Supporting Information

French and Heberlein 10.1073/pnas.0910813106

SI Text

***Drosophila* Strains.** The *Or83b-GAL4* strain was a kind gift of L. Voshall (Rockefeller University, New York), *UAS-NR1*$^{RNAi-1}$ and *UAS-NR1*$^{RNAi-2}$ were generously provided by B. Dickson (Research Institute of Molecular Pathology, Vienna) and T. Tully (Watson School of Biological Sciences, Cold Spring Harbor, NY), respectively. *sgg*^{E3} and *sgg*^{E6} were provided by F. Wolf (Gallo Research Center, Emeryville, CA). *sgg*^{EP1379} and *sgg*^{EP1576} were obtained from the Szeged *Drosophila* Stock Center. All other strains were obtained from the Bloomington *Drosophila* Stock Center.

Olfactory Startle. We quantified the olfactory startle response as the maximum average velocity achieved by a group of 20 to 25 flies during a 1-min pulse of ethanol vapor (100:50 E/A ratio). Movement was analyzed in 10-s intervals (1, 2). Typically, flies achieved their greatest speed within 10 s of initial ethanol exposure. In most figures, experimental startle magnitude is presented as a fraction of the maximum velocity achieved by flies of the same genotype that had never been exposed to ethanol.

Histology, Cell-Death Assays, and Microscopy. For TUNEL assays, flies were exposed to ethanol for 1 h and processed 2 to 3 h after exposure. Fly heads were embedded and cut into 14-μm sections. Sections were then processed using the Roche In Situ Cell Death Detection kit, TMR Red. GFP fluorescence was examined in whole-mount dissected antennae or in fixed cryosections. Images were collected on a Leica TCS SP2 confocal laser scanning microscope and final images were generated by overlaying a *z*-series of eight to 10 2-μm sections.

Western Blot Analysis. Frozen antennae were homogenized in lysis buffer containing 13 parts 1× PBT (1× PBS plus 0.2% Tween-20), 5 parts 4× NuPAGE LDS Sample Buffer (Invitrogen), and 2 parts 0.5 M DTT (DTT). Samples were denatured at 80 °C for 15 min, and then each lane was loaded with protein equivalent to 30 antennae. Electrophoresis and transfer were carried out under standard conditions. Antibodies used were: 1/500 anti-GSK3 kinase domain (clone 4G-1E, Upstate Biotechnology, Lake Placid, NY); 1/10000 anti-α-tubulin (DM1A, Novus Biologicals).

1. Wolf FW, Eddison M, Lee S, Cho W, Heberlein U (2007) GSK-3/Shaggy regulates olfactory habituation in *Drosophila*. *Proc Natl Acad Sci USA* 104:4653–4657.

2. Cho W, Heberlein U, Wolf FW (2004) Habituation of an odorant-induced startle response in *Drosophila*. *Genes Brain Behav* 3:127–137.

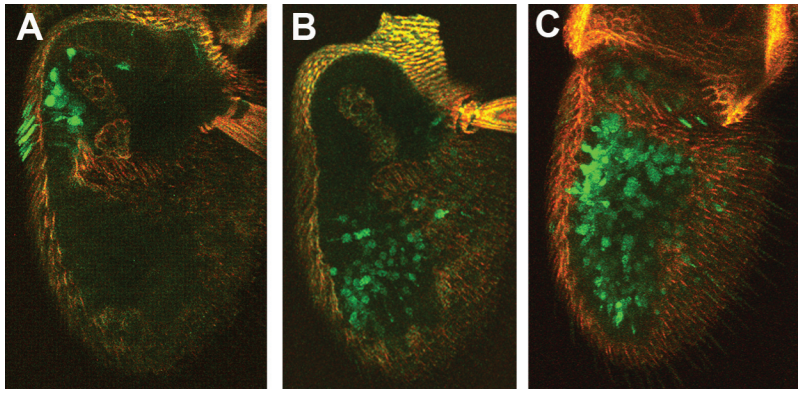

Fig. S1. Expression of olfactory receptors in sensillar subtypes. Confocal reconstructions of antennae from flies expressing *UAS-GFP* in olfactory neurons under the control of the *Or22-GAL4* (*A*), *Or67d-GAL4* (*B*) or *Or83b-GAL4* (*C*) drivers. *Or22a-GAL4* is expressed in basiconic sensilla, while *Or67d-GAL4* is expressed in trichoid sensilla [Couto A, Alenius M, Dickson BJ (2005) Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. *Curr Biol* 15:1535–1547.]. Green, GFP; yellow/orange,autofluorescence.

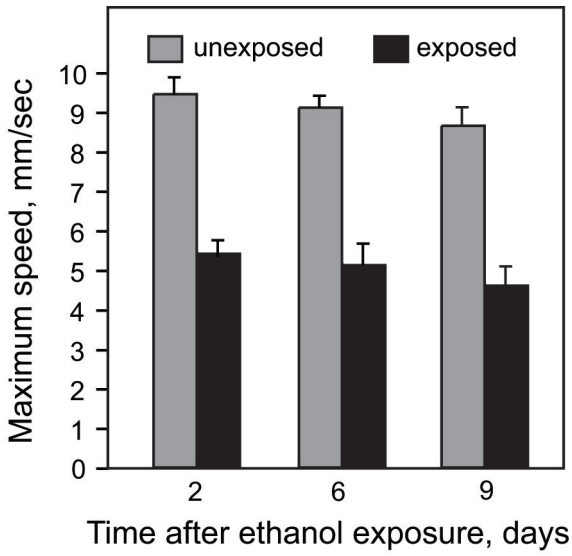

Fig. S2. Damage to the startle is permanent. Flies were tested for olfactory startle 2, 6, and 9 days after a single exposure to ethanol. Recovery of startle was never observed ($n = 4$).

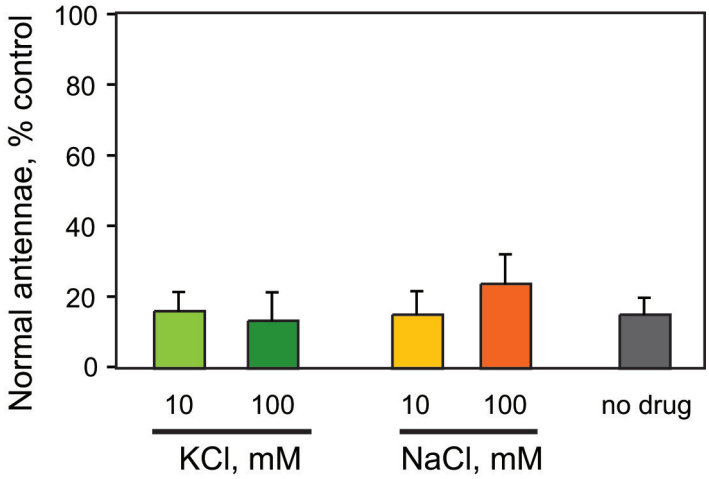

Fig. S3. Neither potassium chloride nor sodium chloride protects against olfactory damage. Flies fed 10-mM or 100 mM KCl- or NaCl-containing food for 5 days before ethanol exposure show no resistance to the damaging effects of ethanol on antennal morphology ($n = 4$). Flies were exposed to ethanol on Day 5 of treatment, and assayed on Day 7.

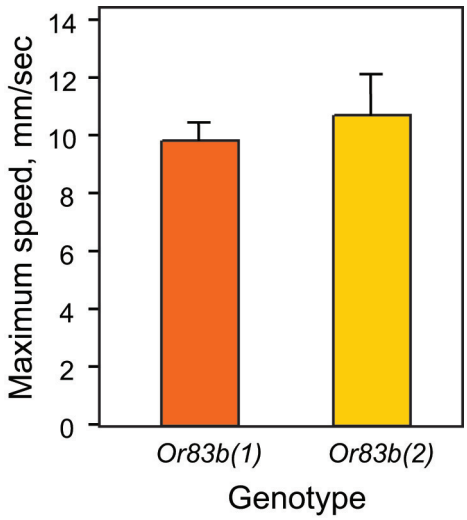

Fig. S4. *Or83b* null alleles have a normal ethanol startle. We tested two null alleles of *Or83b*: *Or83b*(*1*) and *Or83b*(*2*) [Larsson MC, et al. (2004) Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron* 43:703–714.]. Both alleles show a normal olfactory startle in response to ethanol exposure ($n = 4$) (compare with Fig. 1*A*).

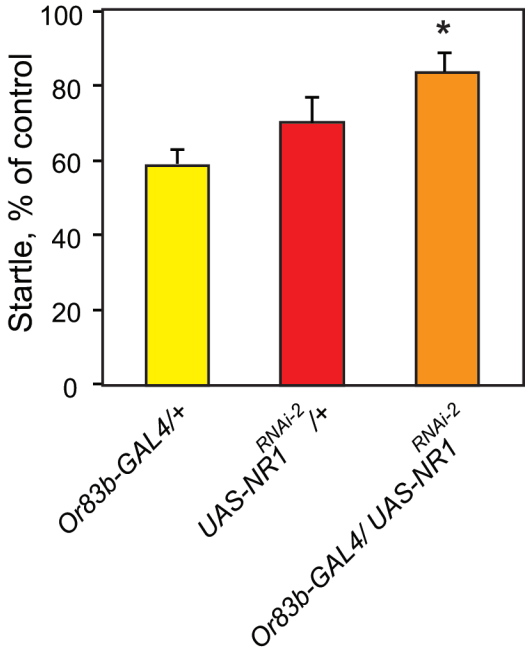

Fig. S5. Reducing NR1 in olfactory receptor neurons protects them from ethanol-induced olfactory damage. A second NR1 RNAi line shows protection of the ethanol startle when expressed in the ORNs. We tested an independently isolated NR1 RNAi line (*UAS-NR1^{RNAi-2}*) and found that, when expressed under the control of *Or83b-GAL4*, this line also leads to protection of the olfactory startle ($n = 5$, $*$, $P < 0.05$).

Table S1. Ethanol-induced ORN death is not cell-autonomous

	UAS-p35		Control (no *UAS-p35*)	
	Surviving ORNs	No surviving ORNs	Surviving ORNs	No surviving ORNs
Or67d-GAL4	47% (8/17)	53% (9/17)	50% (8/16)	50% (8/16)
Or22a-GAL4	71% (12/17)	29% (5/17)	56% (10/18)	44% (8/18)
Or83b-GAL4	82% (14/17)	18% (3/17)	47% (9/19)	53% (10/19)

Cell death in antennae expressing *UAS-p35* under the control of *Or22a-GAL4*, *Or67d-GAL4*, or *Or83b-GAL4*. Expression of p35 in a small group of cells is insufficient to protect against ethanol-induced cell death, indicating that the process is not cell-autonomous. The degree of cell death is correlated with more restricted p35 expression, with *Or83b-GAL4/UAS-p35* having the strongest protective effect and *Or67d/UAS-p35* having the weakest.