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Integrating multiple sensory systems to modulate neural networks controlling posture

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Short title: Spinal cord tonic reactions in paralyzed rats

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Conflict of Interest: VRE, along with (RRR and YG) researchers on the study team hold share-holder interest in NeuroRecovery Technologies, the company providing the electric stimulator for this study. VRE is also the President and Chairman of the Board for the company. VRE, RRR, and YG hold certain inventorship rights on intellectual property licensed by The Regents of the University of California to NeuroRecovery Technologies and it's subsidiaries.

ABSTRACT

In this study we investigated the ability of sensory input to produce tonic responses in hindlimb muscles to facilitate standing in adult spinal rats and tested two hypotheses: 1) whether the spinal neural networks below a complete spinal cord transection can produce tonic reactions by activating different sensory inputs and 2) whether facilitation of tonic and rhythmic responses via activation of afferents and with spinal cord stimulation could engage similar neuronal mechanisms. We used a dynamically controlled platform to generate vibration while weight bearing, epidural stimulation (at spinal cord level S1), and/or tail pinching to determine the postural control responses that can be generated by the lumbosacral spinal cord. We observed that a combination of platform displacement, epidural stimulation, and pinching the tail produces a cumulative effect that progressively enhances tonic responses in the hindlimbs. Tonic responses produced by epidural stimulation alone during standing were represented mainly by monosynaptic responses, whereas the combination of epidural stimulation and tail pinching during standing or epidural stimulation during stepping on a treadmill facilitated bilaterally both monosynaptic and polysynaptic responses. The results demonstrate that tonic muscle activity after complete spinal cord injury can be facilitated by activation of specific combinations of afferent inputs associated with load-bearing proprioception and cutaneous input in the presence of epidural stimulation and indicate whether activation of tonic or rhythmic responses are generated depends on the specific combinations of sources and types of afferents activated in the hindlimb muscles.

Key words: Spinal cord transection, Postural control, Locomotion, Spinal cord stimulation, Vibration

INTRODUCTION

Spinal neuronal networks related to posture and locomotion can produce different motor patterns even when isolated from supraspinal control. These spinal networks can be activated pharmacologically (Barbeau et al. 1987; Chau et al. 1998; Lavrov et al. 2004; Lavrov et al. 2008a, Gerasimenko et al. 2009), via epidural electrical stimulation (Iwahara et al. 1991; Dimitrijevic et al. 1998; Gerasimenko et al. 2001; Gerasimenko et al. 2003; Ichiyama et al. 2005; Musienko et al. 2010; Nandra et al. 2011), intraspinal stimulation (Bamford et al. 2005; Lavrov et al. 2015), and by manipulation of cutaneous-proprioceptive sensory afferents such as with motor training (Mushahwar et al. 2007).

Although several successful strategies to improve locomotor ability, such as manual training, robotic training, assist-as-needed motor training, and spinal cord epidural stimulation, have been translated from animal models into the clinic to improve recovery in spinal cord injured subjects (Edgerton et al. 2001; Dietz and Harkema 2004), one of the critical limitations in gaining greater independence in daily activities after paralysis is postural control of the trunk. While some postural mechanisms are dependent on supraspinal control (Macpherson et al. 1997; Macpherson and Fung 1999; Deliagina and Orlovsky 2002), the spinal networks also play a major role in dynamically modulating a highly coordinated system from the soles of the feet to the head to achieve an erect posture (Edgerton et al. 2004; Deliagina et al. 2006; Lyalka et al. 2005).

The role of these spinally controlled networks is poorly understood for producing these supportive reactions and in transitioning to and from a more rhythmic vs. tonic activity when isolated from supraspinal influences. Several findings suggest that the spinal cord can produce some simple tonic responses that contribute, at least to some degree, to sustaining body weight support in spinal animals. Adult cats, for example, show some postural control after a complete spinal

cord transection and this ability can be improved by training weight-support function (Fung and Macpherson 1999; de Leon et al. 1998; Tillakaratne et al. 2002; Barbeau et al. 1987; Lovely et al., 1986). Other findings show that cats trained to step cannot stand, whereas cats trained to stand cannot step very well (de Leon et al. 1998). These results support task-specific training effects and emphasize the close interaction between spinal mechanisms responsible for stepping and standing. It is unclear, however, whether the abilities to step and to stand are mediated by the same or different neuronal spinal circuits. Unlike spinal cats (Lovely et al. 1986, 1990), spinal rats recover a high level of locomotor or postural ability only with extensive training combined with other interventions such as epidural stimulation and/or pharmacological modulation that enhance the excitability of the appropriate spinal networks (Ichiyama et al. 2005; Edgerton et al. 2001, Edgerton et al. 2008).

The purpose of this study was to investigate the spinal cord mechanisms controlling postural limb responses in the hindlimbs of adult rats after a complete mid-thoracic spinal cord transection. We tested the following hypotheses: 1) the spinal neural networks below a complete transection can produce tonic reactions that can be facilitated by activating different sources and types of afferents, e.g., platform vibration during weight bearing and pinching the tail and 2) the facilitation of tonic and rhythmic responses via spinal cord epidural stimulation and the activation of afferents engage similar neuronal mechanisms.

METHODS

Six adult female Sprague Dawley rats (270-300 g body weight) were used in this study. The experimental procedures comply with the guidelines of National Institute of Health Guide for the Care and Use of Laboratory Animals and the experiments were conducted in accordance

with a protocol approved by the Animal Care Committee at the University of California, Los Angeles.

Surgical procedures

All surgical procedures were performed under aseptic conditions. Rats were anesthetized deeply using a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) administered i.p. and maintained at a surgical level with supplemental doses of ketamine (10% of the initial dose) as needed.

Spinal cord transection procedures

The spinal cord of each rat was completely transected at a mid-thoracic level as described previously (Talmadge et al. 2002; Roy et al. 1991). Briefly, a skin incision was made between T6 to T10 and a partial laminectomy was performed at ~T8-T9. The dura was opened with microscissors, 2-3 drops of lidocaine (1%) were applied, and the spinal cord was completely transected using microscissors and fine forceps. Two surgeons verified the completeness of the spinal cord transection by lifting the cut ends of the transected cord. Gelfoam was placed at the transection site as an anticoagulant and to separate the cut ends of the spinal cord.

Head connector and EMG implantation procedures

Two amphenol head connectors instrumented with Teflon-coated stainless steel wires (AS632, Cooner Wire) were mounted on the skull as described previously (Roy et al. 1991). Briefly, a small skin incision was made on top of the skull and the connectors were secured firmly to the skull using stainless steel screws and dental cement.

Skin incisions were made bilaterally over the bellies of the medial gastrocnemius (MG) and tibialis anterior (TA) muscles. A pair of wires was routed subcutaneously from the head connector to each of the isolated muscles. Bipolar intramuscular EMG electrodes were implanted into each muscle as described previously (Roy et al. 1991). Briefly, two wires were passed through each muscle using a 23-gauge needle, a small notch of the Teflon-coating (~0.5 to 1.0 mm) was removed from each wire to form the recording electrodes, the electrodes were placed in the mid-belly of the muscle, and each wire was secured at the entrance and exit into the muscle with a suture. The EMG wires then were coiled near each implant site to provide stress relief.

Epidural electrode implantation procedures

A partial laminectomy was performed at the L2 vertebral level to expose the dura. The connective tissue between the T13 and L1 vertebrae was incised and one teflon-coated stainless steel wire was passed under the L1 vertebra to reach the S1 spinal level. A small notch was made in the Teflon-coating (~0.5 to 1.0 mm) of wire to expose the stainless steel, which served as the stimulating electrode. The wire (electrode surface facing the spinal cord) then was affixed to the dura at the midline of the spinal cord (S1) above and below the electrode using 9.0 suture as described previously (Ichiyama et al. 2005). The wire was coiled in the back region to provide stress relief. One wire with ~1 cm of the Teflon exposed at the distal end was inserted subcutaneously in the thoracic region and served as a common ground.

All surgical areas were irrigated liberally with warm, sterile saline and closed in layers, i.e., investing fascia and then the skin. All incision sites were cleaned thoroughly with saline solution. Analgesia was provided by buprenex (0.5–1.0 mg/kg i.m., TID). The analgesics were ini-

tiated prior to completion of the surgery and continued for a minimum of 2 days. The rats were allowed to fully recover from anesthesia in an incubator.

Stimulation and recording

All tests were performed on fully awake animals. Stimulation was performed using a Grass S88 Stimulator (Grass Instruments) through a stimulus isolation unit (Grass SIU5, Grass Instruments). Stimulation at the S1 spinal segment was performed at frequencies of 10, 50, 90, and 130 Hz and with a 0.2 ms pulse duration. EMG signals were recorded (2,000 Hz), amplified, and filtered (10 to 1,000 Hz band-pass).

Standing platform testing

Beginning three weeks post-surgery, the spinal rats were placed in an upper body harness support system to test for postural limb reflexes. Weight support was provided to maintain a stable standing posture on a standing platform. The body harness support system was attached to the sensor to record the disturbance in weight support during postural reactions (recordings are presented in Fig. 2). This standing platform can be utilized to generate any arbitrary trajectory in its working space that allows testing the animal with different disturbances (Liang et al. 2006). The platform employs the "NINJA" configuration (Nagai et al. 2003). Each linkage has two active revolute joints and one passive revolute joint that is perpendicular with the two active ones. DC motors drive the active joints through cable driven speed reducers having very low friction resistance. An 8-axis PCI GalilTM 1800 motion control board controls the DC motors. Two patterns were utilized in this study to induce tonic reactions: a vertical up and down trapezoid form displacement and a fast vertical sinusoidal vibration of the platform Vertical up and down dis-

placement produced periodic loading and unloading (Fig. 1). The position of the hindlimbs before testing was adjusted such that the hindlimbs always had stable contact with the platform. The main parameters of the trapezoidal wave are the amplitude, the cycle period, and the time it takes for the platform to move from a neutral position to the upper or lower limit position. For all tests we used 15 mm amplitude and 0.08 sec duration of loading. After an initial evaluation of the effect of the platform displacement on the EMG responses, 1 Hz frequency was chosen based on its effect to completely fill the gaps between the tonic components (Fig. 2). For the remainder of the study we chose a 0.2 Hz frequency of displacement since it clearly produced the rapid components of the EMG response. Fast vertical vibration of the platform was a 10 Hz sinusoidal vibration with 3 mm amplitude. For some tests during this study, tail pinching was applied manually to facilitate tonic responses. To grade the intensity of the tail pinching we used a moderate intensity that activated tonic responses in both the extensor and flexor muscles but without the appearance of the reciprocal activity in the muscles that occurred with a more intense pinching intensity.

Insert Figure 1 about here

Data analyses

Tonic responses were measured as the mean maximal peak-to-peak amplitudes of ten responses during each platform displacement (at 1, 0.5, and 0.25 Hz) or vibration (at 10 Hz) alone and in combination with epidural stimulation with and without tail pinching. During epidural stimulation spinal cord reflexes were analyzed during the intervals between the stimuli applied at 40 Hz. The recordings were divided into three windows based on the latencies of the

responses as described previously (Lavrov et al. 2006; Gerasimenko et al. 2006; Lavrov et al. 2008b, 2008c), i.e., 5 to 10.5 msec for the middle responses (MRs), 10.5 to 13.5 for the late responses (LRs), and 13.5 to 25 ms for the entire polysynaptic complex of responses (PCs).

Statistical analyses

All data are reported as mean ± SEM. Statistical significance was determined using a paired t-test or a one-way repeated measures analysis of variance (ANOVA). Values that were not distributed normally were analyzed using the nonparametric Kruskal-Wallis ANOVA rank test for overall changes and the Wilcoxon sign-rank test to determine significant differences between groups. The criterion level for determination of statistical significance was set as $P < 0.05$ for all comparisons.

RESULTS

Postural responses to platform vertical displacements

All spinal rats exhibited an initial rapid and prolonged response to the platform's vertical displacements (Fig. 2A). The rapid response was stable across all vertical displacement frequencies, whereas the response duration was more stable with increasing frequencies of displacement. Vertical displacements at 0.25 Hz produced complex responses with the response durations being attenuated over time (Fig. 2A). Displacements at 0.5 Hz produced consistent response durations over time (Fig. 2B), whereas displacements at 1 Hz produced a stable tonic response over the entire testing period (Fig. 2C). Generally, the duration (Fig. 2D) and amplitude (Fig. 2E) of the tonic responses progressively increased with increasing frequencies of platform displace-

ment. The response duration was significantly shorter at 0.5 and 0.25 Hz than at 1 Hz, and shorter at 0.25 than 0.5 Hz ($P< 0.001$, ANOVA). The response amplitude was significantly higher at 1 than 0.25 Hz ($P = 0.03$, Wilcoxon Signed Rank Test).

Insert Figure 2 about here

Postural responses to tail pinching

Each vertical platform displacement induced a response in the extensor muscle (MG). Tail pinching during standing on the platform produced responses in both the MG and TA muscles (Fig. 3A). Moderate intensity tail pinching (during platform displacement at 0.2 Hz) produced a tonic response in both muscles, whereas more intense tail pinching also enhanced a rhythmic response, particularly in the MG. In both cases, tail pinching induced a sustained tonic response. After intense tail pinching the amplitude of the responses in the MG to platform displacements increased. The amplitudes of the tonic responses were significantly higher during platform displacement plus tail pinching than during platform displacement alone in both the MG (Fig. 3B, $P = 0.015$) and TA (Fig. 3C, $P = 0.002$) muscles (Paired t-tests).

Insert Figure 3 about here

Postural responses to spinal cord epidural stimulation

During platform vertical displacement at 0.25 Hz, epidural stimulation of the spinal cord (40 Hz at the S1 spinal segment) produced a tonic response in the MG muscle and a response to the platform displacement in the TA that was not observed without epidural stimulation (Fig.

239 4A). When the epidural stimulation was stopped, the duration of MG response to the platform
240 displacement progressively decreased and the TA response ceased. Epidural stimulation did not
241 significantly affect the amplitude of the responses to the platform displacement (Fig. 4A). To de-
242 termine any frequency-dependent effects of epidural stimulation on the muscle responses, epi-
243 dural stimulation was applied at 10, 50, 90, and 130 Hz during passive standing on the platform
244 (no displacement). A progressive and significant increase in mean amplitudes for both the MG
245 (P= 0.01) and TA (P=0.04) muscles was observed with increasing stimulation frequencies (Fig.
246 4B, Kruskal-Wallis ANOVA rank test).
247
248 **Insert Figure 4 about here**
249
250 *Cumulative effects of a combination of platform vertical displacement, tail pinching, and spinal*
251 *cord epidural stimulation on postural responses*
252 Platform vertical displacement alone (0.2 Hz) resulted in an initial rapid and prolonged
253 response in the MG (Figs. 2A and 5Aa). The addition of epidural stimulation enhanced both the
254 amplitude and duration of the response (Fig. 5Ab). The further addition of tail pinching resulted
255 in more robust response (Fig. 5Ac).
256 Epidural stimulation without tail pinching produced primarily MRs between vertical dis-
257 placements with latencies between 5 to 7 ms (Fig. 5B). When tail pinching and epidural stimula-
258 tion were applied during platform displacement, the pattern of the MRs was similar to that ob-
259 served with epidural stimulation alone (Fig. 5B and C), but polysynaptic complexes (PCs) with
260 latencies between 13.5 to 25 ms were observed (Fig. 5C).
261

Insert Figure 5 about here

Interaction of afferent inputs in producing rhythmic and tonic responses in spinal rats

We compared the pattern of activity induced in the MG and TA by tail pinching with and without platform vibration (10 Hz, 3 mm amplitude) to determine if the same spinal networks are responsible for tonic and rhythmic responses. The combination of platform vibration and intense tail pinching initially produced some rhythmic activity in the MG and TA but quickly transformed to a stable tonic response (Fig. 6A and B). In contrast, the same intensity of tail pinching alone produced consistent reciprocal rhythmic responses in both the MG and TA (Fig. 6A and C).

Insert Figure 6 about here

Intense tail pinching during platform vibration elicited ~5 Hz rhythmic responses in the MG when the hindlimbs were suspended above the platform (unloaded position) (Fig. 7Aa). After making contact with the vibrating platform (loaded position), the rhythmic response was quickly transformed into a tonic response. Following unloading, the tonic response was transformed into a modified rhythmic response. The peak-to-peak maximum amplitudes (Fig. 5B, $P = 0.015$) and durations (Fig. 7C, $P = 0.001$) of the responses in the MG were higher when the hindlimbs were loaded than unloaded (Paired t-tests).

Insert Figure 7 about here

In all tested animals tonic responses evoked in TA and MG muscles were stable and consistent across all tests. Activation of the different sensory inputs, alone or combined, in all cases caused clear increasing of tonic response in case of combination of platform displacement with TP, ES, or TP+ES or immediate shift of rhythmic to the tonic activity in case of combination of ES or TP with platform vibration.

DISCUSSION

The main findings of this study are: 1) vibration during load-bearing, epidural stimulation, and tail pinching individually evoked predictable tonic responses in the hindlimb muscles of spinal rats, 2) combinations of these three afferent stimuli produced larger responses than those induced by each of these sensory inputs alone, and 3) either epidural stimulation or tail pinching induced a rhythmic output whereas their combination with platform vibration consistently resulted in a tonic reaction, suggesting that facilitation of tonic and rhythmic responses share some of the same neuronal mechanisms that occur during platform vibration. These data support the hypothesis that after a complete spinal cord transection whether activation of tonic or rhythmic responses are generated depends on the specific combinations of sources and types of afferents activated in the hindlimb muscles.

In the past, spinal neurons and their interconnections have been primarily evaluated by direct recordings from individual neurons in highly reduced preparations in *in vitro* or *in vivo* anesthetized animals. These approaches cannot be assumed to be studying the same circuits within the spinal cord as occurs in actual movements in awake animals *in vivo* after a chronic spinal cord injury and, thus, provide limited clues related to the integration of the identified structures

within the complex spinal circuitry in an *in vivo* non-anesthetized state. The gaps in our knowledge of the role of different afferent inputs relative to specific motor tasks appear to be one of the main limitations in understanding many aspects of postural control. To fill some of these gap, in this study we monitored the EMG responses during specific postural movements (perturbations) induced by a combination of different sensory inputs using a unique platform design. The present results emphasize the importance for further testing our hypotheses in animal models and human subjects with a complete or incomplete spinal cord injury as well as in intact subjects to evaluate the relevant spinal cord mechanisms.

Role of afferents on the tonic responses in the hindlimb muscles of spinal rats

Afferent input from receptors located in the skin, joints, and muscles provide information to the spinal cord circuits and also contribute to balance control mediated by supraspinal mechanisms (Duysens et al. 2000; Deliagina et al. 2000). For example, information from cutaneous receptors of the feet is important for precise balance control and information from tendon and joint receptors are important in the recognition/perception of joint dynamics (Fitzpatrick and McCloskey 1994; Allum et al. 1998). Muscle receptors also provide complex control of balance via spinal mechanisms for the control of muscle stiffness (Allum and Buedingen, 1979). These previous observations combined with the present data suggest that the collective ensemble of the inputs from all of these sensory receptors that converge on the lumbosacral spinal cord segments generates a precise, comprehensive, and "recognizable" perception reflecting the immediate physiological and mechanical state of the spinal networks controlling the hindquarters during postural and locomotor control.

In the present study, activation of sensory input by vibration of the hindquarters during load bearing produced phasic and tonic responses, particularly in the extensor muscle (MG). In addition, the duration and amplitude of these responses increased as the frequency of displacement was increased from 0.25 to 1 Hz. Both epidural stimulation and tail pinching facilitated the tonic responses to platform displacement. The effects of platform vertical displacement presumably were mediated by activation of cutaneous receptors and proprioceptors. The addition of epidural stimulation facilitated these tonic reactions via modulation of spinal interneuronal networks that not only perceive the limb dynamics, but also define the subsequent motor actions via feedforward strategies**.**

In this study we did not identify the specific afferents being activated but investigated the possibility to facilitate tonic and rhythmic reactions via different stimulation protocols and/or sensory inputs. While future studies should be performed to test the effect of activation of different sensory inputs, the mechanisms involved in this response are not likely to be attributable to a specific type of receptor as much as it will be related to which combination of receptors are activated. Our results demonstrate that the effect of combinations of different sensory inputs may produce similar outcomes, emphasizing the nonspecific character of activation based on integration of multiple sensory systems rather than activation of specific types of receptors.

Spinal mechanisms for the control of tonic responses of the hindlimbs of spinal rats

Immediately after spinal cord transection, the sudden loss of the tonic drive provided by descending pathways results in a loss of muscle tone in the hindlimbs. After an initial inhibition, the monosynaptic spinal cord reflexes usually recover during the first few days post-injury in rats (Valero-Cabre et al. 2004, Lavrov et al. 2006). In contrast, spinal cord polysynaptic reflexes ap-

pear only after long-term recovery, i.e., several weeks after the injury. Similarly, the responses mediated by thick-myelinated afferent fibers (A α and A β) remain active and increase their excitability after spinal cord injury, whereas those conveying stimuli by A δ and C fibers are abolished immediately after the injury and recover only partially over time (Valero-Cabre et al. 2004). We have observed the partial restoration of polysynaptic responses related to flexor-extensor reflexes during a 3 to 6 week period post-transection (Lavrov et al. 2006). Recovery of these spinal cord reflexes after injury, however, cannot provide sufficient support and postural control to achieve independent standing in spinal rats (Dunbar et al. 1986).

Cats with a complete spinal cord transection at a lower thoracic level exhibit very poor postural responses and, as a rule, are not able to maintain the dorsal-side-up orientation of their hindquarters (Macpherson et al. 1997; Macpherson and Fung 1999). Cats with a complete mid-thoracic spinal cord transection, however, can be trained to stand and step with weight support (Lovely et al. 1986; Lovely et al. 1990; de Leon et al. 1998; Barbeau et al. 1987). Compared to cats spinalized as adults, cats spinalized shortly after birth appear to recover better locomotor performance even without training (Grillner 1975; Robinson and Goldberger 1986; Shurrager and Dykman 1951) and are reported to have some residual balance control. In addition, acutely decerebrated cats demonstrate considerable levels of dynamic, bilateral equilibrium sufficient to sustain continuous stepping (Musienko at al. 2014).

While there appears to be some differences in the neural mechanisms of the spinal networks that control postural balance during standing and stepping (Grillner 1975; Rossignol 1996), it seems that there must be some sharing of the same spinal cord circuits. For example, both balance control and stepping are critically dependent on propriospinal pathways. Based on the present data, it can be concluded that the same afferent inputs can induce rhythmic or tonic

responses in the spinal cord below a complete transection. Overall these data demonstrate that tonic reactions can be mediated by 1) mechanoreceptors activated by acceleration induced by platform displacement or vibration, 2) tonic electrical activation of spinal networks that can generate either stepping or tonic extensor responses, and 3) nonspecific tonic reactions induced by tail pinching which generates long-latency responses consistent with the activation of spinal polysynaptic networks as observed during stepping on a treadmill when facilitated by 40 Hz epidural stimulation (Lavrov et al. 2008). Thus, it appears that similar electrophysiological mechanisms of interneuronal network activation occur in the generation of both tonic and rhythmic patterns.

Combination of tonic and rhythmic outputs in the hindlimb muscles of spinal rats

It has been reported that supraspinal mechanisms play a predominant role in the control of posture by the fact that even after recovery of spinal postural reflexes, spinal animals cannot maintain appropriate body weight support and balance control (Dunbar et al. 1986). The present data, however, support the idea that the same neural networks can participate in the formation of two different motor patterns in spinal rats, i.e., tonic and rhythmic. The rhythmic activity produced by tail pinching during suspension becomes a tonic response when the hindlimbs are placed on a vibrating platform, indicating that the same neural networks are active but produce different responses based on the combination of sources of afferent information. In addition, depending on the stimulating parameters used, epidural stimulation can generate a predominantly tonic reaction that transforms to a rhythmic output when the treadmill is turned on or when the stimulation strength is increased. These findings suggest that whether a tonic or rhythmic output is generated depends on the source of afferent input and/or the level of activation of a given population of interneurons. It is equally clear, however, that the mechanisms underling the rhythmic

response to tail pinching depend on the physiological state of the networks being activated, e.g., differences in the level of excitation/inhibition of flexor vs. extensor interneurons projecting to a motor pool and whether the hindlimbs are load bearing.

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Figure Legends

Figure 1. (A) Schematic diagram of standing platform: (a) – movable platform; (b) linkages; (c) motors and encoders. (B) Schematic diagram of body weight support system combined with the standing platform. (C) Diagram of the vertical movement platform paradigm: Amp amplitude; T, cycle period; t, the time it takes for the platform to move from a neutral position to the upper or lower limit position.

Figure 2. The effects of platform vertical displacement at 0.25 (A), 0.5 (B), and 1 (C) Hz on muscle activity and weight support in spinal rats. The mean (±SEM) duration and amplitude of the tonic responses at 0.25, 0.5, and 1 Hz are depicted in (D) and (E), respectively. *, +: significantly different from 1.0 and 0.5 Hz, respectively. MG, medial gastrocnemius; TA, tibialis anterior; WS, weight support; Plat, platform vertical displacement. Amplitude scale bars in (C) also apply to (A) and (B).

Figure 3. The effects of tail pinching (TP) on the tonic responses during platform vertical displacements (Plat-Dis) at 0.2 Hz in spinal rats are shown in (A). The mean (±SEM) amplitudes of the tonic responses in the MG and TA muscles during 0.2 Hz vertical displacements are shown

in (B) and (C), respectively. *, significantly different from Plat-Dis alone. Abbreviations, same as in Figure 2.

Figure 4. The effects of epidural stimulation (ES) on the EMG activity of the MG and TA during platform vertical displacements (Plat-Dis) at 0.25 Hz in spinal rats are shown in (A). The effects of ES applied at 10, 50, 90, and 130 Hz on the mean (±SEM) amplitude of the responses during standing without platform vertical displacements are shown in (B). *, +, ‡: significantly different from 10, 50, and 90 Hz stimulation, respectively, for both muscles. Abbreviations, same as in Figure 2.

Figure 5. The effects of platform displacement (Plat-Dis) at 0.2 Hz (Aa), plus epidural stimulation (Stim) (Ab), plus tail pinching (TP) (Ac) on the responses in the MG muscle are shown. Epidural stimulation alone during standing produced mainly monosynaptic responses (MRs) (B), whereas the combination of Stim and TP produced both MRs and polysynaptic responses (PCs), (C). Abbreviations, same as in Figure 2.

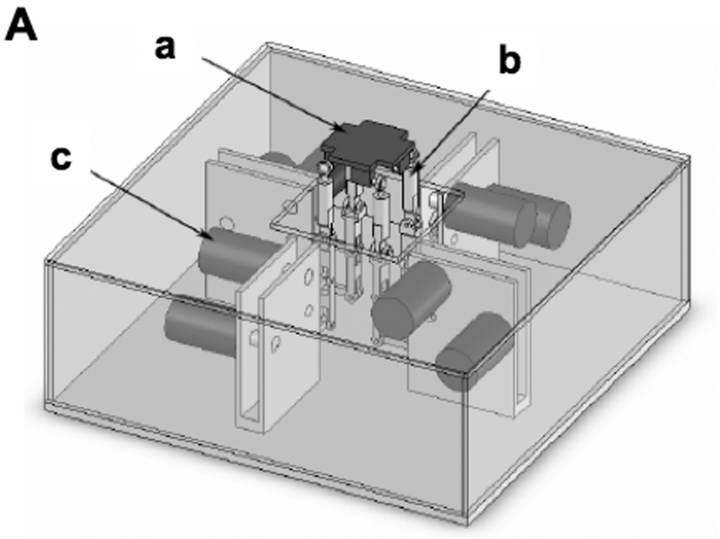
Figure 6. The effects of tail pinching (TP) alone and the combination of TP and platform vibration (Plat-Vib) (10 Hz, 3 mm amplitude) on the responses generated in the MG and TA are shown in (A). The combination of TP and vibration produced a tonic response (A and B), whereas TP alone produced rhythmic responses (A and C). Abbreviations, same as in Figure. 2.

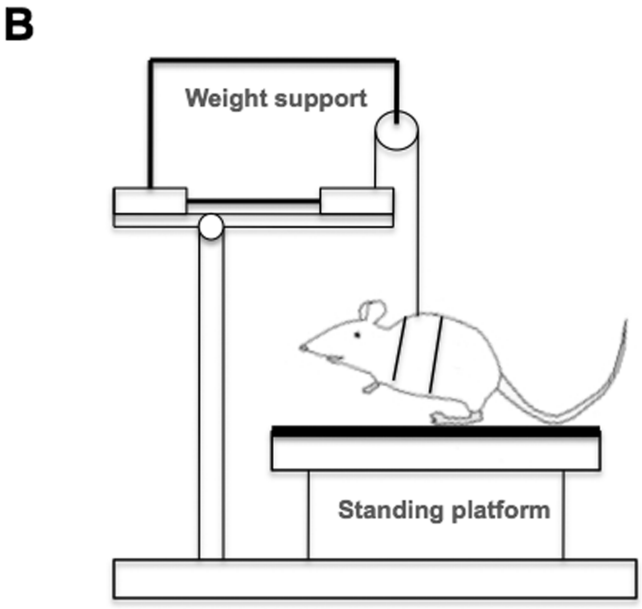
Figure 7. The effects of unloading (a) and loading (b) the hindlimbs of spinal rats on the platform during tail pinching and platform vibration (Plat-Vib) at 10 Hz on the EMG responses in

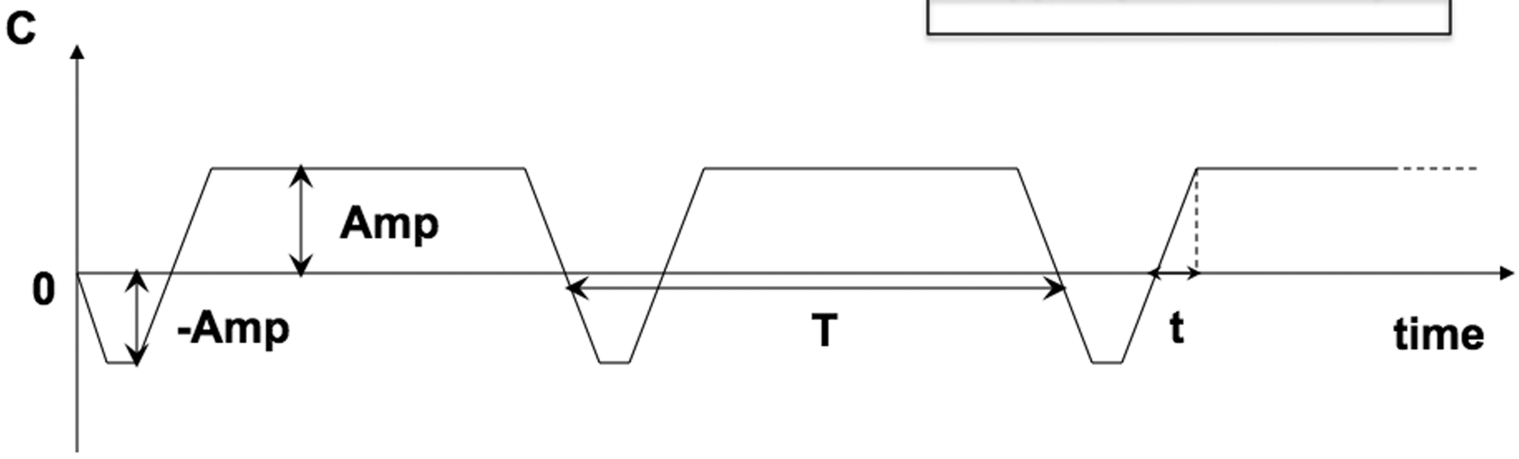
582 the MG are shown in (A). The peak-to-peak maximum amplitude and duration of the EMG re-

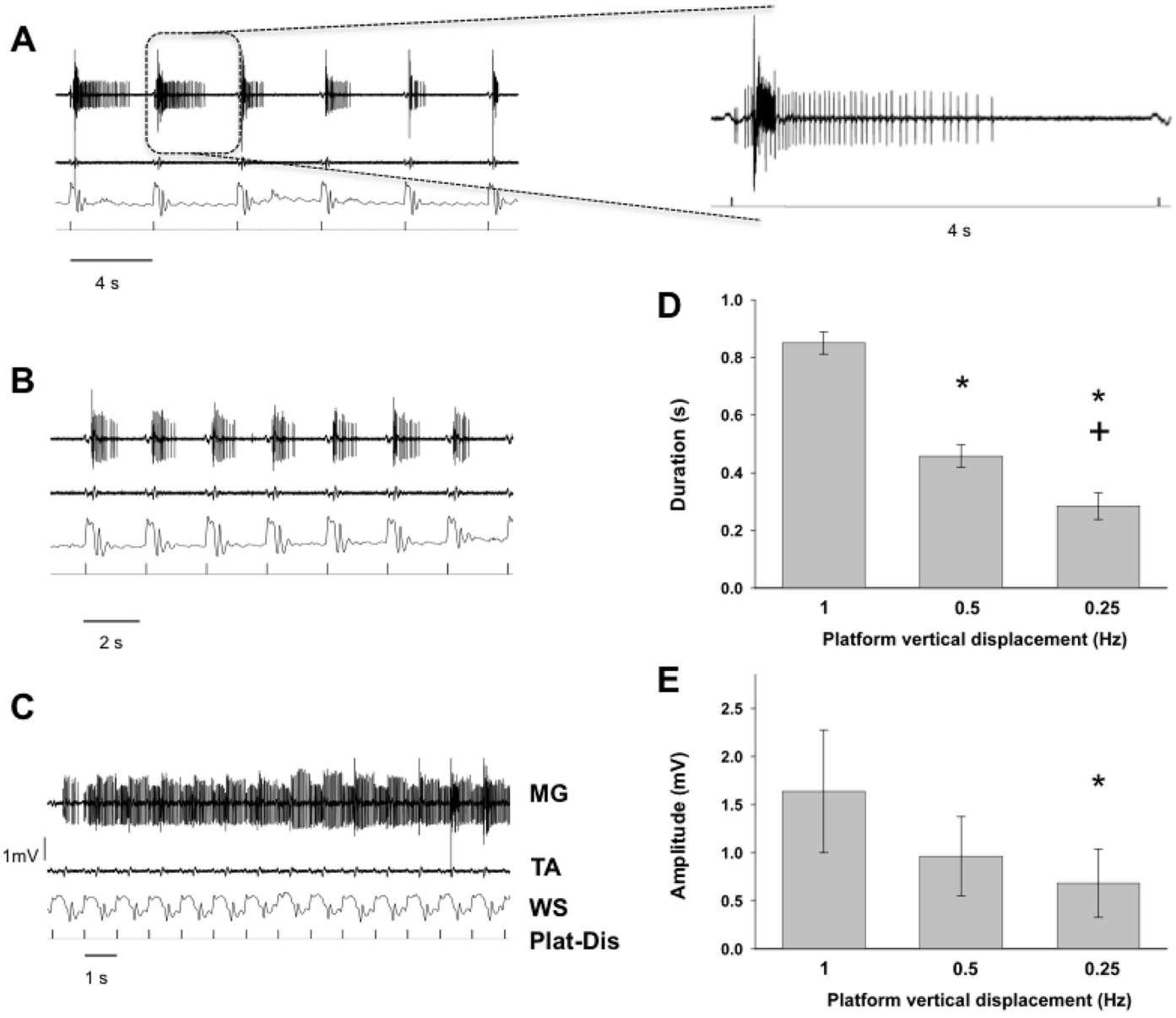
583 sponses of the MG in the unloaded and loaded positions are shown in (B and C).

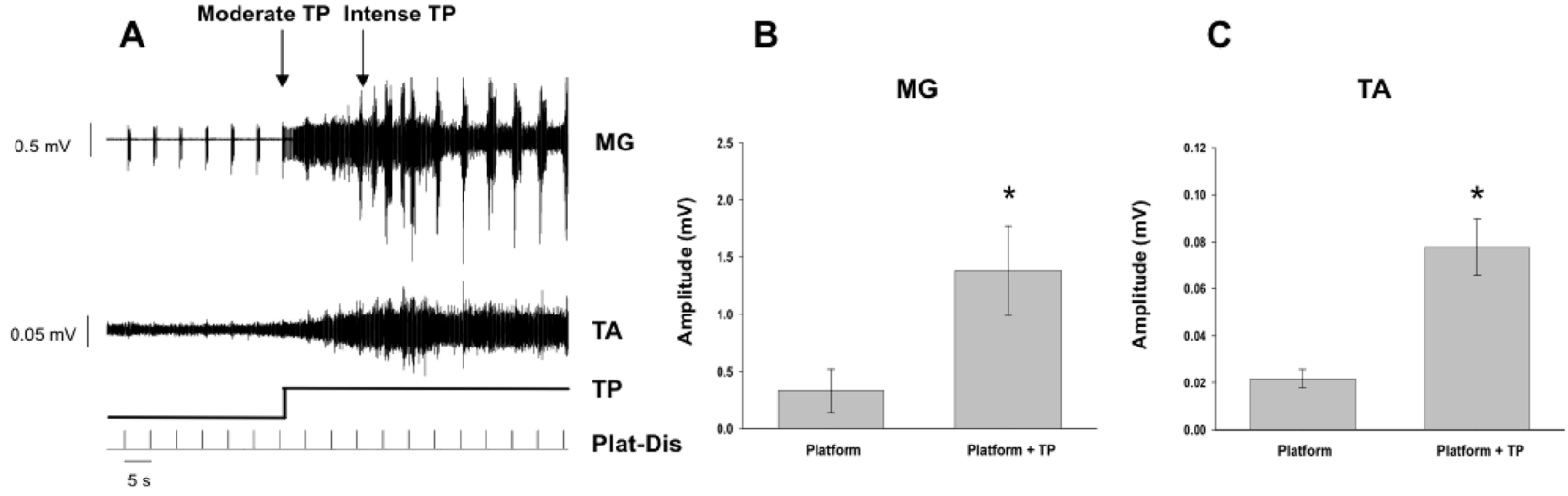
584

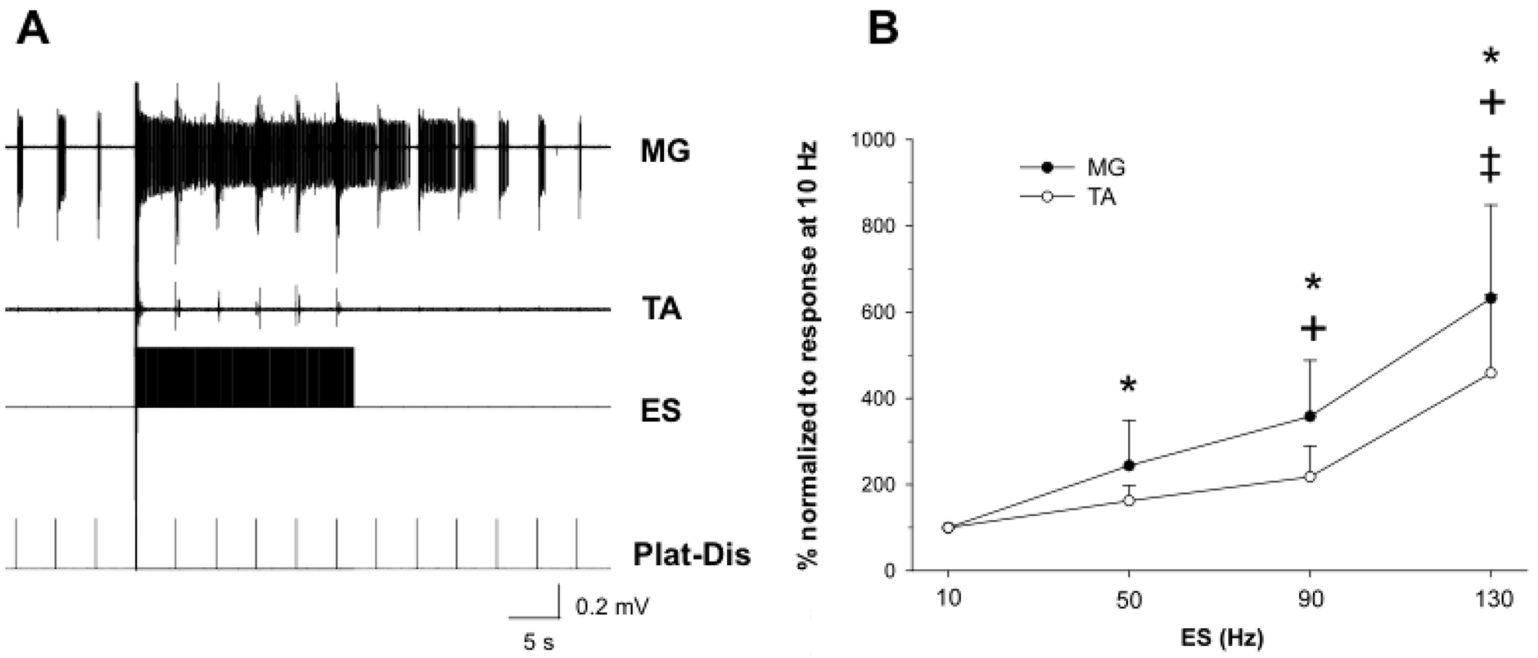

A
a
b
c

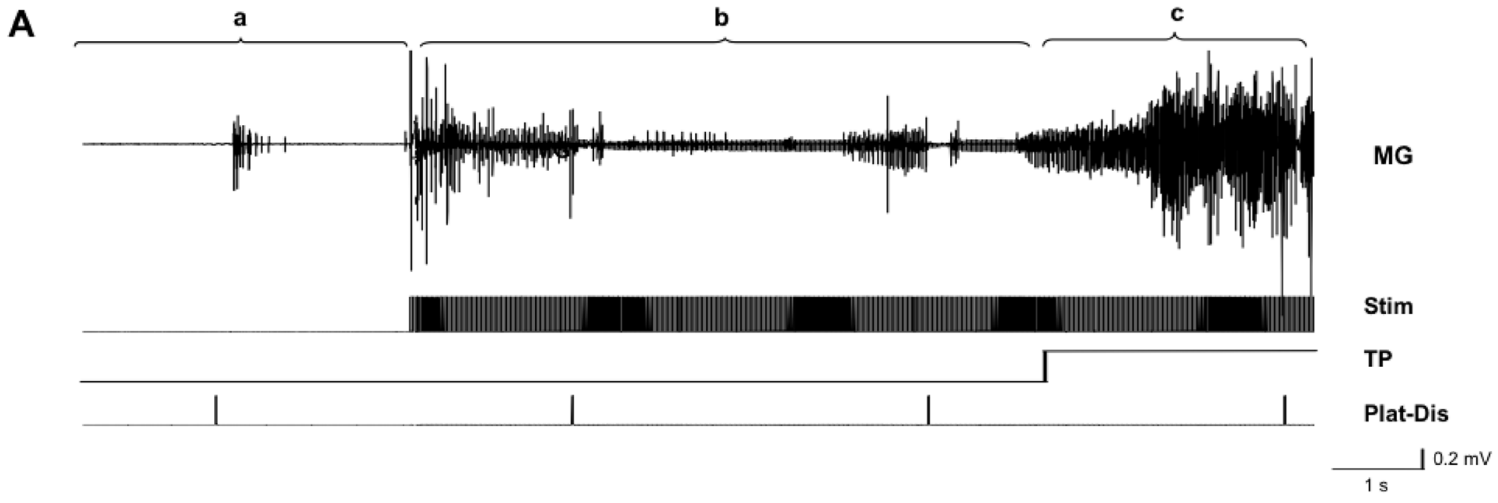

B
Weight support
Standing platform

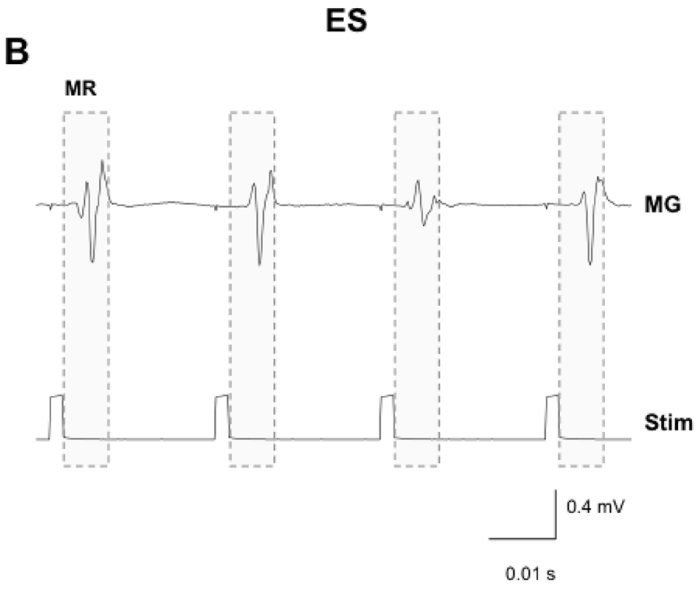

C
Amp
0
-Amp
T
t
time

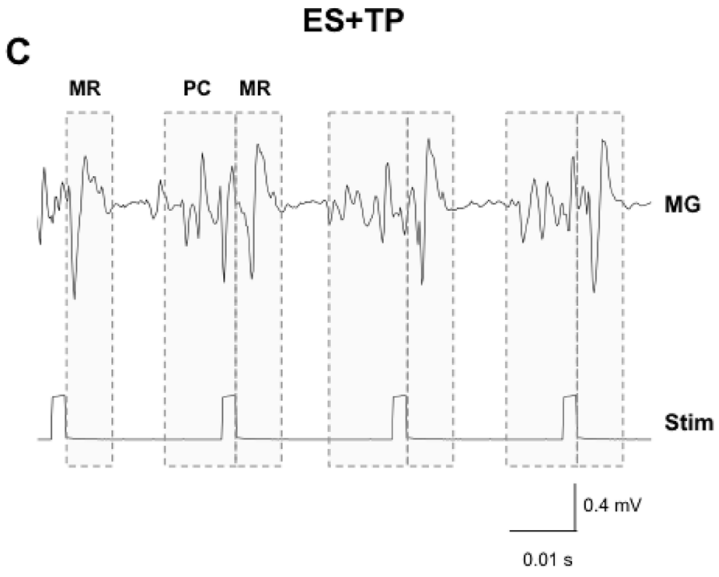

A
4 s
4 s
B
2 s
C
1mV
MG
TA
WS
Plat-Dis
1 s
D
Duration (s)
1.0
0.8
0.6
0.4
0.2
0.0
*
*
+
1
0.5
0.25
Platform vertical displacement (Hz)
E
Amplitude (mV)
2.5
2.0
1.5
1.0
0.5
0.0
*
1
0.5
0.25
Platform vertical displacement (Hz)

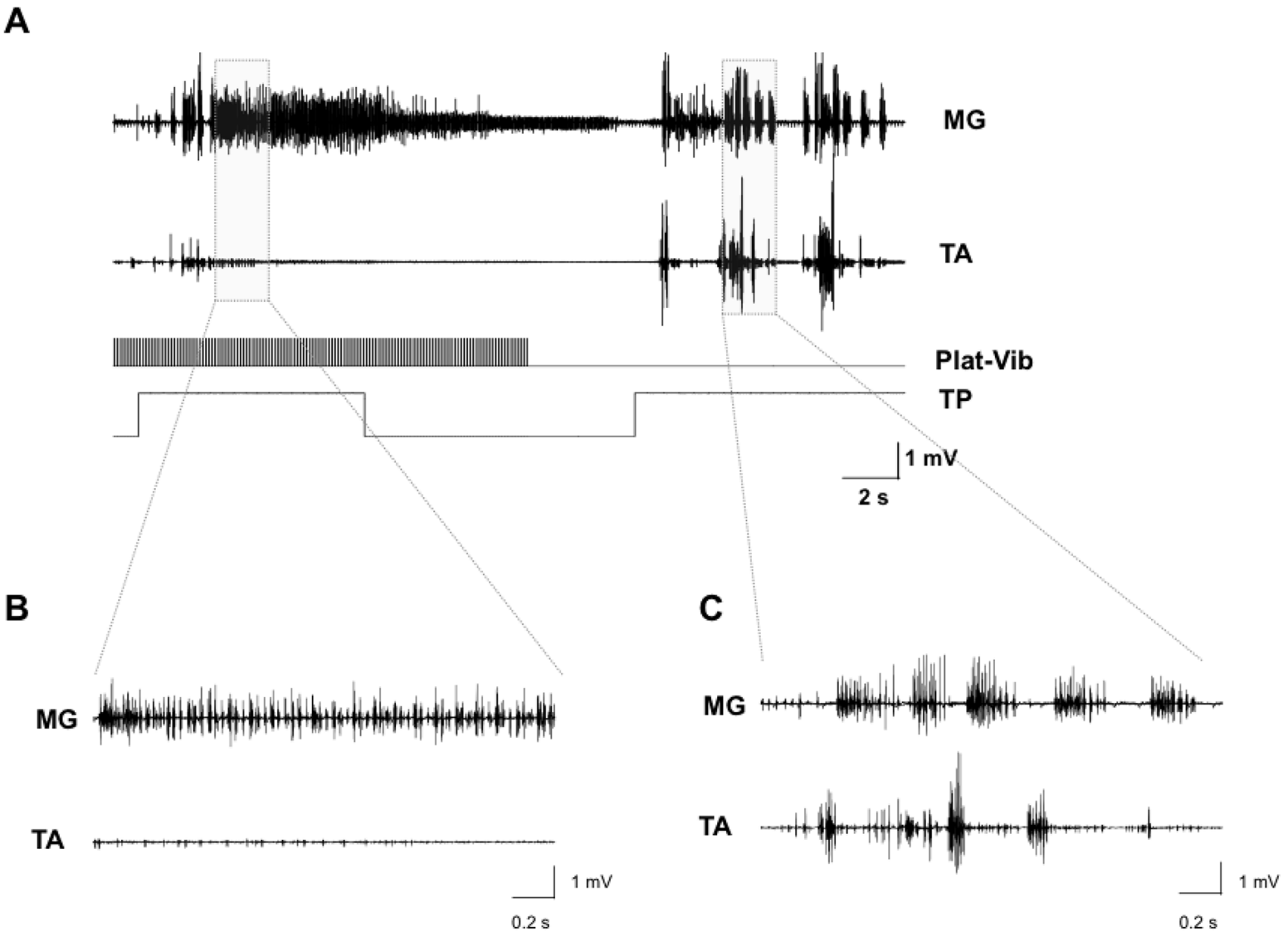

A
Moderate TP
Intense TP
0.5 mV
MG
0.05 mV
TA
TP
Plat-Dis
5 s
B
MG
Amplitude (mV)
2.5
2.0
1.5
1.0
0.5
0.0
*
Platform
Platform + TP
C
TA
Amplitude (mV)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
*
Platform
Platform + TP

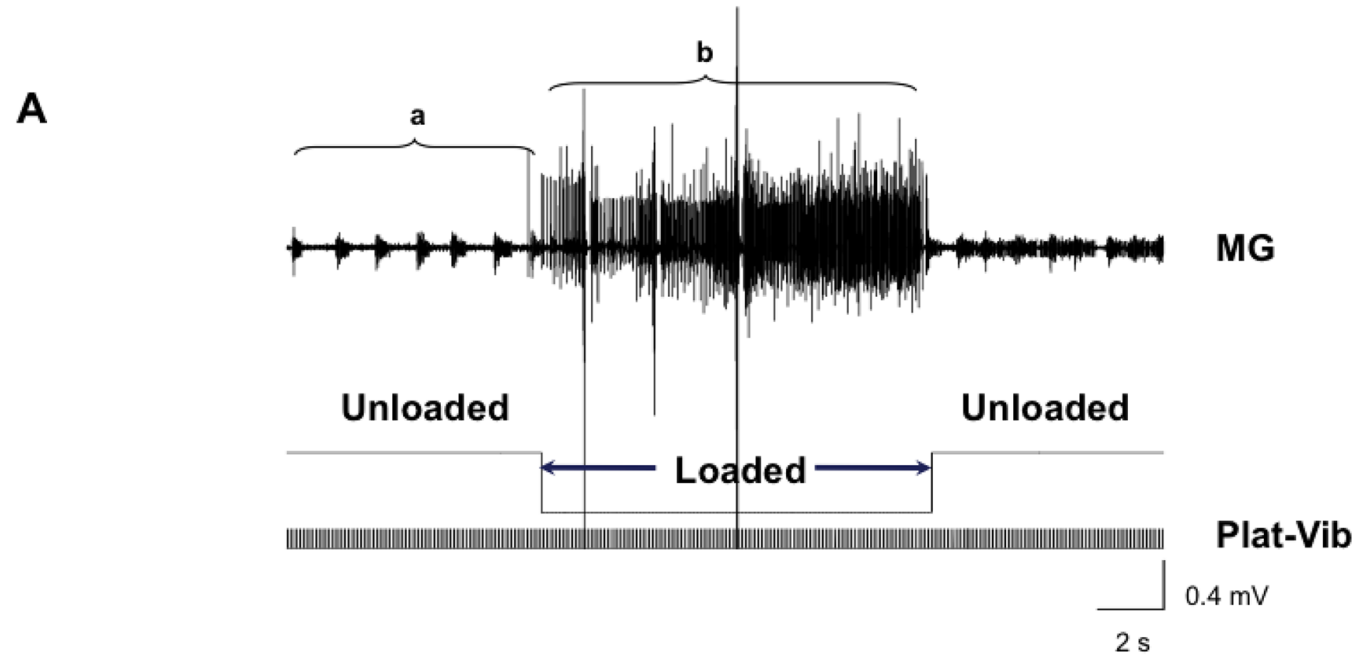

A
MG
TA
ES
Plat-Dis
0.2 mV
5 s
B
% normalized to response at 10 Hz
1000
800
600
400
200
0
MG
TA
*
*
+
*
+
‡
10
50
90
130
ES (Hz)

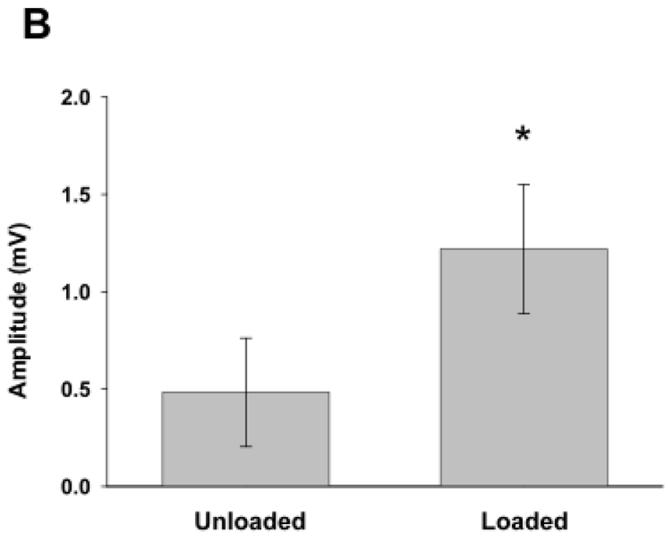

A
a
b
c
MG
Stim
TP
Plat-Dis
0.2 mV
1 s

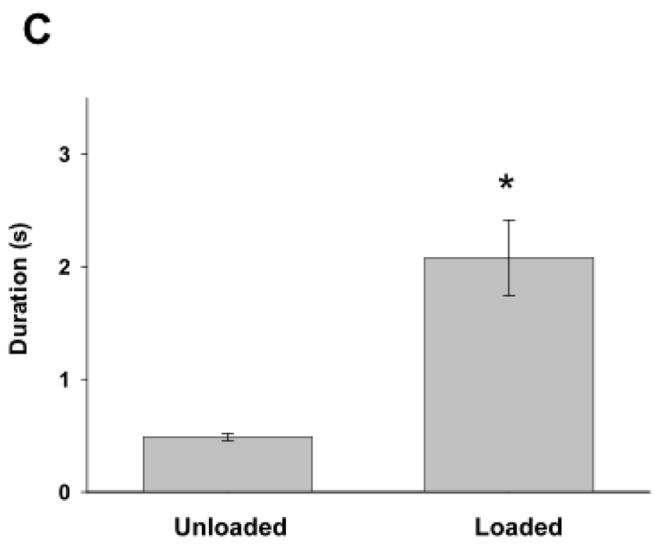

ES
B
MR
MG
Stim
0.4 mV
0.01 s


ES+TP
C
MR
PC
MR
MG
Stim
0.4 mV
0.01 s


A
MG
TA
Plat-Vib
TP
1 mV
2 s
B
MG
TA
1 mV
0.2 s
C
MG
TA
1 mV
0.2 s


A
a
b
MG
Unloaded
Unloaded
Loaded
Plat-Vib
0.4 mV
2 s


B
Amplitude (mV)
2.0
1.5
1.0
0.5
0.0
*
Unloaded
Loaded


C
Duration (s)
3
2
1
0
*
Unloaded
Loaded