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INTEGRATION OF CO_2 AND ODORANT SIGNALS IN THE MOUSE OLFACTORY BULB

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Abstract—Carbon dioxide (CO_2) is an important environmental cue for many animal species. In both vertebrates and invertebrates, CO_2 is detected by a specialized subset of olfactory sensory neurons (OSNs) and mediates several stereotypical behaviors. It remains unknown how CO_2 cues are integrated with other olfactory signals in the mammalian olfactory bulb, the first stage of central olfactory processing. By recording from the mouse olfactory bulb *in vivo*, we found that CO_2-activating neurons also respond selectively to odorants, many of which are putative mouse pheromones and natural odorants. In addition, many odorant-responsive bulbar neurons are inhibited by CO_2. For a substantial number of CO_2-activating neurons, binary mixtures of CO_2 and a specific odorant produce responses that are distinct from those evoked by either CO_2 or the odorant alone. In addition, for a substantial number of CO_2-inhibiting neurons, CO_2 addition can completely block the action potential firing of the cells to the odorants. These results indicate strong interaction between CO_2 signals and odorant signals in the olfactory bulb, suggesting important roles for the integration of these two signals in CO_2-mediated behavioral responses.

Key words: mitral/tufted cells, pheromone, mixture, lateral inhibition, olfactory coding.

CO_2 is one of the major byproducts of cellular metabolism. Although the basal CO_2 level in the air is relatively low (0.038%), local atmospheric CO_2 level can fluctuate dramatically with the metabolic activity of organisms. Ambient CO_2 level can signal the presence of food, predators, or environmental stress and mediate stereotypical behaviors for animals across phyla (Stange and Stowe, 1999; Suh et al., 2004; Thom et al., 2004; Jones et al., 2007; Hallem and Sternberg, 2008; Luo et al., 2009). Recent studies have shown that, for both vertebrates and invertebrates, CO_2 is detected by a specialized subset of olfactory sensory neurons (OSNs) (Suh et al., 2004; Thom et al., 2004; Luo et al., 2009). Although CO_2 is odorless to humans, it is sensitively detected by a specialized olfactory subsystem in mice (Hu et al., 2007; Sun et al., 2009). The CO_2-responsive OSNs in mammals uniquely express several signaling molecules, such as guanylyl cyclase-D (GC-D) and phosphodiesterase 2A (PDE2A) that suggest their use of guanosine 3′,5′-cyclic monophosphate (cGMP) as the second messenger (Fulle et al., 1995; Juilfs et al., 1997; Sun et al., 2009). In addition, they project to a set of the so-called necklace glomeruli at the caudal end of the main olfactory bulb (MOB) (Juilfs et al., 1997; Hu et al., 2007; Walz et al., 2007). Because GC-D^+ OSNs are not known to express signaling components in the canonical transduction pathway within the typical OSNs, such as G_{olf} and ACIII (Juilfs et al., 1997; Meyer et al., 2000), it is likely that GC-D^+ neurons represent a specialized channel dedicated to detecting CO_2 but not typical olfactory signals.

A key question that remains unresolved is whether CO_2-activating bulbar neurons can also respond to other odorants. CO_2 activates bulbar neurons that extend their dendrites into the necklace glomeruli (Hu et al., 2007). However, necklace glomeruli are intermingled with other glomeruli in the main olfactory bulb, suggesting potential interaction between CO_2 signals and olfactory signals in the MOB. A recent tracing study suggests that at least some necklace glomeruli receive inputs from GC-D^- OSNs (Cockerham et al., 2009). Thus, the CO_2-responsive bulbar neurons may also be excited by odorants that activate these GC-D^- OSNs. Although mitral/tufted (M/T) cells—the projection neurons of the bulb—extend their primary dendrite into only one glomerulus, they also interact extensively with their basal dendrites (Shepherd et al., 2004). Inhibitory lateral connections are believed to shape odorant responses (Mori et al., 1999; Tan et al., 2010). However, it remains unclear whether CO_2-activating cells can be inhibited by odorants and conversely whether typical odorant-responsive M/T cells can be inhibited by CO_2. An interesting feature of CO_2 signaling lies in the fact that CO_2 levels are correlated with the metabolic activity of organisms including plants and animals. It is conceivable that CO_2 cues are often simultaneously released with other odorants in natural environment. It remains untested how CO_2-responsive cells in the bulb respond to the mixtures of CO_2 and odorants.

In this study, we carried out *in vivo* physiological recordings from the mouse olfactory bulb to address these questions. We find that a majority of CO_2-responsive neurons are selectively activated by other odorants. Many of these odorants are putative mouse pheromones and nat-

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Abbreviations: CAII, carbonic anhydrase II; cGMP, guanosine 3′,5′-cyclic monophosphate; CNG channel, cyclic nucleotide-gated channel; GC-D, guanylyl cyclase-D; MOB, main olfactory bulb; M/T cell, mitral/tufted cell; OSN, olfactory sensory neuron; PDE2A, phosphodiesterase 2A; PSTHs, peri-stimulus time histograms; 2,5-DMP, 2,5-dimethyl pyrazine.

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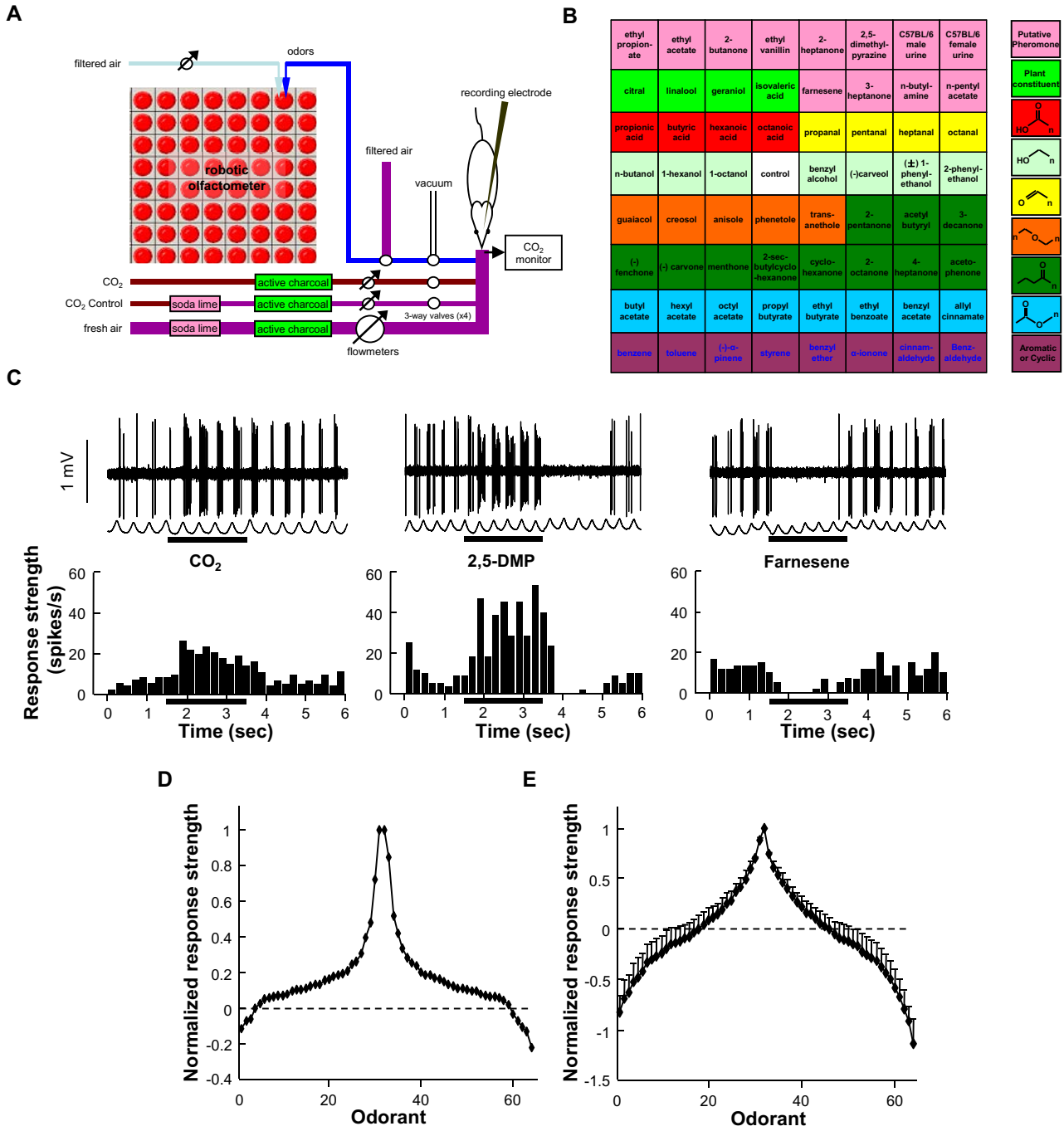

Fig. 1. CO_2-activating neurons in the olfactory bulb respond selectively to typical odorants. (A) A schematic diagram illustrates our method of odorant and CO_2 applications. Sixty-three odorants were stored in a panel containing 8×8 headspace vials and delivered with a custom-made robotic olfactometer. Purified room air from one headspace vial was used as the control for flowrate. CO_2 was delivered separately by a dedicated line and solenoid valves. Purified room air with CO_2 eliminated by soda lime was used as the control for CO_2 pulses. (B) A diverse set of odorants was used in this study. Pink indicates putative pheromone; green, plant leaf constitutive; red, acids; yellow, aldehyde; light green, alcohol; orange, ether; dark green, ketone; blue, acetate; purple, aromatic ring; white, control. Some odorants have multiple functional groups, although only one is indicated. (C) Representative physiological traces (upper) and PSTHs (lower) show the responses of a mitral cell to CO_2 and odorants. This cell was excited by 1% CO_2 and 2,5-dimethyl pyrazine (2,5-DMP, 1% saturated vapor) and inhibited by farnesene (1%). Horizontal bars indicate 2-s CO_2 or odorant pulse. Traces below the firing patterns indicate simultaneously recorded respiratory rhythms. (D) The olfactory tuning curve of the same cell as shown in (C). Response intensities were normalized to the maximal response of the optimal odorant. Odorant identities were aligned to produce a smooth and symmetrical curve, with the maximal excitatory response in the middle. Dashed line, zero response. (E) Mean olfactory tuning curve of nine cells that was completed with the testing of 63 odorants. Same conventions as in (D). Error bars, s.e.m. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.