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Characterization of Calbindin Positive Interneurons within the Ventral Horn of the Mouse Spinal Cord

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Characterization of Calbindin Positive Interneurons within the Ventral Horn of the Mouse Spinal Cord

Taylor L. Floyd and David R. Ladle

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Introduction

Sensory-motor circuits in the spinal cord integrate sensory feedback from muscles and coordinate locomotor behavior. Although we know how the sensory-motor system generally works, the main issue lies in identifying all neurons involved and understanding their interrelationships. Many interneurons contribute to sensory-motor circuits and have been well studied. For example, Renshaw cells (RCs) are inhibitory interneurons that prevent motor neurons from over-activating. A distinguishing feature of RCs is that they are only interneurons within the ventral-most region of the spinal cord expressing the calcium binding protein calbindin (CB). Recent studies have found other subpopulations of ventral horn interneurons outside of the RC area that express CB, but knowledge regarding the function and connectivity of these neurons is limited. We hypothesize CB expression serves a functional purpose for ventral horn interneurons as well as identifying RCs. Here we compare known characteristics of RCs with other ventral horn interneurons that express CB.

Table 1. Ventral Horn CB Positive Interneuron Characteristic Checklist

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Motor neuron contact</th>
<th>Sensory afferent contact</th>
<th>Calcium binding proteins</th>
<th>Premotor neuron</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1 progenitor group</td>
<td>Yes</td>
<td>Yes (until P15)</td>
<td>CB, calretinin, parvalbumin</td>
<td>Yes</td>
</tr>
<tr>
<td>V2 progenitor group</td>
<td>No</td>
<td>No</td>
<td>CB, calretinin</td>
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</table>

Methods

Sensory-motor circuits in the spinal cord integrate sensory feedback from muscles and coordinate locomotor behavior. Although we know how the sensory-motor system generally works, the main issue lies in identifying all neurons involved and understanding their interrelationships. Many interneurons contribute to sensory-motor circuits and have been well studied. For example, Renshaw cells (RCs) are inhibitory interneurons that prevent motor neurons from over-activating. A distinguishing feature of RCs is that they are only interneurons within the ventral-most region of the spinal cord expressing the calcium binding protein calbindin (CB). Recent studies have found other subpopulations of ventral horn interneurons outside of the RC area that express CB, but knowledge regarding the function and connectivity of these neurons is limited. We hypothesize CB expression serves a functional purpose for ventral horn interneurons as well as identifying RCs. Here we compare known characteristics of RCs with other ventral horn interneurons that express CB.

Table 2. Primary Antibodies Used in This Study

<table>
<thead>
<tr>
<th>Antibody Name</th>
<th>Type</th>
<th>Host Species</th>
<th>Dilution</th>
<th>Company</th>
<th>Labeling Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calbindin - polyclonal</td>
<td>rabbit</td>
<td>rabbit</td>
<td>1:10,000</td>
<td>SWANT</td>
<td>Renshaw cells, motor neuron, sensory dorsal root axons</td>
</tr>
<tr>
<td>Parvalbumin - polyclonal</td>
<td>rabbit</td>
<td>rabbit</td>
<td>1:500, preadsorbed at 1:20 with PBB3</td>
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<td>Presumptive inhibitory afferent axons, Napa subtype</td>
</tr>
<tr>
<td>Calretinin - polyclonal</td>
<td>mouse</td>
<td>mouse</td>
<td>1:8000</td>
<td>Zymed</td>
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</tr>
</tbody>
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Future Directions

We will be conducting immunohistochemical experiments to evaluate whether all ventral horn CB positive neurons:

- Express GABA
- Receive motor neuron contacts
- Receive sensory contacts
- Express all three calcium binding proteins
- Act as premotor neurons

What about CB positive GABAergic neurons?

- RCs highly express GABA in the embryonic spinal cord but by birth, many RCs switch from GABA to glycine.
- RCs and BCBs expressing CB also express glycine.
- Many RCs are surrounded by GABA receptors throughout development but hardly any express GABA within the soma.
- Therefore, we believe it is highly improbable for any RCs in this study to express GABA throughout postnatal development.
- If BCBs are shown to express high GABA levels, then the differences in neurotransmitter expression may indicate RCs and BCBs are different cell types.

Conclusions

- Larger amounts of RCs are found in the lumbar spinal cord than BCBs during development.
- After P28, the amount of CB positive neurons in the spinal cord decreases by 29% for RCs and 38% for BCBs.
- Throughout development, the percentage of glycinergic RCs steadily decreases while glycinergic BCB levels stop changing after P28.
- The amount of RCs and BCBs reducing glycine and CB expression may be due to the downregulation of CB in the ventral spinal cord.
- Differences in glycine and GABA neurotransmitter expression may reveal differences between RCs and BCBs.

Acknowledgements

This work was supported by NIH Grant NS072454.

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Image Analysis

GlyT2-GFP and wild type (WT) mice at ages P7 (postnatal day 7), P14, P28, and P45 (3-4 per age group) were anesthetized and perfused transcardially with 10ml of phosphate buffered saline (PBS) solution followed by 20ml of 4% paraformaldehyde. For P0 and P7 pups, anesthesia was induced by exposure to ice cold water while P14, P28 and P45 mice received 0.13% (1.04mg) and 0.15% (1.45mg) of Euthasol respectively. After perfusing, the lumbar spinal cord was dissected and placed in 6% paraformaldehyde for 24 hours then 30% sucrose for another 24 hours. The lumbar region was dissected into three sections (thoracic 3-rostal lumbar 5, caudal lumbar 5-rostal lumbar 5, caudal lumbar 5-sacral 1) and frozen in tissue tek for 24 hours. After freezing the tissue, cords were cut on a cryostat into 20μm thick sections.

Ventral Horn CB Positive Cell Density in the Lumbar Spinal Cord

![Graph showing cell density comparison]

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Image Analysis

GlyT2-GFP and wild type mice were used to observe the Olympus Epi Fluorescence Spot Microscope and confocal images were taken on the Olympus FV1000 scope. Z-stack images were layered over one another to determine CB-positive and CB-parvalbumin - calretinin-overlap. Images were flattened using the Olympus FLAP/FLIP 1.7 software and adjusted in the Adobe Photoshop CS3 program.