Physiology & Behavior 174 (2017) 1–9

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

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

Kyle M. Baumbauer [a,b,*], Joel D. Turtle [c], James W. Grau [c]

[a] School of Nursing, The Center for Advancing Management of Pain, University of Connecticut, Storrs, CT 06269, United States
[b] Department of Neuroscience, Institute for Systems Genomics, UConn Health, Farmington, CT 06033, United States
[c] Department of Psychology, Texas A&M University, College Station, TX 77843, United States

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.

ARTICLE INFO

Article history:
Received 5 October 2016
Accepted 22 February 2017
Available online 24 February 2017

Keywords:
Plasticity
Spinal cord
Instrumental learning
Timing

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

* Corresponding author at: School of Nursing, The Center for Advancing Management of Pain, University of Connecticut, Storrs, CT 06269, United States.
E-mail address: kyle.baumbauer@uconn.edu (K.M. Baumbauer).

http://dx.doi.org/10.1016/j.physbeh.2017.02.028
0031-9384/

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.