Figures and figure supplements

Neuron hemilineages provide the functional ground plan for the Drosophila ventral nervous system

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Figure 1. The different strategies that were used to establish lines that showed selective expression in defined hemilineages. The strategies are based on a screen through the large collection of enhancer lines built from cis regulatory modules (CRMs) of CNS expressed genes. (A) For CRMs whose thoracic expression is confined to a hemilineage, gene-switch constructs are combined with feeding larvae the progesterone mimic (RU486) in the last larval stage. The larval expression of flippase then promotes the excision of a STOP cassette from another transgene allowing a constitutive promotor (Actin5C) to drive continual expression following excision. (B) When the larval expression pattern includes functional larval neurons as well as a hemilineage, expression in the larval neurons is blocked by including a nSynaptobrevin-GAL80 gene. Gene switch cannot be used in this context because it is not suppressed by GAL80. (C) Spatial and temporal specificity is accomplished using a conditional flippase that is the human progesterone receptor ligand-binding domain (hPR) fused to Flippase. Exposure of third instar larvae to RU486 then confines the flip event to the last larval stage. See text for more details.

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Figure 2. Strategy for clean expression of hemilineages into the adult stage. Z-projections of confocal stacks showing the expression pattern driven by R24B02 used in various genetic combinations. Arrowheads show the t1 and t2 clusters of hemilineage 12A. (A, B) Pattern shown by R24B02-GAL4 driving pJFRC2-10XUAS-IVS-mCD8::GFP (pJFRC2) in larval (A) and adult (B) stages. The hemilineage 12A clusters are prominent at the end of larval life, but do not express in the adult. (C) Adult VNS of a cross of Actin5C>dSTOP>GAL4, UAS-Flippase, pJFRC2 to R24B02-GAL4. The persisting expression in the hemilineage 12A clusters is badly obscured by many ‘off-target’ cells (e.g., red arrowheads) presumably arising from embryonic and adult expression patterns in this line. (D, E) R24B02-GeneSwitch flies crossed to pJFRC2 and either maintained without hormone (D) or fed on 1 mM RU486 food for 24 hr as third instar larvae. (F–H) Adult nervous systems of flies of the genotype R24B02-GeneSwitch, Actin5C>dSTOP>GAL4 , UAS-Flippase, pJFRC2 and either raised without hormone mimic (F) or fed RU486 food during the third larval stage (G, H). H shows a higher power view of the hemilineage 12A cells found in T1, T2 and A1; this hemilineage dies in T3. Green: GFP; magenta: N-cadherin.

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Figure 3. nSyb-GAL80 suppresses GAL4 expression specifically in mature neurons. Yellow arrowheads show location of hemilineage 12B cell clusters in the thoracic segments. (A) In the third-instar larva, R57C10-GAL4, a nSynaptobrevin promoter fusion-GAL4 drives expression in primary neurons, but not secondary neurons. Inset: single optical slice showing colocalization of anti-GFP (green) and the pan-neural marker anti-elav (magenta) in primary neurons (e.g., white arrowhead), but not in immature secondary neurons (e.g., blue arrowhead). (B) R15D11-GAL4 drives expression in secondary hemilineage 12B (yellow arrowheads) and various primary neurons. (C) R15D11-GAL4 with nSyb-GAL80: expression is suppressed in primary neurons, and only 12B expression remains. (D) When used to drive UAS-Flippase in a flip-on immortalization strategy, R15D11-GAL4 yields expression in hemilineage 12B, but also in numerous off-target cells (red arrowheads). Genotype: w; UAS-Flippase (attP40)/pJFRC19-13XLexAop2-IVS-myr::GFP (attP40), Actin5Cp4.6>dsFRT>LexAop65 (su(Hw)attP5), R15D11-GAL4 (attP2). (E) Same genotype as (D), but with nSyb-GAL80 on the X chromosome (su(Hw)attP8). Expression in off-target cells is much reduced. A small amount of off-target expression is observed in secondary neurons and descending neurons (e.g., red arrowheads). (F) Adult VNC expression pattern using both nSyb-GAL80 and UAS-Flip-Switch to restrict expression to the targeted cells in (C). No off-target expression is observed. In panels D–F, myr::GFP concentrates in processes rather than in the cell bodies and therefore the cell clusters are difficult to see in Z-projections. E, F: insets are partial projections showing t1 cell body clusters.

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Figure 3—figure supplement 1. UAS-hPR-flp is fully active in the presence of RU486, but inactive without drug. (A) R20B05-GAL4 drives UAS-GFP expression in all secondary neurons. (B) A larva bearing the genotype pJFRC2-UAS>STOP>GFP(attP18)/w; +; UAS-hPR-flp(VK00005)/R20B05-GAL4 and fed on RU486 food for 24 hr prior to dissection. GFP expression is present in nearly all of the expected cells, indicating that the STOP cassette has been excised by UAS-hPR-flp with high efficiency. (C) A larva of the same genotype as (B), but never treated with RU486. No GFP expression is observed, meaning that the hPR-flp was inactive in the absence of drug.

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Figure 4. The anatomy and behavioral consequences of stimulating hemilineages 1A through 5B. Each hemilineage is depicted as a projected confocal Z-stack of the VNS (T1, T2, T3 and the fused abdominal neuromeres) (A, E, G, K, M, P) and a transverse projection through segment T2 (B, F, H, L, N, Q, bracketed region in dorsal view). Yellow arrowheads: hemilineage cell body clusters; white arrowheads: main neurite bundle entering neuropil or crossing midline; magenta asterisk: major off-target expression; green: GFP; magenta: neuroglian. Pictures are video frames of groups of decapitated flies that express TRPA1 in the particular hemilineage and are exposed to a heat ramp to stimulate the neurons. (A-D) Hemilineage 1A. (A, B) The 1A neurons are located dorsolaterally in each thoracic segment, project across the midline and have ventral (v) arbor in the leg neuropil and dorsal (d) arbor in the tectulum neuropil. Arrow: characteristic posterior hook of the ventral arbor. (C) T2 transverse projection showing that nSynaptotagmin::GFP (nSyt, magenta) localizes to the ventral arbor. (D) The response of activating the 1A neurons in decapitated flies with a 24–37˚C heat ramp over a 50 s period. Images show the position of marked flies at the beginning and end of the ramp and the path each moved during the period. (E, F) Hemilineage 1B. The 1B neurons are located in the posterior ventrolateral region of each segment and send arbors in to ventral and dorsal regions of the ipsilateral leg neuropil. (G–J) Hemilineage 2A. (G, H) The 2A neurons are situated ventromedially in the anterior third of the ganglion; they project dorsally and arborize throughout the ipsilateral tectulum. (I) T2 transverse projection showing that nSyt-GFP (magenta) localizes in the more lateral parts of the arbor. (J) Activation of the 2A neurons by the head ramp results in buzzing of the outstretched wings (30˚C, arrow). (K, L) Hemilineage 3A. The 3A interneurons are in a posterior ventrolateral cluster, they enter the leg neuropil near the leg nerve and ramify through most of the ventral half of the leg neuropil. The line had substantial sensory expression (*). (M–O) Hemilineage 3B. (M, N) The 3B interneuron clusters are in posterior T1 and T2 and project dorsally to ramify through the dorsal part of the tectulum. (O) Thermal activation of the 3A primarily evokes flicking and scissoring movements.
Figure 4. Continued

of the wings (arrows). (P–S) Hemilineage 5B. (P, Q) The 5B clusters are positioned ventrolaterally in the posterior part of each thoracic neuromere. They project dorsally and across the intermediate posterior commissure to arborize in dorsal and medial regions of the neuropil. (R, S) Thermal activation of the 5B interneurons cause decapitated flies to splay out their legs (R, 37°C) and tethered intact flies to crouch onto a Styrofoam ball they are holding (S, 32°C).

Genotypes: for most genotypes see Supplementary file 1; for the remainder: (C) LexAop-RFP (su(Hw)attP8)/w; R22G11-LexA (attP40)/+; LexAop-nSyt-GFP (su(Hw)attP1)/+. (I) UAS-nSyt-GFP (attP18)/w, +; R50G08-GAL4 (attP2)/UAS-HA (VK00005).

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Figure 4—figure supplement 1. Dorsal (A) and transverse (B) view of the adult VNS showing a MARCM clone for the T2 lineage 1. The 1B siblings have been pulled anteriorly into T1 where they branch through the ipsilateral leg neuropil. The 1A siblings are projection neurons with ventral (v) arbor in both the ipsi- and contralateral leg neuropils and bilateral dorsal (d) arbors that extend into the dorsal, tectulum neuropil. *: arbor from another lineage.

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Figure 5. The anatomy and behavioral consequences of stimulating hemilineages 6A through 9A. Organization of panels and general symbols are as in Figure 4. (A–D) Hemilineage 6A. (A, B) The 6A neurons are in prominent ventromedial clusters in segments T1 through A1. They ramify through the lateral regions of the tectulum neuropil with a concentration in the dorsal T2 area. (C) T2 transverse projection showing that nSyt-GFP (magenta) localizes in the lateral parts of the arbor. (D) Video frames of decapitated flies at early (1 s) and intermediate (31 s) times in the heat ramp. Middle frames are multiple exposures taken over 1 s periods showing phasic, repetitive movements of single limbs (15–16 s, arrow), and jerky movements of the entire fly (30–31 s, arrow). (E–G) Hemilineage 6B. (E, F) The 6B neurons are located in the posterior medial region of each segment and project through a posterior commissure to arborize in the tectulum and dorsal regions of the leg neuropils. (G) Video frames showing the position of decapitated flies at an intermediate (20 s) and late (36 s) portion of the heat ramp as they are starting to move. The respective dots mark the anterior margin of the thorax at the 2 times. The middle frame shows the position of the fly at the start (white dot) and end (red dot) of the sequence and at 2 s intervals in between; arrow shows direction of movement. (H–J) Hemilineage 7B. (H, I) The 7B neurons are in prominent ventrolateral clusters in segments T1 through A1. They ramify through the lateral regions of the tectulum and send a prominent projection into the T2 leg neuropil (arrow). (J) Frames of a high-speed video of a decapitated fly taking off during heating. It shows the expected sequence of wing elevation (30 ms), jump (38 ms), and flapping (45 ms). (K–M) Hemilineage 8A. (K, L) The 8A cluster is situated in the anterolateral region of each segment and projects into the lateral leg neuropil. (M) Early and late frames during the heat ramp showing only minor positional changes through the period. (N, O) Hemilineage 8B. (N, O) The 8B clusters are in the anterolateral region of each segment and project through an anterior commissure to spread widely through the thoracic neuromeres. A prominent subset of the t3 cells form Figure 5. continued on next page
Figure 5. Continued

A bowtie-shaped structure (arrow) that receives input from haltere afferents. Transverse section (O) is of this input area. (P–R) Hemilineage 9A. (P, Q) The 9A clusters assume an anterolateral position and project into the region of the ventral leg neuropil that receives input from proprioceptors. (R) Video frames from early and late stages of the heat ramp. The decapitated flies responded by splaying out their legs. Genotypes: for most genotypes see Supplementary file 1; for the remainder: (C) nSyb-GAL80 (su(Hw)attP8)/LexAop-RFP (su(Hw)attP8); UAS-flp (attP40)/act>STOP>LexA (attP40); R35A03-GAL4 (attP2)/LexAop-nSyt-GFP (su(Hw)attP1).

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Figure 5—figure supplement 1. Dorsal (A) and transverse (B) view of the adult VNS showing a MARCM clone for the T3 lineage 7. From the cell cluster (arrowhead) the cells project dorsally to elaborate a dense arbor (i) in the ipsilateral tectulum neuropil and then cross via an anterior commissure and extend an anterior output arbor (c). They also send a second projection that crosses the midline and extends ventrally into leg neuropil (cv). Bracket shows level of transverse projection.

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Figure 6. The anatomy and behavioral consequences of stimulating hemilineages 10B through 14A. Organization of panels and general symbols are as in Figure 4. (A–C) Hemilineage 10B. (A, B) The 10B clusters are ventromedially located in the anterior part of the segment. They project across an anterior commissure and produce a dorsal (d) arbor that runs longitudinally, and a ventral (v) arbor that extends into medial leg neuropil. (C) Video frames showing the position of decapitated flies at intermediate (20 s) and late (36 s) portion of the heat ramp. The respective dots mark the anterior margin of the thorax at the 2 times. The middle frame shows the position of the fly at the start (white dot) and end (red dot) of the sequence and at 2 s intervals in between; arrow shows direction of movement which was generally backward. (D–F) Hemilineages 11A and B. (D, E) These hemilineages are laterally located only in T1 (11A) and T2 (11A and 11B). They ramify primarily in the T2 tectulum neuropil and have projections into the leg neuropils of T1 and T3 (arrows). (F) Frames of a high-speed video of a decapitated fly taking-off during heating. Flapping begins prior to the jump. (G–J) Hemilineage 12A. (G, H) The ventrolateral 12A clusters are on the posterior border of segments T1 and T2. They project dorsally and arborize through most of the dorsal tectulum. (I) T2 transverse projection showing that nSyt-GFP (magenta) localizes to medial regions of the 12A projection. (J) Video frames showing progression of behaviors of decapitated flies during the heat ramp. 27°C: flies quiet; 30°C, some walking and showing lateral wing waving (arrow); 35°C: flies showing wing buzzing (blur) although wings (arrows) usually not extended in flight position. (K–N) Hemilineage 12B. (K, L) The 12B clusters are ventrally located at the posterior border of the neuromere. They project across a posterior commissure and arborize widely through the contralateral leg neuropil. (M, N) Video frames of dorsal and lateral views of decapitated flies subjected to a heat ramp. At elevated temperatures the flies often showed tonic extensions of the T2 and T3 legs. (O, P) Hemilineage 13A. (O, P) The somata of the 13A neurons are spread over the anterior ventrolateral region of the neuromere. Their arbors extend through most of the ventral half of the leg neuropil. (Q–T) Hemilineage 13B. (Q, R) The 13B clusters are pulled to the anterior midline and their axons cross the midline in a very ventral anterior commissure and the cells branch through the ventrolateral leg neuropil. (S, T) Video frames of dorsal and lateral views of decapitated flies early and late in the heat ramp. At high temperatures the legs are extended laterally and often are elevated from the substrate (arrow). (U, V) Hemilineage 14A. (U, V) The 14A clusters are also pulled to the midline and their axons cross in a ventral commissure. They project through most of the ventral...
Figure 6. Continued
and lateral leg neuropil. This line also expressed in occasional other lineages (*). Genotypes: for most genotypes see Supplementary file 1; for the remainder: (I) nSyb-GAL80 (su(Hw)attP8)/act>STOP>LexA (attP18); LexAop-RFP (attP40)/UAS-flp (attP40); LexAop-nSyt-GFP (su(Hw)attP1)/R24B02-GAL4 (attP2).

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Figure 6—figure supplement 1. tsh-GAL80 eliminates expression specifically in the VNC. (A) The lineage 11 genotype drives GFP expression in the VNC in lineage 11 (yellow arrowheads, arrows) and occasionally in descending axons (*). (B) The same genotype as in (A), but with the addition of tsh-GAL80. Expression is eliminated in lineage 11, but remains in the descending neurons. Genotypes A: nSyb-GAL80 (su(Hw)attP8)/w; UAS-flp(attP40)/+; R26B05-GAL4 (attP2)/nSyb-LexA (attP2), LexAop>STOP>GFP (VK00005). B: nSyb-GAL80 (su(Hw)attP8)/w; UAS-flp (attP40)/tsh-GAL80, R26B05-GAL4 (attP2)/nSyb-LexA (attP2), LexAop>STOP>GFP (VK00005).

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Figure 7. The anatomy and behavioral consequences of stimulating hemilineages 15B through 24B. Organization of panels and general symbols are as in Figure 4. (A–C) Hemilineage 15B. (A, B) The larger of the two motor lineages. Cell bodies are located in the anterolateral region of the segment and the neurons innervate primarily muscles to distal leg segments. (C–E) Hemilineage 18B. (C, D) The 18B clusters are located in the dorsoanterior regions of T2 and T3. Their axons cross in an anterior commissure and have discrete contralateral (c), intermediate (i) and ventral (v) projections. The ventral projections extend into the leg neuropil. (E) Frames of a high-speed video of a decapitated fly taking-off during heating. Flapping often began prior to wing raising causing the wings to bend (8ms, 25ms). (F–I) Hemilineage 19A. (F, G) The 19A clusters are posterior dorsolateral in the segment and project arbor into the ventral leg neuropil and also to a convergence point at the midline just below the tectulum. This convergence point shows nSyt-GFP (magenta) localization (H, arrow). (I) Video frames from early and midway through the heat ramp. With increased temperature the flies extend their T2 legs and begin to wave them. The frames for a one second period (18–19 s) are superimposed to illustrate movements of the T2 legs (arrow) and stability of the others. (J, K) Hemilineage 19B. (K, L) The major representation of the 19A cells are in the T2 cluster located posterior dorsolateral region of the segment, with a smaller cluster in T3. The axons cross in a posterior commissure and show medial (m) and lateral (l) anterior projections that are confined to the tectulum. The line had massive contamination from haltere afferents (*). (L–N) Hemilineages 20A, 22A. (L, M) The cell bodies for the 20A, 22A clusters are in the posterior ventrolateral region of the segment and the neurons innervate the middle third of the leg neuropil. (N) Video frames of decapitated flies showing that they splay out their legs as the temperature rises. (O–Q) Hemilineage 21A. (O, P) The 21A clusters are in the posterior ventrolateral region of the segment. They project dorsomedially and then arborize over most of the dorsal two-thirds of the leg neuropil. (Q) Video frames of decapitated flies during the heat ramp. Eventually the flies become immobile with their legs frozen at unusual angles (21 s). The frames for an intermediate, one second period (10–11 s) are superimposed to show the transient hyperkinetic leg movements. (R–T) Hemilineage 23B. (R, S) The 23B clusters are in the posterior dorsolateral region of the segment. The neurons produce an extensive ipsilateral arbor and then cross the posterior commissure for their output arbor. (T) Stimulation of the 23B neurons produce uncoordinated leg movements and the flies basically stay in place (36°C). (U–W) Hemilineage 24. (U, V) The smaller of the two motor lineages. Cell bodies are located in Figure 7. continued on next page
the anterodorsolateral region of the segment and the neurons innervate primarily proximal leg muscles. Video frames of decapitated flies subjected to a heat ramp. At elevated temperatures the flies typically showed repetitive leg movements. Genotypes. For most genotypes see Supplementary file 1; for the remainder: (I) UAS-nSyt-GFP (attp18)/w, UAS-RFP(attP40)/+: R32E04_GAL4 (attP2). DOI: 10.7554/eLife.04493.028

Figure 7—figure supplement 1. Dorsal (A) and transverse (B) view of the T2 region of the adult VNS showing a lineage 19 MARCM clone. The 19A siblings project ventrally into the ipsilateral leg neuropil while the 19B siblings project across the posterior commissure (white arrowhead). (C, D) Z-projections of ventral (C) and dorsal (D) regions of the clone that captures most of the arbors of the 19A and 19B hemilineages, respectively. The 19A cells have primarily arbor in their leg neuropil but they also have a prominent projection to the midline. The 19B cells have strong lateral anterior projections (lat) that are both ipsilateral and contralateral as well as weaker, symmetrical medial projections (med). DOI: 10.7554/eLife.04493.029
Figure 8. Generation of a vPR6-specific line by intersecting hemilineage 12A and fru-LexA. (A, B) Parental expression patterns. (A) The fru-LexA expression pattern. The female thoracic pattern is a subset of the male pattern, and many of the neurons have dimorphic arbors. (B) Hemilineage 12A. Genotype: nSyb-GAL80 (su(Hw)attP8)/+; UAS-flp (attP40)/+, R24B02-GAL4/nSyb-LexA, LexAop>STOP>GFP. (C) The intersection of A and B, isolating the vPR6 neurons. Genotype: nSyb-GAL80(su(Hw)attP8); UAS-flip(attP40)/LexAop>STOP>GFP(attP40); R24B02-GAL4(attP2)/fru-LexA. Insets: the arbors and numbers of cells match digital representations of the complete vPR6 pattern in males (left) and females (right), adapted from Yu et al. 2010a.

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Figure 9. Relationship of hemilineages to neuron type and to classes of evoked behavior. (A) Summary of the range of behaviors in decapitated flies elicited by stimulation of the neurons in each of the hemilineage groups using TRPA1 expression and a heat ramp to activate the temperature-sensitive channel. Behaviors are divided into six categories explained in the text. Hemilineages are arranged according to the complexity of their behavioral responses. Most behavioral responses were sustained but a few hemilineages (*) showed a progression of behaviors during the heat ramp. Diagrams show the extent of the hemilineage’s arbor in transverse views of the ventral nervous system at the level of the mesothorax (T2). The numbers of flies analyzed in each group ranged from 15 to 25. (B) Registration of the hemilineage arbors to a common outline and then overlapping the hemilineage groups in which at least 50% of the individuals showed the indicated types of behavior.

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