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Interactions between dorsal and ventral root stimulation on the generation of locomotor-like activity in the neonatal mouse spinal cord

Afferent and efferent input to the locomotor CPG

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Avinash Pujala. Designed research, Performed Research, Wrote Matlab routines for data analysis, Analyzed data, Wrote paper.

Dvir Blivis. Designed research, Performed research, Analyzed data, Wrote paper.

Michael J. O'Donovan. Designed research, Analyzed data, Wrote paper.

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ABSTRACT

We investigated whether dorsal (DR) and ventral root (VR) stimulus trains engage common postsynaptic components to activate the central pattern generator (CPG) for locomotion in the neonatal mouse spinal cord. Ventral root stimulation did not activate the first order interneurons mediating the activation of the locomotor CPG by sacrocaudal afferent stimulation. Simultaneous stimulation of adjacent dorsal or ventral root pairs, subthreshold for evoking locomotor-like activity, did not summate to activate the CPG. This suggests that locomotor-like activity is triggered when a critical class of efferent or afferent axons is stimulated and does not depend on the number of stimulated axons or activated postsynaptic neurons.

DR- and VR-evoked episodes exhibited differences in the coupling between VR pairs. In DR-evoked episodes the coupling between the ipsi- and contralateral flexor/extensor roots was similar and stronger than the bilateral extensor roots. In VR-evoked episodes, ipsilateral flexor/extensor coupling was stronger than both the contralateral flexor/extensor and the bilateral extensor coupling. For both types of stimulation, the coupling was greatest between the bilateral L1/L2 flexor-dominated roots. This indicates that the recruitment and/or the firing pattern of motoneurons differed in DR and VR-evoked episodes. However, the DR and VR trains do not appear to activate distinct CPGs because trains of DR and VR stimuli at frequencies too low to evoke locomotor-like activity, did so when they were interleaved.

These results indicate that the excitatory actions of VR stimulation converge onto the CPG through an unknown pathway that is not captured by current models of the locomotor CPG.

SIGNIFICANCE STATEMENT

In 2005 we showed that stimulation of motor axons can activate the neural circuitry for locomotion in the neonatal mouse spinal cord. This was a surprising result because motoneurons are thought to be purely output elements of the spinal cord. Here we show that

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motor axons reach the locomotor circuitry by a different pathway to that employed by sensory axons. Despite this difference, both types of stimulation activate common locomotor circuitry. An understanding of how motor axons activate these circuits will provide novel insights into the organization of locomotor networks.

INTRODUCTION

In isolated preparations of the neonatal rodent spinal cord, locomotor-like activity can be initiated by bath-applied drugs, or by electrical stimulation of the dorsal roots (Whelan et al., 2000; Delvolvé et al., 2000) or the brainstem (Zaporozhets et al., 2004). Although the mechanisms responsible for such activation are not clear, some progress has been made in understanding how lumbar locomotor networks are activated by stimulation of sacrocaudal afferents (SCA). Lev-Tov and his collaborators have shown that SCA activation of the lumbosacral locomotor CPG is mediated by a set of relay interneurons that project to the lumbar locomotor networks both directly and indirectly through the white matter tracts (Strauss and Lev-Tov 2003; Etlin et al., 2010). These relay interneurons are innervated by sacral sensory fibers, including VGluT1^{+} muscle spindle afferents and VGluT2^{+} afferents. During SCA stimulation, calcium imaging has demonstrated that these neurons deliver both a tonic and a rhythmic drive to the lumbar segments (Etlin et al., 2013). When their ascending axons are interrupted using white matter lesions, SCA stimulation failed to activate the generator indicating their critical role in mediating the effects of dorsal root stimulation (Etlin et al., 2010, 2013).

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Stimulation of ventral roots can also trigger locomotor-like activity (Mentis et al., 2005), modulate the frequency of drug-induced locomotor rhythms (Machacek and Hochman, 2006) and entrain disinhibited bursting (Machacek and Hochman, 2006; Bonnot et al., 2009). These effects persist in the presence of cholinergic antagonists but are blocked or reduced by iono- or metabotropic glutamatergic antagonists (Mentis et al., 2005; Bonnot et al., 2009). Beyond this, nothing is known about how ventral root stimulation triggers locomotor-like activity. Given that both dorsal and ventral root excitation of the locomotor generator are mediated by glutamatergic mechanisms, we investigated the extent to which they share common mechanisms for activating the generator and if the two types of stimulation activate the same generator. We investigated this by pairing or interleaving stimulus trains to dorsal and ventral roots and establishing their ability to trigger locomotor-like activity. We also performed experiments to address the possibility that the effects of ventral root stimulation might be mediated by afferents within the ventral roots (Windle 1931; Coggeshall 1980).

METHODS

Surgical procedures

Experiments were performed on Swiss Webster neonatal mice (Taconic Laboratory; P0–P4) of either sex. The mice were decapitated and eviscerated and the remaining tissue was placed in a dissecting chamber and continuously superfused with an artificial cerebrospinal fluid (ACSF; concentrations in mM: 128 NaCl, 4 KCl, 1.5 $CaCl_2$, 1 $MgSO_4$, 0.5 NaH_2PO_4, 21 $NaHCO_3$, 30 D-glucose) bubbled with 95% O_2 - 5% CO_2. A ventral laminectomy exposed the cord, which was then transected between the 5^{th} and 7^{th} thoracic segments and removed from the vertebral column together with the attached roots and the cauda equina. In some experiments, the cord

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was not transected to preserve connections with the brainstem. During the dissection, the ACSF was initially at a temperature of 6-7°C and was progressively allowed to warm to 23–25°C (room temperature) before transfer of the cord to the recording chamber.

Electrophysiology

Recording

Electrical activity from motoneurons was recorded with tight-fitting plastic suction electrodes into which individual ventral roots were drawn. The recorded signals were analog-filtered (DC/0.01-3 kHz) and amplified (gain: 1000), digitized at 5-10 kHz (Digidata 1322A) and stored on a computer. In some experiments, the activity of ventral and/or ventrolateral white matter funiculi (VF and/or VLF) was also recorded. Episodes of data were analyzed off-line using Clampfit. Locomotor activity was quantified using wavelet analysis (Mor & Lev-tov, 2007). For this purpose, the ventral root (VR) recordings were high pass filtered at 50 Hz, half-wave rectified, and low pass filtered at 5 Hz to obtain the envelope of spiking activity. The stimulus artifacts were removed digitally and the rectified integrated neurograms were resampled at 50 Hz.

Stimulation

To elicit locomotor-like activity dorsal- and/or ventral roots were stimulated with trains (frequency: 1-5Hz, duration: 5-15 s) of square-wave electrical pulses (duration: 200-250 µs). The stimulus intensity was based on the threshold for eliciting fictive locomotion. The threshold (Thr.) for a given spinal root was defined as the lowest current intensity at which that root had to be stimulated to elicit locomotor-like activity in 5/5 attempts. Typically, the threshold for

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dorsal roots (L6-S3) was 10-15μA and for ventral roots (L4-L6) it was 20-35μA. The range of stimulus intensities used in our experiments was 0.8 to 10 times the threshold (0.8-10x Thr.). Descending pathways from the brainstem were excited in the same way, using a bipolar stimulation electrode placed in the ventromedial medulla (Zaporozhets et al, 2004; Blivis et al, 2007). To elicit monosynaptic responses in motoneurons or VF/VLF interneurons, the appropriate dorsal roots were stimulated with a single electrical pulse.

Data Analysis and Statistics

To quantify locomotor-like activity, we employed wavelet analysis (Torrence and Compo, 1998; Grinsted et al, 2004; Wang et al, 2004; Mor and Lev-Tov, 2007). Analysis was performed using modified MATLAB (Math Works) routines originally developed by Torrence, Compo, Grinsted and others (Torrence and Compo, 1998; Grinsted et al, 2004) and adapted for electrophysiological analysis by Mor and Lev-Tov (2007). The code is freely available for download at https://github.com/avinashpujala (The relevant code is in the repositories 'General' and 'Spectral-Analysis'). Tutorials for usage of scripts will be provided upon request.

The wavelet transform (WT) decomposes a signal into a set of 'wavelet' coefficients using a damped and finite function of zero mean, called the mother wavelet. This function is localized in time and frequency. Here, we used the *complex Morlet wavelet*, which is obtained by modulating a sine wave with a Gaussian (Mor and Lev-Tov, 2007).

Cross-wavelet transforms (XWT) were computed to assess the relationship between pairs of time series (Grinsted et al, 2004; Mor and Lev-tov, 2007) as follows:

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143 $$W_{xy} = W_x W_y^* \quad (1)$$

144 Here, W_x and W_y are the wavelet transforms of the two time series x and y and * denotes
145 the complex conjugate. As with the entries in W_x and W_y, the entries in W_{xy} - termed
146 crosswavelet (XW) coefficients - are also complex numbers. Thus, they can be represented as
147 vectors in the 2-dimensional orthogonal space spanned by the real (abscissa) and imaginary
148 (ordinate) number lines. Since the different rows *(i)* and columns *(j)* of W_{xy} correspond to
149 different frequencies (or scales) and times respectively, each coefficient of W_{xy} corresponds to
150 a unique location in frequency-time space. The vector length of a coefficient provides a
151 measure of the combined power (XW power) between the signals x and y at a specific
152 location in frequency-time space, whereas the vector angle of that coefficient indicates the
153 phase difference between the signals at that same location.

154 To examine only the locomotor-like activity component of an evoked episode we set
155 phase values outside the range of $180° \pm 45°$ to zero.

156

157 $$W_{xy}(i,j) = \begin{cases} W_{xy}(i,J), 180° - 45° \le \arg W_{xy}(i,j) \le 180° + 45° \\ 0, otherwise \end{cases} \quad (2)$$

158

159 The power of the XW spectrum was normalized by the *crossvariance* of the two time series as
160 follows (Grinsted et al, 2004)

161 $$\frac{W_{xy}}{\sigma_x \sigma_y} \quad (3)$$

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Here, σ_x and σ_y are the standard deviations of the ventral root recordings x and y. This normalization expressed the values of XW power in units of variance and allowed the comparison of spectrograms generated from different preparations. To exclude noise-related activity from the spectrogram displays, we deleted those entries in W_{xy} whose XW power was not statistically different from values expected for background white noise. Significance was established at the 5% level (for details see Grinsted et al., 2004; Mor and Lev-Tov, 2007).

Figure 1 Near Here

We quantified episodes of locomotor-like activity by computing the *strength* (S) of the activity as illustrated in Figure 1. We used bilateral pairs of flexor-dominated (L1 or L2) and of extensor-dominated (L5) ventral roots for these analyses. If we denote the signals from these ventral roots by flexor Left (fL), flexor Right (fR), extensor Left (eL) and extensor Right (eR) respectively, then alternating bursting is expected between the signal pairs $\{fL, eL\}, \{fLfR\}, \{fR, eR\}$, and $\{eL, eR\}$. Crossed wavelet transforms were computed between each of these pairs. Non-locomotor-like ($>180 \pm 45°$) and non-significant values were removed from the resulting matrices of normalized XW coefficients - $W_{fLeL}, W_{fLfR}, W_{f\mathrm{Re}R}, W_{eLeR}$. The 4 coefficient matrices were then averaged as follows:

$$W_{avg} = \frac{1}{4}(W_{fLeL} + W_{fLfR} + W_{fReR} + W_{eLeR}) \quad (4)$$

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To compute locomotor strength (S) for given episode of locomotor-like activity, we took the vector sum of all the entries in W_{avg} and computed the square root of the length of the resultant vector. Thus, if W_{avg} were an $M \times N$ matrix, then

$$\mathrm{S} = \sqrt{\left| \sum_{m=1}^{M} \sum_{n=1}^{N} W_{avg} \right|} \tag{5}$$

where *m* and *n* are the row and column indices respectively.

The comparison of locomotor strengths for different stimulus intensities (fig. 3) or for interleaved dorsal and ventral root stimulus trains (fig. 9) was made was in the following manner. In each preparation, the strengths were computed for several episodes of fictive locomotion elicited under the various conditions. Then, the strengths were normalized by dividing them by the mean of the strengths for one of the three conditions. For instance, if S_1, S_2 and S_3 are the sets of strengths computed for trials in the conditions $1, 2$ and 3 respectively. The normalization of values in each of the sets was done as follows:

$$\frac{S_1}{\langle S_1 \rangle}, \frac{S_2}{\langle S_1 \rangle}, \frac{S_3}{\langle S_1 \rangle} \tag{6}$$

Where, $\langle S_1 \rangle$ indicates the mean of all values in the set S_1. This procedure was repeated for every preparation, so that every time the average strength for one of the conditions always equaled one. This allowed the data to be pooled across preparations in a way that preserved the strength relationships among the different conditions. Significance testing was done using

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non-parametric tests. For comparison between two conditions we used the Wilcoxon rank sum test, and for more than two conditions we used the Kruskal-Wallis test ($p < 0.05$).

The process of calculating locomotor strength in this manner is similar to computing cross-spectral power densities (CPSD) for pairs of ventral root signals with expected out-of-phase rhythms during locomotor-like activity, then calculating the area under the curve for each of these CSPDs for a range of physiologically plausible frequencies, and finally averaging these areas to obtain a single number. Thus, much like the area under a CSPD, locomotor strength depends on the amplitude of the rhythmic signals, relative to background noise, as well as their spectral composition. However, because of the phase filtering process described in equation (2), unlike the area under a CSPD, the locomotor strength calculation is not influenced by in-phase rhythmic components in pairs of ventral root signals. Even when factoring only out-of-phase $(135° \leq \phi \leq 225°,$ where ϕ is phase difference) rhythmic components, locomotor strength further depends on the variability of the phase values. This is because the complex elements of the coefficient matrix (W_{xy}) generated for a pair of signals ventral root signals x and y are vector-summed during the process of computing the locomotor strength. Thus, locomotor strength is expected be larger for a pair of out-of-phase rhythmic signals that show little variation within the range of phase values ($135° - 225°$) we defined as locomotor-like activity.

To compare the strength of coupling between pairs of ventral root signals (see fig.4) we first computed the crosswavelet transforms for all pairs of ventral roots expected to show alternating rhythmic activity during fictive locomotion. However, instead of averaging the

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resultant matrices of wavelet coefficients (equation 4), we computed from each matrix a scalar value $\mathfrak{p}$ whose magnitude depended both on the amplitude of the discharge as well as the phase coupling between the two sets of rhythms; better quality rhythms with tighter locomotor-like phase coupling yielded higher magnitudes. For the ventral root pair $\{eL, fL\}$, the scalar quantifier $\mathscr{P}_{eLfL}$ would be computed as follows:

$$\mathscr{P}_{eLfL} = \sqrt{\left|\sum_{m=1}^{M}\sum_{n=1}^{N} W_{eLfL}^{alt}\right|} \tag{7}$$

where, as in equation (4) m and n correspond to the row and column indices within the coefficient matrix W_{eLfL} and the superscript alt on W indicates the filtering of values within this matrix based on their phase (see equation 2).

After computing $\mathscr{P}$ values for all ventral root pairs and trials that were elicited using identical stimuli during an experiment we sorted each value into one of 4 groups based on the ventral root pair from which that value was generated. We then standardized these values to make them comparable across preparations by always treating the group corresponding to the ipsilateral flexor and extensor roots $\{if, ie\}$ as the reference group and dividing all values in all groups by the mean of values in the reference group. This procedure is described by the equation below where the ipsilateral reference roots are on the left.

$$\frac{P_{fLfR}}{\langle P_{fLeL}\rangle}, \frac{P_{f\,\mathrm{Re}\,R}}{\langle P_{fLeL}\rangle}, \frac{P_{eLeR}}{\langle P_{fLeL}\rangle}, \frac{P_{fLeL}}{\langle P_{fLeL}\rangle} \tag{8}$$

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Here, P refers to set of all values within a group and $\langle P \rangle$ indicates the mean of all values within that group. So for instance, $P_{fLfR} = \{\mathscr{P}^1_{fLfR}, \mathscr{P}^2_{fLfR}, \mathscr{P}^n_{fLfR}, \mathscr{P}^N_{fLfR}\}$, where the superscripts on $\mathscr{P}$ indicate the trial number.

RESULTS

Ventral root-evoked locomotor-like activity results from antidromic stimulation of motor axons not orthodromic stimulation of sensory axons in the ventral root

In our earlier experiments (Mentis et al., 2005) we found no evidence of afferent axons in the ventral roots when the proximal end of a cut ventral root was back-labelled with fluorescent Dextrans. However, because this method may have failed to identify very small afferent axons in the ventral roots we used a physiological approach to determine if stimulation of afferent axons in the ventral roots was responsible for activating the locomotor CPG by ventral root stimulation. We first established that stimulation of the dorsal root ganglion (DRG) could activate locomotor-like activity with the corresponding segmental ventral root cut (fig.2B). The threshold intensity for evoking locomotor-like activity by DRG stimulation was statistically indistinguishable from a dorsal root stimulation suggesting that it was equally effective at recruiting dorsal root afferents (n = 20, DRG/DR pairs from 5 preparations, Wilcoxon rank sum test, $p < 0.05$). However, locomotor-like activity could never be evoked when the DRG was stimulated with the dorsal root cut even when stimulating at 10 times threshold (fig.2C).

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Figure 2 Near Here

To maximize the chance of evoking locomotor-like activity in each preparation, we stimulated the DRGs of at least three different segments on both sides of the cord (33 different DRGs from 5 different preparations). We focused on the L4 – L6 DRGs because stimulation of the corresponding ventral roots has been shown to be particularly effective at eliciting locomotor-like activity (Bonnot et al., 2009). Finally, locomotor-like activity was evoked when the spinal nerve was stimulated with a cut dorsal root showing that it was mediated by axons travelling in the ventral root (4 out of 5 preparations - 16 of 25 spinal nerves stimulated in total, fig.2D). In the same experiments we also used DC recordings of the ventral root activity to ensure that subthreshold, slow potential activity was not evoked in the ventral root when stimulating the DRG with the dorsal root cut. As shown in fig.2E, stimulation of the right DRG with its dorsal root intact produced an episode of locomotor-like discharge superimposed on a slow depolarization that briefly outlasted the stimulus train. However, when the dorsal root was cut no slow potential activity was detected in response to DRG stimulation (fig.2F). Similar results were observed in the other 4 experiments.

These results, together with the earlier failure to detect ventral root axons using back-labelling experiments (Mentis et al., 2005), suggest that ventral-root evoked locomotor-like activity results from the activation of efferents rather than afferents in the ventral root.

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Properties and comparison of Locomotor-Like activity generated by dorsal and ventral root stimulation

We first examined the relationship between stimulus intensity and the strength of a locomotor-like episode (see Methods) for both dorsal and ventral root stimulation (fig.3). As the stimulus intensity applied to a dorsal or ventral root was increased, a threshold was reached for evoking locomotor-like activity. Below this intensity, locomotor-like activity could not be evoked or was only evoked in a minority of trials. For example, at 0.9 times threshold fewer than 8% of the trials resulted in locomotor-like activity (2/45 VR stimulation trials from 14 preparations, and 5/70 DR stimulation trials from 23 preparations). When stimulated at intensities up to 3 times threshold, the strength of locomotor-like episodes progressively increased (188 VR stimulation trials from 24 preparations, 558 DR stimulation trials from 63 preparations).

Figure 3 Near Here

We found that even small changes in stimulus intensity could produce substantial increases in the strength of locomotion. In the example shown in fig.3A, when the ventral root stimulus intensity was increased from 1 to 1.1 times threshold, the locomotor strength increased from 281 to 457 and similar effects were observed with dorsal root stimulation.

To quantify and compare the locomotor activity generated by either dorsal or ventral root stimulation we examined the distribution of the coherent power (normalized to the power of the ipsilateral flexor and extensor discharge) between the following pairs of ventral roots: ipsilateral L1-L2/L5; contralateral L1-L2/L5; bilateral L1-L2; bilateral L5. Using this analysis, a

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303 difference emerged between the dorsal and ventral root-evoked episodes (300 trials from 45
304 preparations for DR stimulation; 145 trails from 25 preparations for VR stimulation).

305 For both dorsal and ventral root evoked episodes, the coherent power was greatest
306 between the activity of the bilateral L1 and L2 flexor-dominated roots and weakest between the
307 extensor-dominated bilateral L5 roots (Fig.4). During dorsal root stimulation, the coherent
308 power between the ipsi- and contralateral flexor L1/2 and the extensor L5 discharges was
309 similar and weaker than the bilateral flexor coupling. By contrast, when the ventral roots were
310 stimulated, the ipsilateral flexor/extensor coupling was not significantly different from the
311 bilateral flexor coupling but was significantly stronger than the contralateral flexor/extensor
312 coupling.

313 Figure 4 Near Here

314 ***Do motoneuron and dorsal root axons project to the same relay interneurons?***

315 It has previously been shown that activation of the locomotor CPG by sacrocaudal
316 afferent stimulation is mediated by relay interneurons whose axons project through the white
317 matter to the lumbar cord (Strauss and Lev-Tov 2003; Blivis et al., 2007; Etlin et al., 2010, Etlin
318 et al., 2013). The projection through the ventral funiculus is particularly important because
319 cutting this tract compromises activation of the locomotor network by sacrocaudal afferent
320 stimulation (Strauss & Lev-tov, 2003; Blivis et al., 2007; Lev-Tov & O'Donovan, 2009; Etlin et al.,
321 2010; Lev-Tov et al, 2010). The axons of these relay interneurons cross the midline and ascend
322 rostrally, within the ventral funiculus, for several segments. We hypothesized that ventral root
323 stimulation could activate the locomotor CPG if motor axons also activated these relay

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interneurons. To test this possibility, we stimulated the dorsal and ventral roots and measured the responses evoked in a strip of the contralateral VF peeled off from the spinal cord ~3-5 segments rostral to the stimulated roots (fig.5).

Figure 5 Near Here

Dorsal root stimulation evoked a short latency potential in both the ipsilateral ventral root and the contralateral VF. The respective latencies were (7.3 ± 1.4ms ventral root, 12.0 ± 2.1 ms VF; 150 trials from 6 experiments). The longer latency of the VF responses to the DR stimulus was because the recording electrode was 3 – 5 segments from the stimulus location. To establish if these responses were monosynaptic we measured the difference between the minimum and maximum latencies of the responses (defined as jitter) and found that it was statistically indistinguishable for the DR-evoked ventral root (0.5 ± 0.2ms) and VF (0.7 ± 0.4ms) recordings (5 experiments). For comparison, the jitter of monosynaptic EPSPs evoked in muscle identified motoneurons in response to stimulation of the muscle nerve (ventral roots cut) was 0.4 ± 0.1 ms (Shneider et al., 2009). The slightly longer jitters observed in the present study may be due to introduction of a small delay due to the functional low pass filtering of that accompanies electrotonically recorded ventral root and VF potentials. These results suggest that dorsal root afferents project monosynaptically to VF interneurons consistent with earlier immunocytochemical data (Etlin et al., 2013). By contrast, short latency potentials were never recorded from the VF when the ventral root was stimulated. Indeed, only long latency (74 ± 18 ms, 106 trials from 6 experiments) responses could be detected (fig. 5D) and these could be accompanied by an evoked episode of locomotor-like activity (Fig.5E). In three experiments, we

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345 also recorded from the ventrolateral funiculus (VLF), but were unable to detect any short
346 latency responses following ventral root stimulation. We conclude, therefore, that motor axons
347 do not make direct monosynaptic connections with the relay neurons that mediate activation
348 of the CPG by dorsal root stimulation.

349 To establish if dorsal and ventral root trains activated a common set of postsynaptic
350 neurons we stimulated a dorsal and ventral root pair simultaneously at intensities below their
351 locomotor thresholds to see if the combined stimuli would activate the locomotor network. We
352 reasoned that if the threshold for locomotor activation depended on the recruitment of a
353 critical number of postsynaptic cells, some of which were activated by both dorsal and ventral
354 root stimulation, then stimulating the two roots just subthreshold should recruit enough
355 neurons to trigger locomotor-like activity.

356 Figure 6 Near Here

357 Before stimulating a pair of dorsal and ventral roots simultaneously, we first established
358 that stimulation of each root individually could activate the locomotor rhythm and that their
359 respective locomotor thresholds remained stable over a 30-minute period. If, at any time, the
360 threshold deviated from the initially determined value by > 5%, we discarded that root and
361 stimulated a different root. An example of this type of experiment is shown in fig.6. In this
362 experiment, the L6 dorsal and ventral roots were stimulated initially at the threshold for
363 evoking locomotion (20μA for ventral Root, 12μA for dorsal root). Then the stimulus intensity
364 was reduced to 90-95% of this value. We determined experimentally that this stimulus intensity
365 was the highest subthreshold intensity that failed to evoke locomotor-like activity in any of the

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366 control trails. When the two trains were delivered simultaneously at this reduced intensity
367 locomotor activity was never evoked. Similar findings were made with other root combinations
368 (table 1). Indeed, we were surprised that simultaneous subthreshold stimulation of any root
369 pair (DR-VR, DR-DR or VR-VR) failed to produce locomotor-like activity. These findings indicate
370 that either the two pathways for activating the locomotor CPG did not share early components
371 or that our assumptions concerning the threshold for activation of locomotor-like activity were
372 wrong (see Discussion).

373 Table 1 Near Here

374 ***Afferent, efferent, and descending pathways converge onto higher-order interneurons***
375 ***involved in rhythmogenesis***

376 One limitation of the previous experiments is the possibility that sub-threshold
377 stimulation of each pathway fails to propagate beyond the earliest synapses in the pathway.
378 Alternatively, it may be that the initial stimuli in a suprathreshold stimulus train facilitate the
379 pathway allowing the subsequent stimuli to evoke locomotor-like activity. In view of these
380 concerns, we stimulated two roots at suprathreshold intensities but at a frequency (sub-
381 optimal) that did not evoke locomotor-like activity. This allowed us to examine the effects of
382 interleaving the sub-optimal, suprathreshold stimulus trains from two roots to achieve an
383 effective frequency that was double the individual trains.

384 Figure 7 Near Here

385

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We first established that stimulation of either a dorsal or a ventral root at 2.67 Hz produced locomotor-like activity that was recorded from the left and right L1 ventral roots (fig.7A and B). When the roots were stimulated at half this frequency locomotor-like activity was not observed (fig.7C and D), but when these suboptimal DR and VR stimulus trains were interleaved (fig.7E) locomotor-like activity was triggered.

We then interleaved the ventral root trains with stimulus trains applied to the brainstem. Brainstem stimulation is commonly used to evoke locomotor-like activity because it is thought to mimic the physiological activation of the locomotor CPG by descending signals (Atsuta et al., 1990; Zaporozhets et al., 2004; Blivis et al., 2007). As shown in figure 8, locomotor-like activity was evoked by stimulation of the L5 ventral root and the brainstem with 6.67 Hz, but not 3.33 Hz stimulus trains (Fig. 8A-D). Although ventral root stimulation occasionally produced some alternating rhythmic discharge in the bilateral L6 ventral roots when stimulated at 3.33 Hz (Fig. 8D), the overall strength of such activity was much weaker than when the same root was stimulated at 6.66 Hz (Fig. 8B). Interleaving the ventral and brainstem stimuli at 3.3 Hz resulted in robust locomotor-like activity.

To quantify the effects of joint, interleaved stimuli we integrated the power in the region of the spectrogram exhibiting locomotor-like activity to obtain a measure of the strength of the locomotor episode. Since all combinations of the stimulated dorsal- and ventral pairs roots successfully evoked locomotor-like activity, we pooled data after normalizing the samples to account for variation in their median values across preparations (see METHODS).

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406 We found that the strength of locomotor-like activity evoked by dual root stimulation
407 was always within the range of values obtained by stimulation of dorsal and ventral roots alone
408 (fig.9), as long as the stimulation frequency was matched in all three conditions (Kruskal-Wallis,
409 $n = 42$, $p < 0.05$). Collectively, these results indicate that the rhythmogenic circuitry common to
410 afferent- and efferent pathways is also excited by descending pathways when eliciting
411 locomotor-like activity.

412 Figure 9 Near Here

413 In the final set of experiments, we examined the ability of dorsal or ventral root trains to
414 maintain the locomotor rhythm once it had been initiated by stimulation of the other type of
415 root. If both dorsal and ventral root stimulation activate the same CPG, then once locomotor-
416 like activity was initiated by stimulation of one of the pathways it should continue uninterupted
417 when the stimulus is switched from one pathway to another. To test this prediction, we
418 identified roots that could evoke locomotor-like activity reliably and then stimulated each root
419 at a suprathreshold intensity for eliciting locomotor-like activity with 10 or 15 second stimulus
420 trains (4-5 Hz). From ten such trials we noted the time it took to activate at least 1 complete
421 cycle of locomotion to insure that the switch in stimulation would only occur once locomotor-
422 like activity has been successfully initiated.

423 Figure 10 displays the results of an experiment in which the L6 dorsal root and the
424 ipsilateral L5 ventral root were stimulated. The roots were stimulated separately for 15 seconds
425 and evoked alternating locomotor-like activity recorded from the left and right L1 ventral roots
426 (fig.10A and B). We then stimulated each root for 5 seconds before the stimulation was

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switched to the other root for the next 10 seconds (fig.10C and D). We found that rhythmic alternating bursts in the L1 ventral roots continued to occur without interuption despite the switch in the source of stimulation. As a consequence, the cross-wavelet spectrogram generated from the activity of these ventral roots showed a continuous region of significant power with locomotor-like phase values (Fig. 10C and D). In both examples presented here, the switch in stimulation occurred while the right L1 ventral root was bursting and the left L1 was quiescent but similar results were obtained when the roots were switched in the opposite phase.

Figure 10 Near Here

In this example, the stimulated dorsal- and ventral roots elicited locomotor-like activity of similar frequency. Sometimes, however, stimulation of dorsal- and ventral roots elicited locomotor rhythms of different frequencies. In the experiment shown in fig.10E, stimulation of the dorsal root for 10 seconds elicited locomotor-like activity at 0.46 Hz. When the stimulation was switched to the ventral toot for 5 seconds the frequency of the locomotor-like activity almost tripled to 1.4Hz (fig.10F). This did not depend on the order of root stimulation because the frequency fell when the ventral root was stimulated first. Similar results were observed in 12 experiments (32 dorsal-ventral root pairs stimulated).

DISCUSSION

We have shown that inputs from the dorsal and ventral roots do not converge at an early point in their respective pathways to the locomotor CPG. Rather, they appear to converge much closer to the CPG and perhaps within the rhythm generating circuitry itself. In addition, the locomotor-like behavior recruited by dorsal or ventral root stimulation is not identical,

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449 implying corresponding differences in the composition of the rhythmogenic networks recruited
450 by the different types of stimuli.

451 Several studies have shown that activation of the locomotor CPG by stimulation of
452 sacrocaudal afferents (Whelan et al., 2000; Lev-Tov et al., 2000) is mediated by a
453 heterogeneous set of relay interneurons that project to the lumbar segments (Strauss and Lev-
454 Tov, 2003; Etlin et al., 2010). Many of these neurons project their axons into the ventral
455 funiculus (VF) where their activity can be recorded in response to sacrocaudal afferent
456 stimulation. Our recordings revealed that lumbar dorsal root stimulation evoked monosynaptic
457 potentials in the VF, consistent with the presence of VGluT1 and VGluT2 afferent terminals on
458 the somata and dendrites of the neurons projecting into the VF (Etlin et al., 2013). However, no
459 such short-latency potentials were recorded when the ventral roots were stimulated indicating
460 that these cells are not contacted directly by motoneuron terminals.

461 We were surprised that stimulation of pairs of adjacent spinal roots, just subthreshold
462 for evoking locomotor-like activity, showed no evidence of summation. We assumed that the
463 threshold for evoking locomotor-like activity by either dorsal or ventral root stimulation would
464 depend upon the recruitment of a critical number of postsynaptic cells. Accordingly, combined,
465 just-subthreshold stimulation of a pair of roots would recruit the necessary complement of
466 postsynaptic cells to reach threshold for locomotor-like activity. Since this was never observed
467 for any pair combination (DR-DR, VR-VR, DR-VR), this postsynaptic mechanism for the
468 locomotor threshold appears to be incorrect. Dose et al., (2015) also found that combined
469 subthreshold stimulation of 2-3 dorsal roots failed to activate the lumbar CPG in the neonatal
470 rat spinal cord which they attributed to the operation of a “gating system that filters the

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amplitude of afferent stimuli". However, they provided no evidence in support of this mechanism. It seems unlikely that the locomotor threshold is determined simply by the number of axons activated, because combined stimulation of two roots just subthreshold, while significantly increasing the number of stimulated axons does not trigger the locomotor rhythm. We propose instead that locomotor threshold depends on the species of dorsal or ventral root axons that are recruited by the stimulus. For sacrocaudal afferents it is known that the locomotor CPG can be activated by high threshold mechanical, thermal and nociceptive afferents (Blivis et al., 2007) but not by low-threshold muscle afferents (Etlin et al., 2013). In addition, high-threshold capsaicin-sensitive afferents expressing transient receptor potential vanilloid 1 (TRPV 1) have been shown to contribute to the activation of the CPG by sacrocaudal afferent stimulation (Mandadi et al., 2013). Therefore, our inability to observe spatial facilitation between pairs of dorsal roots stimulated at subthreshold intensity may be explained by a failure to activate the appropriate class of high threshold afferent. This does not imply that either the axons or the post-synaptic neurons recruited by the subthreshold stimulus do not contribute to locomotor activity once it is generated. Etlin et al., (2013) split the first coccygeal dorsal root and stimulated one branch at group I-II strength and the other above the threshold for A-δ fibers. Stimulation of the first branch did not evoke locomotor activity whereas stimulation of the second branch did. However, when both were stimulated together the evoked locomotor-like activity exhibited greater power and more cycles compared to stimulation of the second branch alone.

While the axonal composition of dorsal roots is highly varied, the same is not true for ventral roots. Ventral roots comprise the axons of somatic motoneurons, γ-motoneurons that

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493 innervate muscle spindles and in some segments pre-ganglionic autonomic efferents (Biscoe et
494 al., 1982). Unfortunately, little is known about the recruitability of the various axonal classes as
495 a function of stimulus intensity in the neonatal mouse spinal cord. However, it is unlikely that
496 the recruitment of high threshold preganglionic axons is necessary to trigger the locomotor
497 generator because ventral roots that contain very few preganglionic axons (Biscoe et al., 1982)
498 can evoke locomotor activity when stimulated (Bonnot et al., 2009). These observations raise
499 the possibility that the axons of small α-motoneurons or those from γ- motoneurons have to be
500 stimulated to trigger the activity. γ-motoneurons are known to have recurrent collaterals and
501 also to receive recurrent inhibitory input from both α- and other γ-motoneurons. In contrast to
502 α-motoneurons, much less is known about the synaptic connections of γ- motoneurons within
503 the spinal cord and in particular if their only synaptic targets are Renshaw cells.

504 An alternative possibility is that stimulation of two roots causes some form of mutual
505 inhibition of the evoked responses. Consistent with this possibility, we found that stimulation of
506 two roots at 0.9-0.95 times threshold never evoked a locomotor-like episode whereas
507 stimulation of a single root at the same intensity resulted in locomotor-like activity in 8% of
508 trials. However, we believe this difference to be due to technical rather than biological factors.
509 When subthreshold stimulation of a single root does produce a bout of locomotor-like activity,
510 the observed bout tends to be weak and the locomotor strength tends to have a value close to
511 zero. In other words, bouts of locomotor-like activity elicited by subthreshold stimulation are
512 often barely detectable, whether on the basis of visual inspection of activity in the ventral root
513 or on the basis of the computed strength. In addition, subthreshold stimulation often evoked
514 disorganized activity and stimulus-locked firing. It is possible, therefore, that simultaneous

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subthreshold stimulation of a pair of roots increases non-locomotor activity that occludes the detection of what might have otherwise been barely detectable locomotor-like activity. The strength metric is sensitive to such occlusion because non locomotor-like activity can increase the variance of the signal and thereby affect the significance estimation of simultaneously occurring weak locomotor-like activity. In other words, increased variance can act as noise, and thus decrease the signal to noise ratio. And since significance estimation imposes a threshold such that values below the threshold are set to zero, weak bouts of locomotor-like activity that do not cross the significance threshold may result in strength values of zero.

The basic pattern of locomotor-like activity was similar in episodes evoked by dorsal and ventral root stimulation. Nevertheless, the pattern of activity evoked by each type of stimulus was distinct. At equivalent stimulus intensities (times threshold) ventral root evoked episodes generally exhibited a higher frequency of bursting than those evoked by dorsal root stimulation. In addition, the distribution of coherent power between the recorded ventral roots exhibited characteristic features for the two types of evoked episodes. With both types of stimulation, the coupling between the bilateral, flexor-dominated L1/2 roots was much stronger than the coupling between the L5/6 extensor-dominated roots. Clear differences were seen, however, between the coupling of the flexor- and extensor dominated roots between the two types of stimulation. When the dorsal roots were stimulated the power was similar for the flexor/extensor coupling on each side of the cord. Ventral root trains, however, produced episodes in which the flexor/extensor coupling ipsilateral to the stimulated root was as strong as that of the bilateral flexor L1/2 coupling and was ~2x stronger than that of the contralateral flexor/extensor coupling.

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The flexor dominance seen in the ventral root recordings might reflect an asymmetry in the operation of the central pattern generator and its outputs. Consistent with this idea, recording of burst deletions show that flexor deletions are accompanied by tonic extensor activity whereas extensor deletions do not perturb the flexor rhythm recorded from the L1 or L2 segments (McCrea and Rybak 2007; Zhong et al., 2012). This has led to the suggestion that the rhythm generating component of the CPG is asymmetric with a flexor center that generates the locomotor rhythm and an extensor center that bursts because of rhythmic inhibition derived from the flexor center (Shevtsova et al., 2015; Rybak et al., 2015).

Current models divide the locomotor CPG into a rhythm generating circuit and a pattern generating circuit that controls the timing of motoneuron bursts (Shevtsova et al., 2015; Rybak et al., 2015; Rybak et al., 2006a,b; McCrea and Rybak 2007, 2008; Zhong et al., 2012). Accordingly, the differences between dorsal and ventral root episodes might be due to differential activation of components of the pattern generating circuits by the two types of stimuli. However, while this may account for the differences in the strength of coupling between various motoneuron pools it cannot account for the differences in the frequency of DR- and VR-evoked episodes. This difference was particularly evident when the stimulus was switched abruptly from dorsal to ventral resulting in a progressive increase in burst frequency once the ventral root was stimulated (fig.10E and F). It is known that a subset of the V2a (Zhong et al., 2011; Ampatzis et al., 2014) and V0 (Talpalar et al., 2013) neuronal populations are progressively recruited as locomotor speed increases. Moreover, genetic ablation or acute silencing of V1 interneurons slows the locomotor rhythm (Gosgnach et al, 2006). It is possible,

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therefore, that the differences in the frequency of DR- and VR- evoked rhythms might be due to the differential recruitment of these interneuronal populations.

Finally, it is important to emphasize that our results cannot be explained by current models of the locomotor circuitry because they do not include excitatory inputs from motoneurons. This is largely because the nature of the motoneuron excitatory drive to the locomotor CPG is unknown. One possibility is that motoneurons project to a class of excitatory interneurons in addition to Renshaw cells. This suggestion has been made by Machacek and Hochman (2006) and Humphreys and Whelan (2012) who have provided evidence that the excitatory pathway is enhanced by noradrenaline and inhibited by dopamine. At the present time we do not know the identity of this excitatory pathway or the extent to which it is engaged during drug-induced or normal locomotion.

FIGURE LEGENDS

Figure 1. Schematic showing the calculation of Locomotor strength. The five numbered panels illustrate successive operations performed on the four ventral root records. The ventral root records are first high pass (HP) filtered at 50 Hz and the stimulus artefacts digitally removed (1). After rectification and low pass (LP) filtering (2), the cross wavelet spectra are computed (3) for each pair of ventral roots whose activity is expected to alternate (L5L vs L1L; L1L vs L1R; L1R vs L5R; L5R vs L5L). The non-locomotor phases (> 220˚ and < 120˚) are then set to zero (4). The four resulting spectra are then averaged (5) and the strength is calculated as indicated by the formula. The power in the cross wavelet spectrum is indicated by the color map shown to the right of the spectrum.

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In the lowest right hand panel, three locomotor-like episodes are shown with different locomotor strengths. Note the poor development of bursting in the bilateral L5 roots is reflected in the low strengths (146, 153) of the first two episodes. Once the L5 bursting occurs the strength increases to 275.

Figure 2. Stimulation of the DRG does not evoke locomotor-like activity if the corresponding dorsal root is cut. (A) Schematic to illustrate two possible ways in which dorsal root ganglion afferents (blue) might enter a ventral root. (B) Stimulation of the dorsal root ganglion (DRG) with the ventral root cut evokes locomotor-like activity. (C) DRG stimulation fails to evoke locomotor-like activity when the DRG is stimulated with the dorsal root is cut. (D) When the spinal nerve is stimulated with the dorsal root cut, locomotor-like activity is evoked. (E) DC recordings of the ventral roots during an episode of locomotor-like activity triggered by stimulation of the right DRG. (F) When the corresponding dorsal root is cut all ventral root activity is abolished when the DRG is stimulated.

Figure 3. The strength of locomotor-like activity increases with increasing stimulus intensity applied to either a dorsal or a ventral root. (A) Locomotor-like activity elicited by stimulation of the L6 ventral root at 1x (top traces) and 1.1x (bottom traces) threshold intensity. (B). In the same preparation, locomotor-like activity was elicited by stimulation of the L6 dorsal root at 1x (top traces) and 1.1x (bottom traces) threshold intensity. The bar indicates the duration of stimulus train (10 sec). The values of locomotor strength (S, see METHODS) computed for each of the displayed episodes is shown to the top and right of the traces. The displayed values reflect the effect of stimulation intensity (C) Locomotor strengths calculated for dorsal (558

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602 from 63 experiments) and ventral root-evoked episodes (188 from 24 experiments) are plotted
603 as a function of stimulus intensity expressed in multiples of threshold. The horizontal lines show
604 median values and the ends of the vertical lines mark the 25th and 75th quartile of the sample
605 set. The * above a group indicates a statistically significant difference with the group
606 immediately to the left.

607 **Figure 4. Comparison of the locomotor-like activity produced by dorsal sacral (A) and lumbar**
608 **ventral (B) root stimulation.** The average coherent power (+ SD) between the different root
609 pairs is expressed with reference to the normalized power of the ipsilateral flexor and extensor
610 ventral roots. For the dorsal root stimulation episodes 300 trials from 45 preparations were
611 used; for ventral root stimulation 145 trials from 25 preparations were used. The open circles
612 represent the averaged value of the coherent power for each preparation (45 DR, 25 VR). The
613 lines above the graph indicate significant differences between the connected pairs (Kruskal-
614 Wallis followed by chi-squared statistic, $p < 0.01$). The grey lines highlight the significant
615 differences between dorsal and ventral root stimulation.
616 i F-E, L1 vs L5 Ipsilateral to the stimulated root; Bi F-F, left L1 vs Right L1; c F-E, L1 vs L5
617 contralateral to the stimulated root; Bi E-E, L5 vs L5.
618
619 **Figure 5. Stimulation of a dorsal, but not a ventral root evokes a short latency potential in the**
620 **ventral funiculus.** (A) Schematic of the spinal cord showing the stimulation and recording setup.
621 A stimulating electrode is applied to the left L6 dorsal root (blue) and recording electrodes are
622 applied to the ipsilateral L6 ventral root (VR, red) and a strip of the contralateral L3 ventral
623 funiculus (VF, green). Below the schematic are shown 25 superimposed recordings from the

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ventral funiculus (green) and the ventral root (red) in response to dorsal root stimulation (4 times Threshold) once every 30s. The arrow heads show the point at which the minimum and maximum latency difference (jitter) was computed. (B) The left pair of bars show the mean (± SD) onset latencies of the DR-evoked VR (red) and VF (green) responses, whereas the right pair show the average jitter for each type of recording (6 experiments; 25 trails each). * indicates statistically significant difference in onset latency ($p < 0.001$, Tukey's multiple comparisons test). (C) Schematic showing the spinal cord with the left L6 VR (red) stimulated and the contralateral L3 VF (green) recorded. The dashed red line within the L6 segment shows the putative monosynaptic connection between the stimulated VR (red) and the cell bodies of the recorded VF interneurons (green). Below this is the response in the VF recording to 100 stimuli (100 μA) applied to the L6 VR every 30s. The green area defines ± 2 SD of the mean. (D) Long-latency responses evoked in the VF in response to ventral root stimuli. (E) Simultaneous low pass filtered ventral root (VR) and VF recordings of a locomotor-episode in response to a ventral root stimulus train (4 Hz).

Figure 6. Simultaneous stimulation of dorsal- and ventral roots at just sub-threshold intensities does not elicit locomotor-like activity. (A and B) The top traces show examples of locomotor-like rhythms elicited by stimulation of the homonymous dorsal (left, DR stim.) and ventral (right, VR stim.) roots at threshold intensity (1x Thr). The line at the bottom of the traces shows the duration of the stimulus train (10 sec). The activity was high-pass filtered at 50Hz. The traces below this show that locomotor-like activity was not produced by root when the stimulus intensity was set to 0.9x threshold. (C) When the two subthreshold stimuli were

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combined locomotor activity was still not evoked (D) Box plots showing pooled data from several experiments (43 trials from 18 preparations). Stimulation of the dorsal and ventral alone or together does not result in locomotor-like activity. The values of strength were normalized to the mean of the ventral root sample set. The lines in the box plots show the median values, and the edges of the black rectangles show the 25th and 75th quartiles of the data.

Figure 7. Interleaved, suboptimal dorsal and ventral root-evoked responses combine to trigger locomotor-like activity. (A) An episode of locomotor-like activity elicited by stimulation of the left L6 dorsal root with a train of electrical pulses appearing every 375ms. The black traces show ventral root signals from the left (top) and right (bottom) L1 ventral roots, while the spectrogram above displays the corresponding crosswavelet power spectrum. The hot regions in the spectrum with leftward pointing arrays atop indicate locomotor-like activity, while the cooler regions with rightward pointing arrows result from stimulus-locked synchronous responses. The blue vertical ticks below the ventral root traces indicate the stimulus pulses. The trace on the right shows the global power spectrum obtained by summing values in the crosswavelet spectrum along the time dimension for all frequencies. The global power spectrum displayed here has been normalized by the maximum value. The number displayed next to the peak of the global power spectrum (in red) indicates the frequency at which the power peaks. The red polar plot in the lower right hand corner shows the power-weighted distribution of phase angles (indicated by arrows) for all regions of significant power appearing the crosswavelet spectrogram. (B) An episode of locomotor-like activity elicited in the same preparation as in A, but by stimulation of the L6 ventral root with a train of electrical pulses delivered at intervals of 375 ms (vertical red ticks). (C-D) Activity resulting from the stimulation

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of the aforementioned dorsal and ventral root respectively, but with pulse trains with an inter-pulse-interval twice as long as in A and B (i.e., 750ms). The activity evoked at this stimulus frequency is not locomotor-like: although the bursts in the two ventral roots appear somewhat rhythmic, their phases are not tightly coupled and completely out-of-phase. (E) When the suboptimal dorsal and ventral root stimulus trains were offset by 375ms so as to interleave the pulses delivered to the different roots (red and blue vertical ticks below the ventral root traces) and create a combined train with an effective inter-pulse-interval of 375ms, locomotor-like activity was evoked.

Figure 8. Interleaved, suboptimal brainstem and ventral root stimulus trains combine to trigger locomotor-like activity. The layout of this figure is the same as that of fig.7. (A, B) Cross-wavelet spectrograms (top) of locomotor-like activity recorded from the left and right L6 ventral roots (black traces, bottom) evoked in response to stimulation of the brainstem or the L5 ventral root with an optimal 6.7Hz stimulus train. The blue and red bars below the ventral root activity represent the stimuli. The maximum XW power is at 0.6 HZ (Brainstem) and 0.77 Hz (Ventral root) and is shown in the power spectra on the right. The activity between the roots is alternating (arrows to the left, phase plot in the lower right hand corner). (C, D) Stimulation of the brainstem at the sub-optimal frequency of 3.33 Hz evoked synchronized bursting (arrows to the right). Stimulation of the ventral root at the 3.33 Hz evoked weak alternating bursts together with the stimulus locked synchronous signals. (E) Interleaving the 3.33 Hz brainstem and ventral root stimulus trains (alternating blue and red bars) evoked locomotor-like activity.

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690 **Figure 9. Comparison of the strength of locomotor-like activity evoked by optimal, suboptimal**
691 **and interleaved dorsal and ventral root stimulation.** Box and whisker plots compare the
692 strengths of locomotor-like episodes evoked when stimulating at the optimal frequency,
693 suboptimal frequency and interleaved suboptimal frequencies. The horizontal lines within the
694 boxes indicate the median values, the lower and upper edges of the boxes mark the 25th and
695 75th percentile, and the whiskers extend to the most extreme data points. The X indicates that
696 locomotor-like activity could not be elicited. The strength is expressed as normalized values
697 because data from different preparations were pooled. The data were generated from 7
698 ipsilateral pairs of roots and 6 contralateral pairs.
699
700 **Figure 10. Locomotor-like activity initiated by stimulation of one pathway continues without**
701 **interruption when stimulus is abruptly switched to a different pathway.** (A, B) The panels
702 show episodes of locomotor-like activity recorded from the left and right L1 ventral roots,
703 initiated by either dorsal (A. DR stim 15 sec, 4 Hz, 2 x Thr.) or ventral root (B. VR stim 10 sec, 4
704 Hz, 2x Thr.) stimulation. (C, B) These panels show locomotor episodes evoked by sequential
705 dorsal and ventral root stimulation. In panel C, the dorsal root is stimulated for 5 seconds (blue
706 stimulus markers below neurograms) followed by ventral root stimulation for 10 seconds (red
707 stimulus markers below neurograms). In panel D the order is reversed with the ventral root
708 stimulus (5 sec) followed by the dorsal root stimulus (10 sec). In each case the locomotor-like
709 episode was evoked at 0.52 Hz. (E) Locomotor-like activity evoked by stimulation the Right S2
710 dorsal root for 10 seconds followed by stimulation of the Right L5 ventral root for 5 seconds. In
711 this example the switch in the stimulated root resulted in a rapid change in locomotor

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frequency from 0.46 Hz to 1.4Hz without obvious phase perturbation (see phase plot in lower right hand corner). (F) Instantaneous locomotor frequency the evoked locomotor episode shown in panel E.

Table 1. Pairs of ventral and dorsal roots stimulated to assess spatial facilitation. The various combinations of dorsal and ventral roots are shown with the number of pairs of each class used (number).

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Figure 1 Pujala et al. 2 Col

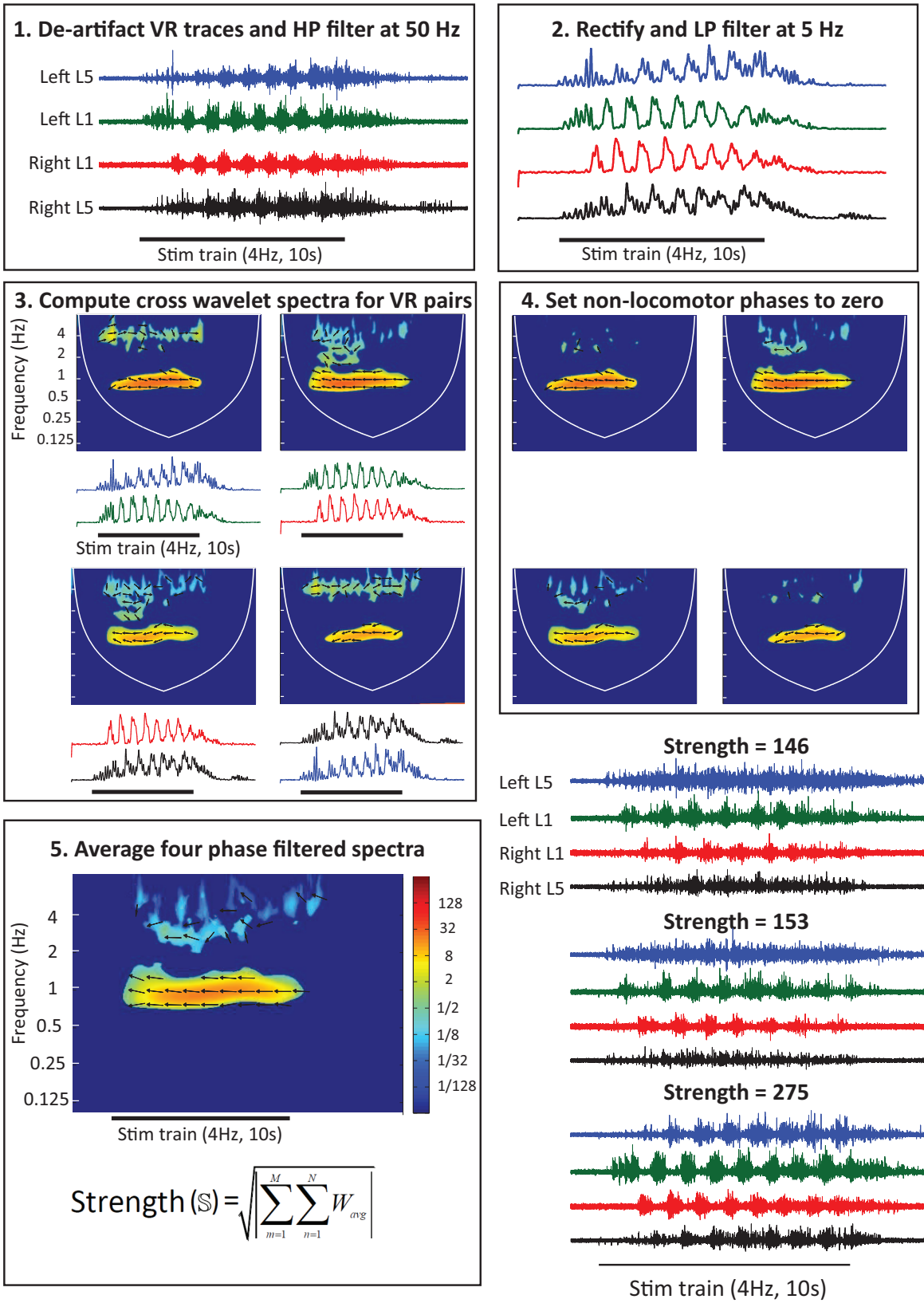

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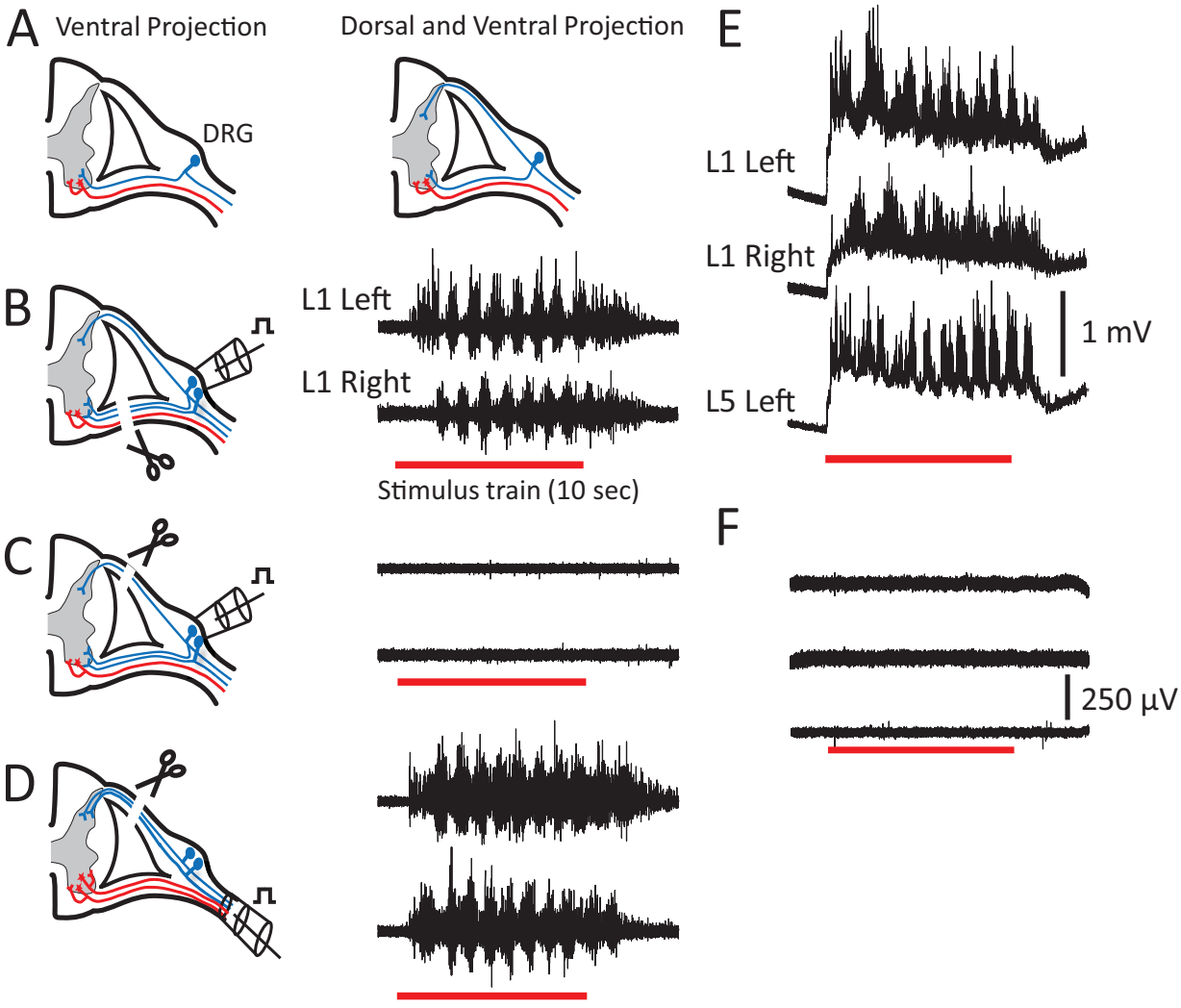
Figure 2 Pujala et al. 2 Col



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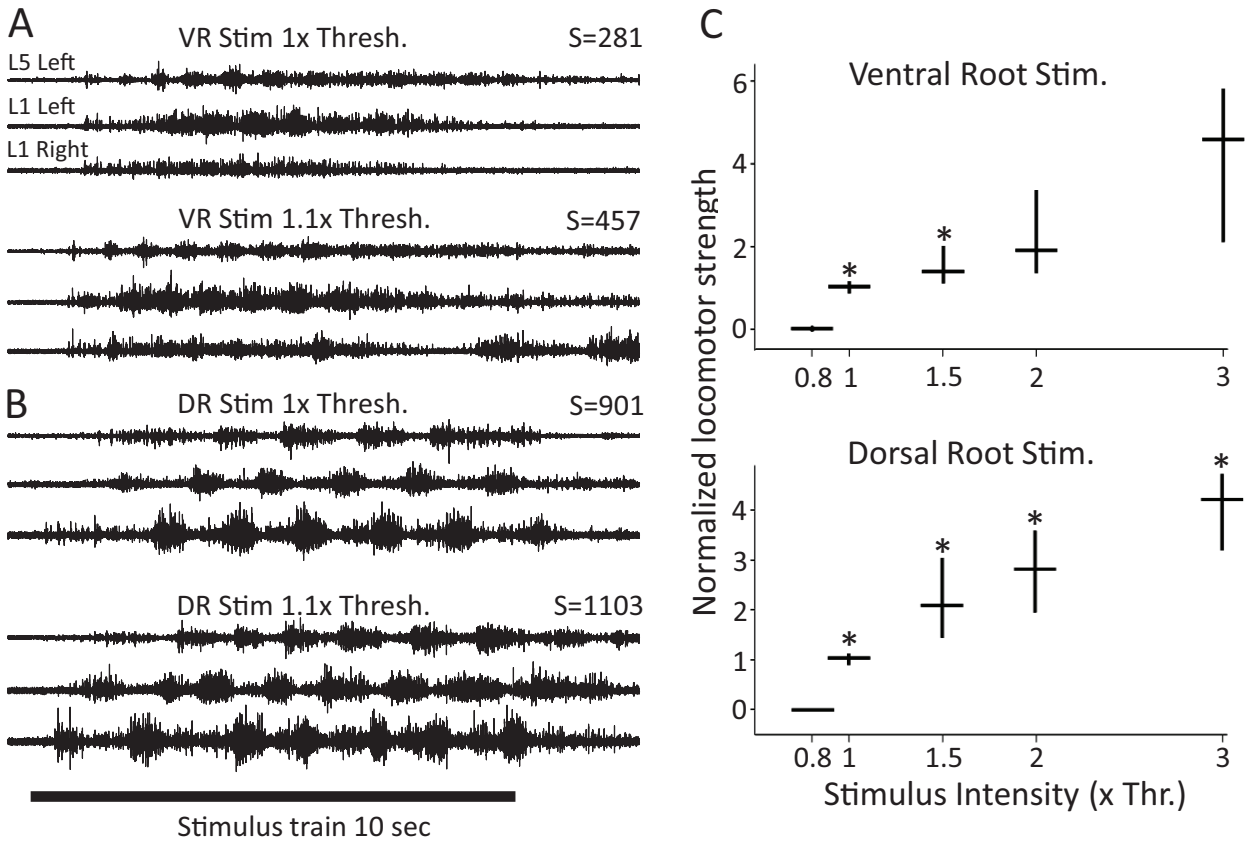

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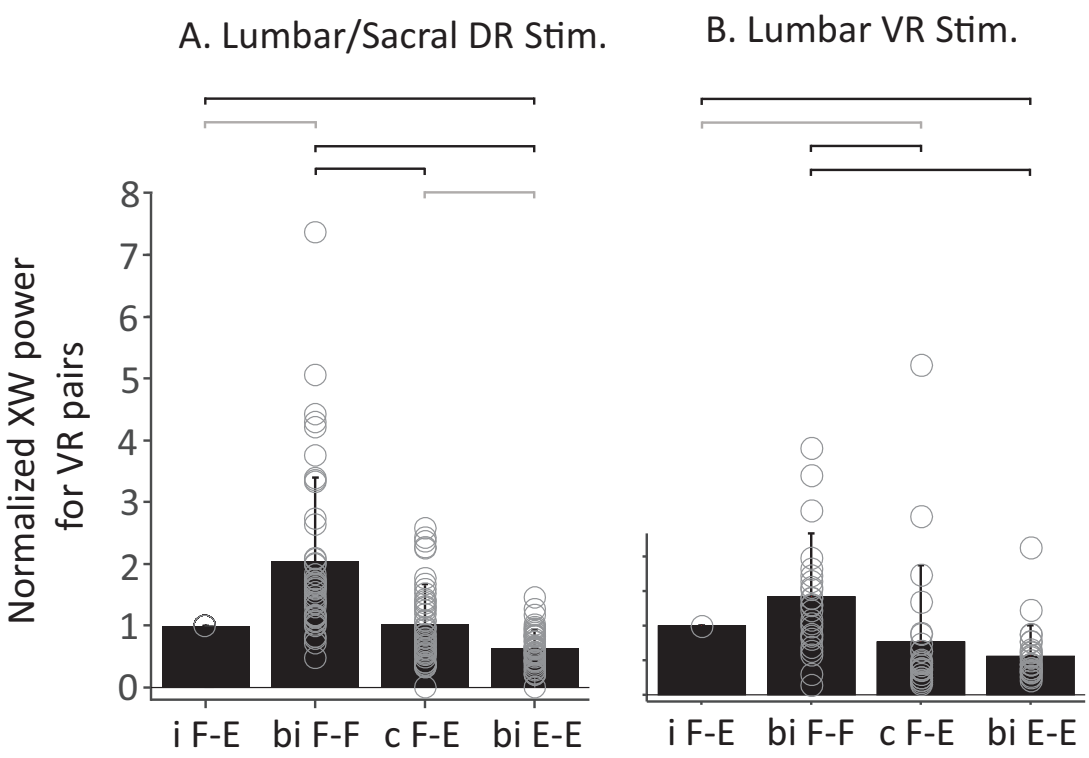

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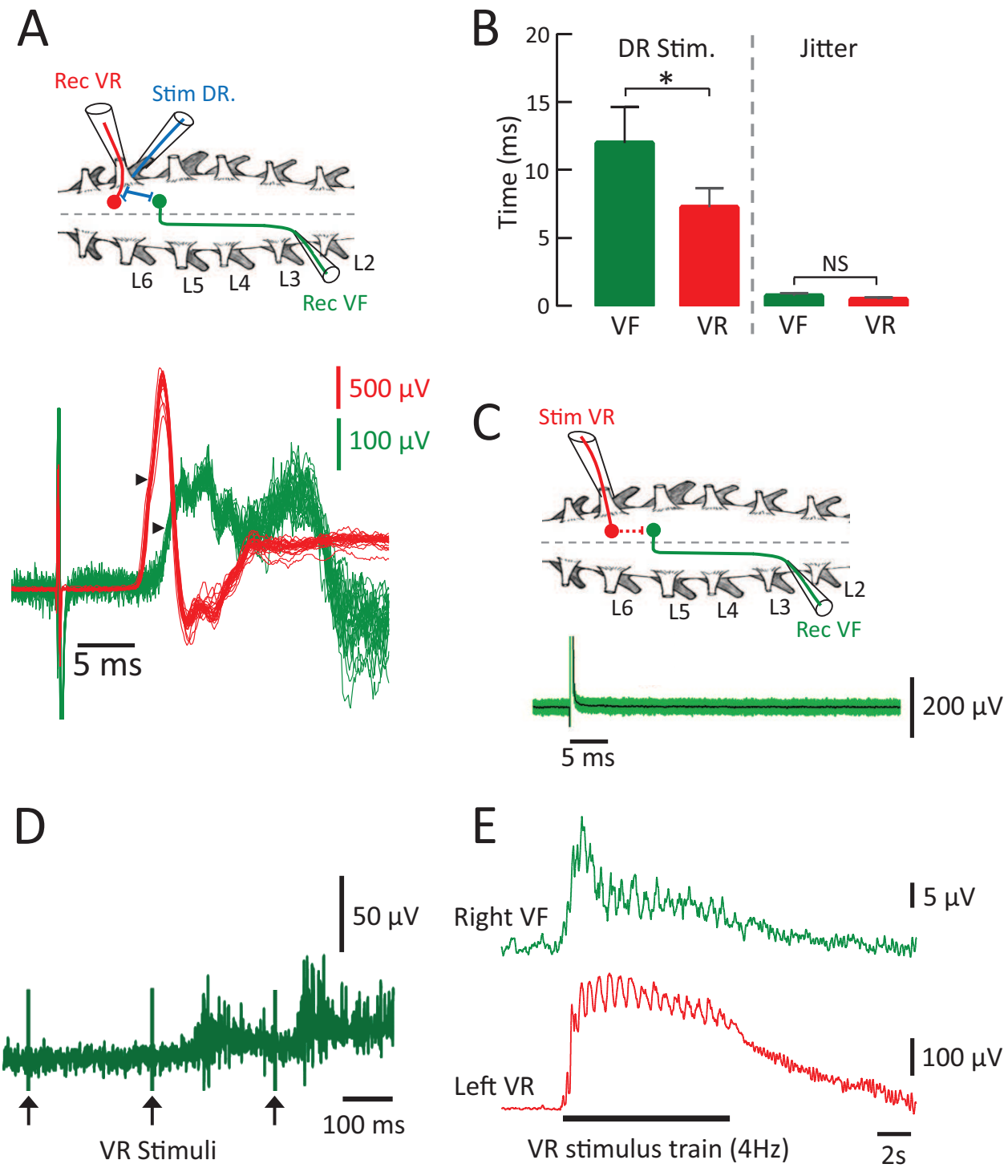

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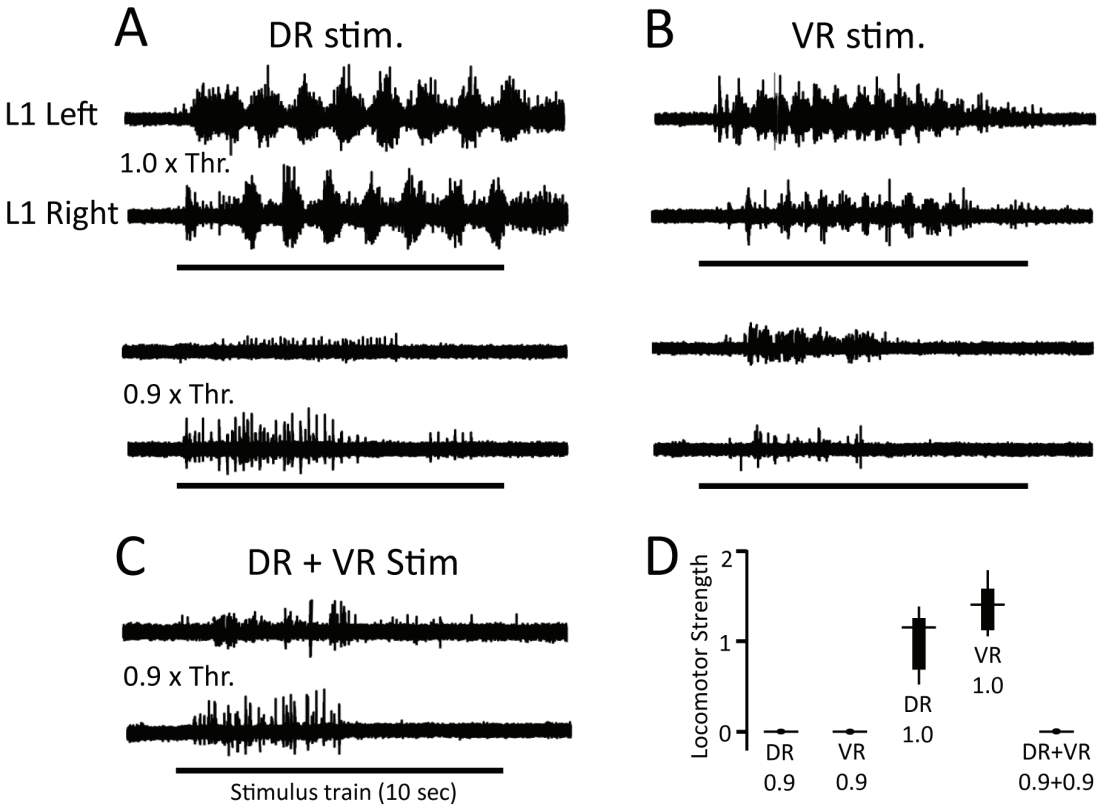

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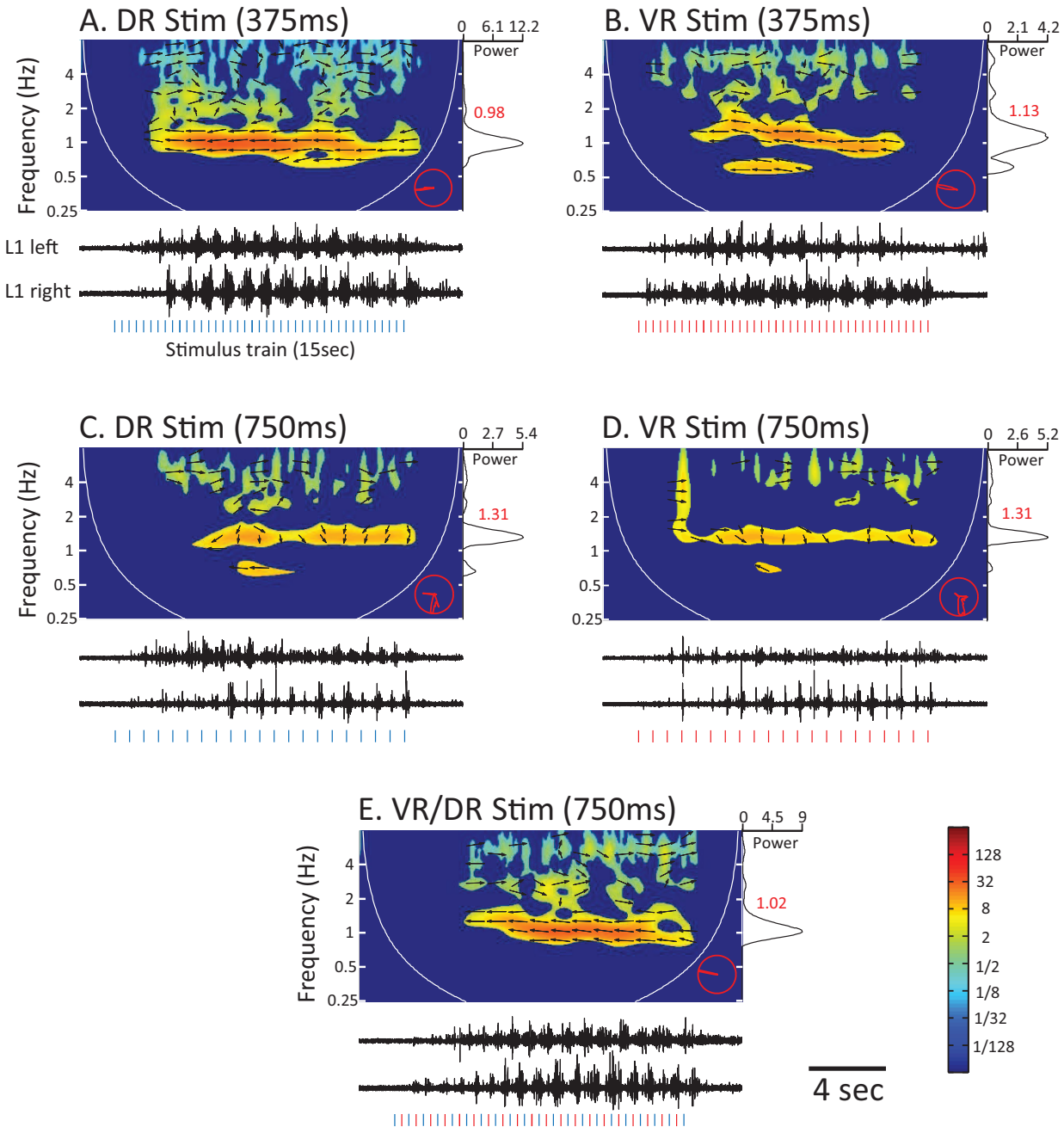
Figure 7 Pujala et al. 2 Col



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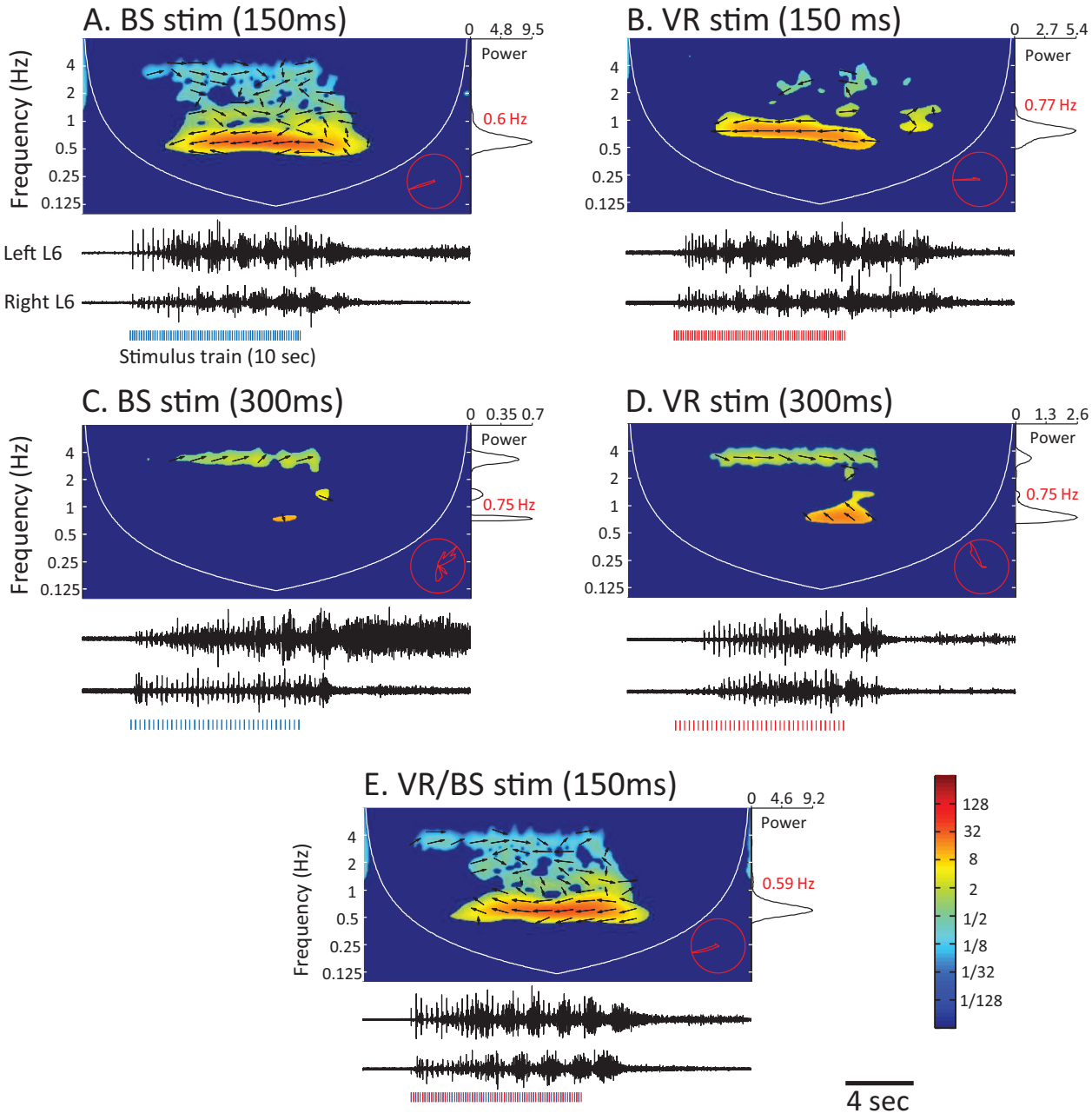

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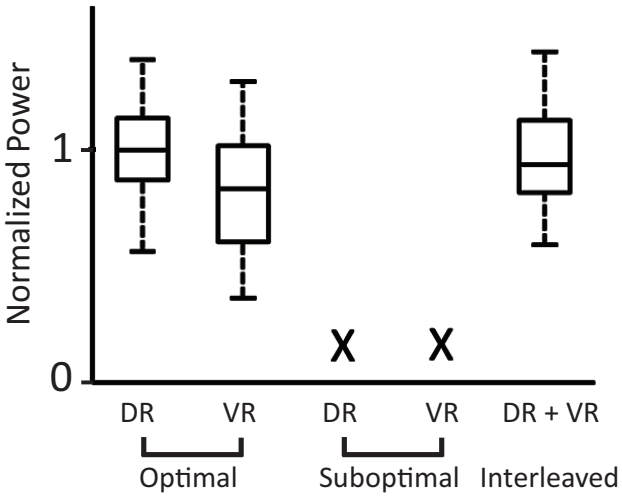

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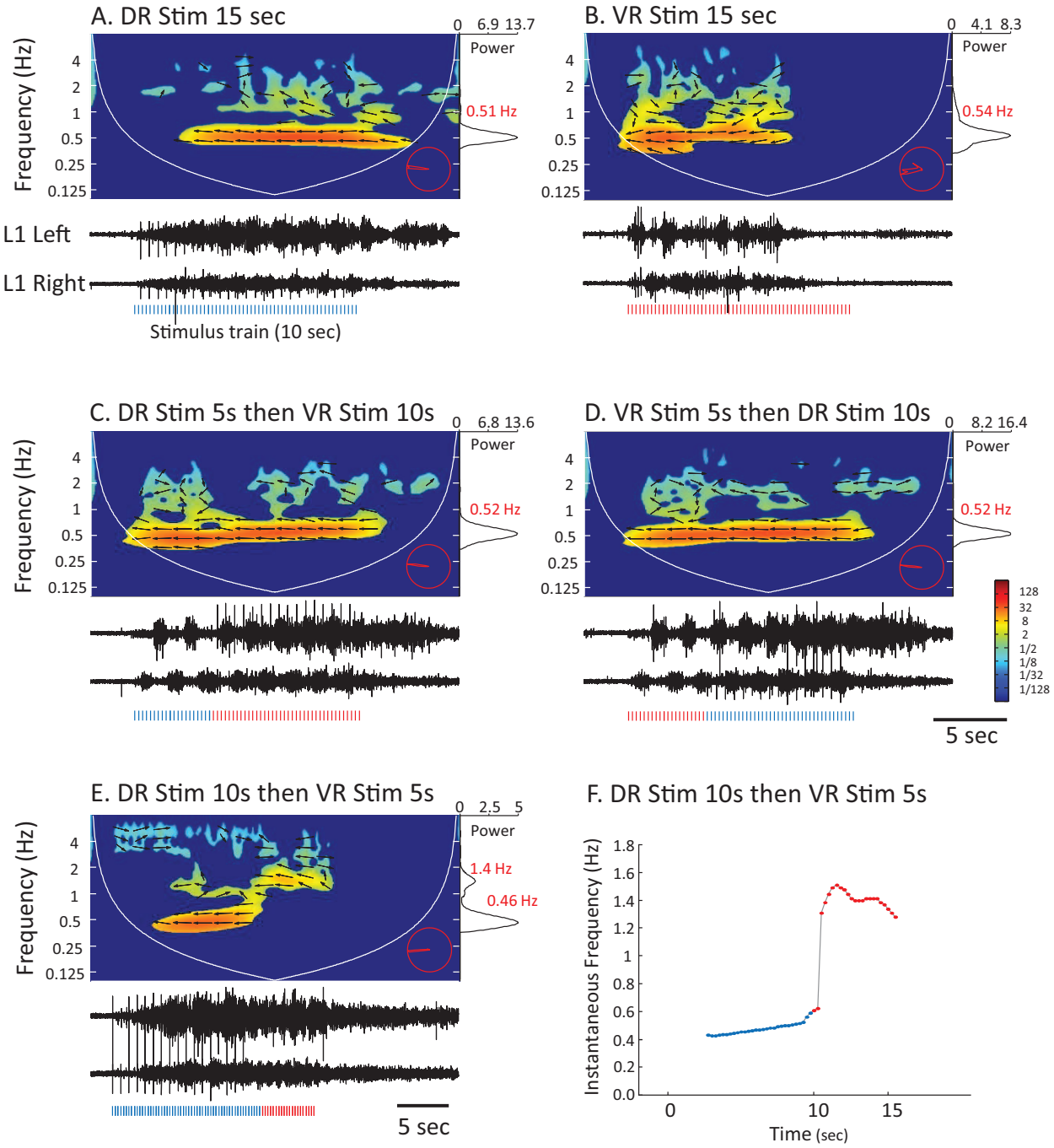
Figure 10 Pujala et al. 2 Col



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Dorsal Root	Ventral Root	Number
L5	Ipsi L4	3
	Ipsi L5	5
	Ipsi L6	2
	Contra L5	2
L6	Ipsi L5	3
	Ipsi L6	6
	Contra L6	2
	Contra L5	1
S1	Ipsi L6	3
	Contra L6	2