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Supplemental Information

Autonomous Circuitry for Substrate Exploration in Freely Moving *Drosophila* Larvae

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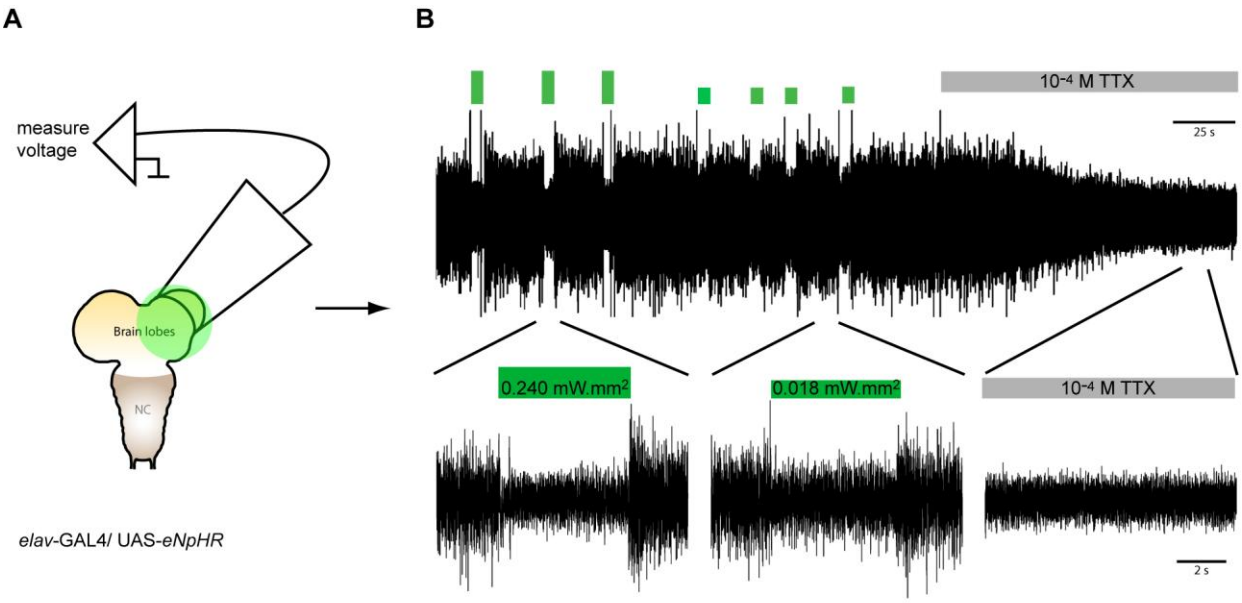
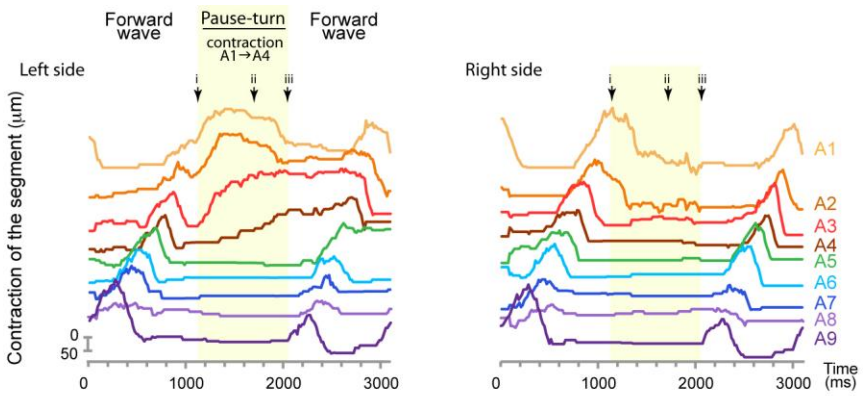
Supplemental Inventory



Supplementary Figure 1. eNpHR-mediated inhibition of brain activity.
(**A**) Diagram of a brain lobe extracellular recording preparation. Early third instar larvae were dissected as described in supplemental experimental procedures for the semi-intact preparation. A suction electrode was attached to one lobe and the preparation was illuminated for 5 s intervals with 560 ± 10 nm light. The nerve cord (NC) and adjacent lobe were not illuminated.
(**B**) Time course of a typical experiment (top). Brain lobe activity consisted of large field potentials (LFPs). In the presence of green light (green bars) LFPs were decreased in a dose dependent manner. Light intensities used were similar to (0.240 mW/mm^2) or less than (0.018 mW/mm^2) those used in behavioral experiments. TTX application reduces LFPs to amplitudes similar to those seen during illumination with a light intensity of 0.240 mW/mm2. Expanded time scale views of light and TTX responses are shown in lower traces.



Supplementary Figure 2. Description of a pause turn.
Contraction of abdominal segments during a pause-turn. The right and left boundaries of each segment were tracked in a movie taken at 60 frames/ s. During the turn, a unilateral wave of muscle contraction propagates from A1 to A4 on the left hand side (i-ii) followed by partial relaxation (iii). Segments A5 to A9 remain relaxed on both sides of the animal.

Supplemental Experimental Procedures

Fly Stocks

The following fly lines were provided by Bloomington Stock Center: *w*^{1118}; *elav*-GAL4; *cha7.4*-GAL4; UAS-mCD8-GFP; UAS-stinger-RFP; and UAS-*shi*^{ts1}. Several lines were described elsewhere: *tsh*-GAL4 [S1]; *tsh*-GAL80 [S2]; UAS-*EGFP-kir2.1* [S3] and *cha3.3*-GAL80 [S4].

BL-Gal4: w-; *elav*-Gal4, *tsh*-Gal80/CyO,P{Dfd-GMR-nvYFP}2; *cha3.3*-GAL80, UAS-mCD8-GFP/ TM6B, P{Dfd-GMR-nvYFP}4, Sb[1] Tb[1] ca[1]
BL+sens-Gal4: *elav*-Gal4, *tsh*-Gal80/CyO,P{Dfd-GMR-nvYFP}2; UAS- mCD8-GFP/ TM6B, P{Dfd-GMR-nvYFP}4, Sb[1] Tb[1] ca[1]
NC-Gal4: w-; *tsh*-Gal4/CyO,P{Dfd-GMR-nvYFP}2; UAS-mCD8-GFP/ TM6B, P{Dfd-GMR-nvYFP}4, Sb[1] Tb[1] ca[1].

The UAS-*eNpHR-YFP* line in the attP40 landing site was generated from a plasmid containing NAchRS-NpHR-ERexp(FCYENEV)-EYFP ("eNpHR-YFP" sequence at http://www.optogenetics.org). The eNpHR-YFP coding sequence was cut out as a BamHI blunt end fragment and cloned into a pUAS-Stinger-attB vector.

Semi-Intact Preparation

Third-instar larvae were dissected and pinned in a Sylgard-coated dish in HL3.1 saline containing (in mM) 70 NaCl, 5 KCl, 0.8 CaCl2, 4 MgCl2, 10 NaHCO3, 5 trehalose, 115 sucrose, and 5 HEPES, pH7.15. The animals were filleted taking special care not to damage the 3 anterior and 3 posterior segments (Figure 3a). Under these conditions peristaltic movements continued for at least 30 minutes at room temperature (22 ^{o}C). The peristaltic waves were recorded with a Sharp Viewcam Z camera fitted to a Leica S8APO dissection scope. After 5 minutes the brain lobes and SOG were removed and the animals were recorded for another 20 minutes. The frequency and duration of peristaltic waves was quantified with VCode 1.2.1.

Immunohistochemistry

Nervous systems of early third instar larvae raised at 22 °C for 4 days were dissected in PB (100mM NaH_2PO_4/Na_2HPO_4) pH 7.2, fixed with 4% formaldehyde in PB for 20 min at room temperature (RT) and rinsed in PBS plus 0.3 % Triton X-100 (PBT) 3 X 15 min. Specimens were then incubated with primary antibody in PBT overnight at 4^{o}C in a wet chamber (chicken anti-GFP (Abcam) 1 ⁄ 2000; anti-SCR 6H4.1 1/50, anti-Fas II ID4 1/10, anti-Chaoptin 24B10 1/100 (Developmental Studies Hybridoma Bank, USA)), washed in PBT 4 X 15 min, and incubated with secondary antibody in PT for 3 h at RT (Alexa488 anti-Chicken 1:700; Alexa633 anti-Mouse 1:250 (Invitrogen)). Secondary antibodies were washed 4 X 30 min in PBT and specimens were mounted in Vectashield (Vector Laboratories) between two aluminum-foil spacers, to avoid distortion of nerve cords, under number 1 cover glasses.

To image patterns of GFP expression in whole animals, 3rd instar larvae were killed in 90% ethanol and placed between a slide and a coverslip in a drop of glycerol 80%. Images were collected using a Leica SP5 confocal laser scanning microscope.

Electrophysiology

Third instar larvae were dissected and pinned out in a sylgard-coated petri dish filled with physiological saline as above for semi-intact preparations. Suction electrodes were prepared from thick-walled borosilicate glass (GC100F-10; Harvard Apparatus). In each experiment, the diameter of the pipette was approximately the same or slightly smaller than the diameter of individual brain lobes. We gently pulled individual brain lobes into the suction electrode and performed extracellular recordings with a Model 1700 extracellular amplifier (A-M Systems, Sequim, WA). We used a BX51wi compound microscope (Olympus), to visualize the preparation and a MP225 micromanipulator (Sutter Instruments) to maneuver the suction electrode. Light pulses were delivered by manually controlling the shutter of an attached mercury arc-lamp (as for behavior experiments). We restricted illumination to single brain lobes using a combination of high power objectives and a magnifier for the microscope illuminator. The light intensity was measured with a PM100 optical power meter attached to a sensor S130A, 400nm-1100nm (Thorlabs Karlsfeld, Germany). Data were digitized with a Powerlab 8/30 (ADinstruments) and analyzed using Chart 7 (ADinstruments).

Statistical Analysis

Statistical analysis was performed employing the Prism Graphpad 5.0b software package (2009). Normality was tested with a Kolmogorov-Smirnov test with Dallal-Wilkinson-Lilliefor *p* value. A Bartlett's test for equal variances was also performed before deciding if a parametric or non-parametric test was going to be used to analyze the data. For the frequency distribution of turning angles, the angles were distributed between 0 and 360^{o} and grouped in intervals of 5^{o}. A Watson's U^2 test for non parametric two-samples was performed.

Supplemental References

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S2. Clyne, J. D., and Miesenböck, G. (2008). Sex-specific control and tuning of the pattern generator for courtship song in *Drosophila*. Cell *133*: 354-363.

S3. Baines, R.A., Uhler, J.P., Thompson, A., Sweeney, S.T., and Bate, M. (2001). Altered electrical properties in *Drosophila* neurons developing without synaptic transmission. J Neurosci. *21*: 1523-1531.

S4. Kitamoto, T. (2002). Conditional disruption of synaptic transmission induces male-male courtship behavior in *Drosophila*. Proc. Natl. Acad. Sci. USA *99*: 13232-13237.