Analysis of the Mammalian Central Pattern Generator through the Characterization of Lumbar Interneuronal Activity

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In spinal cord injury rehabilitation, one of the main methods used involves re-training the patients' spinal circuitry to produce movement. Located in the spinal cord, the central pattern generator (CPG) is the term used to describe the neural circuitry which drives locomotion within vertebrates. Understanding the composition and architecture of the CPG will assist the development of rehabilitation approaches for spinal cord injury.

Models of the spinal CPG include the half center model, unit burst model and the two-layer model where the rhythm is set independently of the muscle activation patterning. The purpose of this study was to explore if lumbar interneuronal firing activity is compatible with the proposed two-layer model of the CPG in spinalized cats.

We analyzed neuronal firing activity during spontaneous disruptions of muscle activity resulting in silent or tonic muscle activity during air-stepping. These episodes, called deletions, were detected using the time differences between successive electromyography (EMG) onset times for each trial. Deletions were further classified as non-resetting or resetting. In a resetting deletion, the perturbed muscle activity returns at a non-integer multiple of the pre-deletion locomotor cycle period while it returns at a multiple of the original period in a non-resetting deletion. A separate rhythm generating layer in the CPG circuitry is hypothesized to be responsible for this preservation of the locomotor cycle "clock". Neuronal activity was classified as rhythmic, silent or tonic throughout a deletion period.

Neural activity was recorded by two 64 site microelectrode arrays (model A8x8-5mm-200-200-177, Neuronexus) inserted at a depth of 3000 μm in two lumbar segments along the rostral caudal axis. Electromyography activity of seven hind-limb muscles was recorded bilaterally. In-vivo extracellular recordings of neurons of the intermediate zone and ventral horn of the lumbar spinal cord were taken simultaneously with hind limb Electromyography (EMG) in a spinal adult air stepping cat. Recorded activity was analyzed using our in-house MATLAB code. Additional analysis was all conducted via custom MATLAB algorithms. The coefficient of variation squared was used as a measure of the spike train behavior (MATLAB function, gammafit). Additional interneuronal analysis was performed in MATLAB between spikes via generalized linear model (GLM), depth analysis and cross correlation histograms to evaluate their relationships.

Through the characterization of the firing of 356 total interneuronal units during non-resetting and resetting deletions, we found that there was a significant portion of units which maintained their rhythmicity throughout non-resetting deletions. Additionally, we found that most units which had their rhythmicity disrupted were recorded during resetting deletions. Believed to encode the rhythm generator layer, the units which maintained rhythmicity during non-resetting deletions showed an inconclusive relationship that was neither inhibitory or excitatory through the GLM. This evidence supports the hypothesis that some interneurons encode the clock feature of the rhythm generator layer in the two-layer CPG model by maintaining rhythmicity throughout a deletion, but the relationship of those encoding interneurons is still continually being assessed.

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Evidence for a Two-Layer Central Pattern Generator from the Characterization of Spike Train Activity

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ABSTRACT

In mammals the central patter generator (CPG), located in the spinal cord, is responsible for the activation of flexor and extensor motor neurons. It is necessary to understand the architecture of the spinal CPG in order to advance therapeutic interventions for spinal cord injury. The two-layer model of the CPG has been proposed against other models such as the unit burst generator (UBG) model and the single layered half center model. Evidence for the two-layer CPG model comes from muscle activity re-starting on an integer multiple of its pre-deletion period, where "deletions" are spontaneous disruptions of the locomotor cycle where muscle activity goes silent or tonic. EMG of 7 hind limb muscles was recorded along spinal interneural activity in adult cats[2]. Through characterization of interneural spike activity, we found that during non-resetting deletions there are a proportionally higher amount of spike train units which maintained their rhythmicity throughout a deletion episode. This contrasts with the resetting deletions which had higher proportions of spike train units that went from being rhythmic to silent. These findings support the two-layer CPG model by suggesting that the some interneurons encode the clock feature of the RG layer by maintaining rhythmicity throughout a deletion.

BACKGROUND

The two-layer Central Pattern Generator is a model which drives locomotion in vertebrates.

- Rhythm generator (RG) layer acts as a rhythmic clock
- Pattern formation (PF) layer controls the distribution of excitation and inhibition signals to motor neuron pools coordinating muscle activity[3]

Evidence for a two-layer CPG model was initially gathered from deletion episodes during fictive locomotion.

- A deletion is a reduction of at least one rhythmic burst of activity in multiple neuron pools[3]
- Non-resetting (NR) deletions occur in the PF layer which disturbs the firing of neuron pools. Resetting (R) deletions occur in the RG and perturb the locomotor "clock".

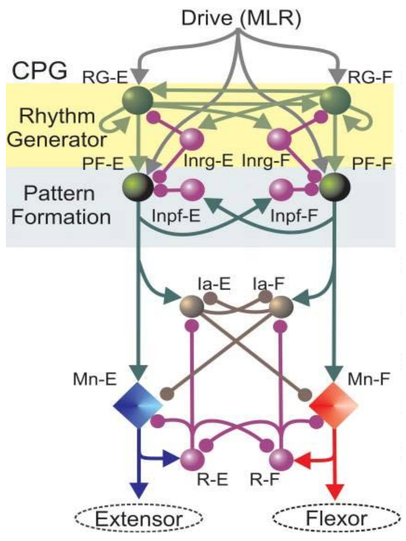


Figure 1: Two-Layer Central Pattern Generator Model

Model of the central pattern generator in which populations of interneurons are circles and motoneurons are diamonds

METHODS

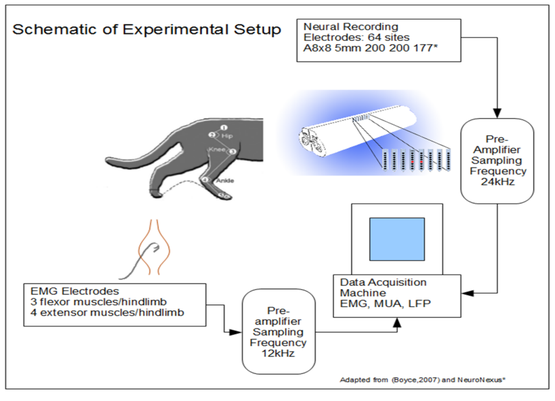


Figure 2: Schematic of Experimental Setup

- Muscle and interneuronal activity during air-stepping in spinalized cats was examined to determine the presence of deletion episodes during locomotor bouts[4]
- Electromyography (EMG) was taken of 3 flexor and 4 extensor muscles in the hind limb of a spinalized cat
- Spike train activity was recorded, characterized and plotted against its respective EMG trial.

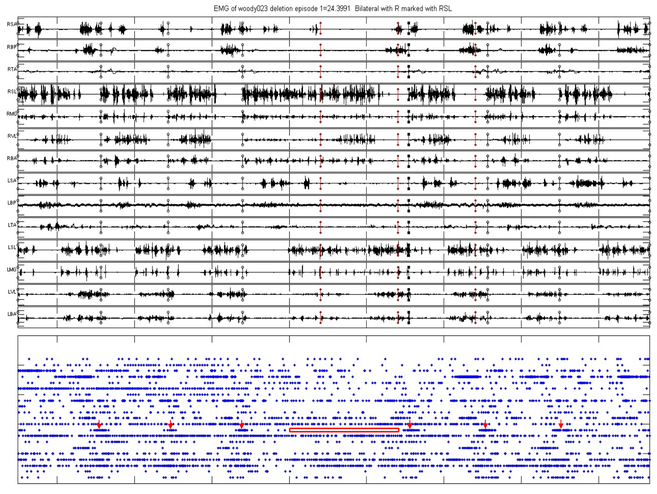


Fig. 2: Resetting Deletion

Top: EMG of Sartorious, Biceps Femoris Posterior, Tibialis Anterior, Medial Gastrocnemius, Vastus Lateralis, Biceps Femoris Anterior, Soleus
Bottom: Spike train activity

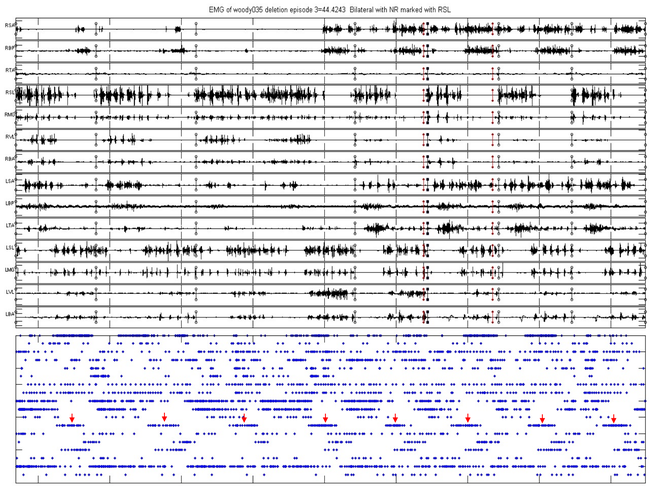


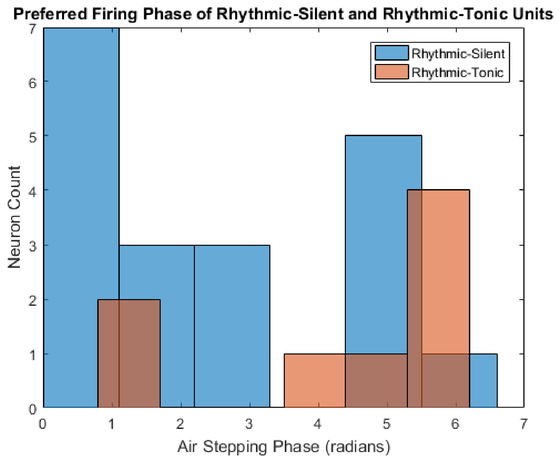
Fig. 3: Non-Resetting Deletion

Top: EMG of Sartorious, Biceps Femoris Posterior, Tibialis Anterior, Medial Gastrocnemius, Vastus Lateralis, Biceps Femoris Anterior, Soleus
Bottom: Spike train activity

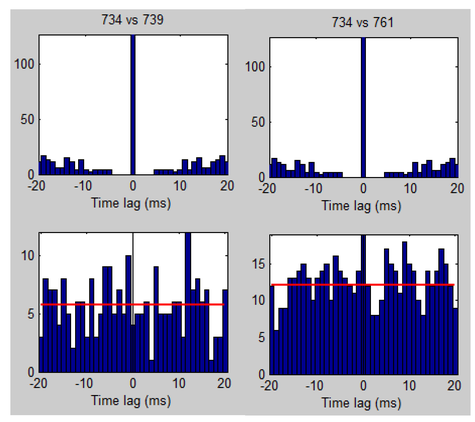
RESULTS

Resetting			
Characterization	Count	Total	Percentage
Rhythmic	22	139	15.8
Rhythmic-Silent	63	139	45.3
Rhythmic-Tonic	39	139	28.1
Tonic	15	139	10.8
Non-Resetting			
	Count	Total	Percentage
Rhythmic	35	72	48.6
Rhythmic-Silent	22	72	30.6
Rhythmic-Tonic	6	72	8.3
Tonic	9	72	12.5

High proportion of rhythmic activity in NR deletions compared to rhythmic to silent activity in R deletions is evidence of RG



Firing phase of RT units favors extension phase throughout NR deletions while RS units favor the flexion phase



Cross correlation histograms between flexor and extensor related units hypothesized in PF layer show trend of inhibitory coupling

CONCLUSION

- High proportion of rhythmic spike train activity during non-resetting deletions supports evidence of the RG
- Asymmetrical interaction between perturbed units suggests participation in the pattern formation layer
- A majority of the perturbed flexor and extensor related units during NR deletions are in reciprocal inhibition with one another

ACKNOWLEDGEMENTS & REFERENCES

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