Afferent signaling and interneuronal activity during non-fictive locomotion in the in vitro spinal cord - hindlimb rat preparation

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During locomotion, the spinal cord receives sensory input that must be processed and used to refine and reinforce locomotor output. To study this sensory input and processing, we developed a neonatal rat spinal cord-hindlimb preparation (**SCHP**) consisting of an isolated neonatal rat spinal cord with hindlimbs intact and oriented dorsal-up to locomote on a surface (Hayes et al 2009). The SCHP combines the neural accessibility of in vitro preparations with sensory-modulated, non-fictive locomotion. Further, the spinal cord can be mechanically stabilized to allow intracellular and extracellular recordings in a controlled solute environment. Using the advantages of the SCHP, we studied the modulation of sensory activity in dorsal roots (**DRs**) and interneurons during non-fictive monoamine-induced locomotion and in response to mechanical perturbations.

During 5HT, DA, and NA locomotion, DRs often exhibited rhythmic bursting. Their rhythmicity was modulated with locomotor strength and enhanced by ground contact. Assistive locomotor-like limb movements could also initiate locomotion and DR rhythmicity in sub-locomotor-threshold 5HT or DA, or increase ongoing DR rhythmicity. Toe and tail pinches could reset ongoing 5HT and DA locomotion, or initiate brief locomotor bouts with DR rhythmicity in the absence of drugs. Thus, afferents in the SCHP have strong access to the spinal locomotor network, allowing them to alter or induce locomotion.

We also observed locomotor-related rhythmic dorsal root potentials (**DRPs**), representing presynaptic inhibition of afferent input. L2 DRPs tended to be in-phase with flexor activity, while L5 DRPs tended to be out-of-phase. DRP amplitude was most pronounced during robust locomotion and was clearly facilitated during limb loading and ground contact, suggesting that the extent of presynaptic inhibition varies with locomotor conditions. Part of the locomotor-related DRPs were $GABA_A$ receptor independent as they persisted after application of bicuculline. Large rhythmic DRPs were also evoked or enhanced by assistive limb movements and toe or tail pinches, with or without drugs present.

Finally, we performed the first whole-cell patch recordings from mammalian spinal interneurons during unrestrained locomotion. We observed dorsal horn interneurons that were nearly quiescent at rest, but received rhythmic drive potentials and fired repetitively during locomotion at a rate proportional to locomotor strength. In response to toe pinch, these neurons received strong inhibition followed by rebound firing. Although preliminary, intracellular recordings during locomotion are an important step in the study of spinal sensory processing.

Keywords: CPG, Sensory Neurons, Presynaptic Inhibition