Supporting Information

Ghezzi et al. 10.1073/pnas.1005439107

SI Materials and Methods

Benzyl Alcohol Vial Coating and Treatment. Preexposure to benzyl alcohol was performed using 30-mL glass vials coated with benzyl alcohol as described in Ghezzi et al. (1). Benzyl alcohol coating was performed by adding 200 μL of a solution of 0.4% benzyl alcohol in acetone to the 30-mL glass vials. The vials were continuously rotated for 45 min at 22 °C to evaporate the acetone, leaving a thin coat of evenly distributed benzyl alcohol. All benzyl alcohol experiments included an acetone-only control. One group of 10 to 15 age-matched female flies was placed in a benzyl alcohol vial and exposed to the benzyl alcohol until sedation (10–15 min, approximately). A second group was placed in the acetone-only control for the same time but did not sedate (1). Both groups were returned to food vials and allowed to recover for 24 h before testing.

Benzyl Alcohol Vapor Chamber Treatment. For the tolerance assays, single flies were exposed to benzyl alcohol using a custom-built benzyl alcohol vapor chamber (Fig. S1). The vapor chamber was constructed by placing an upside-down 50-mL conical tube over a 30-mL Corex tube containing 3-mL benzyl alcohol. A 100-μm nylon mesh cell strainer (Fisherbrand, Fisher Scientific) placed between the tubes formed a vapor-permeable barrier between the open end of both tubes. The benzyl alcohol-containing tube was placed in a 35 °C heat block to volatilize the benzyl alcohol. A 5-mm hole was drilled into the conical end of the tube to allow access to the chamber. Single age-matched female flies were placed in the chamber through the hole and allowed to sedate during a 20 min benzyl alcohol exposure. The hole was plugged using a 20-μL micropipet "yellow" tip. After this period, flies were immediately removed from the chamber and tested for behavioral recovery, seizure susceptibility, and ability to follow a high-frequency stimulus.

Single Fly Electrophysiological Set-Up. Individual adult female flies were anesthetized in ice for 3 min and placed on a small mount of bee's wax on a microscope slide. The legs of the fly were immobilized by embedding them in the wax in a natural standing position (horizontal, dorsal side up). The head and thorax were glued together to prevent movement by application of small amounts of loctite Super Glue (Henkel Corporation) using a pulled thin capillary tube. After drying for 3 min, a small drop of SignaCreme conducting gel (Parker Laboratories, Inc.) was applied to each eye using a syringe and a 27-gauge half-inch needle. Using micromanipulators (Narishige), a 200-μM diameter uninsulated tungsten-wire electrode (FHC Inc) electrolytically sharpened to approximately 5 μm was placed into the conductive cream on each of the compound eyes. When inserted, the electrode tips were immersed in the conducting cream and made contact with the surface of the eye, but did not pierce the eye. A 75-μm diameter recording electrode electrolytically sharpened to approximately 5 μm was inserted through the dorsal cuticle into the right-uppermost dorsolongitudinal muscles (DLM) that lies just beneath the cuticle. Finally, a 200-μm diameter reference electrode, electrolytically sharpened to approximately 5 μm, was inserted into the abdomen (2). For electrophysiological recordings of benzyl alcohol sedated flies, the use of ice anesthesia was not necessary as flies were already anesthetized. Furthermore, to reduce the set-up time to a minimum, stimulating electrodes were inserted midway into the fly's brain, through the anterior face of the head, between the first antennal segment and the edge of the eye. Application of conducting gel to the eyes and gluing of the head was not necessary in this preparation as the electrodes were inserted inside the cuticle and provided stability to the head.

Measurement of Benzyl Alcohol Concentrations. Benzyl alcohol concentration was measured in the sedated flies immediately after removal from the vapor chamber using liquid-phase gas chromatography. Fifteen sedated flies were transferred from the vapor chamber into 0.2-mL gas chromatography vials containing 150 μL of acetone and capped immediately. A dilution series of benzyl alcohol in acetone was prepared for the generation of a standard curve. Samples (in triplicates) and standards were loaded to a 310 G gas chromatograph (SRI Instruments). Data were collected and analyzed using SRI instruments Peak Simple software according to the manufacturers instructions. Final benzyl alcohol concentration was determined by interpolating the benzyl alcohol peak area values from each samples to the standard curve with known benzyl alcohol concentrations and converted to molarity of benzyl alcohol per fly, assuming a 0.88-μL water volume per fly (3).

1. Ghezzi A, Al-Hasan YM, Larios LE, Bohm RA, Atkinson NS (2004) *slo* K(+) channel gene regulation mediates rapid drug tolerance. *Proc Natl Acad Sci USA* 101:17276–17281.
2. Lin M, Nash HA (1996) Influence of general anesthetics on a specific neural pathway in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 93:10446–10451.
3. Cowmeadow RB, Krishnan HR, Atkinson NS (2005) The *slowpoke* gene underlies rapid ethanol tolerance in Drosophila. *Alcoholism Clin Exp Res* 29:1777–1786.

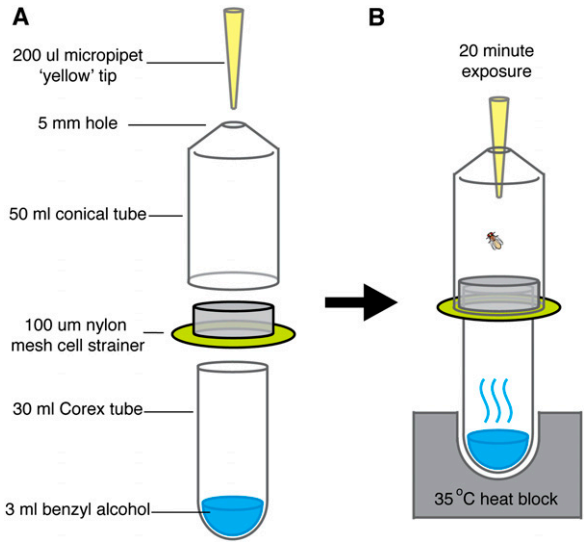

Fig. S1. The benzyl alcohol vapor chamber. (*A*) Components used to build the chamber consist of a 30-mL Corex tube containing 3 mL of benzyl alcohol, a 100-μm nylon mesh cell strainer, a 50-mL conical tube with a 5-mm hole drilled at the conical end, and a 200-μL micropipet tip. (*B*) Once the chamber is assembled, a fly is placed inside the 50-mL conical chamber, and the micropipet tip is used to plug the hole. The nylon mesh prevents the fly from entering the benzyl alcohol-containing tube but allows the heated benzyl alcohol fumes to permeate into the chamber. A 20-min exposure is sufficient to sedate a wild-type fly.