Karolinska Institutet

Institutionen för Neurovetenskap

Organization of the spinal locomotor network in zebrafish

Pattern of recruitment and origin of excitatory drive

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Abstract

Locomotion is of vital importance for animals to survive in a complex environment. Locomotor movements are generated by neuronal networks that reside in the spinal cord and are composed of motoneurons and interneurons. The aim of this thesis was to investigate the organization of the spinal circuitry with emphasis on the recruitment and origin of excitation therein.

We used an *in vitro* adult zebrafish preparation to investigate the recruitment pattern of the motoneurons and the underlying mechanisms. We found that the motoneurons in the adult zebrafish are organized into four pools with a specific topographic location in the spinal motor column. These motoneuron pools are recruited from ventral to dorsal with increasing swimming frequencies. In contrast to the larval zebrafish, the recruitment order of motoneurons in adult zebrafish is not set by their input resistance, but instead by a combination of their biophysical properties and the strength of the synaptic drive to motoneurons.

V2a interneurons constitute an important class of excitatory interneurons that make monosynaptic connections with motoneurons. To test if these interneurons provide the excitatory drive underlying locomotion, we used larval zebrafish. Photoablation of a part of the V2a interneuron population decreased the excitability of the spinal network. In zebrafish with ablated V2a interneurons the threshold for inducing locomotion was increased both pharmacologically and with electrical stimulation. These animals also showed an increase in the rostro-caudal delay. These results indicate that V2a interneurons represent an intrinsic source of excitation necessary for the normal expression of swimming activity.

Finally, we used optogenetic tools to determine if activation of V2a interneurons is sufficient to elicit swimming activity in larval zebrafish. For this transgenic zebrafish were used in which ChR2 was selectively expressed in V2a interneurons. Light activation of V2a interneurons induced synaptic excitatory inputs in other V2a interneurons that was sufficient to induce rhythmic locomotor activity. These results show that selective optogenetic activation of V2a interneurons is able to generate swimming activity in larval zebrafish.

The work presented in this thesis has provided new insights into the organization of the zebrafish spinal locomotor circuitry. We have gained novel insights into the mechanisms governing recruitment of motoneurons. In addition, we have uncovered the excitatory interneurons at the origin of the excitatory drive necessary for the generation of locomotion.

Key words: zebrafish, locomotion, motoneuron, recruitment order, V2a, Chx10, excitatory interneuron, ChR2

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