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Patterning and organization of motor neuron dendrites in the *Drosophila* larva

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ABSTRACT

Precise patterns of motor neuron connectivity depend on the proper establishment and positioning of the dendritic arbor. However, how different motor neurons orient their dendrites to selectively establish synaptic connectivity is not well understood. The *Drosophila* neuromuscular system provides a simple model to investigate the underlying organizational principles by which distinct subclasses of motor neurons orient their dendrites within the central neuropil. Here we used genetic mosaic techniques to characterize the diverse dendritic morphologies of individual motor neurons from five main nerve branches (ISN, ISNb, ISNd, SNa, and SNc) in the *Drosophila* larva. We found that motor neurons from different nerve branches project their dendrites to largely stereotyped mediolateral domains in the dorsal region of the neuropil providing full coverage of the receptive territory. Furthermore, dendrites from different motor neurons overlap extensively, regardless of subclass, suggesting that repulsive dendrite–dendrite interactions between motor neurons do not influence the mediolateral positioning of dendritic fields. The anatomical data in this study provide important information regarding how different subclasses of motor neurons organize their dendrites and establishes a foundation for the investigation of the mechanisms that control synaptic connectivity in the *Drosophila* motor circuit.

Introduction

The proper wiring of motor circuits during development is critical for normal locomotive behavior. Precise patterns of motor neuron connectivity depend on the selective connection between the axon and muscle target and the establishment of a unique dendrite arborization pattern that determines the specificity and degree of synaptic input. In the mouse and chick, spinal motor neurons are organized into distinct columns and pools and the positional identity of motor neurons correlates with the muscles they innervate (Jessell, 2000; Landmesser, 1978; Tsuchida et al., 1994). Motor neuron pools that target different muscle subsets establish distinct dendrite arborization patterns within the spinal cord and respond to sensory stimulation with different latencies (Vrieseling and Arber, 2006). Consequently, the selectivity of synaptic input is directly influenced by the differential orientation and positioning of motor dendrites in the spinal cord (Vrieseling and Arber, 2006). Therefore, understanding how different subclasses of motor neurons establish and position their diverse dendritic morphologies within the central nervous system (CNS) will be useful in deciphering how motor circuits are assembled.

Due in large part to the simplicity and morphological stereotypy of its neuromuscular system, *Drosophila melanogaster* has served as an invaluable tool in the study of motor circuit formation (Chisholm and Tessier-Lavigne, 1999; Collins and DiAntonio, 2007). In *Drosophila*, neurons and glia in the embryonic CNS are derived from progenitor cells called neuroblasts, which undergo multiple rounds of asymmetric cell division to generate a diversity of cell types (Goodman and Doe, 1993). Approximately 400 neurons, including an estimated 38 motor neurons, are generated from 30 distinct neuroblasts within each half-segment (or hemisegment) of the embryonic ventral nerve cord (VNC) (Schmid et al., 1999). Abdominal hemisegments in the embryo are comprised of 30 highly stereotyped body wall muscles, each of which is innervated by one or more of the 38 unique motor neurons (Landgraf et al., 1997). Motor axons exit the VNC through one of three main nerves (intersegmental nerve, segmental nerve, or transverse nerve) before innervating their specific target muscle(s) (Landgraf et al., 1997; Sink and Whitington, 1991). The intersegmental nerve is comprised of three nerve branches (ISN, ISNb, and ISNd) that target the internal muscles, while the segmental nerve is comprised of two nerve branches (SNa and SNc) that target the external muscles (Fig. 1). The origin and axonal projection patterns of the embryonic motor neurons that comprise the five main nerve branches have been well characterized and provide a reliable baseline for the identification and characterization of larval motor neurons in this study (Landgraf et al., 1997; Schmid et al., 1999).

Previous studies have shown that embryonic motor neurons with similar dendritic morphologies are generally derived from a common neuroblast and their axons innervate muscles that are functionally related (Landgraf et al., 1997). Unlike in the vertebrate spinal cord, the

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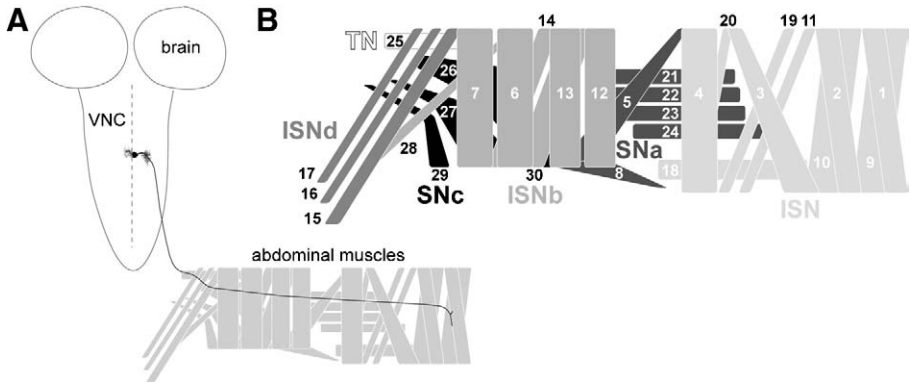

Fig. 1. The neuromuscular system of the *Drosophila* larva. (A) The larval CNS is comprised of the brain and ventral nerve cord (VNC), which is segmentally reiterated and bilaterally symmetrical with respect to the ventral midline (dotted line). A representative MARCM clone (MN9-Ib) is shown. (B) The stereotyped organization of the peripheral body wall muscles. In each abdominal hemisegment, motor axons from the six main nerve branches (ISN, ISNb, ISNd, SNa, SNc, and TN) innervate the 30 muscles.

selective connection between the motor axon and muscle target is not correlated with the cell body position of the neuron, but rather by the positioning of motor dendrites within the neuropil (Landgraf et al., 2003a). Dendritic fields of embryonic motor neurons are partitioned into distinct spatial domains within the VNC resulting in a myotopic map of the peripheral body wall muscles. This myotopic map is established independent of muscles and glia, suggesting that cell-autonomous mechanisms regulate the patterning and orientation of motor dendrites. In addition, dendrite–dendrite interactions between different motor neurons do not influence the organization of dendritic territories during embryogenesis (Landgraf et al., 2003a). However, the extent to which contacts between neighboring dendrites from the same or different subclass of motor neuron affects the establishment of dendritic fields during later developmental stages is not well known. Furthermore, it is unclear whether the spatial distribution of motor dendrites that forms the basis of the myotopic map is maintained during larval development.

By later larval stages, motor axon terminals are fully differentiated and neuromuscular synapses are well established and uniquely identifiable (Hoang and Chiba, 2001). However, there is a significant gap in our understanding of how motor dendrites establish their synaptic connections. A complete anatomical description of larval motor neurons is necessary to provide a foundation for the investigation of the mechanisms that control synaptic connectivity of motor circuits. In this study, we used mosaic analysis with a repressible cell marker (MARCM) to genetically label larval motor neurons with green fluorescent protein (GFP) to characterize their dendritic morphologies with single-cell resolution. We found that motor neurons elaborate largely stereotyped dendritic arbors that orient themselves within defined mediolateral domains in the central neuropil according to their corresponding nerve branch. Furthermore, dendrites from different motor neurons overlap extensively, suggesting cell-autonomous mechanisms control the mediolateral positioning of dendrites. The data in this study provide the first comprehensive description of larval motor neurons and provides further insight into the organizational principles of motor circuits in *Drosophila*.

Materials and methods

Generation of motor neuron clones

To generate motor neuron clones, we utilized the MARCM system (as described in Lee and Luo, 1999). *yw; FRT82B* flies were crossed to *elav-Gal4, UAS-mCD8::GFP, hs-FLP; FRT82B, tub-Gal80* flies. Clones were generated using *hs-FLP* (*hsp70 promoter-flippase*) to induce recombination at *FRT82B* and by visualizing mCD8::GFP expression driven by *elav-Gal4*, which drives Gal4 expression in all postmitotic neurons (Lin and Goodman, 1994). In brief, embryos were collected for 2 h and allowed to develop for 3–5 h at 25 °C before heat shock. Embryos were heat-shocked for 30 min at 38 °C, allowed to recover at room temperature for 30 min, then heat-shocked for an additional 45 min at 38 °C. Heat-shocked embryos were allowed to develop at 25 °C to third instar larval stage. Larvae with CNS clones were then selected before being dissected, fixed, and immuno-processed with the appropriate antibodies (see below). Fixed preparations were mounted on poly-L-lysine coated coverslips, dehydrated in an ethanol series, cleared in xylenes, and then mounted in DPX medium before imaging (Grueber et al., 2002).

Immunocytochemistry

Third instar larvae with motor neuron clones were immunolabeled with rat anti-mCD8 antibody at 1:200 dilution (Invitrogen, Carlsbad, CA) and monoclonal anti-Fasciclin II antibody (1D4) at 1:200 dilution (Developmental Studies Hybridoma Bank, University of Iowa). Cy2-and Rhodamine Red X (RRX)-conjugated secondary antibodies were used at 1:200 dilution (Jackson ImmunoResearch Laboratories, West Grove, PA). Single-cell motor neuron clones were visually identified and confocal image stacks were obtained using a Leica TCS SP2 (Leica Microsystems, Bannockburn, IL) or an Olympus FluoView FV1000 (Olympus, Tokyo, Japan). Z-series stacks were reconstructed into single collapsed images using ImageJ software (National Institutes of Health, Bethesda, MD). Three-dimensional and orthogonal views were created using Volume Viewer in ImageJ.

Quantitative analysis

To quantify the average mediolateral position and width of the dendritic arbor, we plotted the relative positions of the most medial dendritic branch and most lateral dendritic branch with respect to the ventral midline and DL fascicle for each neuron using Adobe Photoshop (Adobe Systems Inc.). Because of the variability in the width of the neuropil for each preparation (between approximately 60–110 μm), values were normalized with the ventral midline position assigned a value of 0 and the DL fascicle position assigned a value of 1. Neurons that were rotated off-center with respect to the y-axis were not subjected to this analysis.

Results

Patterning of motor neuron dendrites in the larval CNS

To examine the dendritic arborization patterns of motor neurons in the larval CNS in detail, we used MARCM to genetically label single motor neuron clones with a membrane-targeted GFP (mCD8::GFP) (Lee and Luo, 1999). Although MARCM provides unparalleled single-