Supporting Information

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SI Materials and Methods

Electrophysiology. Sound-evoked potentials (SEPs) were recorded as previously described (1–3). Each fly was mounted in a trimmed 200-μL pipette tip with its head protruding and held in place with modeling clay. The tip was then placed on a custom holder mounted to a movable stage. Guided via a dissecting microscope, two electrolytically sharpened tungsten electrodes were inserted—a recording electrode into the joint between the first and second antennal segments and a reference electrode into the head capsule near the left supraorbital bristles. Electrode signals were passed to and amplified by a DAM50 differential amplifier (World Precision Instruments), then digitized and normalized via an instruNet 100B Analog/Digital Data Acquisition I/O system (GW Instruments) onto a G3 Macintosh computer using SuperScope II (GW Instruments) software.

Acoustic stimuli were converted to an analog signal using the Data Acquisition I/O system, fed to a PCA4 amplifier (Pyle Audio Inc.) connected to an 8-inch (20.3-cm) 8Ω speaker (Radio Shack) placed in an acoustic foam-lined box. Sound was delivered to the fly via 0.25-inch (63.5-mm, inner diameter) Tygon tubing with one end mounted close to, but not touching, the speaker cone. A plastic pipette tip, cut to a 5-mm circular opening and plugged loosely with cotton to reduce echo, was inserted into the other end and mounted close to the fly; antennae were kept within the hemisphere circumscribed by the pipette opening to maintain near-field acoustic conditions (4).

Circadian Locomotor Analysis. Circadian rhythm analysis was performed as described elsewhere (5). In the first experiment, locomotor activity of control and noise-exposed adult male and female Canton-S and 40AG13 flies (3–5 d posteclosion) was monitored with Trikinetics Activity Monitors for 5 d of light/dark (LD; 12 h/12 h; Fig. S7 A, I–2) followed by 7 d of constant darkness (DD) at 25 °C. To eliminate the effect of behavioral disruption from placement in the apparatus and acclimatization, all data taken from the first 14 h were discarded, as were data from flies that died during the course of the experiment. In the second experiment, activity of control and noise-exposed adult male 40AG13, 40AG13/Cyo, and nrv3/40AG13 flies (3–5 d posteclosion) were studied with the same apparatus for 8 d of LD (Fig. S8 A, I–3), starting immediately after trauma ended. Data were analyzed offline using numerical analysis software (GNU Octave) and statistical software (Prism, GraphPad). Activity levels were quantified as the averaged number of crossings per unit time (Figs. S7 and S8). For the first experiment, data for males and females were pooled, and differences between traumatized and control flies tested via Student t test (two-tailed; α = 0.05) for data binned at 12 h and 1 d for the first 7 d of the experiment. In the second, male-only, study we also discarded any data from flies that died during behavioral monitoring. Differences between genotype/treatment groups were tested with one-way ANOVA (α = 0.05) with Bonferroni post hoc comparisons between control and noise-exposed animals within each genotype.

Nrv3 Immunostaining. Antennae were dissected in PBS, fixed in 4% paraformaldehyde in PBS for 30 min, embedded in Tissue-Tek Optimal Cutting Temperature (OCT) Compound (Ted Pella Inc.), and then cut into 25-μm sections in a cryostat. Antennae were stained with polyclonal antibodies against Nrv3 (generated in guinea pig); [1:200 in PBS + 0.1% (vol/vol) Triton X-100 (PB)] + BSA; ref. 6] and incubated overnight. After primary antibody staining, antennae were washed in PBS + BSA for an hour before FITC (1:200) secondary antibody incubation. Confocal images were taken using a Leica SP2 Confocal Microscope. The Nrv3 antibody was a gift from Greg Beitel at Northwestern University (Evanston, IL).

Fig. S1. Physiological effects of acoustic trauma are evident across a broad range of test stimulus sound pressure levels. Mean SEPs with varying stimulus sound-pressure level (dB SPL) vs. trauma treatment and days posttreatment with varying stimulus SPL (dB). (A) 65 dB. Canton-S flies: n = 65 (sham), 68 (trauma) for 0 d; 40 (sham), 39 (trauma) for 7 d. 40AG13 flies: n = 65 (sham), 73 (trauma) for 0 d; 38 (sham), 37 (trauma) for 7 d. (B) 75 dB. Canton-S flies: n = 67 (sham), 67 (trauma) for 0 d; 41 (sham), 39 (trauma) for 7 d. 40AG13 flies: 65 (sham), 75 (trauma) for 0 d; 39 (sham), 37 (trauma) for 7 d. (C) 85 dB (data presented in Fig. 1). Canton-S flies: n = 69 (sham), 67 (trauma) for 0 d; 42 (sham), 39 (trauma) for 7 d. 40AG13 flies: 66 (sham), 75 (trauma) for 0 d; 39 (sham), 37 (trauma) for 7 d. (D) 95 dB. Canton-S flies: n = 66 (sham), 66 (trauma) for 0 d; 41 (sham), 39 (trauma) for 7 d. 40AG13 flies: 64 (sham), 76 (trauma) for 0 d; 40 (sham), 37 (trauma) for 7 d. Significance tested by t test (*P < 0.05; ***P < 0.001; ****P < 0.0001). Error bars indicate SEM. For additional description of graph features, see Fig. 1. TPT, time posttrauma.
Fig. S2. Nrv3 protein expression is limited to Johnston’s organ (JO) sensory neurons. Confocal image of a cryosection through the antenna showing Nrv3 labeling in the plasma membrane of JO neurons (solid arrow), in the cytoplasm of the neuronal soma (asterisk), and in the inner dendritic segment (dashed arrow). The nerve from the third antennal segment (labeled by AN) also shows Nrv3 expression.

Fig. S3. Canton-S flies do not exhibit gross JO abnormalities immediately posttrauma. Longitudinal TEM sections of JO in sham (A and B) and trauma-exposed (C and D) Canton-S flies. (Scale bars: 1 mm.)
Fig. S4. Acoustic trauma does not produce JO morphological defects 7 d posttrauma. (A, 1) Longitudinal and (A, 2) cross-sectional TEMs of sham 40AG13/CyO flies. (B, 1) Longitudinal and (B, 2) cross-sectional TEMs of traumatized 40AG13/CyO flies. (C, 1) Longitudinal and (C, 2) cross-sectional TEMs of sham nrv3/40AG13 flies. (D, 1) Longitudinal and (D, 2) cross-sectional TEMs of traumatized nrv3/40AG13 flies. (Scale bars: 1 mm.)

Fig. S5. JO neurons show no gross morphological changes 7 d posttrauma. Sample TEM sections from (A) sham 40AG13/CyO flies. (B) Trauma-exposed 40AG13/CyO flies. (C) Sham nrv3/40AG13 flies. (D) Trauma-exposed nrv3/40AG13 flies. (Scale bars: 1 mm.)
Fig. S6. Noise trauma changes mitochondrial morphology. Sample TEM sections with highlighted mitochondria (purple) and mitochondria alone from sham (A and C) and traumatized (B and D) 40AG13/CyO flies. The reductions in mitochondrial size between sham (E and G) and noise-exposed (F and H) nrv3/40AG13 flies are even more prominent. (Scale bars: 1 μm.)
Fig. S7. Exposure to acoustic trauma does not greatly affect circadian locomotor patterns. Activity levels pooled in 1-h bins show behavior of Canton-S (A, 1) and 40AG13 (A, 2) flies during 5 d of LD (shaded rectangles) and 2 d of DD. Both control (blue lines) and noise-exposed (red lines) show similar anticipatory “morning” and postdark phase “evening” activity peaks. Activity levels measured in 12-h (B, 1 and B, 2) and 1-d (C, 1 and C, 2) intervals were not significantly different between control (dark bars) and traumatized (white bars) flies. Combined N for Canton-S: 27 flies each for control and traumatized groups, for 40AG13: 23 control flies, and 26 traumatized flies. Error bars represent mean count numbers ± SEM.
Fig. S8. Circadian behavior of nrv3 heterozygote flies is not affected by exposure to noise trauma. Activity levels pooled in 1-h bins show behavior of 40AG13 (A, 1), 40AG13/CyO (A, 2), and nrv3/40AG13 flies during 7 d of LD (shaded rectangles). Both control (blue) and noise-exposed (red) lines show similar anticipatory morning and postdark phase evening activity peaks. Activity levels measured in 12-h (B, 1–3) and 1-d (C, 1–3) intervals were not significantly different between control (dark bars) and traumatized (white bars) flies. Total N values: 40AG13, 17 control and 17 traumatized flies; 40AG13/CyO, 18 control and 17 traumatized flies; and nrv3/40AG13, 17 control and 18 traumatized flies. Error bars represent mean count numbers ± SEM.