Supplemental text and tables

Differing strategies used by motor neurons and glia to achieve robust development of an adult neuropil in Drosophila

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Supplementary Figure legends

Figure S1. Gal4 drivers label Dll+ thoracic astrocytes and EGs in the adult.

(A-C) Adult VNS expressing mCD8:GFP (green) (A1-A4) or nGFP (green) (B1-C4) under the control of *alrm-Gal4* (A1-C4) , *R56F03-Gal4* (B1-B4) or both (C1-C4) and immunostained with anti-Repo (blue) and anti-Dll (red). (B2) boxed region shows EG processes surrounding an astrocyte. Note that all of the glia surrounding adult neuropils express either *alrm-Gal4* or *R56F03-Gal4,* and all NG express Dll.

(D-E) Adult VNS expressing H2B::RFP (red) under the control of *alrm-Gal4* (D1-D4) or *R56F03-Gal4* (E1-E4) immunostained with anti-pros (bleu). Note: As for other region of the CNS Prospero is expressed in astrocyte and not in EG.

Arrowhead: EG, Arrow: Astrocytes. ProNm: Prothoracic Neuromere, AMNn: Accessory Mesothoracic FS: Frontal Section, TS: Transverse Section.

Figure S2. Developmental origin of the NG.

(A) L3 CNS in which glia surrounding the six immature leg neuropil express mCD8::GFP under the control of *R31F10-Gal4* and are costained with anti-BRP (blue), anti-Dll (red). A2 shows the thoracic region at higher magnification

(B) Adult VNS expressing mCD8:GFP (green) under the control of *R31F10-Gal4* costained with anti-BRP (blue).

(C) Adult VNS with neuropil glia expressing the multicolor flybow system FB1.1 (yellow, red and green) under the control of a lineage tracing method activated with *R31F10-Gal4* and immunostained with anti-Pros (blue) (see **Methods** for details).

ProNm: Prothoracic Neuromere, AMNn: Accessory Mesothoracic Neuromere, MesoNm: Mesothoracic Neuromere, MetaNm: Metathoracic Neuromere,. FS: Frontal Section T1, T2, T3 : Three thoracic segments.

Figure S3. Developmental origin of the NG.

(A) L3 CNSs with neuropil glia precursors expressing mCD8::GFP under the control of *Dll-Gal4* and labeled with anti-BRP (blue), anti-NCad (red).

Figure S4. Expression and function of Dll during NG development

(A-D) L3 CNSs expressing mCD8:GFP under the control of *repo-Gal4* (A1-A4) , *alrm-Gal4* (B1-B4) , *R56F03-Gal4* (C1-C4) and *Dll-Gal4* (D1-D4). (A1-A4) Arrows indicate the different classes of NG: LSG: Larval surface glia (Dll-), LCG: Larval cortex glia (Dll+), LNG: Larval neuropil glia (Dll++), NGp: Neuropil Glia precursors (Dll+++). (B1-B4) Arrows indicate astrocytes. (C1-C4) Arrowheads indicate EG; arrows indicate astrocytes. (D1-D4) Asterisk indicates interneurons expressing Dll.

(E-F) Adult VNS expressing mCD8::GFP under the control of *Dll-Gal4* (E1-E3) or *repo-Gal4* (F1-F4) costained with anti-BRP (blue) and anti-Dll (red) (E1-E3) or anti-Repo (blue) and anti-Dll (red) (F1-F4). (E1-E3) Arrows indicate adult NG (GFP-), note Dll is expressed in the axonal sensory neuron and in one interneuron per neuromere. (F1-F4) Arrows indicated the different classes of NG: ASG: Adult Surface Glia (Dll-), ACG: Adult Cortex Glia (Dll+), ANG: Adult Neuropil Glia (Dll++).

(G-H) L3 T2 hemisegment containing a *WT* (G1-G4) and a *Dll-/-* (H1-H4) Lin A MARCM clones expressing mCD8::GFP under the control of *VGlut-gal4* and *repo-Gal4* and immunostained against anti-Dll (red) and anti-BRP (blue). (C2, D2) the arrows indicate a Lin A neuropil glia precursors (NGp).

(I-L) *WT* (I1-J3) and *Dll* (L1-L3) *cis*^{2} MARCM Lin A clones expressing mCD8::GFP and mCD8::RFP under the control of *repo-Gal4* and *VGlut-QF,* respectively (I1-I2, K1-K2) or UAS-mCD8::GFP, QUAS-mCD8::GFP and UAS-H2B::RFP under the control of *repo-Gal4*, *VGlut-Gal4* and *VGlut-QF* (J1-J3, L1-L3) and co-stained with anti- BRP (blue).

ProNm: Prothoracic Neuromere, AMNn: Accessory Mesothoracic Neuromere, MesoNm: Mesothoracic Neuromere, MetaNm: Metathoracic Neuromere,. FS: Frontal Section T1, T2, T3: Three thoracic segments.

Figure S5. Number of astrocytes produced by each of the three NGB.

(A) Average number of Lin A (N=10), Lin D (N=4), Lin Z (N=13) astrocytes in adult VNS.

(B-C) Number of astrocytes in individual Lin A, Lin D, Lin X samples. Error bars indicate standard deviation.

Figure S6. Lin A *cis*^{2} MARCM in T2 and T3 segments.

(A-D) *WT* Lin A *cis*^{2} MARCM clones expressing mCherry (A1-B2) or mCD8:RFP (C1- D2) and mCD8::GFP (A1-D2) under the control of *alrm-Gal4* (A1-B2), *R56F03-Gal4* (C1-D2)and *VGlut-QF* (A1-D2) respectively.

Supplemental Table 1. Number of NG

	Number of Ensheathing glia	Number of Astrocytes
L1 Larva: Brain hemispheres	~12	~15
L1 Larva: Abdominal hemi-segments	3	6
Adult: Brain hemispheres	~1335	~1550
Adult: Prothoracic Neuromeres	~140	~139
Adult: Accessory + Mesothoracic Neuromeres	~210	~200
Adult: Metathoracic Neuromeres	~160	~160

Supplemental Table 2. *cis*^{2}-MARCM experiments with astrocyte and EG drivers

	Number of clones
Lin A clones with astrocytes	>12
Lin Z clones with astrocytes	4
Lin D clones with astrocytes	15
Lin A clones with EG	>4
Lin D clones with EG	0
Lin Z clones with EG	9

Supplemental Table 3. Number and final location of astrocytes born in T1.

LIN A born in T1	ProNm astrocyte number	AMesoNm astrocyte number	MesoNm astrocyte number	total	ProNm targeting	AMesoNm targeting	MesoNm targeting	controlateral targeting
exp1	54	3	0	57	yes	yes	yes	yes
exp2	80	23	1	104	yes	yes	yes	yes
exp3	86	6	0	92	yes	yes	no	no
exp4	46	0	0	46	yes	yes	yes	no
exp5	64	6	0	70	yes	yes	yes	no
exp6	55	1	0	56	yes	yes	no	no
exp7	95	7	0	102	yes	yes	yes	no
exp8	89	8	0	97	yes	yes	yes	no
exp9	85	7	1	93	yes	yes	yes	yes
exp10	93	0	0	93	yes	no	no	no
exp11	47	15	0	62	yes	yes	yes	no
Lin D born in T1	ProNm astrocyte number	AMesoNm astrocyte number	MesoNm astrocyte number	total	ProNm targeting	AMesoNm targeting	MesoNm targeting	controlateral targeting
exp1	17	1	0	18	yes	yes	no	no
exp2	46	0	0	46	yes	yes	no	no
exp3	19	1	0	20	yes	no	no	yes
exp4	44	0	0	44	yes	no	no	no
exp5	16	0	0	16	yes	no	no	no
exp6	18	0	0	18	yes	no	no	no
exp7	12	0	0	12	yes	no	no	no
exp8	13	2	0	15	yes	yes	yes	no
exp9	34	12	0	46	yes	yes	yes	yes
exp10	45	1	0	46	yes	yes	no	no
exp11	31	1	0	32	yes	no	no	no
exp12	51	0	0	51	yes	yes	no	no
exp13	64	0	0	64	yes	no	no	no
Lin Z born in T1	ProNm astrocyte number	AMesoNm astrocyte number	MesoNm astrocyte number	total	ProNm targeting	AMesoNm targeting	MesoNm targeting	controlateral targeting
exp1	28	1	0	29	yes	yes	no	yes
exp2	71	0	0	71	yes	no	no	no
exp3	56	8	0	64	yes	yes	no	no
exp4	61	5	0	66	yes	yes	no	no