Figure legends

Figure 1. Location and morphology of *Gr21a*-expressing cells in the *Drosophila* antenna.

(A) Expression of mCD8::GFP in the antenna driven by *Gr21a-Gal4*. A stack of 40 1μm confocal sections of the third antennal segment, using reflection of 633 nm light (red) to visualize cuticular structures, including tracheae. I, II and III are antennal segments, ar, arista. Scale bar is 50 μm. (B) Detail of the region boxed in (C) from another preparation to show neuronal morphology. od, outer dendrite, id, inner dendrite, s, soma, a, axon, Sac, sacculus. The arrow points to the region under the sacculus, which is characterized by rows of large basiconic sensilla of the ab1 and ab2 type. Scale bar is 20 μm. (C) Schematic drawing of the antenna showing the distribution of *Gr21a* expressing cells compared to (D) the distribution of physiological sensillum types in the area occupied by large basiconic sensilla (modified from de Bruyne *et al.*, 2001). Dots represent ab1 sensilla (green), ab2 sensilla (magenta) and ab3 sensilla (yellow). Other basiconic sensilla are indicated as grey circles. S, sacculus. Note that ab1 sensilla are more numerous than ab2 sensilla in the group under the sacculus and there are no ab3 sensilla. Distribution pattern and number of Gr21a positive cells suggest Gr21a is expressed in ab1 sensilla.

Figure 2. Calcium imaging of *Gr21a*-expressing neurons in *Drosophila*

antennae and antennal lobes of the brain.

(A-B) CCD camera image of cameleon expression prior to odor application in the antenna (A) and glomerulus V of the antennal lobe (B) as used for analysis of calcium induced changes in fluorescence. Dotted white lines outline the 2nd and 3rd segment of the antenna and the brain with antennal nerves. Typical areas over which the calcium signal was integrated are outlined in red. (e) indicates the position of the esophagus. Scale bar is 50 μm. (C-D) Examples of the time course of changes in fluorescence ratio ($\circledcirc F^R$), relative to background (F^R), in antenna (C) and the V glomerulus (D). Three stimuli are shown: A puff of synthetic air (black), paraffin oil headspace (green) and 10% CO_2 (red). Time is indicated (seconds) relative to the start of the 3 sec stimulation (indicated in grey) (E) Dose-response relationship for CO_2 stimulation in antenna and antennal lobe (n=6). Error bars are SEM. The inset shows an example of responses from one preparation (mean time courses for three repetitions). (F) Calcium responses from antennae upon stimulation with vapor from several odorants (1% v/v in paraffin oil), compared with the lowest response to CO_2 (0.1%), demonstrate *Gr21a* expressing cells respond specifically to CO_2. Error bars are SEM (n=6). (G) For comparison, responses from the four neurons (A B, C and D) in ab1 sensilla as measured in increased action potential frequency during 500 ms stimulation ($\circledcirc f$). Data are partly from de Bruyne *et al.* 2001, with permission. The response spectrum of Gr21a expressing neurons matched that of ab1C neurons. (H-I) Time course of the change in fluorescence is shown for the left glomerulus (red),

right glomerulus (green) and esophagus (black). Stimulation is with CO_2, for a preparation with both antennae intact (H) or the left antenna removed (I).

Figure 3. Extracellular recordings from ab1 sensilla

(A) Example of a 1500 ms trace showing activity from four neurons. Action potentials are labeled according to shape and amplitude differences as in de Bruyne *et al.* (2001). Spikes fired by the C neuron are indicated as vertical lines. Only neuron C responds to stimulation with 1% CO_2. The apparent delay is largely due to the travel time of odorant to the preparation. Changes in firing rate of the B neuron are not consistent across preparations. (B) Dose response relation for ab1C neurons stimulated with varying concentrations of CO_2 (n=5). (C) Same data as in D, demonstrating a linear relationship of response with CO_2 concentration. (D) A linear relationship also exists between action potential firing rates and changes in fluorescence ratio for ab1C neurons. Spike rates were calculated from the equation in (C) for the lower three doses tested in figure 2E. Calcium signals accurately reflect action potential firing.

Figure 4. Behavioral responses to CO_2

(A) Olfactory avoidance behavior was tested in a T-maze. Comparison of three stimuli (n=8): no stimulus on either side (control), 8% CO_2 from a pressurized gas tank on one side (CO_2) or 8% pressurized synthetic air on one side (AIR). CO_2 was clearly avoided by many flies (Chi square, $p<0,05$) and this was not due to effects

related to pressurized gas per se. Error bars are SEM. (B) Dose response relation shows significant avoidance above 0.1% (Student *t* test, p<0,05) and saturation of this response above 0.3%. Dotted lines indicate the SEM of control experiments (n=56). For 0.3, 1, and 8.3% n=32, all others n=24. (C) Relating avoidance behavior to spike activity in ab1C cells. Spike rates were calculated from the equation in figure 3C for CO_2 concentrations tested in figure (B). Relevant olfactory coding for avoidance behavior is at rates below 50 spikes/s.

Figure 5. Inducing apoptosis in ab1C neurons by expression of *rpr* eliminates antennal response to CO_2 and severely affects avoidance behavior.

(A-B) Confocal sections of the third antennal segment of flies carrying the *Gr21a-Gal4* construct combined with *UAS-mCD8::GFP* (A) or with *UAS-mCD8::GFP* and *UAS-rpr* (B). The ab1C neurons are ablated by *rpr*-induced apoptosis. (C) Electroantennogram responses from the proximo-medial region of the antenna. Mean amplitudes during 1 second stimulations are shown (n=15). Error bars are SEM. Flies carrying *UAS-rpr* construct combined with the *Gr21a-Gal4* driver are compared with siblings that do not carry the driver. The CO_2 (6%) response in flies with ablated ab1C neurons is reduced to the same level as the synthetic air control (Student *t* test, $p < 0.001$). (D) Single sensillum recordings from ab1 sensilla. Responses to 1% CO_2 from a control fly carrying only the *Gr21a-Gal4* driver and to 10% CO_2 from a *Gr21a-Gal4; UAS-rpr*

fly demonstrate the absence of CO_2 sensitivity in the latter. In the fly with ablated ab1C neurons only three spike classes can be identified, fired by the A, B and D neurons. Responses to stimulation with ethyl acetate and methyl salicylate, specific ligands for the A and D neurons respectively, are still present. (E) Avoidance of CO_2 (0.3%) was tested for the sibling flies mentioned under (D) as well as for flies homozygous for the *Gr21a-Gal4* or *UAS-rpr* P-elements. For full genotypes see materials and methods. The *rpr* construct and the *Gr21a* driver constructs do not affect CO_2 response whereas avoidance of CO_2 was significantly reduced in flies carrying *Gr21a-Gal4* and *UAS-rpr* compared to their siblings without *UAS-rpr* (Kruskal-Wallis, $p < 0.05$).