Supplemental Figures

Supplemental Figure 1. Segmental designation.

Four panels of L3F VNC's stained to reveal the segmental pattern of the serotonergic system. The first panel (top left) is marked with *tph*GAL4/UAS-GFP and serotonin and shows the overall serotonergic pattern. Of question is the identity of the most posterior segment, which has single cells per hemisegment and is marked A8 in this image. The posterior commissure in each thoracic segment is indicated with an arrow. The top right panel is stained for fasII, which marks the longitudinal axonal bundles. This does not indicate the identity of the terminal serotonergic segment except that it is outside the 'normal' axonal bundle pattern. The two lower panels are stained for fasIII, which marks

the thoracic segments and in addition, the lower right panel is stained for FMRFamide (arrowheads) that also marks the thoracic segments. Counting from the third thoracic segment in each case reveals that the last serotonergic segment is A8.

Supplemental Figure 2. The medial and lateral branch pattern of A7 differs from that of other segments in the abdominal VNC.

Panels show two views of lateral A7 and medial A7 branch pattern. Both the contralateral and ipsilateral patterns are very different from the patterns seen in the other segments of the VNC. The two primary branches are not present. Instead a single branch extends into the contralateral neuropil, which disperses into a cloud of serotonergic varicosities. The ipsilateral projections are also more elaborate than those found in other segments of the VNC. Also included is the A6 medial pattern.

Supplemental Figure 3. *Derailed* expression misdirects crossing of serotonergic axons along the anterior commissure of the adjacent posterior segment.

(A) *Drl* expression driven by *eg-GAL4*. A single A6 serotonergic neuron remains following *rpr-hid* ablation. The commissural axon can be seen crossing the midline along the anterior commissure of the adjacent posterior segment. The white dotted rectangle denotes the area enlarged in (B).

(B) A6 and A7 region enlarged from (A). Arrow denotes the posterior commissure of A6, defined by BP102 staining. The arrowhead denotes the anterior commissure of A7. (B') is a wild type for comparison.

(C) BP102 staining alone from (A). Arrowheads denote the anterior commissures. The arrows denote the posterior commissures. (D) Serotonin staining alone from (A).

Supplemental figure 4. The normal medial pattern resumes after *robo2* mis-expression ceases.

(A) *Rpr-hid* and *robo2* expression driven by eg-GAL4. During L1 low levels of egGAL4 expression drive *robo2*, and serotonergic axons remain repulsed from the midline. This can be seen in the laterally localized cluster of serotonergic varicosities of a L1 single medial serotonergic cell. The white dotted line denotes the midline. In larval stages after L1, *robo2* is presumably no longer overexpressed and the serotonergic neurons are able to extend their branches into the ipsilateral neuropil. The circular primary branch structure is created over time on the ipsilateral side. A two day old larvae (L2) has established primary-like branches, but the branches have not yet completed the circular primary branch structure. An L3F has completed the circular primary branch-like structure, as seen in the isolated serotonergic medial cell.

(B) Continued expression of *robo2* in L3F by driving *robo2* with *eg-GAL4* and *ddc-GAL4* prevents axons from filling the neuropil. (C) Same as (B) but including neuropil staining (BP102).

Genotypes: *UAS-robo2 x eg-GAL4, UAS-robo2 x eg-GAL4;ddc-GAL4*, *UAS-rpr-hid*

Supplemental Movie 1.

3D rendering of larval abdominal VNC segments with a single medial serotonergic cell expressing GFP. A 360 degree rotation of the segments demonstrates the clear circular

medial pattern that is seen in all medial serotonergic cells in the abdominal VNC (except A8).

Supplemental Movie 2.

3D rendering of larval abdominal VNC segments with a single lateral serotonergic cell expressing GFP. A 360 degree rotation of the segments demonstrates the clear lateral pattern that is seen in all lateral serotonergic cells in the abdominal VNC (except A8).

Cover Candidate

Dorso-ventral views of a *Drosophila* larval A3 CNS segment stained for serotonin (red) and GFP (green). In each panel, only one of the two serotonergic neurons is labeled with GFP showing that each has a unique branch pattern. While each has a largely separated zone of branching, competition for space between the neurons does not seem to play a role in patterning.

Figure

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