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Fixed spaced stimulation restores adaptive plasticity within the spinal cord: Identifying the eliciting conditions

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HIGHLIGHTS

- Variable/unpredictable stimulation undermines learning and adaptive spinal function.
- Fixed spaced (temporally predictable) stimulation promotes learning and adaptive spinal function.
- Fixed spaced stimulation reverses the effects of variable stimulation.
- Low intensity stimulation between 0.5 and 5 Hz has a therapeutic effect.
- Fixed space stimulation may have clinical relevance for enabling recovery following injury.

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ABSTRACT

Prior work has shown that neurons within the spinal cord are sensitive to temporal relations and that stimulus regularity impacts nociceptive processing and adaptive plasticity. Application of brief (80 ms) shocks (180–900) in a variable manner induces a form of maladaptive plasticity that inhibits spinally-mediated learning and enhances nociceptive reactivity. In contrast, an extended exposure (720–900) to stimuli given at regular (fixed spaced) intervals has a restorative effect that counters nociceptive sensitization and enables learning. The present paper explores the stimulus parameters under which this therapeutic effect of fixed spaced stimulation emerges. Spinally transected rats received variably spaced stimulation (180 shocks) to the sciatic nerve at an intensity (40-V) that recruits pain (C) fibers, producing a form of maladaptive plasticity that impairs spinal learning. As previously shown, exposure to 720 fixed spaced shocks had a therapeutic effect that restored adaptive learning. This therapeutic effect was most robust at a lower shock intensity (20 V) and was equally strong irrespective of pulse duration (20–80 ms). A restorative effect was observed when stimuli were given at a frequency between 0.5 and 5 Hz, but not at a higher (50 Hz) or lower (0.05 Hz) rate. The results are consistent with prior work implicating neural systems related to the central pattern generator that drives stepping behavior. Clinical implications are discussed.

1. Introduction

Prior work has shown that environmental stimulation can bring about a lasting change in spinal function (see [1–3] for reviews). The impact of environmental stimulation is particularly evident following spinal cord injury (SCI) when communication between the brain and spinal cord is interrupted. When descending modulation is disturbed, spinal neurons can become increasingly sensitive to the effects of stimulation and exhibit a state of over-excitation within the sensory circuitry of the dorsal horn, a phenomenon known as *central sensitization* [4–7]. Central sensitization can be induced by peripheral tissue damage, inflammation, application of chemical irritants (e.g., capsaicin, formalin), or by electrical stimulation at an intensity that engages peripheral nociceptive fibers. At a cellular level, nociceptive sensitization has been shown to have a lasting effect on spinal function that depends on a form of NMDA receptor (NMDAR) mediated plasticity [8–12]. The sensitization of pain (nociceptive) circuits within the spinal cord is associated with increased reactivity to mechanical stimulation and a strengthening of the nociceptive signal relayed to the brain (when ascending fibers are spared) [12–14], and may also contribute to impaired recovery following injury [15,16]. Given these effects, and the relationship between

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central sensitization and chronic pain, central sensitization has been characterized as a form of maladaptive plasticity [17].

How noxious stimulation affects spinal function depends upon both behavioral and environmental variables. In rats that have undergone a transection at the second thoracic vertebra (T2), shock applied to one hind leg whenever the limb is extended (controllable stimulation) brings about a progressive increase in flexion duration that minimizes net shock exposure, a form of adaptive plasticity known as instrumental learning [18–20]. Subjects that have received the same amount of shock independent of leg position (uncontrollable stimulation) do not exhibit an increase in flexion duration and later fail to learn when tested with controllable stimulation applied to the opposite (contralateral) leg. This learning impairment lasts 24–48 h and has been linked to the induction of central sensitization [10,16,17,21–23]. Supporting this, exposure to uncontrollable stimulation induces enhanced mechanical reactivity (EMR) and experimental treatments that induce central sensitization impair instrumental learning [10,16,24]. Moreover, using electrophysiological stimulation of the sciatic nerve, we have begun to define the circumstances under which afferent neural activity has an adverse effect on spinal function [25]. This work has shown that electrical stimulation only interferes with instrumental learning when shocks are given at an intensity (40 V) that recruits a robust C-fiber response and when stimuli occur at a low frequency (0.25–2.5 Hz). Interestingly, natural C-fiber activity has a variable signature, which may serve as a kind of neural code [26–29].

More recently, we discovered that the impact of noxious stimulation on spinal function also depends upon temporal regularity and the amount of stimulus exposure. When 180 brief (80 ms) shocks are given to the tail or sciatic nerve at 0.5 Hz, both regular [fixed time (FT)] and variable [variable time (VT)] stimuli (0.2–3.8 s, rectangular distribution) engage a learning impairment and enhanced mechanical reactivity (EMR) [EMR 10, 24]. However, if stimulus number is increased 3 fold (to 540 or more), only VT stimulation induces a learning impairment and EMR [24,25,30,31]. Further work revealed that fixed spaced stimulation engages a protein synthesis-dependent form of BDNF and NMDA-mediated plasticity and implicated an oscillatory system [central pattern generator (CPG)] within the rostral lumbar spinal cord [30,32,33].

These observations suggest that spinal systems can discriminate whether stimulation occurs in a regular or irregular manner (implying a sense of time), and that continued exposure to fixed spaced (540+) stimulation can eliminate the learning impairment and EMR induced by a brief (180 shocks given over 6 min) exposure to noxious stimulation [25,30,32]. The implication is that FT stimulation can have a restorative effect that counters the maintenance of maladaptive plasticity. To explore this possibility, we exposed spinally transected rats to 180 shocks given on a VT schedule, a shock schedule that produces a lasting learning impairment [22,25,30]. We then attempted to reverse this effect by administering 720 fixed spaced shocks. We found that the application of more shock, if given in a temporally predictable manner, eliminated the learning impairment induced by VT stimulation [25, 30]. Importantly, this restorative effect is only observed if the shocks are given in a regular manner (FT stimulation). We further showed that an extended exposure to FT stimulation can reverse both the learning impairment and EMR induced by capsaicin [31]. In addition, exposure to 720 fixed spaced shock was shown to have a lasting (24 h) protective effect that blocked the induction of the EMR and learning impairment induced by variable shock or the peripheral application of capsaicin [24,30,31].

The observation that fixed spaced stimulation has a restorative effect, that eliminates the learning impairment and EMR induced by peripheral nociceptive input, is clinically important because treatment will typically follow the induction of nociceptive sensitization. For this reason, we sought to detail the eliciting conditions that produce this therapeutic effect. We addressed this issue using electrophysiological procedures analogous to those used to explore the stimulus conditions that produce a maladaptive effect [25]. In all of the experiments, we first induce a learning deficit by exposing rats to 180 variably spaced shocks. We then present 720 fixed spaced shocks and vary stimulus intensity (Experiment 1), burst duration (Experiment 2), or frequency (Experiments 3 and 4).

2. Methods

2.1. Animals

Subjects were male Sprague–Dawley rats obtained from Harlan (Houston, TX). Rats were 70–90 days old and weighed 350–400 g at the time of spinal cord transection. They were housed in pairs with free access to food and water, and were maintained on a 12–12 h light-dark cycle. All experiments were carried out in accordance with NIH standards for the care and use of laboratory animals (NIH publications No. 80-23), and were approved by the University Laboratory Animal Care Committee at Texas A&M University. Every effort was made to minimize suffering and limit the number of animals used.

2.2. Spinalization surgery

Prior to surgery, the fur over the thoracic portion of the vertebral column was shaved and disinfected with betadine solution. Rats were anesthetized with isoflurane gas. The rat's head was rendered immobile in a stereotaxic apparatus with a small (5 × 4 × 2.5 cm) gauze pillow under the subject's chest. An anterior to posterior incision over the second thoracic vertebrae (T2) was made, the tissue just rostral to T2 was cleared using rongeurs, and the cord was exposed and cauterized. The remaining gap in the cord was filled with Gelfoam (Pharmacia Corp., Kalamazoo, MI) and the wound was closed with Michel clips (Fisher Scientific, Waltham, MA). Following closure of the wound, the surface of each leg was shaved for electrode placement. Intraperitoneal injections (3 mL) of 0.9% saline solution were administered post-operatively to prevent dehydration. Following surgery, rats were placed in a temperature-controlled environment (25.5 °C) and monitored until awake. All rats were checked every 6 to 8 h during the 18–24 h post-surgical period. During this time, hydration was maintained with supplemental injections of saline, and the rats' bladders and colons were expressed as necessary.

Spinal transections were confirmed by inspecting the cord under a 10× dissection scope and by observing the behavior of the subjects after they recovered (paralysis below the level of the forepaws and no supraspinally-mediated pain responses).

2.3. Sciatic nerve exposure and stimulation

Twenty-four hours following surgery, spinalized subjects were placed in a restraining tube with their rear legs exposed. Their legs were positioned so that they were lying flat and extended away from their body. An incision was made on the lateral surface of the leg (counterbalanced) to expose the biceps femoris and vastus lateralus muscles. These muscle groups were dissected away, exposing the sciatic nerve within the popliteal fossa. Bipolar hook electrodes were then placed around the sciatic nerve, with the electrodes 5 mm apart. A test pulse was delivered from the stimulator (model S9; Grass Medical Instruments, Quincy, MA) to ensure contact between the nerve and electrodes. Once the electrodes were in place, the appropriate stimulation treatment was administered. Warm mineral oil was applied as needed to prevent dehydration of the exposed nerve. Immediately following sciatic nerve stimulation, the leg was closed with Michel clips and the subject was prepared for instrumental testing.

2.4. Instrumental testing

The apparatus used was similar to that described in previously published work [18]. Briefly, subjects were loosely restrained in Plexiglas tubes with their hindlimbs suspended above a saline solution contained in a rectangular plastic dish (11.5 cm [w] × 19 cm [l] × 5 cm [d]) positioned 7.5 cm below the restraining tube. Holes were drilled into the anterior portion of the tubes to allow for ventilation. Two slots were cut 4 cm apart and 1.5 cm from the posterior end of the tube to allow both hind legs to hang freely. To monitor leg position, a stainless steel rod (7 cm [l], 0.46 mm [w]) was attached to the pad of one foot (contact electrode) extending past the toes. The contact electrode was attached to the plantar surface of the rat's foot with porous tape (Orthaletic, 1.3 cm [width]; Johnson and Johnson, New Brunswick, NJ), with one end positioned directly in front of the plantar protuberance. Heat-shrink tubing electrically insulated the rod from the paw. A fine wire (0.01 mm [36 AWG], magnet wire single beldsol) was attached to the end of the rod at a point under the insulation. This wire extended from the rear of the foot and was connected to a digital input board that was monitored by a Macintosh G4 computer. To minimize lateral leg movements, a piece of porous tape was wrapped around the leg above the tarsus and attached under the front panel of the restraining tube.

Two electrodes were then inserted into one hindleg. The first electrode was constructed from stainless steel wire (0.05 mm [30 AWG]) and was inserted through the skin over the tibia 1.5 cm from the tarsus. The second was made of fine wire (0.01 mm [36 AWG], magnet wire single beldsol) and was inserted perpendicular to the leg, through the body of the tibialis anterior muscle 1.7 cm above the first electrode. Legshock was applied by attaching one lead from a constant current AC shock generator (Model SG-903; BRS/LVE, Laurel MD) to the electrode inserted into the tibialis anterior muscle. The second lead was attached to the wire implanted in the skin over the tibia. Shock (60 Hz, AC) intensity was adjusted for each subject to a level that produced a 0.4 Newton (N) flexion response. This value was determined prior to instrumental training by looping a monofilament plastic line (6 lb. test strength; Du Pont, Wilmington DE) around the rat's ankle. The end of the line was attached to a strain gauge (Fort-1000; World Precision Instruments, New Haven, CT) and fastened to a ring stand. The strain gauge output was fed through a calibrated multimeter that allowed for a conversion from voltage to force. To determine the necessary flexion force a single 300 ms shock was applied to the leg and the shock intensity was adjusted to elicit the prescribed flexion force. After flexion force was set, the monofilament line was removed from the rat's paw and the saline solution was adjusted so that the contact electrode sat 4 mm beneath the surface of the salt solution. Once each animal was prepared, the 30 min instrumental testing session began. Whenever the subject's leg was in the down position, the end of the rod contacted the saline solution and completed an electrical circuit. When the circuit was closed, shock was delivered to the tibialis anterior muscle, which elicited a flexion response. The flexion response raised the contact electrode out of the saline solution, which broke the circuit and terminated the shock.

2.5. Measures of instrumental learning

Testing sessions were divided into 30 one-minute bins to examine learning across time. Response number and response duration were collected by the computer during these sessions, and were separately averaged across each one-minute bin. Every time the contact electrode left the solution, the number of responses was increased by one. The computer also recorded the amount of time the electrode remained out of the solution. Response duration served as the primary measure of learning and was calculated for each one-minute bin using the equation: response duration = (time out of solution) ÷ (response number + 1).

2.6. Statistics

Baseline behavioral responding was analyzed using a one-way analysis of variance (ANOVA) and response durations were analyzed using a mixed-design ANOVA or analysis of covariance (ANCOVA). Where appropriate, Tukey's Honestly Significant Difference (HSD) was used to conduct post hoc analyses. In all cases $p < 0.05$ was used to determine statistical significance.

3. Results

3.1. A restorative effect is observed when stimulus intensity is reduced

Prior work has demonstrated that the effect of FT and VT stimulation interacts with shock number [24,25,30–33]. Exposure to 180 variable intermittent shock induces a lasting learning impairment, and this remains true when shock number is increased 25 fold (to 4500 shocks). In contrast, the impact of FT stimulation varies as a function of shock number; 180–360 shocks induces a learning impairment while a longer period of stimulation (540–4500 shocks) has a restorative effect. Here we explore whether the effect of FT stimulation varies as a function of shock intensity. We have previously shown that the *adverse* effect of variably spaced shock does not emerge until shock intensity is increased to a level that recruits a robust C-fiber response (40 V). The present experiment evaluates the stimulus intensity needed to induce a *restorative* effect with FT stimulation. Elsewhere, we have related this effect to the activation of a spinal oscillator residing in the lumbar CPG that is thought to set the tempo of stepping [32]. Reasoning that less intense stimulation may be capable of engaging the spinal CPG, we hypothesized that the beneficial effect of FT stimulation may emerge at a lower shock intensity.

Spinally transected rats (n = 10/condition) had their sciatic nerves exposed followed by placement of bipolar hook electrodes as described above. DC sciatic nerve stimulation was employed (rather than tail or leg shock) in order to examine key stimulus parameters and to compare against previous work. Subjects were given 180 variable space shocks (40 V, 0.5 ms pulse width, 50 Hz pulse frequency, 80 ms burst duration, ISI range = 0.2–3.8 s; mean ISI = 2 s) immediately followed by 720 fixed space shocks (0.5 ms pulse width, 50 Hz pulse frequency, 80 ms burst duration, 2 s ISI) at 0, 5, 10, 20, or 40 V. Subjects were administered 720 fixed space shocks because previous work has shown that this number of shocks is capable of reversing the effects of variable stimulation [30]. Rats in the 0 V condition had electrodes placed around the sciatic nerve but received no stimulation. After sciatic stimulation, rats were placed in the instrumental apparatus and were tested with 30 min of controllable AC shock on the contralateral leg.

To ensure that any learning deficit observed was not caused by the inability of shock to elicit the target flexion response, and to assure that our experimental procedures did not affect the shock-elicited flexion response on the test leg, we analyzed the amount of stimulation required to produce a 0.4 N flexion force and subjects' initial flexion durations. Mean (±SE) shock intensity ranged from 0.50 ± 0.01 to 0.54 ± 0.01 mA, and average initial flexion duration ranged from 0.14 ± 0.03 to 0.16 ± 0.03 s. Independent ANOVAs showed that these small differences were not statistically significant for flexion duration, $F(4, 49) = 1.22$, $p > 0.05$, but reached significance for shock intensity, $F(4, 49) = 2.89$, $p < 0.05$. In the analyses that follow, we control for the latter effect using an ANCOVA.

As in past studies [18,19,22,25,30,31,34], our primary measure of learning was response duration. Rats that received 180 variable spaced shocks alone exhibited a learning deficit. Exposure to fixed spaced stimulation restored the capacity to learn and this effect was most robust at a shock intensity of 20 V (Fig. 1). To control for baseline differences in shock reactivity, we entered the shock intensity required to elicit a 0.4 N flexion response as a covariate in an ANCOVA performed on the response duration data. Despite accounting for a significant degree of the

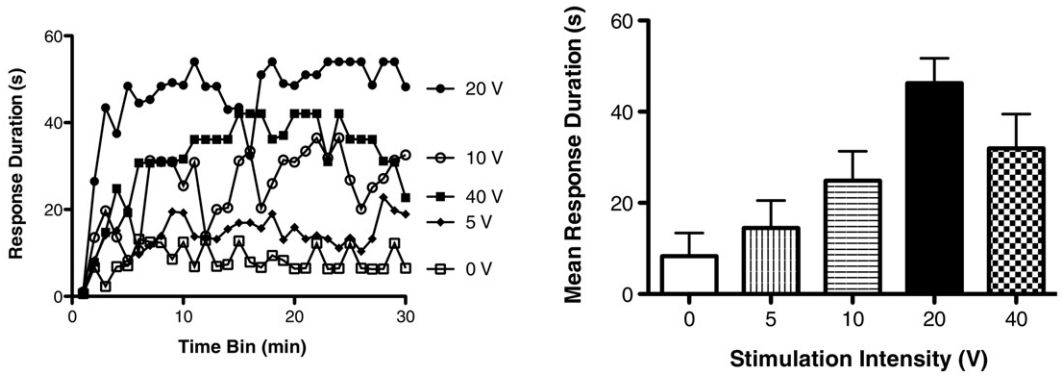

Fig. 1. The voltage-dependent impact of fixed space sciatic stimulation on expression of the learning deficit. Following sciatic nerve exposure and isolation, rats received 180 variable space shocks directly to the sciatic nerve, followed by 720 fixed space shocks at 0, 5, 10, 20, or 40 V. Testing occurred immediately after stimulation. The left panel depicts response durations across time, while the right panel illustrates mean response duration collapsed across trials. Asterisks indicate significantly different performance compared to rats in the 0 V condition ($p < 0.05$), and error bars indicate ± SE (n = 10 rats/condition).

overall variance, $F(1, 44) = 4.27$, $p < 0.05$, entering shock intensity did not affect our other statistical outcomes. The ANCOVA revealed significant main effects of Voltage and Time, as well as a significant Voltage × Time interaction, all Fs > 1.47, $p < 0.05$. Additional analysis using trend analyses revealed a significant quadratic function for the Voltage × Time interaction, $F(4,44) = 3.36$, $p < 0.05$. The quadratic trend indicates that there was a non-monotonic effect of shock intensity, yielding a significant inflection at 20 V.

We also analyzed the number of responses made by subjects in each condition. As in past studies, subjects that failed to learn exhibited the highest rate of responding. In the present experiment, rats that received no FT stimulation (0 V condition) exhibited an average of 121.64 ± 19.84 responses per minute, whereas rats that received FT stimulation exhibited 41.03 ± 18.78 responses per minute. An ANOVA confirmed that the intensity of FT stimulation affected response rate and that the magnitude of this effect varied across time, all Fs > 3.21, $p < 0.05$. These observations are important because they show that the failure to learn does not reflect an inability to perform the target response. Subjects that did not learn repeatedly experienced the response-outcome relation, but this experience did not produce an increase in response duration. Because a similar trend was observed in the subsequent experiments, and because response duration provides a reliable index of learning that avoids some interpretative problems (see [18]), we focus on this measure in the subsequent experiments.

3.2. The restorative effect of fixed stimulation is observed across a range of burst durations

Our studies have routinely used shock stimuli that are 80 ms in duration [18,22,25,30]. This duration allows for the delivery of 4–5 shock pulses within each burst of stimulation. With regard to the learning impairment, we have examined the effect of pulse number and found that single pulses are just as effective as bursts containing 4–5 pulses [25]. The present experiment examines whether the same is true for the restorative effect of FT stimulation. Evidence single pulses are effective will simplify our derivation of the optimal frequency range in the next experiment. Burst duration is also of interest because this factor can influence the profile of neurochemical release [35].

To examine the effects of burst duration, rats (n = 6/condition) were given 180 variable spaced stimuli (40 V, 0.5 ms pulse, 50 Hz pulse frequency, 80 ms burst) followed by 720 fixed spaced shocks (20 V, 0.5 ms pulse, 50 Hz pulse frequency) administered for a duration of 0 (unshocked), 20 ms (one pulse), or 80 ms (4–5 pulses). Using a recording oscilloscope (TDS1001; Tektronics Inc., Beaverton, OR), we verified that 20 ms of stimulation produced a single pulse and that 80 ms of stimulation produced a minimum of 4 pulses (at 50 Hz). Following the stimulation period, subjects had their legs prepared for instrumental testing as described above.

The average shock intensity required to produce the target flexion response ranged from 0.42 ± 0.08 to 0.51 ± 0.02 mA. Initial response durations ranged from 0.16 ± 0.02 to 0.19 ± 0.01 s. Individual ANOVAs performed on each measure failed to detect any significant differences based on group assignment, all Fs < 2.46, $p > 0.05$.

Subjects' response durations are depicted in Fig. 2. Rats that received 0 ms of fixed shock exhibited a learning deficit while rats given 20 or 80 ms fixed shock did not. An ANOVA revealed significant main effects of Burst duration and Time, as well as a significant Burst duration × Time interaction, all Fs > 1.50, $p < 0.05$. The significant interaction term emerged because rats in the 0 ms condition exhibited significantly shorter response durations than rats in the 20 or 80 ms conditions ($p < 0.05$).

3.3. Fixed spaced stimulation has a restorative effect between 0.5 and 5 Hz

Stimulation frequency has a significant impact on neural and behavioral plasticity. High frequency stimulation (e.g. 100 Hz) reliably elicits long-term potentiation (LTP) while low frequency stimulation results in long-term depression (LTD) [36,37]. Previously, we demonstrated that variable stimulation with a mean frequency of 0.25 to 2.5 Hz induces a robust learning impairment, while stimuli presented at lower (0.1 Hz) or higher (5.0 Hz) frequencies does not [22,25,32]. Here, we examine how stimulus frequency, from 0.05 to 50 Hz, affects the therapeutic effect of fixed spaced stimulation. If the restorative effect is related to the entrainment of the CPG tied to stepping behavior, the optimal frequency should lie in the range of locomotor behavior (approximately 0.5–5 Hz; [38,39]).

Spinally transected rats (n = 8/condition) were administered 180 variable shocks (40 V, 0.5 ms pulse, 50 Hz pulse frequency, 80 ms burst) directly to the exposed sciatic nerve immediately followed by 720 fixed shocks (20 V, 0.5 ms pulse, single pulse) at 0, 0.05, 0.5, 5, or 50 Hz. Following the stimulation period, subjects were tested as described earlier.

Administration of 720 stimuli at 0.5 Hz requires approximately 30 min to complete, whereas administration of 720 shocks at 0.05 Hz requires 240 min. Because the duration of restraint could potentially affect learning, half the subjects given stimuli at a frequency of 0.5 Hz or greater were restrained for 30 min while the remaining subjects were restrained for 240 min. All rats received stimulation at the end of the restraint period to keep the time between delivery of the last shock and testing equal across all conditions. An ANOVA showed that the duration of restraint had no effect on instrumental performance, $F(1, 54) = 1.26$, $p > 0.05$. Given this, we collapsed the data across this variable in the subsequent analyses.

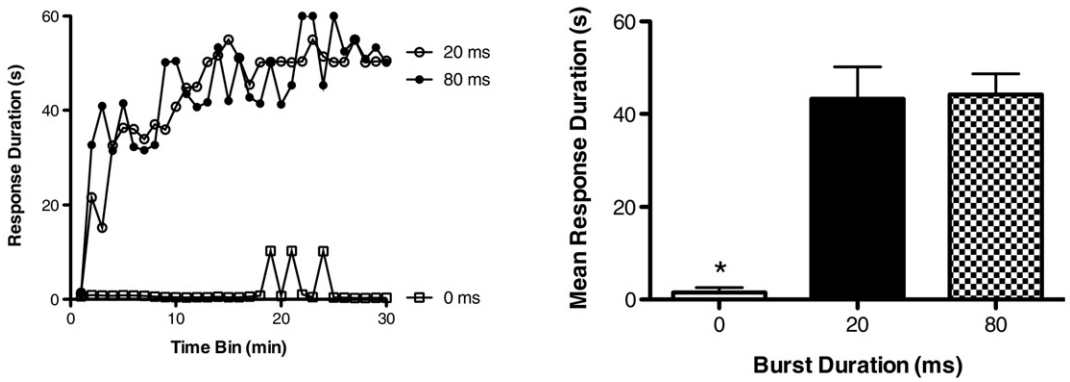

Fig. 2. The impact of burst or single pulse stimulation on instrumental performance. Rats received 180 variable space shocks immediately followed by 720 fixed space shocks with 0, 20, or 80 ms burst durations. The 20 ms burst allowed for 1 pulse to be administered to the sciatic nerve, while the 80 ms burst allowed for 4–5 pulses. Following stimulation rats were prepared for instrumental testing. The left panel depicts response durations across time, while the right panel illustrates mean response duration collapsed across trials. Asterisks indicate significantly different performance compared to rats in the 20 and 80 ms conditions ($p < 0.05$), and error bars indicate ± SE (n = 6 rats/condition).

The mean shock intensity needed to produce a 0.4 N flexion response ranged from 0.49 ± 0.02 to 0.55 ± 0.03 mA and initial response durations ranged from 0.15 ± 0.01 to 0.18 ± 0.01 s. Independent ANOVAs performed on each measure did not detect any significant differences based on group membership, all Fs < 1.37, $p > 0.05$.

Fixed spaced stimulation restored learning in a frequency-dependent manner. Rats administered 0, 0.05, or 50 Hz of Fixed shock failed to learn at test, while rats given 0.5 or 5 Hz learned (Fig. 3). An ANOVA revealed significant main effects of Frequency and Time, as well as a significant Frequency × Time interaction, all Fs > 1.59, $p < 0.001$. This significant interaction emerged because rats in the 0, 0.05, and 50 Hz conditions performed poorly relative to subjects in the 0.5 and 5 Hz conditions. ($p < 0.05$).

3.4. The restorative effect of 5 Hz stimulation emerges only when shocks are given in a regular manner

The above findings demonstrate that the operational range of FT stimulation is from 0.5–5 Hz. This range corresponds to the frequency of hind limb stepping and, in this way, the results are consistent with the hypothesized link to the locomotor CPG [38,39]. Our claims here, however, must be constrained because a key comparison is lacking. In prior work, we showed that 720 FT shocks given at 0.5 Hz has a restorative effect while the same number of shocks given in a variable manner does not. For 5 Hz stimulation, we do know whether FT and VT stimulation have different effects. Indeed, when stimulus frequency is increased, spinal systems may not be able to discriminate these two forms of stimulation. The present experiment evaluates this possibility.

Twenty-four hours after spinal transection a learning deficit was induced with 180 variable space stimuli to the exposed sciatic nerve (40 V, 0.5 ms pulse, 50 Hz pulse frequency, 80 ms burst). Then, subjects were randomly assigned to one of two experimental conditions (n = 12/condition). Half of the subjects received 720 stimuli to the sciatic nerve at 5 Hz with a fixed ISI of 0.2 s, while the other half received the same number of stimuli, but the ISI was varied rectangularly between 0.02 and 0.38 s (20 V, 0.5 ms pulse width, single pulse).

The average shock intensity needed to produce a 0.4 N flexion response ranged from 0.56 ± 0.04 to 0.58 ± 0.01 mA and initial response durations ranged from 0.22 ± 0.02 to 0.23 ± 0.02 s. Individual ANOVAs performed on each measure did not detect any significant differences based on group membership, all Fs < 1.0, $p > 0.05$.

High frequency stimulation restored learning in a schedule-dependent manner. Rats administered fixed space stimulation learned at test, whereas rats administered variable space shock showed a learning impairment (Fig. 4). An ANOVA revealed a significant main effect of Time as well as a significant Condition × Time interaction, all Fs > 6.88, $p < 0.05$.

The results imply that spinal systems can discriminate FT and VT stimulation applied at 5 Hz and that the former has a greater restorative effect. The pattern of results does, however, appear somewhat different from those observed using 0.5 Hz stimulation [30], where we found that rats that had received additional VT stimulation exhibited very poor test performance. This difference is not entirely unexpected because VT

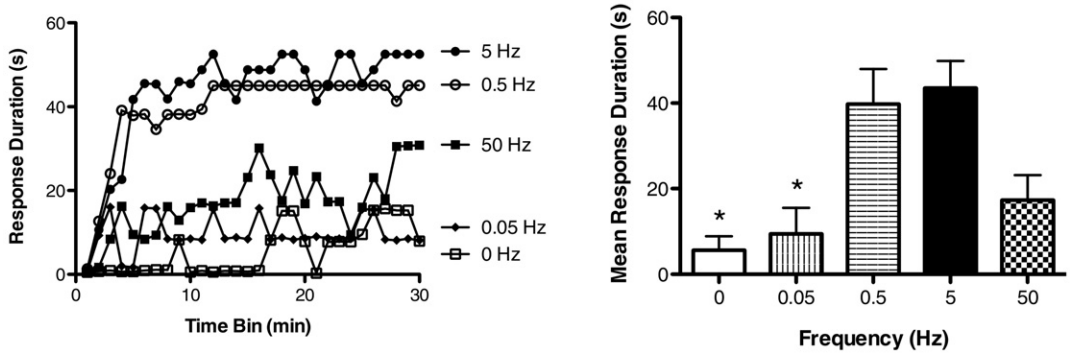

Fig. 3. The effect of stimulation frequency on instrumental performance. Rats received 180 variable space shocks prior to 720 fixed space shocks at 0, 0.05, 0.5, 5, or 50 Hz. Instrumental performance was assessed immediately following sciatic nerve stimulation. The left panel depicts response durations across time, while the right panel illustrates mean response duration collapsed across trials. Asterisks indicate significantly different performance compared to rats in the 0.5 and 5 Hz conditions ($p < 0.05$), and error bars indicate ± SE (n = 8 rats/condition).

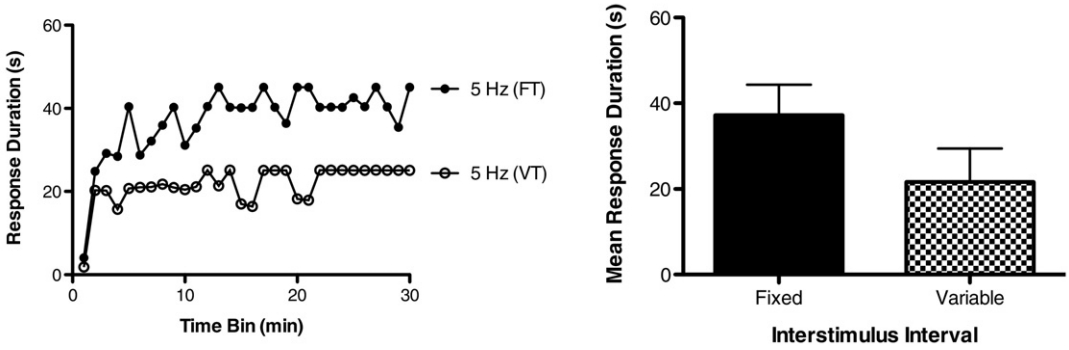

Fig. 4. The ability to differentiate fixed from variable space stimulation at high frequency. Rats received 180 variable space shocks prior to either 720 fixed space shocks at 5 Hz or 720 variable space shocks at an average of 5 Hz. The left panel depicts response duration across time, while the right panel illustrates mean response duration collapsed across time. Error bars indicate ± SE (n = 12 rats/condition).

stimulation given at an average frequency of 0.25 Hz or higher does not induce a learning impairment [22].

4. Discussion

We have previously shown that intermittent nociceptive stimulation can have different effects on spinal function depending upon its temporal distribution; an extended exposure (720+) to shock given in a variable manner induces a form of maladaptive plasticity that inhibits instrumental learning whereas regular stimulation has a restorative effect that enables learning [25,30,31]. Using electrophysiological stimulation of the sciatic nerve, we have explored the stimulus parameters under which variable stimulation inhibits adaptive learning [25]. Using similar procedures, the present paper examined the circumstances that give rise to the restorative effect of FT stimulation.

As expected, VT stimulation alone induced a learning impairment. FT stimulation of the sciatic nerve for 24 min (0.5 Hz, 720 shocks) restored the capacity to learn. This effect was most robust at a shock intensity (20 V) below that which maximally engages C-fiber activity [25]. In contrast, more intense shocks (40 V) are needed to induce a learning impairment. Further, variable stimulation has an adverse effect independent of whether the shock pulses are 20 (1 pulse) or 80 (4 pulses) ms in duration [25]. This is consistent with studies demonstrating that both types of stimuli elicit the release of substance P and our own work linking the development of the learning impairment to this neuropeptide and the activation of C fibers [25,35,40]. Because past work suggested multi-pulse (burst) stimuli may be needed to elicit the release of BDNF [35], and because the restorative effect of FT stimulation has been linked to BDNF, we hypothesized that only longer (80 ms) FT shocks would have a restorative effect. Contrary to our expectations, single and multi-pulse shocks were equally effective. Finally, we showed that FT stimulation has a therapeutic effect when it is applied within a frequency range of 0.5–5 Hz. Importantly, we verified that the restorative effect of 5 Hz stimulation was only observed when shocks were given in a regular manner. Other work has shown that shocks given at a lower (0.25) frequency are also effective [32], implying that the operational range is approximately 0.25–5 Hz. This frequency range falls within the tempo of stepping and, in this way, the findings are consistent with our claim that spinal timing is coupled to the engagement of the CPG that drives locomotor behavior [38,39]. The adverse effects of variable stimulation also emerge at roughly the same (0.25 Hz) frequency, but dissipate at higher (>2.5 Hz) frequencies [25].

The relative effectiveness of FT and VT stimulation across three key parameters (shock number, intensity, and frequency) is illustrated in Fig. 5. This figure shows the mean response duration observed during the 30 min test of instrumental learning. For studies examining the learning impairment after VT shock, we have demonstrated that it emerges after 180 shocks, given at an intensity of 40 V with a mean frequency of 0.25 and 2.5 Hz [25]. For FT shock, the question concerned the restoration of learning. We have shown that this requires 540 regularly spaced shocks, with a minimum intensity of 20 V presented at a frequency between 0.25 and 5 Hz.

It is evident from Fig. 5 that nociceptive stimulation has a negligible effect on spinal function when a small number (36) of shocks are given and/or if they are presented at a low frequency (<0.1 Hz). The only caveat concerns the potential of savings across stimulation sessions; while we have shown that interposing a temporal gap (24 h) does not disrupt the development of the FT restorative effect [25,30], we have not tested whether there is an additive effect of VT stimulation across days. Fig. 5 also shows how FT stimulation can be applied while minimizing the development of maladaptive plasticity. In particular, shocks given at a lower intensity (20 V), and at a higher frequency (5 Hz), should counter nociceptive sensitization with little risk of abetting its development.

The functional implications of our studies are summarized in Fig. 6, which shows how spinal systems gate nociceptive impulses depending upon their temporal distribution and relation to the proprioceptive context. Behavioral control is detected when the onset of a nociceptive stimulus is correlated with a particular behavioral response. We have argued that the latter is indicated by proprioceptive cues related to limb position and movement [2]. The detection of behavioral control inhibits the development of maladaptive plasticity and promotes the performance of responses that minimize net exposure to noxious stimulation. In the absence of behavioral control, an opponent process is engaged that sensitizes nociceptive reactivity and inhibits instrumental learning [2,10,18,22,25,30,31]. With further stimulus exposure, spinal systems can determine whether the stimuli occur in a variable or regular manner. If regularity is detected (a form of temporal predictability), it engages processes that inhibit the induction and maintenance of maladaptive plasticity [24,25,30,31]. Recent work also suggests that regular stimulation can promote the performance of periodic motor behaviors [18,19,41], including stepping [42–47] and tail waving (pendulate tail) [32].

Further work is needed to detail how these spinal processes work, how they interact, and the neurobiological mechanisms involved. In the case of instrumental learning and behavioral control, less is known regarding some key parameters, including the minimum amount of stimulation (shock number and intensity) needed to induce a lasting effect. Past work suggests that optimal learning occurs at intermediate shock intensities that generate a flexion force between 0.4 and 0.6 N [18]. Above and below these values, poor learning is observed. We also know that the lowest shock intensity that generates learning can induce a learning impairment, if given in an uncontrollable manner [18]. These observations suggest that spinally-mediated instrumental learning is driven by C-fiber input.

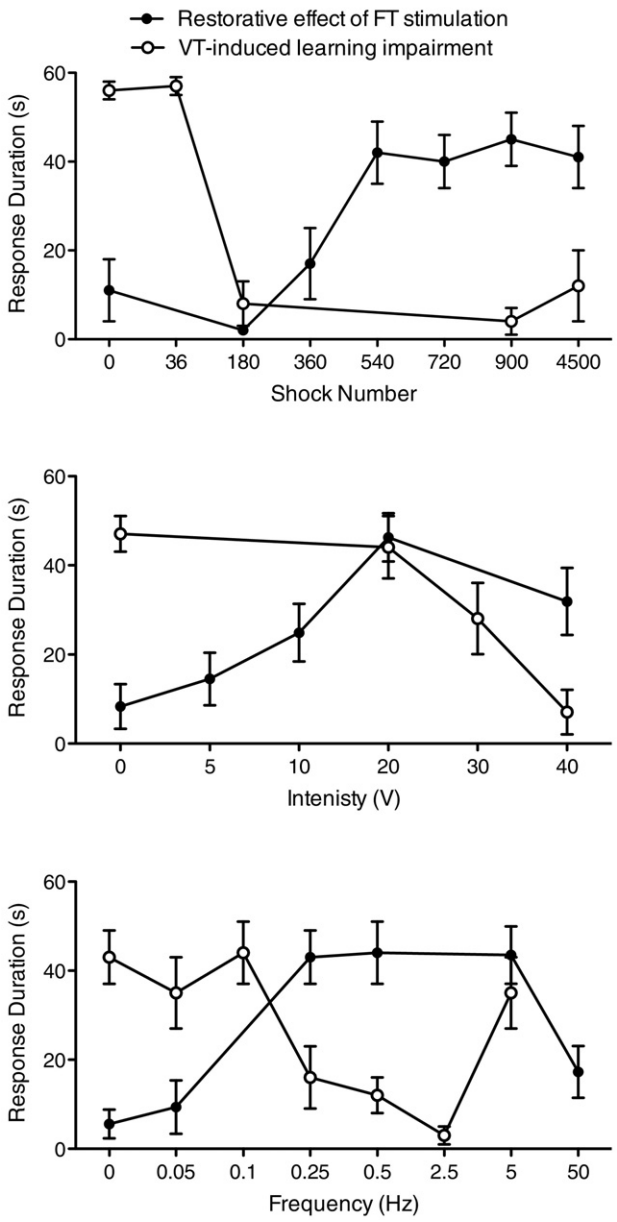

Fig. 5. Summary of how stimulus parameters affect the development of maladaptive plasticity following VT stimulation and the restorative effect of FT stimulation. Each point represents mean response duration (±SE) during testing with response-contingent (controllable) stimulation. Exposure to noxious intermittent shock induces a learning impairment that emerges after 180 shocks, at an intensity of 40 V, and when mean frequency lies within 0.25 and 2.5 Hz. The effect of FT stimulation was derived from studies examining the restoration of learning after the learning deficit had been induced. The induction of a restorative effect requires 540 or more shocks, emerges at a shock intensity of 20 V and is evident when shock frequency lies between 0.25 and 5 Hz. Summary data were derived from the present experiments and prior published work [22,25,30,32,33].

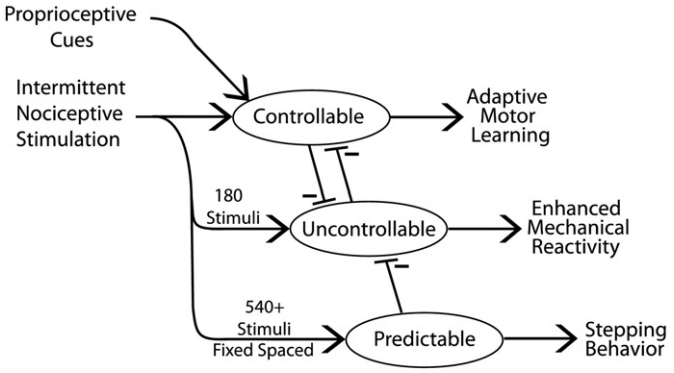

Fig. 6. Functional relations, and eliciting conditions, for controllable, uncontrollable, and predictable stimulation. Controllability is hypothesized to depend upon the relation between proprioceptive signals indicative of position/movement and engages adaptive motor learning that reduces net exposure to noxious stimulation. In the absence of a regular response-shock (outcome) relation (uncontrollable), intermittent nociceptive stimulation induces a form of maladaptive plasticity that inhibits response-outcome learning and sensitizes reactivity to mechanical stimulation. Experience with controllable stimulation can inhibit the induction and maintenance of this effect. Temporal regularity (predictability) is abstracted when nociceptive stimuli are given at regular temporal intervals (fixed spaced). This effect, which requires extended training (540 or more shocks) inhibits the adverse effects of uncontrollable stimulation and promotes oscillatory behavior (e.g., stepping and tail movements).

While both behavioral control and temporal predictability can counter the development of maladaptive plasticity, these processes differ in some key ways, and this may have important clinical implications. As noted previously [2], instrumental learning emerges quickly with very little training (within minutes), which suggest that the learning is biologically prepared [2]. In contrast, learning about the distribution of intermittent nociceptive stimuli requires considerable training (540 + stimuli) and, for this reason, is more likely to have an initial maladaptive effect. Interestingly, while instrumental learning appears to build upon pre-existing stimulus-response (S-R) pathways, learning about temporal relations is surprisingly unconstrained; spinal systems can abstract temporal relations when the site of stimulation is randomly varied, stimuli are randomly omitted, or when bouts of training are separated in time (savings) [32,33]. Learning about time appears to have a "cognitive-like" flexibility that is common for brain-dependent learning but seldom assumed with respect to the spinal cord.

We have shown that variable intermittent shock administered a day after a contusion injury impairs long-term recovery and promotes tissue loss at the site of injury [15,48]. This effect is observed after just 6 min of stimulation (180 shocks). Administering shocks in a controllable manner mitigates this effect [15], presumably because this learning emerges quickly and effectively gates how nociceptive inputs are processed. In the absence of behavioral control, nociceptive stimulation appears to promote hemorrhage, which enlarges the region of secondary injury [49]. Because learning about temporal regularity requires extensive training, and because the initial exposure to FT shock (the first 180–360 stimuli) has the same effect as VT stimulation, we would anticipate that FT stimulation soon after a contusion injury would also induce hemorrhage and promote tissue loss at the site of injury. This observation suggests that temporal regularity may be more clinically relevant during the chronic stage of recovery, when FT stimulation could potentially counter nociceptive sensitization and chronic pain. Further, FT stimulation may promote stepping behavior by initiating oscillatory activity in the locomotor CPG. Indeed, FT stimulation is regularly used to promote stepping behavior [41,45,50], and activation of the locomotor CPG through step-training has been shown to reduce nociceptive sensitization [51]. Recent evidence also indicates that the induction of maladaptive plasticity (through the peripheral application of capsaicin) interferes with locomotor training [52]. These observations suggest that step-training and FT stimulation may have a common effect because both engage a common system (the locomotor CPG) and that maladaptive plasticity interferes with this process. The latter implies that the processes associated with maladaptive plasticity may inhibit those involved in the derivation of regularity. These observations are consistent with our recent finding that a surgical cut at L3, designed to disconnect the neural region that mediates instrumental learning (L3-S2) [53] from the processes (the CPG) that generates the tempo of stepping [33], disrupts learning about stimulus regularity. Subjects that have undergone a L3 cut cannot discriminate variable and fixed spaced stimulation, and as a consequence, both shock schedules induce a learning impairment.

We have also shown that FT and VT stimulation have divergent effects on nociceptive processing. When shock number and intensity is equated, an extended exposure to VT stimulation induces a lasting EMR whereas FT shocks produce hyporeactivity [24]. Further, FT stimulation has been shown to block, and reverse, the EMR induced by capsaicin. These observations are important for two reasons. First, they imply that FT stimulation may be used to attenuate nociceptive sensitization and chronic pain. Second, our work helps to define the stimulus conditions that generate central sensitization. Indeed, the work suggests that this process may develop over a wider range of circumstances, if noxious stimulation occurs in a variable manner. The work also helps to explain why regular stimulation has a non-monotonic effect on nociceptive circuits, inducing a general enhancement (wind-up) early-on, followed by a general reduction in nociceptive reactivity (wind-down) when stimulation is continued. Our results suggest that, if the stimuli are given in an irregular manner, early wind-up will be followed by a lasting nociceptive sensitization [2,10,24].

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