Supporting Information

Hu et al. 10.1073/pnas.0914912107

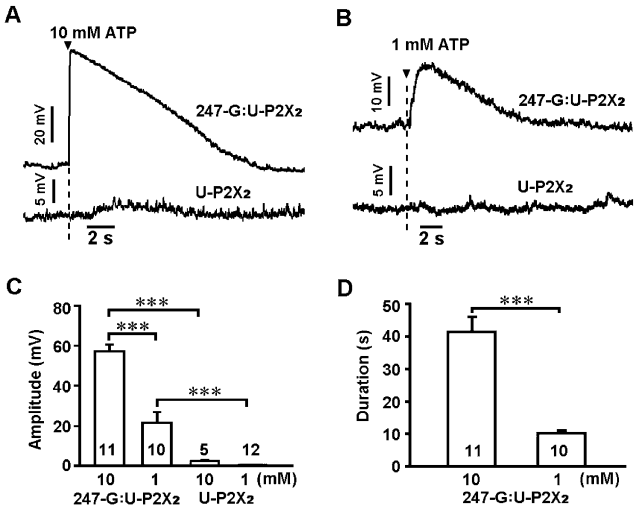


Fig. S1. Activation of $P2X_2$-expressed Kenyon cells (KCs) by exogenous ATP. $P2X_2$ is expressed in KCs under the control of 247-Gal4 and ATP is applied to KCs by local puffing, and the membrane potential of KCs of 247-Gal4:UAS-$P2X_2$ and UAS-$P2X_2$ flies was monitored by whole-cell recording under current clamp. (*A* and *B*) Examples of depolarizations of KCs in 247-Gal4:UAS-$P2X_2$ flies evoked by puffing 10 or 1 mM ATP, respectively, and no significant effect on KCs in UAS-$P2X_2$ flies in response to the same ATP puffing. (*C*) Average peak amplitudes of KC depolarizations observed in two groups of flies illustrated in *A* and *B*. (*D*) Average durations of KC depolarizations observed in 247-Gal4:UAS-$P2X_2$. (***, $P < 0.001$; *t* test). Number in bar chart indicts sample size.

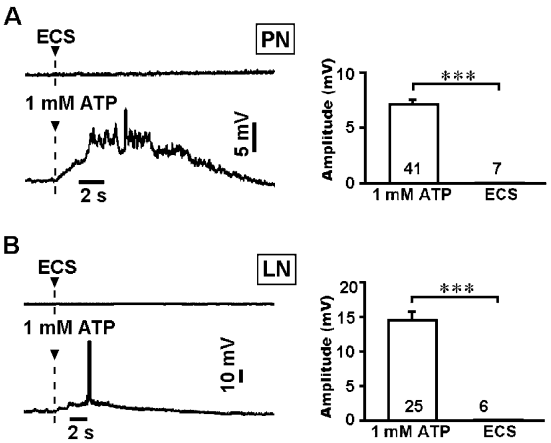


Fig. S2. No responses in projection neurons (PNs) and local interneurons (LNs) evoked by the puffing pressure. (*A*) (*Left*) Example responses of PNs in 247-Gal4:UAS-$P2X_2$ flies caused by local puffing of 1 mM ATP or extracellular solution (ECS) at βγ-lobes. (*Right*) Average peak amplitudes. (± SEM; ***, $P < 0.001$; *t* test). (*B*) (*Left*) Example responses of LNs in 247-Gal4:UAS-$P2X_2$ flies caused by local puffing of 1 mM ATP or ECS at βγ-lobes. (*Right*) Average peak amplitudes. (± SEM; ***, $P < 0.001$; *t* test). Number in bar chart indicts sample size.

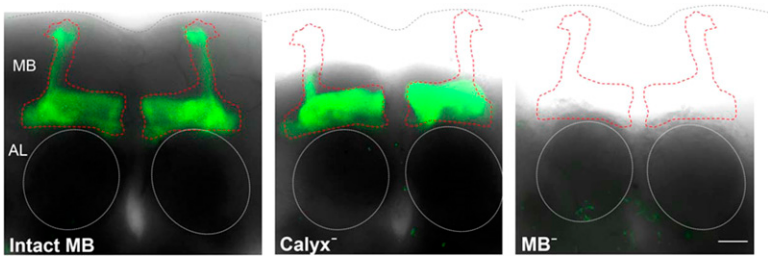


Fig. S3. Photograph to show how calyx (*Center*) or mushroom body (*Right*) was ablated. Dash lines indicate the formal brain regions: gray for brain borderline, red for mushroom body, white for antennal lobe. (Scale bar, 20 μm.)

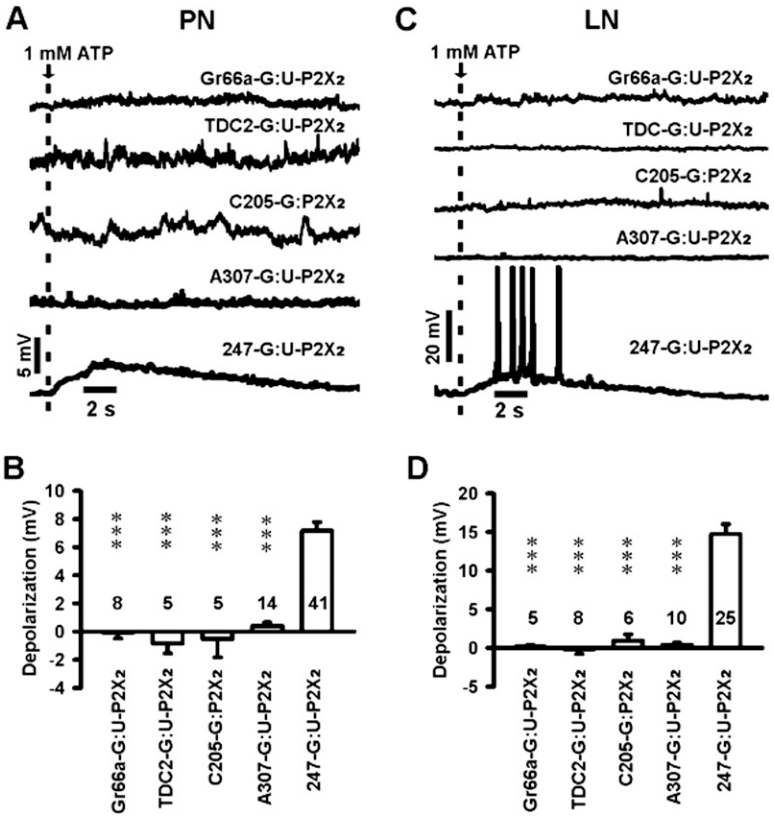


Fig. S4. No significant responses in PNs and LNs were induced by ATP-activation of other type neurons and brain regions expressing $P2X_2$. (*A* and *B*) Representative responses of PNs/LNs evoked by 1 mM ATP puffing at βγ-lobes of $P2X_2$-expressed KCs of 247-Gal4 flies, or taste neurons (Gr66a-Gal4), octopaminergic neurons (TDC2-Gal4), central complex (C205-Gal4), or giant fiber system (A307-Gal4) in the fly brains. (*C* and *D*) Average peak amplitudes of PN and LN responses in *A* and *B*. Number in bar chart indicts sample size.

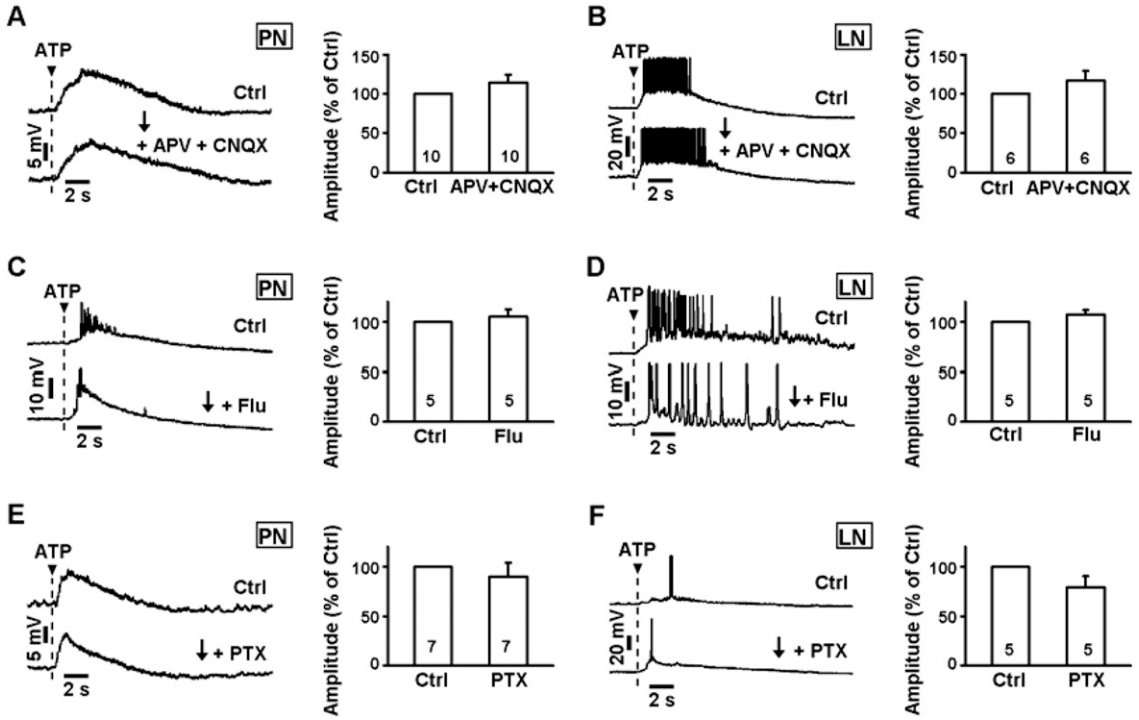
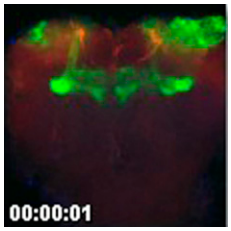
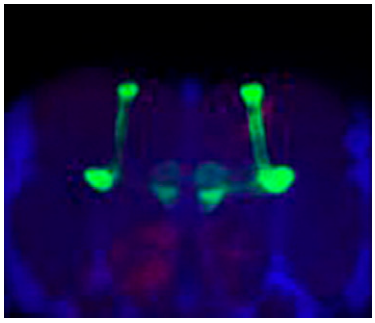


Fig. S5. Responses in PNs and LNs evoked by mushroom body-activation were not affected by blocking receptors for glutamate, dopamine/octopamine, or GABA. (*A*–*F*) Representative responses and average peak amplitudes of PNs and LNs evoked by 1 mM ATP puffing at βγ-lobes of KCs, before and after the treatment with GluR antagonists APV and CNQX (*A* and *B*), dopamine D_1 and D_2 and OCT-receptor antagonist Flu (*C* and *D*), and $GABA_A$ receptor antagonist PTX (*E* and *F*). No significant change was observed by the treatments. Number in column indicts sample size.



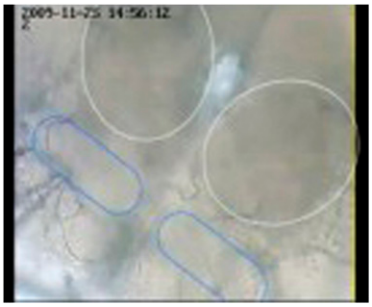
Movie S1. Z-stack confocal section series of the neuron shown in Fig. 4*A*.

Movie S1



Movie S2. Z-stack confocal section series of the neuron shown in Fig. 4*B*. The candidate feedback neuron is in the right half; an antennal lobe local interneuron is shown in the left half.

Movie S2



Movie S3. Profile of ejected solution after the puffing. To provide further characterization of the ATP puffing method, this movie shows a visual profile of ejected solution containing blue dye (Trypan blue, 0.1%) near the site of application under the same puffing conditions (0.3 s, 4 psi, pipette tip opening of 1 μm). The oval shape represents the βγ-lobes of mushroom bodies and the round shape represents the antennal lobes. As shown in this movie, solution ejected toward the mushroom bodies was very restricted to the desired target region, with no visible diffusion to the antennal lobes.

Movie S3