bonferroni ts < 0.23, p > 0.05). After 5 min of training, the 1,800-s shocked group exhibited shorter response durations than
Figure 7. Performance over time during testing in Experiment 4. The change in response duration (top panels) and response number (bottom panels) observed across the testing phase is shown for unshocked subjects and subjects tested at 24, 48, 96, and 192 hr. The data are independently depicted for unshocked (open squares) and 360 s (closed squares) or 1,800 s (closed triangles) shocked subjects. The rightmost panels depict the group mean (±SEM) differences across testing.
the unshocked controls (t = 3.15, p < .05). No other differences appeared significant (rs < 1.70, p > .05). We also compared the number of responses made by subjects tested at 192 hr. An ANOVA found a significant main effect of time, F(29, 609) = 11.18, p < .01 but none of the other between subjects or within-subjects terms approached significance (Fs < 1.05, p > .05).

In summary, we found that just 360 s of intermittent tailshock undermined learning for up to 48 hr, a time course that bears a surprising similarity to that observed in studies of learned helplessness in intact rats (Maier, 1990; Maier & Seligman, 1976). Rats given tailshock for 1,800 s exhibited a longer lasting effect that was clearly evident 4 days after shock treatment. Even 8 days after shock treatment, rats given 1,800 s of shock showed slightly slower acquisition. Although intermittent shock clearly has a long-lasting effect in spinalized rats, it does not appear to permanently ruin behavioral potential. This suggests that the deficit does not reflect an irreversible process (e.g., cell death).

**Experiment 5**

We have previously explored the activation of spinal antinoceptive systems using relatively long (2–25 s) continuous constant current AC tailshocks (Meagher et al., 1993). These studies revealed that shock does not begin to engage spinal antinoceptive systems through intraspinal circuits until shock intensity is set to a very high level (e.g., 1.5 mA or higher). Our studies of instrumental learning have used brief (80 ms) intermittent shock schedules. Despite this difference in the temporal parameters, Experiment 3 showed that the behavioral deficit emerged at about the same shock intensity as spinal antinoception, suggesting that the two phenomena may be related. Indeed, both might reflect an unconditioned response that attenuates the consequences of nociceptive stimulation. In one case, this is evidenced through an attenuation of reflexive withdrawal from a noxious stimulus (inhibition of tail withdrawal from radiant heat [the tail-flick test]); in the other, it is demonstrated through a loss in behavioral potential (the disruption of instrumental learning). If these effects are mediated by a common intraspinal modification, they should be similarly affected by pharmacological manipulations. Supporting this, we have shown that the expression of the behavioral deficit, as well as spinally mediated antinoception, can be blocked by intrathecal administra-

tion of an opioid antagonist (Joyner & Grau, in press). Similarly, we would expect that the stimulus parameters that induce a robust antinoception would also undermine instrumental learning, and vice versa.

Alternatively, it is possible that the rules that govern the induction of the behavioral deficit and antinoception differ in some fundamental ways and that only intermittent shock induces the deficit. Indeed, studies in intact rats have shown that learned helplessness is observed after intermittent, but not continuous, shock (Maier, Sherman, Lewis, Terman, & Liebeskind, 1983). Such an outcome follows naturally from the proposal that subjects must learn that shock and their behavior are independent, for abstracting this relation presumably requires multiple trials. Yet, many would assume that the cognitive processes needed to abstract such a relation require a brain and that spinal cord systems might abide by a simpler rule. At this level of the nervous system, continuous shock (at a level sufficient to engange antinoceptive systems) may produce a behavioral deficit. Experiment 5 evaluated this possibility by comparing the relative impact of 15 s of shock presented as one continuous shock or as a series of brief (80 ms) intermittent shocks spaced over a period of 360 s. Other groups received 360 s of continuous shock or remained unshocked.

**Method**

Twenty-four rats were used in Experiment 5. After spinal transection, rats were prepared for tailshock as described in Experiment 2. One group of rats (n = 6) received 360 s of 1.5 mA AC tailshock generated by the computer program described earlier. Another group was given 360 s of continuous 1.5 mA AC shock, and a third received 15 s of continuous 1.5 mA AC shock (the cumulative amount of shock received during 360 s of intermittent shock). The final group remained unshocked for the entire restraint period. Twenty-four hours after tailshock, subjects were tested as detailed earlier.

**Results**

Before testing, the mean (±SEM) shock intensity required to elicit a flexion force of 0.4 N ranged from 0.56 ± 0.02 mA to 0.70 ± 0.02 mA. Initial response durations ranged from 0.16 ± 0.02 s to 0.29 ± 0.06 s. Neither group difference approached significance (Fs < 1, p > .05).

The impact of shock exposure on response duration is illustrated in the top left panel of Figure 9. The top right panel depicts the group means for response duration. As usual, previously unshocked rats exhibited an increase in response duration as a function of training. Prior exposure to intermittent shock, but not continuous shock, blocked this learning. An ANOVA confirmed that the main effects of pretreatment condition (group) and time were significant (Fs > 7.93, p < .01). There was also a significant Group × Time interaction, F(87, 580) = 1.63, p < .01, indicating that the change in response duration depended on prior shock treatment. Post hoc comparisons of the group means showed that rats that received intermittent shock differed from both the unshocked controls and the continuous shocked groups (ps < .05). No other differences were significant (ps > .05).

Rats given intermittent tailshock also made more contacts with the salt solution than both unshocked rats and rats given continuous tailshock (see Figure 9). An ANOVA revealed a significant main effect of pretreatment condition (group), F(3, 20) = 4.64,
BEHAVIORAL DEFICIT IN SPINAL RATS

Figure 9. Performance over time during testing in Experiment 5. The change in response duration (top panels) and response number (bottom panels) observed across the testing phase is shown for unshocked subjects and 360 s intermittent shocked, 15 s continuous shocked, or 360 s continuous shocked subjects. The right-hand panels show group means (±SEM).

The analysis also found a significant main effect of time, F(29, 580) = 2.62, p < .01, but failed to yield a significant Group × Time interaction, F(87, 580) = 1.18, p > .05. Post hoc comparisons of the group means showed that rats given 360 s of intermittent shock responded more than subjects in the other three conditions (ps < .05). No other differences were statistically significant (ps > .05).

Discussion

Only intermittent shock induced a behavioral deficit in spinalized rats, an outcome that parallels prior studies in intact subjects (Maier et al., 1983). Rats given intermittent shock failed to learn, but the same amount of continuous shock had no apparent effect. Even more surprising, continuous shock failed to induce a behavioral deficit when its duration was increased 24-fold, from 15 s to 360 s. Thus, a relatively small amount of shock presented intermittently over a 6-min period induced a robust behavioral deficit. But when the gap between the shocks was omitted, the deficit failed to develop.

Our results imply that events tied to shock onset play an essential role in the induction of the behavioral deficit. At a behavioral level, this suggests that multiple trials are required. In terms of learned helplessness, this is true because it takes multiple trials to learn that shock and behavior are independent. Although a cognitive description of this mechanism seems out of place when applied to the spinal cord, it is possible that the rules identified by helplessness theory capture some basic functional regularities regarding adaptive neural systems (for further discussion, see Eisen-
stein & Carlson, 1997). Specifically, that mechanisms designed to
downregulate processes tied to the onset of a biologically mean-
ingful event must only be engaged as a last resort—after adaptive
behaviors designed to minimize net exposure to potentially dam-
aging stimuli have failed.

**Experiment 6**

We suggested earlier that the induction of the behavioral deficit
might be related to the activation of spinal opioid systems that
diminish nociceptive reactivity (Joynes & Grau, in press). The
findings from Experiment 5 would appear to argue against this
possibility, as a continuous shock schedule, similar to that known
to induce antinociception on the tail-flick test, had no effect on
learning. However, the procedure used in prior studies of antino-
ception differs in some important ways. For example, instead of
a single tailshock, three shocks are normally applied, and the net
duration examined in earlier studies differs from the values tested
in Experiment 5 (Meagher et al., 1993). Perhaps we have under-
estimated the importance of temporal spacing in our studies of
spinally mediated antinociception, and this effect too would be less
robust when subjects are given a single shock. Alternatively,
different rules may govern the induction of the behavioral deficit
and antinociception. This raises the possibility of a dissociation:
that continuous, but not intermittent, shock will induce antinoc-
ception on the tail-flick test, a pattern opposite to that observed
for the behavioral deficit.

**Method**

Thirty rats were used in this experiment. Twenty-four hours after spinal
transsection, rats were placed in the tubes and given three baseline tail-flick
tests, each separated by a 2-min interval. After these baseline tests, tail
electrodes were attached, and the rats (n = 6) were given either 300 s
or 1,800 s of 1.5-mA intermittent tailshock, 15 or 360 s of 1.5-mA
continuous tailshock, or were unshocked. The tail electrodes were then
removed, and tail-flick latencies were assessed again three times at 2-min
intervals. To limit the contribution of peripheral effects on nociceptive
reactivity, the radiant heat source was applied to the tail at a location 2 cm
proximal to the tail electrodes. Tail-flick latencies were automatically
recorded by a computer (Apple Macintosh).

**Results and Discussion**

Before shock treatment, mean (±SEM) baseline nociceptive
thresholds ranged from 3.87 ± 0.13 s to 4.11 ± 0.18 s. These
group differences did not approach statistical significance, F(4,
25) < 1, p > .05.

**Figure 10** depicts the impact of intermittent versus continuous
tailshock on tail-flick latency. We found that exposure to 15 s of
continuous tailshock induced antinociception and that the magni-

tude of this effect increased when shock duration was lengthened
to 360 s. In contrast, exposure to intermittent shock had little effect
on nociceptive thresholds. An ANOVA confirmed that there was a
significant difference across groups, F(4, 25) = 11.51, p < .01.
Post hoc comparisons showed that rats that received 15 s or 360 s
of continuous tailshock displayed significantly longer tail-flick
latencies than all other groups (ps < .05). The group that received
360 s of continuous tailshock also had significantly longer tail-
flick latencies than the group that received 15 s of continuous
tailshock (p < .05). No other differences approached significance
(ps > .05).

As in prior studies (e.g., Meagher et al., 1993; Terman, Shuvit,
Lewis, Cannon, & Liebeskind, 1984), exposure to continuous
tailshock induced antinociception on the tail-flick test, and the
magnitude of this effect grew as the duration of exposure was
increased from 15 to 360 s. When the 15-s shock period was
broken into 80-ms chunks and distributed over 360 s, it had no
effect on tail-flick latencies. Even when the net exposure to inter-
mittent shock was increased 5-fold, (75 s of shock distributed over
a 30-min period), there was no change in tail-flick latencies.
Evidently, the rules that govern the induction of antinocicep-
tion and the behavioral deficit differ in a fundamental way. Con-
tinuous shock, which produces a strong antinociception and re-
duces mechanical reactivity, does not induce a behavioral deficit.
Brief intermittent shocks, which produce a robust behavioral def-
cicit, do not engage the spinal antinociceptive systems. At a func-
tional level, these two consequences of shock exposure appear
independent.

**Experiment 7**

Experiments 3–6 were motivated, in part, by the hypothesis that
intermittent shock produces a behavioral deficit because it under-
mines nociceptive reactivity. The results of these experiments
suggest otherwise, for intermittent shock yielded little antinocicep-
tion. Moreover, other studies suggest that exposure to intermittent
shock can actually enhance behavioral reactivity to a mechanical
stimulus (a von Frey hair) applied to the base of the paw (Fergus-
on, Crow, Dhruv, Washburn, & Grau, 2000). Paradoxically, this
observation suggests that intermittent shock might disrupt learning
because it augments, rather than diminishes, the affective impact of
our reinforcer (shock onset). This is possible because the relation-
ship between instrumental learning and shock intensity is non-
monotonic, with either weak (e.g., 0.2 mA) or strong (e.g., 0.8 mA)


- legshocks failing to support learning and optimal learning occuring at shock intensities (roughly, 0.4–0.6 mA) that elicit a flexion response

  of 0.4–0.6 N (Grau et al., 1998). Figure 11 (left panel)
illustrates how these assumptions would affect the capacity for learning. Notice how optimal learning (indicated by a positive capacity) should occur at intermediate values. Increasing shock intensity beyond this operational window continues to increment a process that causes performance to deteriorate and which, at sufficiently high values, would eliminate the capacity for learning (a capacity less than zero). Such a nonmonotonic relation is a common characteristic of many learning paradigms (the Yerkes-Dodson Law) and is often explained in a similar fashion, with the deterioration of learning being tied to the build-up of a disruptive process (e.g., Campbell & Masterson, 1969; Meagher et al., 2001; Walker, Cassella, Lee, Delima, & Davis, 1997).

The fact that intermittent shock exposure enhances tactile reactivity suggests that intermittent shock could shift the function that relates shock intensity to learning leftward. If this occurred, intermediate shocks would no longer support learning (see Figure 11, right panel). This account of the behavioral deficit makes a very odd prediction: that rats previously exposed to noncontingent shock would learn if we tested them with less intense shocks. If this were true, it would be a mistake to claim noncontingent shock induces a behavioral deficit, for at lower shock intensities it would have a facilitatory effect. The present experiment evaluates this possibility by testing previously shocked spinal rats at a lower shock intensity.

**Method**

Twenty-four rats were used in this experiment. After we established the shock intensity required to induce either a 0.2- or 0.4-N flexion response, subjects (n = 6) were given 6 min of intermittent tailshock at a 0.5 Hz frequency or no shock. They were then tested for 30 min with contingent shock applied at an intensity that generated a weak (0.2 N) or moderate (0.4 N) change in flexion force.

**Results**

Before testing, the mean (±SEM) shock intensity required to elicit a flexion force of 0.2 N ranged from 0.27 ± 0.10 mA to 0.35 ± 0.08 mA. The mean shock intensity needed to elicit a flexion force of 0.4 N ranged from 0.42 ± 0.07 mA to 0.53 ± 0.12 mA. Initial response durations ranged from 0.12 ± 0.04 s to 0.25 ± 0.20 s. Neither group difference approached significance ($F_1, 20 = 211.10, p < .01$). As expected, an ANOVA revealed that a more intense shock was needed to generate a 0.4-N flexion force relative to a 0.2-N flexion force.
The impact of shock exposure on response duration is illustrated in the top left panel of Figure 12. The top right panel depicts the group means for response duration. As with other experiments, unshocked rats tested at the 0.4 N flexion force criteria exhibited an increase in response duration. Unshocked rats tested at 0.2 N failed to learn. Previously shocked rats failed to learn, and this was true regardless of test force. An ANOVA confirmed main effects of both test force (0.2 N vs. 0.4 N) and shock condition (intermittent shock or no shock) in addition to a significant Force × Shock Condition interaction ($F_{34.00, 580} = 34.00, p < .01$). The within-subjects terms revealed a significant main effect of time, $F(29, 580) = 6.28, p < .01$. All of the higher order interactions with time were also significant, indicating that the change in flexion duration observed over time depended on both test force and prior shock treatment ($F_{34.00, 580} = 6.54, p < .01$). Post hoc comparisons of the group means showed that unshocked rats that were tested at the shock intensity needed to produce a 0.4-N change in flexion force displayed significantly longer response durations than all the other groups ($p < .05$). No other differences were significant ($p > .05$).

Rats that received intermittent tailshock or were unshocked and tested at the 0.2-N flexion force criteria made more contacts with the salt solution (see Figure 12, bottom panels). An ANOVA revealed a significant effect of shock condition, $F(1, 20) = 10.76, p < .01$. Neither the main effect of test force nor its interaction with shock treatment approached significance ($F_{1, 20} < 1, p > .05$). The ANOVA also found a significant main effect of time, $F(29, 580) = 5.98, p < .01$, and a significant Time × Shock Condition interaction, $F(29, 580) = 1.96, p > .01$. Post hoc comparisons of the group means showed that rats given intermittent shock responded more than unshocked subjects regardless of test force ($p < .05$). No other differences were statistically significant ($p > .05$).

**Discussion**

As reported in prior studies, subjects tested with a moderate shock (that generated a 0.4-N flexion response) learned, whereas those tested with a weak shock (that produced a 0.2-N response) did not. Rats that had previously received 360 s of 1.5-mA AC intermittent shock failed to learn, and this was true regardless of the intensity of the test shock. These observations suggest that prior shock treatment does not undermine learning simply because it increases the nociceptive impact of our reinforcer, shock onset. Rather, noncontingent shock appears to generally undermine the

---

*Figure 12.* Performance over time during testing in Experiment 7. The change in response duration (top panels) and response number (bottom panels) observed across the testing phase of Experiment 7. The left panels depict the data for subjects that were unshocked and tested at 0.2 N, unshocked and tested at 0.4 N, tailshocked and tested at 0.2 N, or tailshocked and tested at 0.4 N. The group mean (±SEM) differences are shown in the right panels for the unshocked and shocked subjects. In some cases, SEMs are too small for the error bars to be visible.
capacity for instrumental learning and, in this sense, produces a behavioral deficit.

Experiment 8

We have shown that intermittent and continuous noxious shocks can engage distinct mechanisms, one that undermines instrumental learning but not nociceptive reactivity and another that undermines nociceptive reactivity but not learning. The latter mechanism may undermine nociceptive reactivity, in part, by decreasing the afferent impact of the nociceptive signal. Indeed, such a mechanism is assumed by the gate control theory (Melzack & Wall, 1965), and it is one of the mechanisms thought to underlie the pain inhibitory effects of transectional electrical nerve stimulation. If the afferent signal is inhibited, it is possible that the exposure to continuous shock would attenuate the nociceptive impact of intermittent shock and thereby have a paradoxical protective effect. This reasoning leads to a very odd prediction: that rats given a continuous tailshock during the period in which intermittent legshock is administered should not exhibit a behavioral deficit. The present experiment tests this prediction.

Method

Twenty-four rats were used in Experiment 8. A day after surgery, rats were prepared for the administration of legshock and tailshock. Two groups (n = 12) received 6 min of continuous 1.5-mA AC tailshock, and the remaining subjects did not receive tailshock. Half of the subjects in each tailshock condition (n = 6) concurrently received intermittent legshock to one hind leg at an intensity that generated an initial flexion force of 0.4 N. The legshocks were 80 ms in duration and presented at an intermediate rate (0.5 Hz, range = 0.2-3.8 s). At the end of this shock period, we established the shock intensity needed to elicit a flexion response on the contralateral leg and tested the capacity for learning for 30 min with response-contingent shock. Which leg served as the test leg was counterbalanced across subjects.

Results and Discussion

Before testing, the mean (±SEM) shock intensity required to elicit a flexion force of 0.4 N ranged from 0.53 ± 0.05 mA to 0.59 ± 0.06 mA. Initial response durations ranged from 0.17 ± 0.11 s to 0.25 ± 0.11 s. Neither group difference approached significance (Fs < 1, p > .05).

As shown previously, rats given just intermittent shock failed to learn, whereas those given just a continuous shock acquired the instrumental response. Rats given continuous shock during the period of intermittent shock also acquired the instrumental response, suggesting that continuous shock can have a protective effect. Figure 13 depicts the data for response duration (top panels). The ANOVA on the response duration data revealed significant main effects of tailshock (continuous vs. no shock), legshock (intermittent vs. no shock), as well as a significant Tailshock × Legshock interaction (Fs > 9.12, p < .05). The within-subjects analyses also revealed a significant main effect of time and a significant Time × Legshock interaction (Fs > 2.44, p < .01). None of the other interaction terms were significant (Fs < 1.47, p > .05). Post hoc analyses showed that the group that received intermittent legshock alone displayed significantly shorter response durations than the other groups (ps < .05). No other differences were significant (p > .05).

Rats that received intermittent tailshock alone also made more contacts with the salt solution than did the other groups (see Figure 13, bottom panels). An ANOVA conducted on response number found significant main effects of tailshock and legshock, in addition to a significant Tailshock × Legshock interaction (Fs > 9.50, p < .01). The within-subjects terms failed to yield any significant differences between the groups (Fs < 1.45, p > .05). Post hoc analyses found that the group that received intermittent legshock alone made a significantly greater number of responses than did all the other groups (ps < .05). No other differences were significant (p > .05).

As seen in previous experiments, spinalized rats exposed to continuous tailshock did not exhibit a behavioral deficit, whereas rats given intermittent shock failed to learn the instrumental flexion response. However, rats exposed to continuous tailshock at the same time as intermittent tailshock did not show any signs of the behavioral deficit.

General Discussion

Identifying the physiological mechanisms that mediate the behavioral deficit requires an accurate map of the circumstances under which this system is engaged, how long it lasts, and how it is affected by other unconditioned consequences of shock exposure. The present experiments addressed these issues by examining the shock parameters that induce the behavioral deficit, its duration, and its relation to shock-induced antinociception. We discovered that just 6 min of intense intermittent shock applied to the tail or the leg can induce a behavioral deficit that lasts for days. Continuous shock does not induce a deficit but instead produces an antinociceptive effect that can attenuate the development of the behavioral deficit.

We first showed that a computer program could be used to simulate the shock schedule produced by a master subject and that a robust behavioral deficit was observed after just 6 min of noncontingent legshock. We also found that when subjects were given the minimum number of shocks (approximately 180), the magnitude of the subsequent deficit varied as a function of the shock frequency and was greatest at an intermediate frequency (between 0.1 and 2.5 Hz).

In Experiment 1, as in all prior studies, noncontingent shock was applied to the leg. As a consequence, our inducing agent repeatedly elicited a flexion response. Does the induction of the behavioral deficit depend on this feature of our paradigm, or would stimulation at another locus be equally effective? A priori, there were reasons to expect specificity, for the repeated performance of a response could induce motor fatigue. Experiment 1 side-stepped that problem by testing subjects on the contralateral leg. Experiment 2 further evaluated the specificity of the behavioral deficit by examining whether shock to the tail would induce a behavioral deficit. In spinalized rats, tailshock elicits very little motor activity. Yet it produced a robust behavioral deficit. This suggests that neither direct muscle stimulation nor the performance of a flexion response during the induction period is essential. The induction of the behavioral deficit seems instead to be tied to the magnitude of the afferent barrage. In behavioral terms, it is an unconditioned response to noxious stimulation. From this perspective, the
The master–yoke difference is due to a process engaged by the response–outcome relation experienced by the master subjects, a process that appears to inhibit the development of the unconditioned response that undermines behavioral potential (Crown et al., in press). Recent studies suggest that the same may be true for intact organisms (Sparks et al., 2000).

We knew from prior studies that continuous shock to the tail can induce an antinociceptive effect that inhibits reactivity to noxious stimuli. We reasoned that this effect could potentially underlie the learning deficit observed after noncontingent shock. Indeed, in intact animals, studies suggest that some components of the behavioral deficit observed after noncontingent shock can be explained by an opiate-mediated antinociception that undermines the reinforcing value of subsequent noxious stimuli (Grav, Hyson, Maier, Madden, & Barchas, 1981; Maier et al., 1980). Perhaps a similar effect occurs within the spinal cord. If it does, then the induction of both the antinociception and the deficit should be predicted from a common set of parameters. We knew from prior work that shock-induced antinociception is not observed until shock intensity is set to a very high level (> 1.5 mA). Given this, we predicted that the same would be true for the behavioral deficit. Experiment 3 verified this observation and showed that just 6 min of noncontingent tail shock induced a robust behavioral deficit. Experiment 4 showed that this effect lasts for 48 hr and that increasing shock exposure produced a longer lasting effect. Experiment 5 further explored the hypothesized relation between the behavioral deficit and shock-induced antinociception by comparing the impact of continuous versus intermittent shock. Because long continuous shocks induce a strong antinociception, we reasoned that they would also induce a behavioral deficit. But when the gap between the shocks was omitted, the deficit disappeared. What makes this finding especially surprising is that this remained true even though some of our experimental conditions dramatically increased net shock exposure. Experiment 6 then compared the impact of these same shock schedules on nociceptive thresholds and verified that continuous tailshock produced a robust...
antinociception. Again intermittent shock had a different effect—producing no evidence of antinociception. Indeed, we have subsequently discovered that intermittent shock exposure can actually enhance behavioral reactivity to a mechanical stimulus (Ferguson et al., 2000). In the present study, continuous tailshock also had a different effect, producing a decrease in behavioral reactivity that is consistent with its antinociceptive consequences. These results suggest that the behavioral deficit and antinociception reflect independent consequences of shock exposure and that the behavioral deficit is not due to the induction of antinociception.

Experiment 7 evaluated this hypothesis and found no evidence of learning when previously shocked rats were tested with weaker shocks. This suggests that shock treatment does more than simply shift the parameters under which learning occurs; it appears to generally undermine the capacity for learning.

The discovery that the behavioral deficit and shock-induced antinociception depend on independent systems raised the possibility that these mechanisms might interact—that the induction of one effect would influence the other. For example, if the induction of antinociception closes a nociceptive gate within the spinal cord (Melzack & Wall, 1965), this effect could attenuate the development of the behavioral deficit. Supporting this, Experiment 8 showed that the application of continuous tailshock can prevent intermittent legshock from inducing a behavioral deficit.

Relation to Central Sensitization

The fact that the deficit occurs within a particular stimulus range is intriguing, for the same is true for the phenomenon of wind-up. Physiological wind-up occurs as a result of the cumulative depolarization of nociceptive fibers that brings about the removal of the Mg\(^{2+}\) block of the N-methyl-D-aspartate (NMDA) channel (Dickenson, 1990; Dickenson, Chapman, & Green, 1997). This increases the postsynaptic Ca\(^{2+}\) current flow through NMDA channels and can engage mechanisms that induce a form of long-term potentiation (LTP). Within the spinal cord, LTP could support long-lasting behavioral modifications, including those that underlie instrumental learning.

An NMDA-dependent increase in neural excitability can be engaged in a variety of ways, including peripheral inflammation and nerve injury (Coderre, 2001; Dubner & Ruda, 1992). These insults engage an afferent barrage that, like intermittent shock, increases neural excitability within the spinal cord, the phenomenon of central sensitization. This process can enhance the impact of nociceptive A\& and C-fibers by facilitating their postsynaptic impact. It can also strengthen input from non-nociceptive A\& fibers and thereby allow these fibers to have access to the nociceptive pathway (Herrero et al., 2000). This modification is thought to underlie the phenomenon of allodynia, the pain elicited by a light touch in the region surrounding an area of inflammation (Woolf & Costigan, 1999). If this type of modification occurs in myelinated afferent fibers that signal limb position, it could underlie instrumental learning by providing a mechanism that enables a specific afferent input (the afferent signal that the limb is in a particular position) to trigger a flexion response. This is similar to the mechanism posited by Konorski and Miller (1937a, 1937b) in their initial description of the Pavlovian-instrumental distinction. Those familiar with the learning literature will recognize this as a kind of pairing-specific enhanced sensitization, a mechanism thought to contribute to both instrumental and Pavlovian conditioning in the invertebrate *Aplysia* (Hawkins, Abrams, Carew, & Kandel, 1983; Raymond, Baxter, Buonomano, & Byrne, 1992; Walters & Byrne, 1983).

The fact that both intermittent shock and inflammation enhance mechanical reactivity, and that the consequences of both manipulations can be attenuated by pharmacological agents that block the gamma-aminobutyric acid A or kappa opioid receptor, led us to suggest that a common system was involved. If central sensitization underlies the behavioral deficit, then other manipulations that produce this effect should undermine instrumental learning. We have tested this hypothesis by examining the impact of a variety of inflammatory agents (carrageenan, formalin, complete Freund's adjuvant) that are known to induce central sensitization. All undermined the capacity for instrumental learning (Ferguson, Crown, & Grau, 2001). These findings suggest that peripheral inflammation can induce the behavioral deficit. Intermittent shock simply provides a convenient tool for engaging this process, a tool that allows us to produce central sensitization without inducing tissue damage. It, like shock-induced wind-up, represents a non-natural model system that is hoped to further the understanding (and potential treatment) of a natural process.

Yet, the reader may now feel that we have used a common process (LTP-mediated excitation) to achieve disparate ends, and in a sense, this is true. On the one hand, we have suggested that the acquisition of an instrumental response may be mediated by a form of LTP that brings about a change in a specific neural pathway, laying down a kind of spinal memory (Joyner, Janjua, & Grau, in press). On the other hand, the behavioral deficit induced by peripheral inflammation and intermittent shock also seems to be the result of an NMDA-dependent process that causes a much more widespread modification in neuronal functioning. How can instrumental learning and the behavioral deficit both rely on the same process? One possible explanation comes from work on hippocampal LTP, in which studies have shown that selective increases in neuronal excitability can occur only against a relatively quiet background (McNaughton, 1983). If the system is saturated (through overstimulation), LTP-dependent learning is disrupted (Moser & Moser, 1999). Given this, we propose that central sensitization from intermittent shock or inflammation represents a spinally mediated version of LTP saturation that undermines the capacity for learning.

From this perspective, central sensitization (if prolonged and diffuse) could be viewed as an adverse consequence of a process that is designed to normally encourage protection of a damaged region. Normally, the brain inhibits this process through descending pathways. When this protective process is removed, natural insults (inflammation) and shock stimuli induce greater central sensitization. This saturates nociceptive systems within the spinal cord, reducing the capacity for selective adaptations to specific events (learning) and initiating other processes that can undermine behavioral potential (Joyner et al., in press). Thus, a common mechanism may contribute to both instrumental learning and the development of the behavioral deficit: the activation of the NMDA receptor by excitatory amino acids. Learning reflects a selective, adaptive modification that preserves behavioral potential at other synapses. The deficit reflects a saturation-like effect caused by excessive NMDA-mediated excitation that effectively blocks selective NMDA-dependent learning. If this is true, then learning and the behavioral deficit should be blocked by an NMDA antag-
onist. We have already shown that learning is blocked by an NMDA antagonist (e.g., Joyner et al., in press), and we recently discovered that the same is true for the behavioral deficit (Ferguson, Crown, & Grau, 2002).

Clinical Implications

If exposure to noncontingent shock induces a destructive process that hurts behavioral potential within the spinal cord, engaging this process could hurt the recovery of function after a contusion injury of the spinal cord. Moreover, if the injury disrupts the descending pathways that normally inhibit this destructive process, the injury would allow the process to run unchecked. As a result, nocuous input would induce greater central sensitization, initiating destructive processes that hurt behavioral potential, enhance cell death, and promote the development of neuropathic pain syndromes.

We have begun to explore these clinical implications by testing the impact of intermittent shock on recovery after a mild contusion injury (Grau, Garcia, Ferguson, Crown, & Miranda, 2001). We have discovered that just 6 min of intermittent shock given the day after an injury severely retards behavioral recovery, producing a deficit in locomotive function that is evident 6 weeks later. How can we prevent this destructive process? The work reviewed above suggests that pharmacological treatments that block kappa opioids or the NMDA receptor could be helpful, and there is some clinical evidence that NMDA antagonists may have a protective effect (Wada et al., 1999). We would also predict that bicuculline would be protective (e.g., Ferguson et al., 2000); to our knowledge, this treatment has not been evaluated.

Our work also suggests some truly novel approaches to treatment. For example, we have shown that exposure to contingent shock can engage a process that can prevent the development of the deficit and, in conjunction with a pharmacological treatment, attenuate the deficit after it has been induced (Crown & Grau, 2001). These manipulations could provide a noninvasive technique to maintain and foster behavioral potential within the spinal cord. The present results also suggest that a continuous shock stimulus, such as that provided by transcutaneous electrical nerve stimulation, may help protect spinal cord systems.

Future Directions

Our aim was to elucidate the circumstances under which the application of a controlled noxious stimulus would induce a behavioral deficit that undermines the capacity for instrumental learning. We showed that just 6 min of intermittent shock produces a robust effect that lasts for days. This work lays the foundation for further work exploring the underlying physiological systems. What neurochemical mechanisms mediate the learning and underlie the deficit? Why does intermittent shock, but not continuous shock, induce a deficit? Physiological studies are also needed to evaluate the role of central sensitization in producing the behavioral deficit. Finally, more research is required to evaluate the clinical implications of these findings.

References


Received December 10, 2001
Revision received April 2, 2002
Accepted May 17, 2002