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DRG microstimulation evokes postural responses in awake, standing felines

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Abstract

Objective.—We have demonstrated previously that microstimulation in the dorsal root ganglia (DRG) can selectively evoke activity in primary afferent neurons in anesthetized cats. This study describes the results of experiments focused on characterizing the postural effects of DRG microstimulation in awake cats during quiet standing.

Approach.—To understand the parameters of stimulation that can affect these postural shifts, we measured changes in ground reaction forces (GRF) while varying stimulation location and amplitude. Four animals were chronically implanted at the L6 and L7 DRG with penetrating multichannel microelectrode arrays. During each week of testing, we identified electrode channels that recruited primary afferent neurons with fast (80–120 m s^{-1}) and medium (30–75 m s^{-1}) conduction velocities, and selected one channel to deliver current-controlled biphasic stimulation trains during quiet standing.

Main results.—Postural responses were identified by changes in GRFs and were characterized based on their magnitude and latency. During DRG microstimulation, animals did not exhibit obvious signs of distress or discomfort, which could be indicative of pain or aversion to a noxious sensation. Across 56 total weeks, 13 electrode channels evoked behavioral responses, as detected

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by a significant change in GRF. Stimulation amplitude modulated the magnitude of the GRF responses for these 13 channels ($p < 0.001$). It was not possible to predict whether or not an electrode would drive a behavioral response based on information including conduction velocity, recruitment threshold, or the DRG in which it resided.

Significance.—The distinct and repeatable effects on the postural response to low amplitude (<40 μA) DRG microstimulation support that this technique may be an effective way to restore somatosensory feedback after neurological injuries such as amputation.

Keywords

neuroprosthetics; somatosensory; dorsal root ganglia

Introduction

People that use myoelectric and body-powered prostheses often cite dissatisfaction with the lack of sensation afforded by these devices [1]. Tactile and proprioceptive feedback are required for many aspects of sensorimotor control, including manipulation of objects and coordination of limb movements. Deficiencies in somatosensory feedback limit the utility of prosthetic limbs, requiring visual feedback during motor tasks. Reduced sensory feedback (Rossini *et al* 2011) and concomitant altered sense of agency (Ehrsson *et al* 2008) also cause amputees to report an uncomfortable foreignness when operating their devices (Smurr *et al* 2008). These challenges may reduce how often amputees use their prostheses to complete day-to-day activities, (Rossini *et al* 2011) which frequently leads to device abandonment (Datta *et al* 2004).

The aforementioned limitations also apply to the next generation of advanced prostheses currently in development. Despite innovations in robotics that have yielded anthropomorphic upper-limb prosthetic limbs with many powered degrees of freedom, such as the Luke Arm (DEKA Corp.) and the Modular Prosthetic Limb (Johns Hopkins University Applied Physics Laboratory), body-powered systems provide superior sensory awareness through their body-activated cable systems [2]. As such, these body-powered prostheses are often preferred over advanced prostheses [3]. Additionally, users of lower-limb prosthetics face challenges related to the lack of sensory feedback, including an increased rate of falls and abnormal patterns of muscle activity during gait [4–11]. Ultimately, without somatosensory feedback, even the most advanced prostheses will remain extracorporeal tools with limited functionality, rather than fully integrated functional limbs.

Most studies that have evaluated somatosensory neuroprostheses have focused on evoking tactile sensations [12–18]. While some of these studies have reported evoked proprioceptive sensations [19, 20], the origin of these sensations and the ability to independently control them remains uncertain. As most somatosensory neuroprostheses rely on electrical stimulation of mixed nerves to evoke sensations [19, 21, 22], it is unclear whether any evoked proprioceptive sensations result from direct activation of sensory afferents or are an indirect result of efferent activation giving rise to kinesthesic percepts from muscle contractions. If these proprioceptive sensations do arise from efferent activation, it may

be difficult or impossible to restore them in cases of high-level amputations with limited residual muscle.

Our lab has shown that microstimulation of the dorsal root ganglia (DRG) can elicit selective recruitment of somatosensory afferent fibers in both acute and chronic studies with anesthetized felines [23–25]. Importantly, these studies have demonstrated that approximately half of the evoked responses have conduction velocities consistent with Group I proprioceptive afferents (i.e. 80–120 m s^{-1}) [26]. These results suggest that the DRG may be a viable neural target to restore sensory feedback, and particularly kinesthesia, in people with peripheral nerve injury or limb amputation. As these studies were all performed in anesthetized animals, it remains unclear whether DRG microstimulation can convey physiologically meaningful and task-relevant information that will result in downstream effects on motor control, such as postural corrections.

The primary aim of this study was to extend our previous results into a behavioral paradigm to investigate whether low amplitude DRG microstimulation drives behavioral responses in awake, behaving felines. We hypothesized that microstimulation would activate proprioceptive afferents to repeatedly and reliably evoke postural shifts during quiet standing and that stimulation amplitude would modulate the extent of the behavioral response. In a companion paper, we examine the reflexively evoked motor responses during DRG microstimulation during the same experimental setup to understand the neurophysiological origins of the evoked postural responses [27].

Methods

Experiment overview

Four adult male cats were trained to stand quietly on a platform while equally distributing their weight across force-sensing pegs located under each leg. Once the cats were proficient at this task, we performed an aseptic surgical procedure to implant 32-channel microelectrode arrays at the left L6 and L7 DRG. These DRG have been shown to contain dermatomes for the distal leg [28, 29]. A five-pole nerve cuff was wrapped around the left sciatic nerve. GRFs were monitored to characterize hindlimb responses to DRG microstimulation. A fifth cat underwent surgical procedures, although because of challenges with array insertion in the DRG, there were only evoked electroneurographic (ENG) responses from a limited number of stimulating electrode channels ($N = 7$), and none of those channels ever evoked a behavioral response to stimulation. As such, data from that animal are not reported here. All experiments were performed under the approval of the University of Pittsburgh Institutional Animal Care and Use Committee and the US Army Animal Care and Use Review Office.

Surgical instrumentation and implantation

Anesthesia was induced with a ketamine/acepromazine cocktail and maintained via inhaled isoflurane (1%–2%). Vital signs including heart rate, core temperature, SpO_2, and $ETCO_2$, were monitored throughout the procedure.

The left hindlimb and back of each animal were shaved and cleansed with iodine. An incision was made along the mid-line of the back and the paraspinal muscles overlying the dorsal and transverse processes of the lumbar vertebrae were resected. A laminectomy was performed to expose the DRG at the left 6th and 7th lumbar segments. Floating Microelectrode Arrays (FMAs, MicroProbes for Life Science, Gaithersburg, MD) were inserted into the L6 and L7 DRG of cats F, G, and H and the L7 DRG of cat I. The L6 DRG of cat I was implanted with a 32-channel Utah Electrode Array (UEA, Blackrock Microsystems, Inc., Salt Lake City, UT). A high-speed pneumatic inserter was used for all arrays. The FMAs contained 32 stimulating electrode channels that varied in length from 0.7 to 1.5 mm with a pitch of 400 μm. Each FMA contained an additional 2 reference and two ground electrodes (3.0 mm length) placed at the corners. The UEA contained 32 electrode channels in a 4 × 8 grid and were 1.5 mm long with a pitch of 400 μm. A stainless-steel wire was inserted into a hole in the iliac crest and secured with a bone screw to act as an additional ground electrode and the return electrode for stimulation.

Electroneurogram (ENG) signals were recorded from the left sciatic nerve using a five-contact nerve cuff (Ardiem Medical, East Indiana, PA). The cuff was 40 mm long with neighboring contacts separated by 4 mm. Signals were recorded in a virtual tripole configuration from the second and fourth contacts, using the first, third, and fifth contacts as a common reference.

Leads for all implanted electrodes were tunneled percutaneously to an external backpack. The backpack was attached to a custom titanium base that was sutured to dorsal fascia and the iliac crests. The backpack housed a custom printed circuit board to which all of the electrode leads were attached. A header on the top side of the board was used to establish a connection between the implanted electrodes and the external recording and stimulation equipment during anesthetized and behavioral experiments. A lid was used to cover the backpack outside of experiments.

Anesthetized experiments and channel selection

Anesthetized experiments were performed weekly, with the goal of identifying the threshold and conduction velocities of recruited afferents. During each experiment, stimulation was delivered on each channel and antidromic propagation of compound action potentials (CAPs) along the sciatic nerve was recorded. At the beginning of each session, anesthesia was induced and maintained by intramuscular injection of dexmedetomidine (0.04 mg kg^{-1}), and vital signs were monitored for the duration of the experiment. In a subset of cats (cats G, H, I), isoflurane (1%–2%) was used to maintain anesthesia throughout the experiment and vital signs were monitored continuously. At the conclusion of each experiment, atipamezole (0.4 mg kg^{-1}) was administered to reverse anesthesia.

ENG signals were recorded from the sciatic nerve cuff via a Grapevine Neural Interface Processor (Ripple, Salt Lake City, Utah), using a differential headstage (Surf-D) with an input range of 5 mV and a resolution of 0.2 μV. Digitization was performed directly on the headstage at 30 kHz. Stimulation was performed using two IZ2 16-channel stimulus isolators (TDT, Alachua, FL) and custom LabVIEW software in cats F, G, and H, or Nano 2+Stim 32-channel headstages (Ripple, LLC) for cat I. Stimulation artifacts were blanked

in software using a 1 ms window, which did not interfere with our ability to measure conduction velocities up to 120 m s^{-1}. Following blanking, ENG data were high-pass filtered at 300 Hz (2nd order Butterworth filter) and a binary search was used to determine the threshold amplitude for evoking a response in the sciatic nerve through stimulation on each electrode channel in the DRG. Additional details of the binary search procedure can be found in [25].

Of the DRG electrode channels that evoked ENG responses each week, a single channel was chosen for stimulation during behavioral experiments based on a combination of conduction velocity and threshold for evoking an ENG response. As the goal of this experiment was to recruit Group I proprioceptive afferents, channels that evoked responses with conduction velocities exceeding 80 m s^{-1} were preferentially selected for behavioral stimulation [26]. In order to achieve a selective response limited to a small population of afferents, channels that demonstrated a low recruitment threshold (generally below 20 μA) were preferentially chosen. Finally, a preference was given to unexplored channels. In some animals, a given channel was selected for exploration more than once when it met recruitment criteria and there were limited viable channels remaining. Electrodes were only reselected when more than four weeks had lapsed since its previous exploration, or when it produced different conduction velocities and/or recruitment thresholds. Afferent recruitment via DRG microstimulation has been shown to be stable over the duration of a few weeks, after which changes in recruitment properties occur [23]. Because of this, the instances in which channels were re-selected were treated as independent observations, targeting different afferent fibers.

Behavioral apparatus, task, and training

Following the recruitment threshold testing and DRG electrode channel selection at the beginning of each week, we examined the change in GRFs elicited by two different stimulation amplitudes. To evoke a behavioral response while maintaining focal activation of a small population of afferents, stimulation amplitudes were titrated within the range of 1.0–3.0 times the recruitment threshold, corresponding to a range between 6 and 60 μA. Recruitment thresholds were measured on the first day of behavioral experimentation each week. We use the terms ‘low’ and ‘high’ amplitude stimulation to distinguish between the two amplitudes of stimulation that were tested each week. Initially, we defined these to be 1.5 and 2.0 times the recruitment threshold, for the low and high amplitude conditions, respectively. Once several stimulation trials had been observed, stimulation amplitudes were titrated. If the animal demonstrated an overt reaction to stimulation (e.g. looking at the hindlimb), amplitudes were reduced. If there was no observable change in GRF, the stimulation amplitudes were increased. Amplitude was never increased above 3.0 times the stimulus threshold. Stimuli were balanced, biphasic pulses, with a 200 μs cathodic phase and a 400 μs anodic phase, applied at a frequency of 100 Hz with a train duration of 100 ms. This train duration was used to mimic the transient, sensory responses evoked by postural perturbations applied in previous studies [30].

Cats were trained to stand quietly on a platform with four instrumented pegs that measured GRFs (figure 1). A six-axis load cell in the peg supporting the implanted leg measured

horizontal and vertical GRFs and moments. Single-axis load cells in the pegs supporting the other legs measured vertical GRF. Force signals were sampled at 1 kHz, filtered using a second order, low-pass Butterworth filter with a cutoff frequency of 50 Hz, and recorded via a National Instruments USB-6251 data acquisition unit (National Instruments, Austin, TX). For analysis, the baseline force level within a 5 ms window just prior to stimulation was subtracted from each trial to adjust for slight postural deviations.

Before stimulation was delivered, the cat was required to maintain an approximately even weight distribution (50% ± 30%) between left and right and front and back limbs for a randomized duration (1–3 s). Following the stimulation command signal, 3.5 s elapsed before a success tone signaled the end of the trial, and a food reward was dispensed through an automated pumping system. This complete sequence was repeated for the duration of each testing session, interspersed with other trials that included movement of the support surface and catch trials that prevented the animal from predicting the onset of stimulation. Stimulation-only trials accounted for approximately 16.7% of the 100–150 total trials each day. Before surgical instrumentation and commencement of stimulation experiments, cats underwent daily training until they were able to consistently perform at least 100 trials in a single experimental session.

Data collection and processing

To characterize and compare the kinetic responses to stimulation across animals and stimulation electrodes, we measured the amplitude and latency of the first peak of each component of the GRF signal for the left hindlimb, as well as the weight distributions between front and back or left and right limbs for each trial.

Occasionally, erroneous movements, such as minor postural adjustments, occurred during a trial. These movements were not representative of the animal's response to stimulation and were thus removed in order to improve accuracy. These signals were identified by their deviation from the within-week, trial-averaged response to stimulation. The mean and standard deviation for the vertical GRF under the implanted limb were calculated at each point in the signal and trials were removed if any point exceeded 2.5 standard deviations from the mean at that point. This method resulted in 12.06% ± 10.98% ($\mu \pm \sigma$) aberrant trials rejected across all experiment weeks. Additionally, three weeks of data were excluded because there were only five or fewer high or low amplitude stimulation trials, which made it impossible to accurately characterize the response to stimulation.

Statistics

The main goal of this project was to explore the effects of low-amplitude DRG microstimulation on posture during quiet standing and to explore the effects of stimulation amplitude on the evoked postural responses. To characterize the relationship between stimulation amplitude, time post-implantation, and the evoked postural responses, a MANCOVA combined with a multivariate linear regression was used to relate the effects of experimental variables to the postural responses. The behavioral response for each stimulation trial was captured in the amplitude of the peak force in the rear left paw (F_{LH}) and the percent change in the weight distribution ratios for both the anterior–posterior

(R_{AP}) and left–right (R_{LR}) directions. Over the course of each implant, many channels were selected for exploration in the platform experiments. Because the spacing of the electrodes for each array is 400 μm, the recruitment volume of each electrode is most likely distinct from neighboring electrodes. Therefore, each week was treated as an independent observation, including weeks in which a previously explored channel was re-selected for use in the behavioral experiment. As mentioned above, the microelectrode likely activated different afferent fibers given the lack of recruitment stability over long timescales [23] and changes in the recruitment properties observed in the antidromic responses. To examine the effects of stimulation parameters on postural responses, only weeks in which there was a detected response to stimulation were used in the analysis. The modeled variables include the high amplitude stimulation ratio, the number of days implanted at the beginning of each week, and the trial number within the day.

The effects of stimulation amplitude on the mean response, in terms of amplitude and latency, of the implanted limb were compared using paired t-tests with a Benjamini–Hochberg correction for multiple comparisons. This correction method was selected as a strong control method for family-wise error rate in lieu of more conservative methods (i.e. Bonferroni). To further understand the effect of stimulation amplitude, the high amplitude and low amplitude conditions were compared within weeks using the following equations:

$$\text{Amplitude Ratio} = \frac{\text{Amp}_{\text{High}}}{\text{Amp}_{\text{Low}}}$$

$$\text{Response Ratio} = \frac{\text{Response}_{\text{HighStim}}}{\text{Response}_{\text{LowStim}}}.$$

A linear regression was used to quantify the relationship between response ratio and amplitude ratio across weeks.

The effects of DRG level on the response to stimulation was studied using a MANCOVA of the trial-averaged response amplitude for each component of the GRF. This was done controlling for the suprathreshold stimulation amplitude ratio and including both high and low amplitude stimulation conditions. Furthermore, the implant duration was controlled for using the number of weeks post implantation surgery.

While stimulation evoked ENG responses in the sciatic nerve during each week of the experiment, behavioral responses only occurred for a subset of weeks. To understand which features of channel selection influenced the occurrence of responses, we modeled the occurrences of weeks in which there was a detected response to stimulation using a logistic regression model. A trial with a positive response to stimulation was defined as having a peak GRF within the first 500 ms after stimulation onset that significantly ($p < 0.001$) deviated from the baseline GRF 200 ms before stimulation onset. Responses were detected for each component of the GRF separately to avoid compounding their separate noise levels. The following continuous variables were included: the number of weeks implanted prior to each week of experiments, the conduction velocity of the antidromic response, the

threshold for evoking an antidromic response, and the ratio of the high amplitude stimulation condition to the anesthetized threshold (referred to as the high amplitude stimulation ratio). Additionally, two categorical variables were used: the animal, and the spinal level of the implanted electrode (i.e. L6 or L7).

A key assumption of this experiment is that the interface is stable within a week. To test this assumption, we used a 1-way ANOVA to compare the peak amplitude of the implanted limb's response to the high amplitude stimulation condition across days within a week.

Results

Behavioral responses to DRG microstimulation were recorded in four awake, behaving cats. Primary afferent neurons were recruited up to 17 weeks post implantation, as detected in the antidromic response during recruitment threshold experiments. Across four animals, 56 weeks of data were analyzed. A postural response, evidenced by a change in the z-component of the GRF, to the high stimulation amplitude condition was detected in 12 out of these 56 weeks (table 1).In eight of these 12 response weeks, a response was also detected in one or more of the GRF horizontal components ($F_{\text{LH-X}}$ and $F_{\text{LH-Y}}$). In one additional week, Cat H week 12, a response to stimulation was detected only in the *x*-component, corresponding to axes in figure 1, with no detectable response in the *z*-component of the GRF. Because fast-conducting neurons were targeted in the majority of the experiments, it is possible that afferents targeted in the weeks in which no behavioral response was obtained were uninvolved in the postural response and conveyed unrelated information (e.g. cutaneous, temperature, etc). It is also possible that our inability to control all synaptic inputs on motor pools resulted in the information being discounted. Figure 2 shows characteristic examples of how DRG microstimulation at low amplitudes (14 to 38 μA) evokes unloading responses in two animals on three different weeks and how these effects are modulated by the stimulation amplitude. In week 5 for Cat H, there was an initial decrease in GRF under the left hindlimb that did not affect weight distribution in the anteroposterior axis but caused a shift to the right side, which was rebalanced approximately 500 ms after stimulation onset. This long latency rebalancing response is consistent with engagement with more complex circuits in higher-order centers in the brain rather than direct reflexive activity [31] in response to stimulation. As such, our analyses focus primarily on the initial response within the first 200 ms after stimulation onset. In week 9 for Cat H, there was a slight unloading in the implanted limb that quickly returned to the pre-stimulation load force. However, the compensatory postural changes were not rebalanced for over 500 ms after stimulation. Additionally, the initial rapid rightward shift in balance indicates that the contralateral limb likely was also engaged during the reflexive response. In week 6 for Cat I, the animal unloaded the implanted limb and shifted its weight to the right hindlimb. Once again, rebalancing did not occur within the first 500 ms after stimulation.

One week (Cat F, week 3) was excluded because the EMG response to stimulation during behavioral experiments exhibited several features that were unique to that particular DRG electrode. This electrode was the only electrode to exhibit 'twitch-like' responses in the muscle that were entrained to the stimulation pulse-train. That is, a compound muscle action

potential was evoked at low-latency by each microstimulation pulse. In contrast, muscle responses evoked by stimulation on all other DRG electrodes did not follow the stimulation pulses 1 for 1. Instead, the EMG responses emerged gradually over the course of the 100 ms pulse-train, consistent with temporal integration of afferent input at the motor neuron pool. The FMAs consisted of microelectrodes with multiple lengths, and the microelectrode used on this particular week had a longer length, which may have resulted in insertion through the DRG into the ventral root. While the cause is uncertain, the unique attributes of the early response led us to exclude it from the results reported here.

As the ground reaction force predominantly consisted of the vertical component, with the other components being substantially smaller, we focus on the vertical component of the GRF response. We characterized the response to stimulation by calculating the amplitude and timing of the first peak in the vertical component of the GRF for the twelve weeks in which stimulation evoked a change in the vertical component of the GRF. To understand the effect of stimulation amplitude on the magnitude of the change in GRF, we compared the averaged peak in the GRF under the left hindlimb for the high amplitude stimulation condition to the low amplitude stimulation condition for each week. We found that increasing the stimulation amplitude significantly increased the average peak magnitude of the unloading response when comparing high amplitude stimulation to low amplitude stimulation ($p < 0.001$). This is illustrated in figure 3(a), in which 7 out of 12 weeks demonstrated a larger response to high amplitude stimulation. Response peak latencies are shown in figure 3(b) with an average latency of 0.16 ± 0.07 s which is consistent with reflexive activity. While a subset of weeks showed differences in latency between low and high amplitude stimulation, a paired *t*-test showed no effect of the stimulation amplitude on the response latency across weeks ($p = 0.464$). Corresponding figures for the horizontal components of the GRF are illustrated in supplementary figure 1 (stacks.iop.org/JNE/17/016014/mmedia).

To understand the experimental factors that are most predictive of a behavioral response, we modeled the occurrences of weeks in which there was a detected response to stimulation using a logistic regression model (table 2). We analyzed experimental factors including the number of weeks implanted, the conduction velocity of the evoked antidromic response in the anesthetized experiment, the threshold for evoking the antidromic response, and the high amplitude stimulation ratio. The number of weeks implanted was not found to be predictive of a response ($p = 0.850$), demonstrating a level of stability for the neural interface because responses were not skewed toward the beginning of the implant duration.

We hypothesized that faster conduction velocities would be correlated with the occurrence of a response to stimulation, as the fastest conducting afferents (primary muscle spindles and Golgi tendon organs) are likely to convey proprioceptive information and engage low-latency spinal reflex pathways. Conduction velocity of the evoked responses at threshold ranged from 30 to 120 m s^{-1} ($\mu \pm \sigma = 88.22 \pm 30.60$ m s^{-1}). An electrode channel evoking a group 1 afferent response (i.e. conduction velocity between 80 and 120 m s^{-1}) was used in 8 of the 12 response weeks, with the remaining 4 all being group 2 or $A\beta$ afferents (i.e. conduction velocity between 30 and 75 m s^{-1}). Of the 56 weeks tested, 35 relied on channels that recruited group 1 afferents at threshold, and 21 involved group 2

afferents. The suprathreshold stimulation ratio for the high amplitude stimulation condition was included to determine if simply recruiting more of the surrounding neural tissue was more likely to evoke a response. Neither of these metrics (i.e. conduction velocity and high amplitude stimulation ratio) were predictive of response occurrences ($p = 0.192$ and 0.785 respectively). We also examined categorical variables, including the animal and DRG (i.e. L6 or L7). Adjusting for the number of weeks in which stimulation evoked an antidromic ENG response, we determined that no animal was significantly more likely to respond to stimulation ($p = 0.316$). Stimulation at the L6 DRG resulted in a behavioral response in five of 34 weeks tested (14.7%), compared to stimulation at the L7 DRG, which resulted in eight responses out of 22 weeks tested (36.3%). The presence of responses to stimulation in both DRG indicate that neurons at both DRG are involved in the postural response that contributed to the lack of predictive power between DRG level and response likelihood ($p = 0.121$). Lastly, behavioral responses were observed using an FMA in 12 weeks and a UEA in 1 week. Response magnitudes were largest for the week in which stimulation was applied using a UEA ($p \ll 0.001$ for both the high and low stimulation conditions). Latencies were similar ($p = 0.423$ and 0.135 for high and low stimulation conditions respectively) to the other response weeks. However, it was not a goal of this study to compare the performances of these array types and as only one of the animals was implanted with a UEA (Cat I), further testing would be necessary to evaluate its performance for interfacing with afferents in the DRG compared to FMAs.

To understand how responses to stimulation differ by spinal level, we compared the trial-averaged GRF response amplitudes for weeks in which stimulation was applied at the L6 DRG to weeks in which stimulation was applied at the L7 DRG, while controlling for the effects of suprathreshold stimulation amplitude ratio and the time since implantation (table 3). Both high and low amplitude stimulation conditions were separated and used for this analysis. While the responses to stimulation were not significantly different in terms of the GRF's vertical component, stimulation at the L6 DRG resulted in responses that were significantly greater in magnitude for both of the horizontal components compared to stimulation at the L7 DRG ($p = 0.008$ and 0.003 for the x and y components respectively).

To better understand the effect of stimulation amplitude on the postural response, we compared the averaged magnitude of the response peaks with stimulation amplitude. While there was no correlation between the recruitment threshold for a particular week's stimulation channel and the change in the vertical component of the GRF when compared to other weeks, a comparison within each week showed a clear difference between the magnitude of responses for low and high amplitude stimulation. Figure 4 illustrates this relationship, where high amplitude stimulation evokes a larger response than low amplitude stimulation in all weeks (indicated by a response ratio that was greater than 0). Furthermore, this figure illustrates that the response amplitude increases linearly with the stimulation amplitude ($p = 0.005$). Splitting the weeks by fiber type, there was no significant difference ($p = 0.092$) between groups for the response ratio—stimulation ratio relationship. Week 12 of Cat H was excluded from this portion of the analysis as there was no detectable change in the vertical component of the GRF.

The effect of stimulation amplitude on the change in vertical GRF under the implanted hindlimb is further demonstrated in table 4, which shows the results of a multivariate regression comparing the postural responses within responder weeks. The postural response for each stimulation trial was broken down into the force under the implanted left hindlimb, $F_{LH\text{-}Z}$, and the anteroposterior and left–right weight distribution ratios, R_{AP} and R_{LR}, respectively. The amplitude of the primary peak was detected for each trial and used in the regression. In addition to looking at the effects of high amplitude stimulation ratio, we also calculated the effect of the time post-implant to quantify any effect of chronic changes in the electrode-tissue interface on postural responses and the effect of the trial number within each day to quantify effects of fatigue and/or habituation. The high amplitude stimulation ratio had the greatest effect on the postural response, followed by the time since implant. The trial number within each day had a significant effect on the change in GRF of the implanted limb, which could be indicative of either fatigue or adaptation to stimulation. While trial number influenced the loading force of the implanted limb, there was no significant effect on the weight distribution. A one-way ANOVA comparing responses across days within each week confirmed that there were no significant changes in peak response amplitudes extending across days ($p < 0.05$), suggesting that there were no substantial changes in the electrode interface and pattern of recruitment of primary afferents from day to day.

Discussion

The primary goal of this study was to quantify how posture could be affected by DRG microstimulation that was designed to target proprioceptive sensory afferents projecting from the hindlimbs. We demonstrated that stimulation evoked rapid postural responses in awake, behaving cats without causing pain or distress. These behavioral effects occurred in response to trains of only ten stimulus pulses at amplitudes of 15–40 μA. A previous study modeling recruitment of primary afferent fibers in the DRG predicted that 10–20 medium and large diameter fibers are recruited in response to stimulus pulses in the range of 10 μA [32]. However, because this model didn't account for the encapsulation layer that is likely to form around electrodes implanted chronically, the number of activated neurons is likely to be less for this range of stimulation amplitudes. While the responses to stimulation near activation threshold often looked like single units, the complex waveforms recorded at higher amplitudes prevented us from separating the underlying action potentials and obtaining a more accurate measure of the number of afferents recruited. It is well-documented that electrical stimulation of entire peripheral nerves can drive reflexive responses (e.g. tibial nerve h-reflexes) [33–35], and that whole-nerve or muscle stimulation in cats can drive behavioral effects during walking [36–38]. This study, however, demonstrates that reflexive postural responses can occur even during microstimulation and activation of a small population of sensory afferents. Further, we showed that small increases in the amplitude of stimulation can have significant effects on the magnitude of the postural response. These results demonstrate the potential of DRG microstimulation to controllably drive reflex limb motions, which could improve the control of critical reflex components of limb motion for prosthetic applications.

Proprioception is critical for maintaining balance control and gait stability [39], but most studies that have focused on restoring somatosensory feedback via peripheral nerve

stimulation have primarily reported percepts of touch and paresthesia, with infrequent and inconsistent reports of kinesthesia [16, 19, 40, 41]. The lack of proprioceptive perception is especially surprising given that Group I proprioceptive afferents have the largest diameters and corresponding lowest thresholds of any sensory afferents in the periphery when driven by extracellular stimulation [42]. In addition, these studies have primarily reported on the perceptual qualities evoked by stimulation, and have not focused on the reflex-driven effects that should arise during proprioceptive stimulation. Our previous studies in anesthetized animals have demonstrated that DRG microstimulation causes direct activation of proprioceptive afferents at threshold, but similarly, we have not previously demonstrated that this activation results in any downstream effect within the nervous system [23, 24, 25]. To address this previous limitation, we endeavored to stimulate in awake animals via electrodes that evoked responses with high conduction velocities (i.e. primary muscle spindles and Golgi tendon organs). We expected to see that stimulation of afferents in the L6 and L7 DRG would engage reflex pathways and produce rapid changes in GRF. Comparing the responses to stimulation at each of these locations, while DRG level was not found to be a significant predictor of an electrode's ability to produce a response, the response rate at the L7 DRG was approximately twice that of the L6 DRG for this study (rate = 0.363 and 0.147 respectively). Additionally, stimulation at the L6 DRG tended to evoke larger responses, for all components of the GRF, compared to those evoked by stimulation at the L7 DRG.

Statistical analysis demonstrated that the conduction velocity of the recruited response at threshold was not a significant predictor for the presence or absence of a behavioral response, and stimulation of both fast (80–120 m s^{-1}) and medium (30–75 m s^{-1}) velocity afferents can drive postural effects. These results suggest that DRG microstimulation may have engaged multiple different reflexive pathways to drive behavioral responses. This is especially true given that stimulation was applied at supra-threshold levels that may have activated additional afferents. In fact, across the thirteen postural responses that were evoked in this study, while nearly all involved unloading of the implanted hindlimb, there were complex and varied shifts in center of pressure (figure 2(b)) towards the other limbs. These shifts were consistent within each week, but their heterogeneity across weeks may reflect the diversity in activation of different sensory pathways. Along with conduction velocity, we found that threshold was not a significant predictor of whether stimulation drove a behavioral response, suggesting that even low amplitude stimulation can effectively engage reflex pathways. In fact, stimulation amplitudes as low as 15–20 μA were capable of driving postural responses. The variability in the postural responses within a week, as illustrated by the standard error bars in figure 3(a), may result from the variation in proximity of the stimulating electrode to afferents involved in the postural response. In some cases, the larger activation radius from high amplitude stimulation may recruit additional afferents involved in the postural reflex, while in others no additional posture-related afferents are activated. While the magnitude of postural responses decreased over the duration of the implant, the lack of predictive power between stimulation thresholds and the presence or absence of a postural response suggests that variability in the electrode-tissue interface over time is not a significant driver of the presence or lack of a postural response. This is especially important for electrode interfaces, in which glial scar formation and encapsulation can result in increases in threshold [43]. While the response did not change across days within each

week of testing, the trial number for within-day testing did have a significant effect on the magnitude of the postural response. More specifically, within a single day, the response magnitude decreased with repeated testing. This suggests some level of either fatigue or, more likely, habituation to stimulation. As with time since implant, these effects were small compared to the effect of increasing from low-to high-amplitude stimulation, suggesting that stimulation amplitude can modulate the behavioral response in spite of these factors.

A potential limitation of the experimental setup concerns the stability of the neural interface at the DRG. Micromotion of the electrodes with respect to neural structures could affect the neural interface and alter the population of fibers recruited by stimulation. In this study, we assumed that the neural interface was stable over the course of a single week and that the antidromic responses measured in the anesthetized experiments remained consistent throughout the behavioral experiments for that week. Evidence from our previous study suggests that responses may not be stable over the course of months, but were often stable over the duration of one week [23]. This level of stability was further confirmed in this study as the evoked postural responses to stimulation remained consistent over the duration of each week. Significant changes in the electrode interface likely would have resulted in substantial changes in the postural response from day to day within each week, and we did not see those changes here.

Another limitation of this study is the lack of characterization of recruitment of additional neurons as stimulation amplitude was increased above threshold. In the weekly testing that was done to measure recruitment thresholds, conduction velocity of the evoked response was recorded at threshold, but supra-threshold stimulation was used during behavioral experiments. When supra-threshold stimulation amplitudes were applied, additional neurons were likely to have been recruited. Because our conduction velocity measurements were limited to threshold, we do not know the conduction velocity or modality of those additional neurons. Those additional neurons likely played a role in the evoked behavioral response. In fact, the increased magnitude of the postural response that occurred with increased stimulation suggests that this recruitment of additional neurons played an important role in the response. Furthermore, because the comparison of responses between fiber types resulted in no significant differences, it is difficult to attribute these responses solely to proprioceptive afferents, as the medium velocity fibers activated may have included cutaneous afferents. Despite these limitations, we demonstrated that low amplitude stimulation through a single electrode can evoke a behavioral response in an awake behaving animal.

The consistent activation of unloading responses observed in this study may be a result of stimulation of particular reflex arcs in the spinal cord (e.g. the flexor withdrawal/crossed-extension reflex) or may be an artifact of the particular behavioral paradigm we examined here (i.e. quiet standing). It is also possible that these responses are aversive reactions to noxious stimuli, although we believe this is unlikely, as the animals did not present behaviors suggestve of pain (e.g. vocalizations or licking) and continued to perform the task consistently over many weeks. Future work should focus on performing similar experiments under more complex conditions such as during postural perturbations or gait to fully characterize the behavioral response to DRG microstimulation.

In this study, we demonstrated that a neural interface at the DRG can evoke behavioral effects, even at very low stimulation amplitudes, and that these behavioral effects are modulated by the amplitude of stimulation. Further work is necessary to examine how other stimulation parameters, such as pulse frequency and stimulation duration, can be used to further control these evoked responses. Additionally, this study reveals that additional criteria may be necessary for predicting which electrode channels are capable of recruiting neurons involved in the postural reflex. In a companion paper, we report on the evoked EMG responses during these experiments and their relationship to changes in force under the implanted left hindlimb [27]. Other signals, such as recordings from primary somatosensory cortex may provide additional useful information for evaluating electrodes' capability of evoking a postural response. We hypothesized that stimulation amplitude could be used to modulate response magnitude and compared the two suprathreshold stimulation amplitudes for each week. Stimulation amplitude was shown to have a significant correlation with the response magnitude, confirming our original hypothesis. This is likely a result of recruiting additional nearby afferents which are similarly involved in the postural response. Modulation of the postural response is a desirable characteristic of a somatosensory neuroprosthesis, as this will allow for imparting appropriately sized corrections based on the magnitude of postural perturbations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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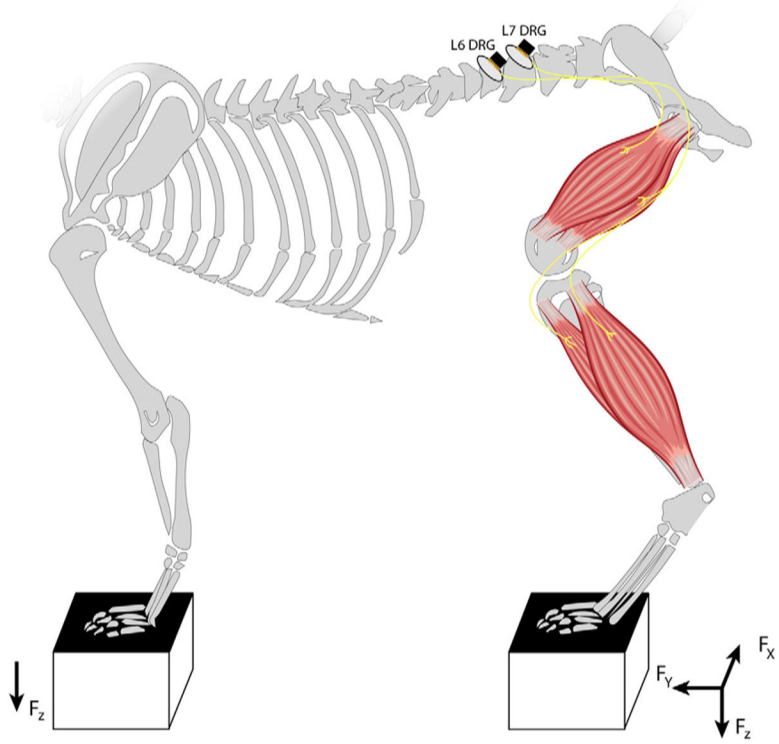

Figure 1.

Schematic diagram of electrode placement and platform axes. MEAs were placed on the left L6 DRG and the left L7 DRG of five cats. Nerve innervations are represented by yellow lines exiting the DRG. Paw loading force was calculated using F_Z for the respective pedestal.

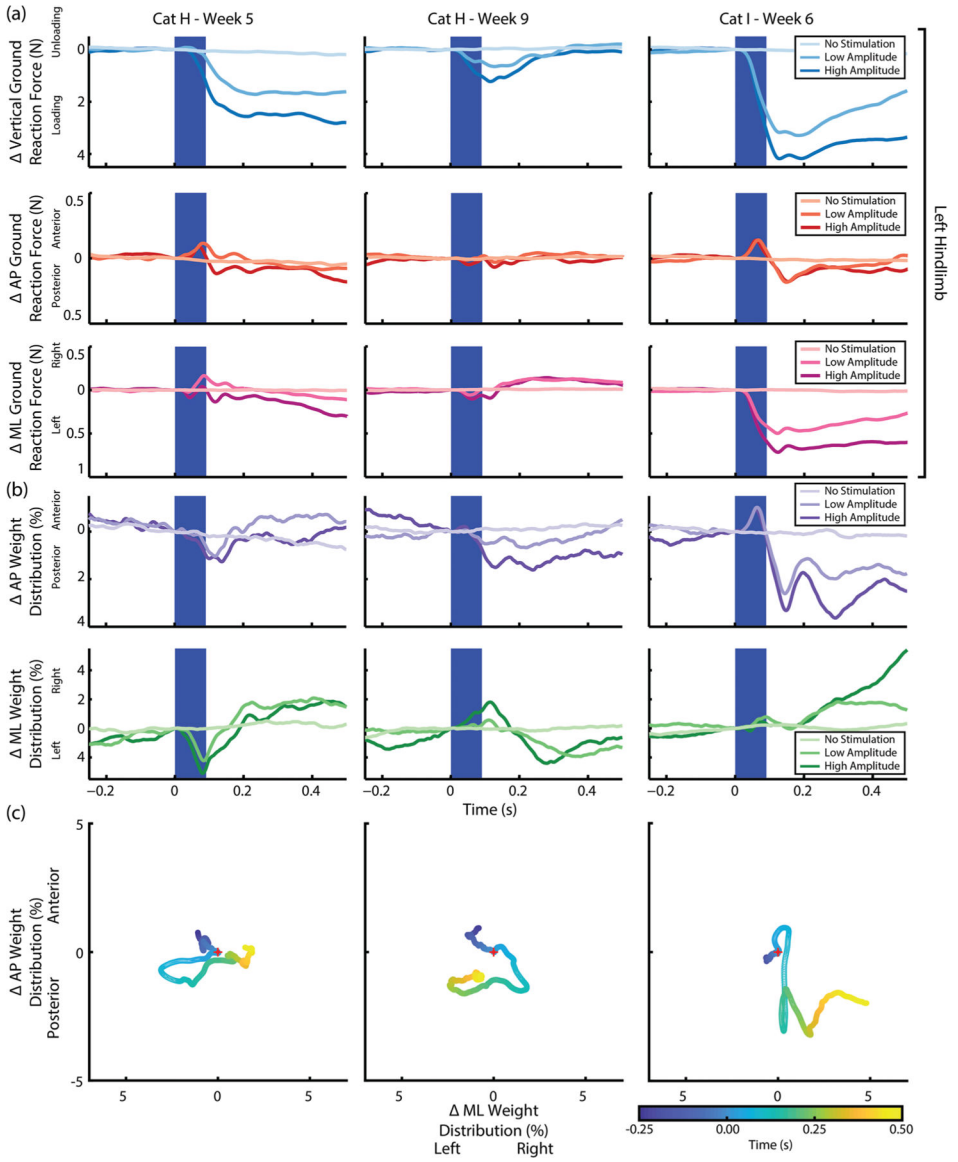

Figure 2.

Trial-averaged postural responses to stimulation. (a) Changes in the ground reaction forces of the implanted limb. (b) Changes in weight distribution in anterior-posterior and left-right directions. (c) Change in anterior-posterior and left-right weight distribution over time for the high amplitude stimulation condition. Stimulation onset is represented by the red plus sign.

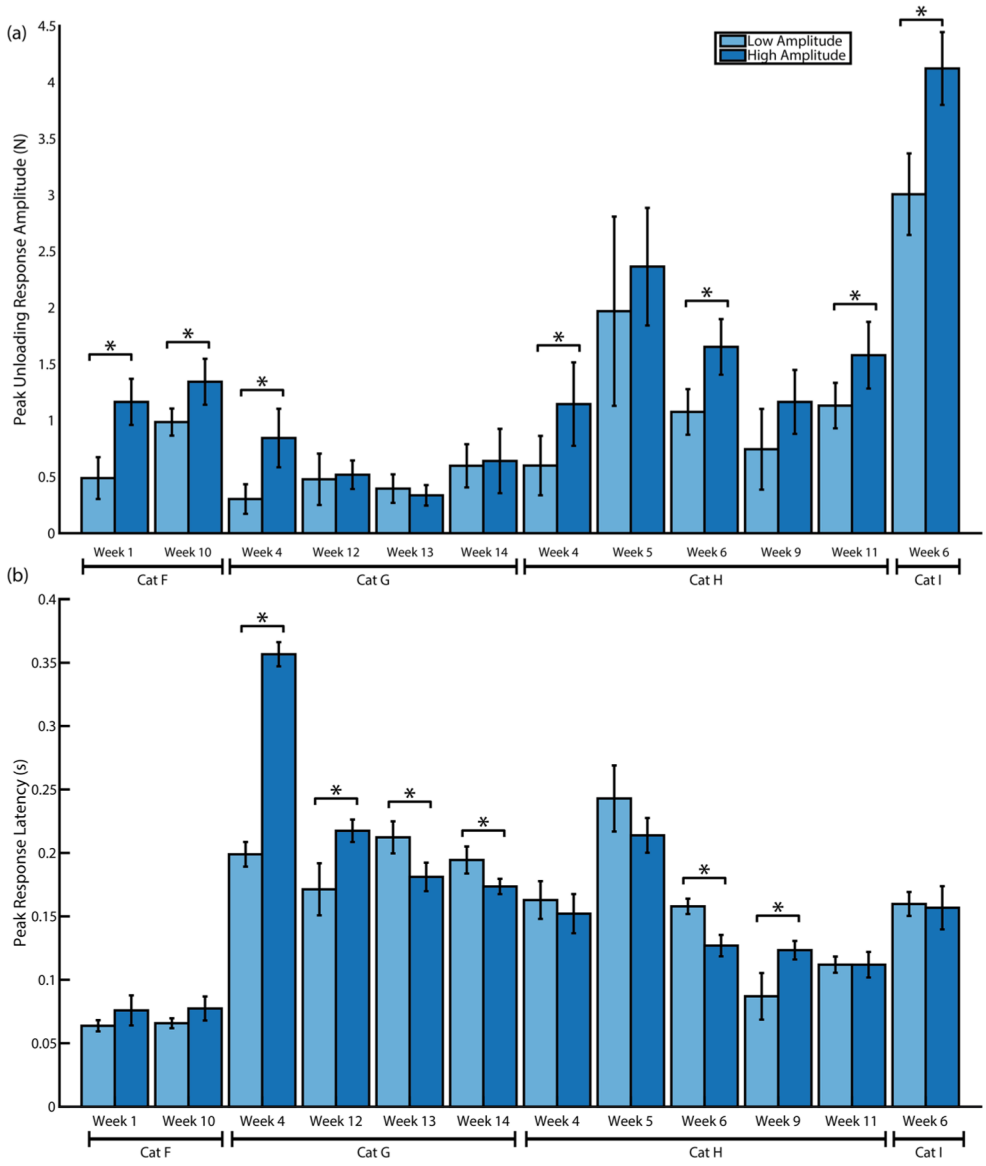

Figure 3.

Postural response amplitudes and latencies for all response weeks. (a) Peak response amplitude for unloading response (*z*-component of ground reaction force) and (b) latency from stimulation onset are shown for each week and both stimulation conditions. The animals corresponding to each experiment are indicated beneath the figure. *Denote statistically significant differences ($p < 0.05$ with Benjamini-Hochberg correction).

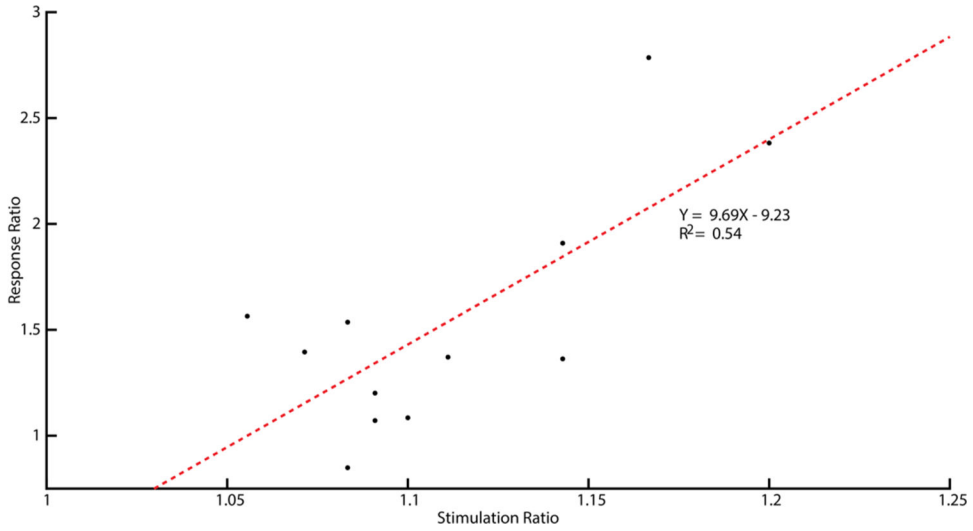

Figure 4.

Comparison of postural response magnitudes and stimulation amplitudes. Response ratio measures the change in paw loading force for the high amplitude stimulation compared to the low amplitude stimulation. Similarly, stimulation ratio measures the difference in stimulation amplitude for the high amplitude stimulation condition compared to the low amplitude stimulation condition. The trend line is represented by the dashed red line. An *F*-test hypothesizing the fit of the model in comparison to an intercept-only model was performed and found to be statistically significant ($p = 0.005$), indicating that the response to stimulation is linked to stimulation amplitude. Week 12 of Cat H was excluded from this portion of the analysis as there was no detectable change in the vertical component of the GRF.

Table 1.

Response week DRG levels, conduction velocities, ENG recruitment thresholds, and stimulation amplitudes.

							Detected response		
Animal	**Week**	**DRG**	**Conduction velocity (m s^{-1})**	**Anesthetized threshold (μA)**	**Low amplitude (μA)**	**High amplitude (μA)**	***X***	***Y***	***Z***
F	1	L6	40	12	15	18			●
F	10	L6	70.6	12	21	24	●	●	●
G	4	L7	120	7	12	14	●		●
G	12	L6	60	13	20	22		●	●
G	13	L6	80	13	24	26			●
G	14	L7	48	12	22	24		●	●
H	4	L7	80	8	14	16			●
H	5	L7	120	15	22	24			●
H	6	L7	120	13	24	26			●
H	9	L7	120	19	36	38	●		●
H	11	L7	80	14	28	30	●		●
H	12	L7	120	12	26	24	●		
I	6	L6	120	10	18	20	●		●
						Totals	6	3	12

Table 2.

Response week predictors for responses detected at high amplitude stimulation. None of these variables were found to be predictive of response weeks.

	MEAN value						
Variable	**0**	**1**	**Min**	**Max**	***t***	**df**	**Sig.**
Weeks implanted	8.488	8.231	1	17	0.191	22.108	0.850
Conduction velocity (m s^{-1})	78.443	90.662	30	120	−1.369	14.635	0.192
Threshold (μA)	11.744	12.308	4	31	−0.503	32.885	0.619
High amplitude stimulation ratio	1.953	1.931	1.10	3.00	0.274	39.329	0.785
	χ^2	df	Sig.				
Animal	3.539	3	0.316				
DRG level	2.405	1	0.121				

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Table 3.

MANCOVAs for averaged GRF responses controlling for stimulation amplitude ratio and weeks implanted. Multivariate significance is calculated using Pillai's trace.

DRG level								
			L6 (n = 5)		L7 (n = 8)			
F	**Multivariate sig. (Pillai's)**	**GRF resp. amplitude**	**Mean**	**SD**	**Mean**	**SD**	**F**	**Univariate significance**
F(3,20) = 5.250	0.008[a]	X	0.226	0.310	0.039	0.132	F(1,22) = 8.494	0.008[a]
		Y	0.149	0.065	0.049	0.104	F(1,22) = 11.502	0.003[a]
		Z	1.474	1.531	1.135	0.699	F(1,22) = 0.657	0.426

[a]: Significance at the $p < 0.05$ level.

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Table 4.

Multivariate regression of postural response variables. Stimulation ratio represents the stimulation amplitude divided by the threshold amplitude.

Covariate	Resp.	Sign(Coeff.)	Log10(abs(Coeff.))	Std. Err.	Significance
Stimulation ratio	$F_{\text{LH-Z}}$	+	−0.038	0.068	0.000[a]
	R_{AP}	+	−3.997	0.000	0.000[a]
	R_{lr}	+	−3.794	0.000	0.000[a]
Days implanted	$F_{\text{lh-z}}$	−	−4.874	0.001	0.000[a]
	R_{AP}	−	−9.552	0.000	0.011[a]
	R_{LR}	−	−9.113	0.000	0.002[a]
Trial number (within day)	$F_{\text{LH-Z}}$	−	−5.652	0.002	0.041[a]
	R_{AP}	−	−9.589	0.000	0.075[a]
	R_{LR}	−	−9.341	0.000	0.076[a]

[a]: Significance at the $p < 0.05$ level.