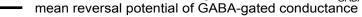
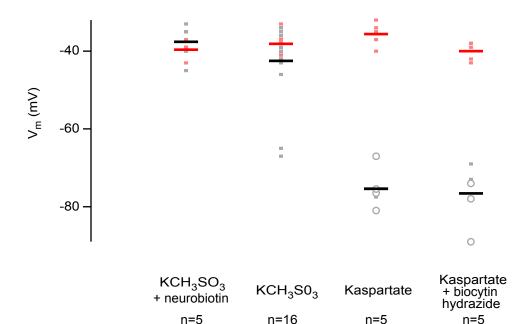
## Supplemental Figure 1. Effect of patch pipette internal solution composition on the reversal potential of GABA-gated conductances in Drosophila antennal lobe neurons.

GABA was iontophoresed onto the somata of LNs recorded in whole-cell mode, and the reversal potential  $E_{GABA}$  was determined, along with the spike threshold for that cell. With a KCH<sub>3</sub>SO<sub>3</sub>-based internal solution containing 0.5% neurobiotin (N-(2-aminoethyl) biotinamide hydrochloride, from Vector Labs),  $E_{Cl}$  should be -52mV, but  $E_{GABA}$  was closer to -40mV. Without neurobiotin,  $E_{GABA}$  was similarly depolarized, although in this case nominal [CI]<sub>i</sub> was zero. This suggests that methanesulfonic acid may affect  $E_{Cl}$  in *Drosophila* neurons, at least at the soma. Using a potassium aspartate-based internal,  $E_{Cl}$  was much more hyperpolarized. In many cells recorded with this internal, the cell could not be hyperpolarized to  $E_{GABA}$ , because antennal lobe neurons cannot be held stably below -75mV. In these cases, an open symbol ( $\circ$ ) marks a  $V_m$  still depolarized to  $E_{GABA}$  that was the most hyperpolarized potential where a cell could be held. Adding 0.5% biocytin hydrazide (Molecular Probes) to this internal did not change  $E_{GABA}$ . PN odor tuning was not substantially different with the Kaspartate+biocytin hydrazide internal versus the KCH<sub>3</sub>SO<sub>3</sub>+neurobiotin internal (supplemental Fig. 2).

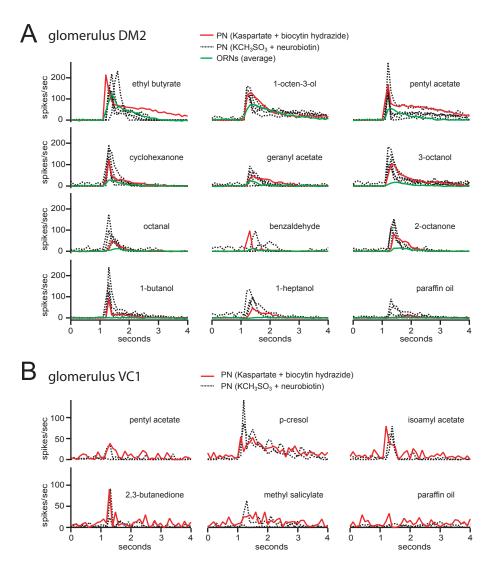
spike threshold
mean spike threshold
reversal potential of GABA-gated conductance (E<sub>GABA</sub>)





## Supplemental Figure 2. PN odor tuning is similar with a KCH<sub>3</sub>SO<sub>3</sub>-based or a Kaspartate-based patch-pipette internal solution.

Peristimulus-time histograms plot spike rate for PNs in glomerulus DM2 (A) or glomerulus VC1 (B). The tuning of PNs recorded with the Kaspartate-based internal was generally within the range of responses recorded with the KCH<sub>3</sub>SO<sub>3</sub> internal. Similar results were observed for PNs in five other glomeruli. The lack of a significant effect implies that GABA<sub>A</sub> conductances were still contributing to PN odor responses even using the KCH<sub>3</sub>SO<sub>3</sub> internal. Consistent with this, the effect of picrotoxin on odor responses is similar using the two different internals (compare Wilson et al., 2004, and Fig. 3). This is most likely because KCH<sub>3</sub>SO<sub>3</sub> is changing  $E_{CI}$  only near the soma. It may also reflect a contribution of shunting inhibition in the KCH<sub>3</sub>SO<sub>3</sub> internal. In (A), average peristimulus-time histograms for the DM2 ORNs are plotted together with the PN data. Some data from (A) is reproduced from Wilson et al. 2004.



Supplemental Figure 3. GABA was periodically iontophoresed into the antennal lobe neuropil, and the amplitude of the GABA-evoked hyperpolarization was monitored over time in whole-cell recordings from antennal lobe LNs. Graph plots mean hyperpolarization amplitude ( $\pm$ SEM) as a % of control. A low concentration of picrotoxin (1µM) was sufficient to block 95% of the GABA response (n=4).

