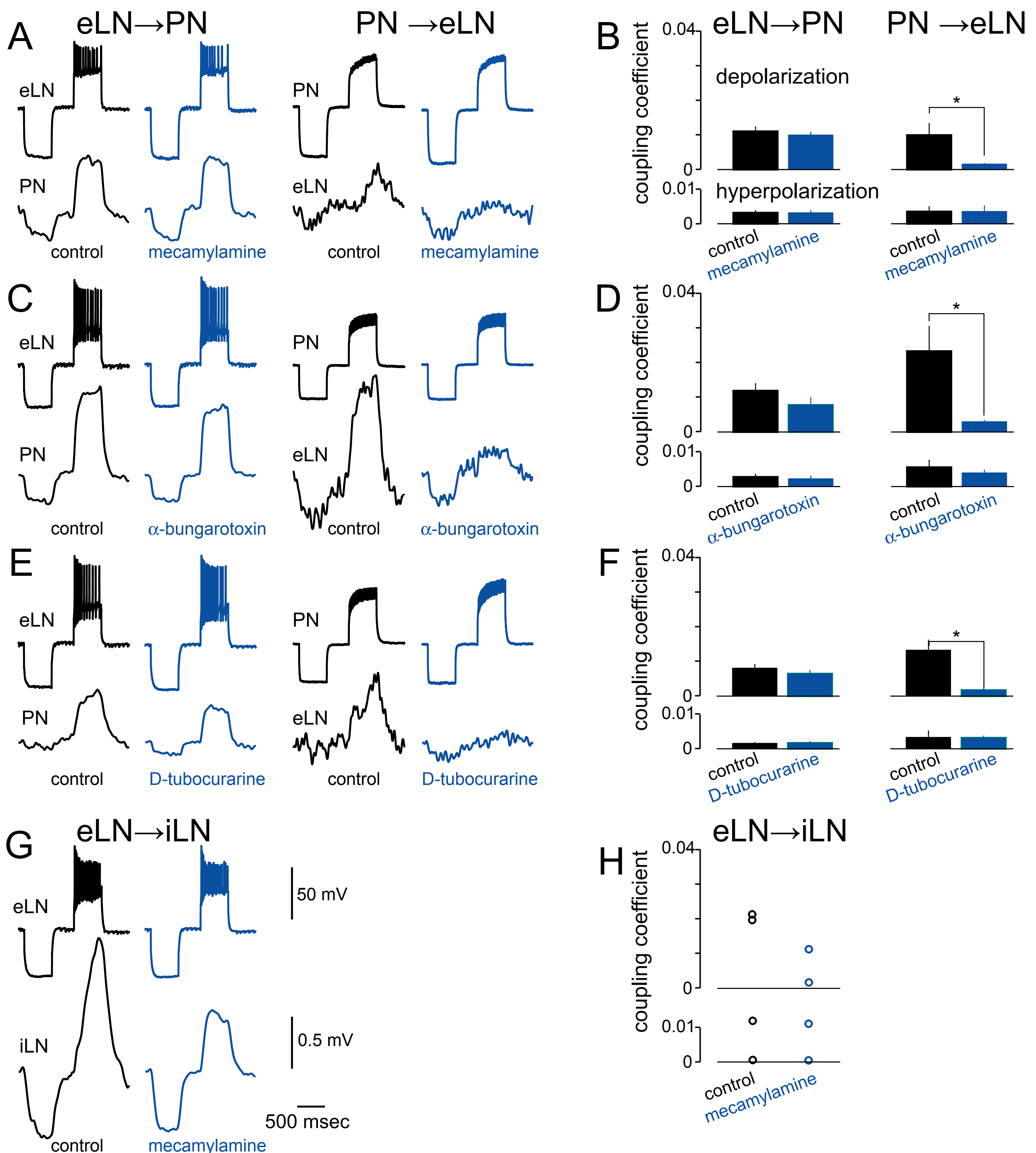

Figure S1: Effects of nicotinic antagonists on excitatory synapses between eLNs and other cells.

A-B. Mecamylamine (50 µM) did not affect transmission of depolarizing steps from eLNs to PNs, but significantly decreased transmission of depolarizing steps from PNs to eLNs (n=4, p<0.05, paired t-test).
C-D. α-bungarotoxin (5 µM) had no significant effect on the transmission of depolarizing steps from eLNs to PNs, but significantly decreased transmission of depolarizing steps from PNs to eLNs (n=6, p<0.05, paired t-test). There was a trend toward a decrease for eLN-to-PN synapses, but in some experiments input resistance also decreased.
E-F. D-tubocurarine (50 µM) had no significant effect on the transmission of depolarizing steps from eLNs to PNs, but significantly decreased the transmission of depolarizing steps from PNs to eLNs (n=3, p<0.05, paired t-test).
G-H. Mecamylamine (50 µM) substantially decreased transmission of depolarizing steps from eLNs to iLNs (n=2).
These results indicate that eLN-to-PN synapses are largely electrical, whereas PN-to-eLN synapses and eLN-to-iLN synapses are largely nicotinic cholinergic synapses, with an additional electrical component.

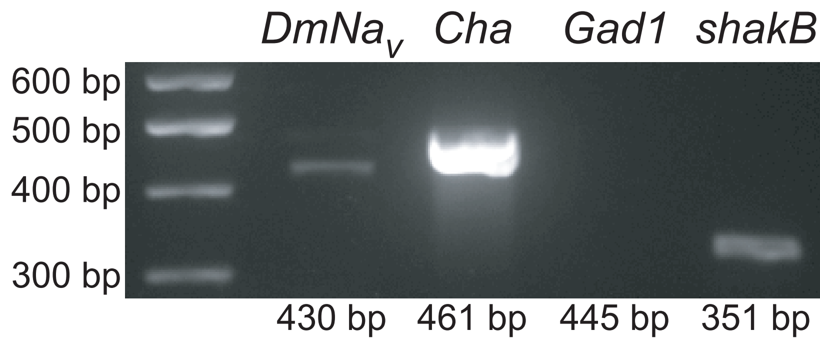

Figure S2: The gap junction subunit *shakB* is expressed in PNs.

We used RT-PCR to identify *shakB* transcripts in PNs. PNs were labeled with GFP using the broadly-expressed PN-specific driver *GH146-Gal4* (Stocker et al., 1997; genotype *GH146-Gal4,UAS-CD8:GFP*). Patch electrodes (6-8 MOhm) filled with the potassium asparate-based internal solution used for whole-cell recordings were targeted to a GFP-positive PN soma. A seal was formed using gentle negative pressure, and then the electrode was retracted to detach the soma from the rest of the cell. Each PN was collected by a separate electrode and the tip of the collection electrode was broken in a solution containing RNase free water and RNase inhibitor (SUPERase-In, Ambion). This procedure was repeated 10-15 times for each reaction tube, so that 10-15 PN somata were included in each sample. cDNAs produced in the initial RT-PCR reaction were further amplified by two separate PCR reactions (25 cycles each) with separate primers sets for each reaction for increased specificity. The primers were designed so that the genomic DNA product of the PCR reactions would be more than 3000 bp (except for Cha, whose genomic DNA product is 795 bp) and thus not amplified by PCR. This allowed detection of mRNA without using a DNA digestion step. As a positive control, we verified that our sample contains transcripts for the sodium channel α-subunit (*DmNa$_v$*) and for choline acetyltransferase (*Cha*). As a negative control, we verified that it does not contain a GABA biosynthetic enzyme (*Gad1*). Predicted transcript sizes are listed below the image. Similar results were obtained in a total of 9 independent experiments.

Primers were as follows:
shakB: first PCR (f) TATCACAATTGGTGGGCGTA, (r) CATCTTTGAACGACGAACGA; second PCR (f) GAGCTGCTCGCCCTTATAAAT, (r)GACCCTCATTCGAGGAGAGA;
DmNa$_v$: first PCR (f) GATGGCACGAGGTTTCATTT, (r) GAATCGAAGCTGGTGTAGCC; second PCR (f) GGAATTGGCTGGACTTCGTA, (r) AGGCACACGTAATCGTCGTC;
Cha: first PCR (f) CCTGCAACTGGCTCACTACA, (r) GCATTATGTCCAGCGAGTCC; second PCR (f) GTCGCGTAGACTGCATCAGA, (r) GGCACTCGTATCCTCACAGG;
Gad1: first PCR (f) CTCATGCCCACAACAGTGAC, (r) GGTGAACATCACCAGTGTGC; second PCR (f) GTGCTGCTGGACTTTGTGAA, (r) AGCCGTGCTCCTTGTAGTTC.
Note that primers for *shakB* do not distinguish between *shakB.neural* and *shakB.lethal*.
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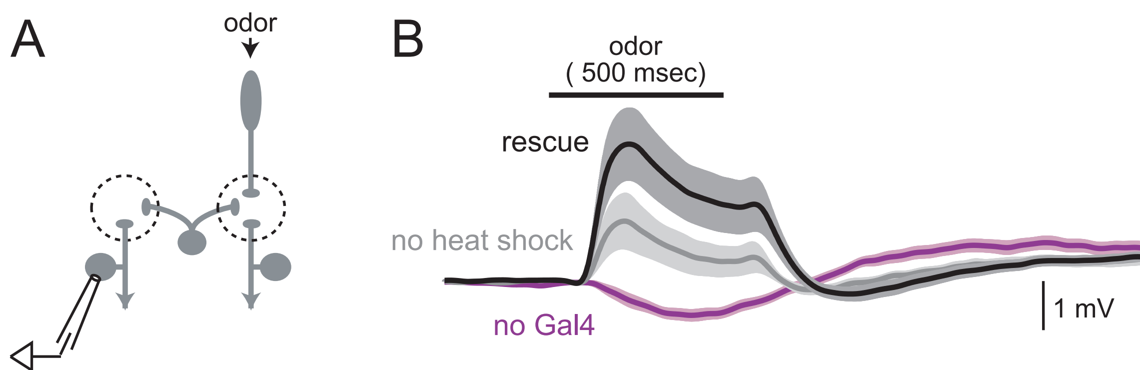

Figure S3. Rescuing *shakB* in adulthood can restore odor-evoked lateral excitation.

In these experiments, we used a *shakB.neural* transgene placed under the control of a heat-shock promoter to selectively rescue expression in adult flies. *shakB2* mutant flies harboring *hsp70-Gal4* and *UAS-shakB.neural* were cultured at 20°C. Directly after eclosion, adult flies (both males and females) were placed in glass vials with small openings in the lids to permit air circulation, and vials were immersed up to the bottom of the lids in a 37ºC water bath for 1 hr. This procedure was repeated twice with a 1-hr interval.

A. One or two days after heat shock, we removed the antennae and recorded from random PNs while stimulating the maxillary palps with odor. Because ~90% of PNs are postsynaptic to the antennae, we presume that the large majority of PNs we recorded are antennal PNs. A small minority of PNs in each genotype exhibited spontaneous EPSPs, always at a high rate, and we discarded these PNs because we assumed they were palp PNs.

B. In these flies, robust odor-evoked lateral excitation was observed in many (although not all) presumptive antennal PNs. In control flies where the *hsp70-Gal4* transgene was omitted, we never observed odor-evoked lateral excitation in any presumptive antennal PNs. In flies that were not heat-shocked but both transgenes were present (*hsp70-Gal4* and *UAS-shakB.neural*), we observed a small amount of odor-evoked lateral excitation, probably due to trace levels of Gal4 even at low temperatures, but this was substantially smaller than in heat-shocked flies. All pair-wise comparisons between the magnitude of depolarization in the three conditions are significantly different ($p<0.05$, Mann-Whitney U-tests). Genotypes were *shakB*^{2};*UAS-shakB.neural*/*hsp70-Gal4* (n=40 heat-shocked [black], n=30 not heat-shocked [gray]) and *shakB*^{2};*UAS-shakB.neural/*+ (n=15 [magenta]). Traces are mean ± SEM across recordings. The *hsp70-Gal4* transgene was originally described by Brand, A. H., and Perrimon, N. (1993). *Development* 118, 401-415.

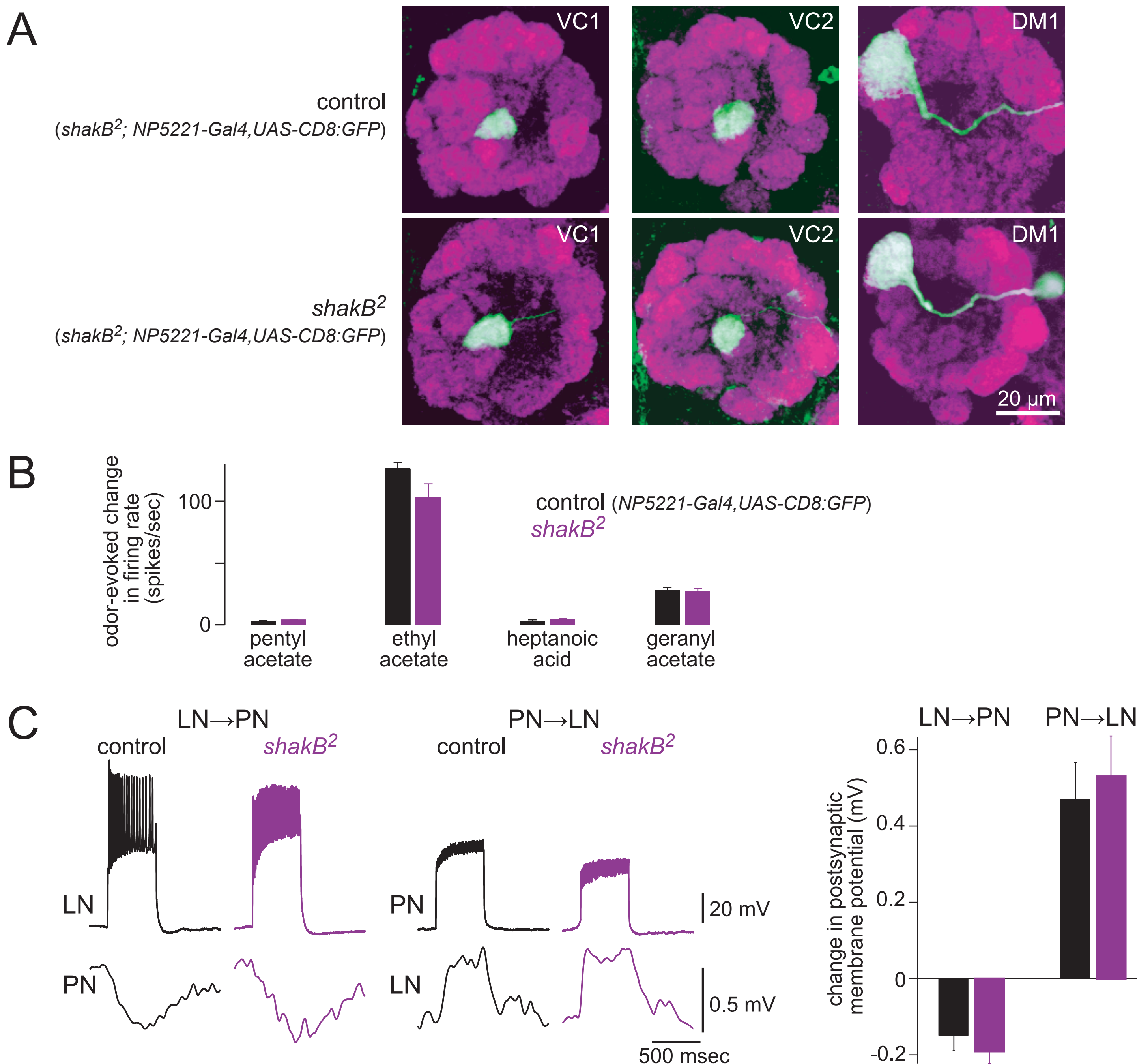

Figure S4. The *shakB*^{2} mutant shows normal PN morphology, ORN responses, and inhibitory synapses.
A. PN morphology is normal. Each image shows a section through the antennal lobe (dorsal is up, medial is left, *z*-projection of a 15-22 µm stack). A single PN is filled with biocytin and labeled with streptavidin-conjugated fluorphore (green). Neuropil is labeled using nc82 antibody (magenta). In both control and mutant flies, the PN dendrites are confined to a single glomerulus.
B. ORN responses are normal. We performed extracellular recordings from ab2A ORNs, choosing these because their spikes are among the easiest to record and sort. Odor-evoked change in firing rates in these ORNs were not significantly different in control versus mutant ORNs (p>0.05, Mann-Whitney U-tests; n=5 control, n=6 mutant, firing rates averaged over the 500-msec stimulus period, all odors 10^{-2} dilutions).
C. Inhibitory synapses are normal. We performed dual recordings from PNs and inhibitory LNs, injecting depolarizing current into the LN and measuring the strength of the PN response (left), or injecting depolarizing current into the PN and measuring the strength of the LN response (right). Postsynaptic responses are averages of 50 trials. The mean strength of these connections is not significantly different in control versus mutant flies (p>0.05, Mann-Whitney U-tests; n=35 control pairs, 19 *shakB*^{2} pairs). For the purpose of this experiment, inhibitory LNs are defined as LNs which do not produce an excitatory PN response. Control recordings were performed in a variety of genotypes (*krasavietz-Gal4,UAS-CD8:GFP*, *LCCH3-Gal4,UAS-CD8:GFP*, *nanchung-Gal4,UAS-CD8:GFP*; described in Chou et al., 2010).

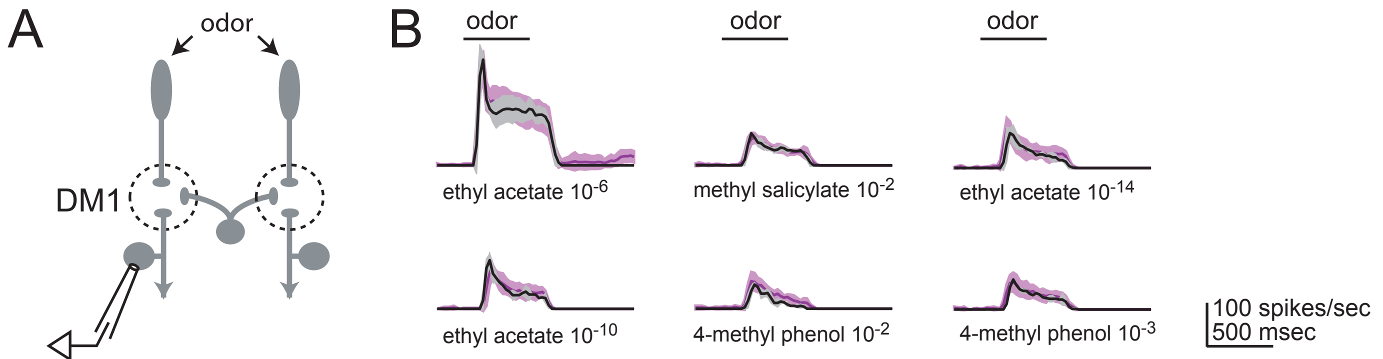

Figure S5: The *shakB²* mutation does not change DM1 PN spiking responses to odors.

We recorded from GFP-labeled PNs in glomerulus DM1 (genotype: *NP5221-Gal4,UAS-CD8:GFP*) and compared odor-evoked spike rates in control versus *shakB²* flies. Peristimulus-time histograms show mean ± SEM, averaged across experiments. There was no significant difference between genotypes (*n*=2 control and 5 mutant).

There are two factors that are likely to account for this negative result. First, the strength of odor-evoked lateral excitation in this glomerulus is unusually weak (Figure 7). Thus, we would not expect to see a large effect of removing lateral excitation to DM1. Second, there is only one GFP-positive DM1 PN in this genotype (Tanaka et al., 2004), suggesting that there may be only one DM1 PN in total. Consistent with this idea, in experiments using specific glomerular labeling with photoactivatible GFP (expressed under the Cha promoter) we found only one PN when photoactivation was targeted to DM1 (W.W. Liu and R.I. Wilson, unpublished observations). Thus, we would not expect any phenotype due to the elimination of sister PN interactions. This negative result is consistent with the conclusion that ORN responses and antennal lobe morphology are grossly normal in the *shakB²* mutant (see Figure S4).