Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Neurobiology

Neural detection of gases — carbon dioxide, oxygen — in vertebrates and invertebrates

Minmin Luo[1,2], Liming Sun[1] and Ji Hu[1]

Carbon dioxide (CO_2) and oxygen (O_2) are important cues that can signal the presence of food, predators, and environmental stress. Here we will review recent studies on the mechanisms of how the olfactory system detects these two molecules. In both vertebrates and invertebrates, the two molecules are detected by subsets of specialized olfactory neurons. In addition, the signal transduction cascades for sensing these two gases appear to be different from those for sensing typical odorants. CO_2 and O_2 signals can evoke stereotypical innate behaviors such as attraction and avoidance in many animal species. Future studies on the neural pathways underlying CO_2 and O_2 sensing may shed light on the circuit mechanisms of these behaviors.

Addresses
[1] National Institute of Biological Sciences, Beijing 102206, China
[2] Department of Biological Sciences and Biotechnology, Tsinghua University, 100084 Beijing, China

Corresponding author: Luo, Minmin (luominmin@nibs.ac.cn), Sun, Liming (sunliming@nibs.ac.cn) and Hu, Ji (huji@nibs.ac.cn)

Current Opinion in Neurobiology 2009, **19**:354–361

This review comes from a themed issue on
Sensory systems
Edited by Leslie Vosshall and Matteo Carandini

Available online 27th July 2009

0959-4388/$ – see front matter

DOI 10.1016/j.conb.2009.06.010

Introduction

The olfactory system of both vertebrates and invertebrates can detect numerous small volatile chemicals in the air. In the last two decades dramatic progresses have been achieved on our understanding of the molecular and cellular mechanisms of olfactory sensing. Studies in last five years provide major insights on how the olfactory systems detect two interesting gas molecules: carbon dioxide (CO_2) and oxygen (O_2). CO_2 is one of the major byproducts of cellular metabolism. Its concentration is low at atmospheric background levels (~0.038%) but can fluctuate dramatically because of the activity of organisms, such as animal respiration and the decomposition of organic material. CO_2 can thus serve as a valuable environmental cue. For example, floral CO_2 indicates food-source profitability for the hawkmoth *Manduca sexta* [1]. Although CO_2 is odorless to humans, it is now known to be detected by the olfactory system of mammals, insects, and worms. In addition, CO_2 can elicit a variety of innate behaviors such as avoiding stressful environment and locating food source [1,2••,3••,4•]. Maintaining appropriate O_2 levels is important for animal survival. Although O_2 levels are rather constant in the air (~20%), they can be quite variable in soil, the natural habitat of nematode worms. Studies have shown that *Caenorhabditis elegans* can sense different O_2 levels and adapt their behaviors accordingly [5••,6•,7].

A common feature revealed by recent studies is that atmospheric CO_2 and O_2 are detected by subsets of specialized sensory neurons. Mice detect CO_2 by a population of olfactory sensory neurons (OSNs) that project to the necklace glomeruli in the olfactory bulb [3••,8]. Insects such as fruit flies and moths detect CO_2 via a single class of OSNs on the antenna that project to one single, identified glomerulus in the antennal lobe (AL) [2••,9]. Acute CO_2 avoidance in *C. elegans* depends on the activity of the paired BAG neurons in the head [4•]. Recent studies reveal that O_2 fluctuations are detected by BAG and URX neurons in *C. elegans* [10••,11]. These cellular and genetic studies have revealed unique receptors and intracellular signaling cascades for CO_2 and O_2 sensing and thus enriched our understanding of the complexity of the olfactory system. Additionally, the identification of defined subsets of neurons mediating behaviors triggered by these two gas molecules may contribute to future studies on the neural circuits underlying some important behaviors.

CO_2 sensing by the vertebrate olfactory system

For decades it had remained unclear whether the mammalian olfactory system can sensitively detect atmospheric CO_2. CO_2 has no odor quality to humans [12]. Most human subjects do not report any clear olfactory sensation even when they are challenged with CO_2 pulses at the concentration of 30%. CO_2 at higher concentrations produces a pungent, painful sensation that is mediated by the trigeminal system rather than the olfactory system [12,13]. However, some early studies did suggest that some vertebrates may be able to detect CO_2 through their noses. For example, an early psychophysical study shows that rats can detect CO_2 at ~0.5% [14], which is well below the typical CO_2 concentrations that produce a trigeminal sensation in humans. In addition, 0.5–1% CO_2 evokes field potentials in the olfactory epithelium of both bullfrogs and rats [15,16]. CO_2-evoked physiological responses in

bullfrogs can be abolished by blocking the enzymatic activity of carbonic anhydrase with acetazolamide, suggesting that carbonic anhydrase may be critical for CO_2 sensing [17].

Our recent study demonstrates that CO_2 can be sensitively detected by mice and this detection is carried out by the necklace olfactory subsystem [3••]. We showed that carbonic anhydrase-2 (CAII) is richly expressed in a small subset of neurons (~1% of total) that also express guanylyl cyclase-D (GC-D) and phosphodiesterase 2A (PDE2A). These OSNs project their axons to the necklace glomeruli — a set of interspersed glomeruli that form the shape of a 'necklace' at the caudal end of the main olfactory bulb (MOB) [8,18–20] (Figure 1a). Because carbonic anhydrase is implicated in CO_2 sensing [16,21], we tested whether GC-D$^+$ neurons can respond to CO_2 by carrying out calcium imaging and targeted electrophysiological recordings from the GC-D$^+$ neurons *in vitro*. GC-D$^+$ cells were strongly activated by CO_2, but GC-D$^-$ neurons were not [3••,22•]. Physiological recordings *in vivo* further revealed that bulbar neurons associated with the necklace glomeruli respond to external CO_2 sensitively at concentrations as low as 0.1% [3••]. By performing behavioral assays using a go/no-go paradigm, we found that mice can be trained to detect CO_2 and that their detection threshold is ~0.066%, which is very close to the atmospheric background CO_2 level (0.038%). CAII mutant mice cannot detect CO_2 but can sense typical odorants without any obvious deficits [3••]. In contrast, CNGA2-knockout mice, which are largely anosmic to typical odorants, can detect CO_2 normally [3••]. These results together support the conclusion that mice detect CO_2 sensitively through the necklace olfactory pathway.

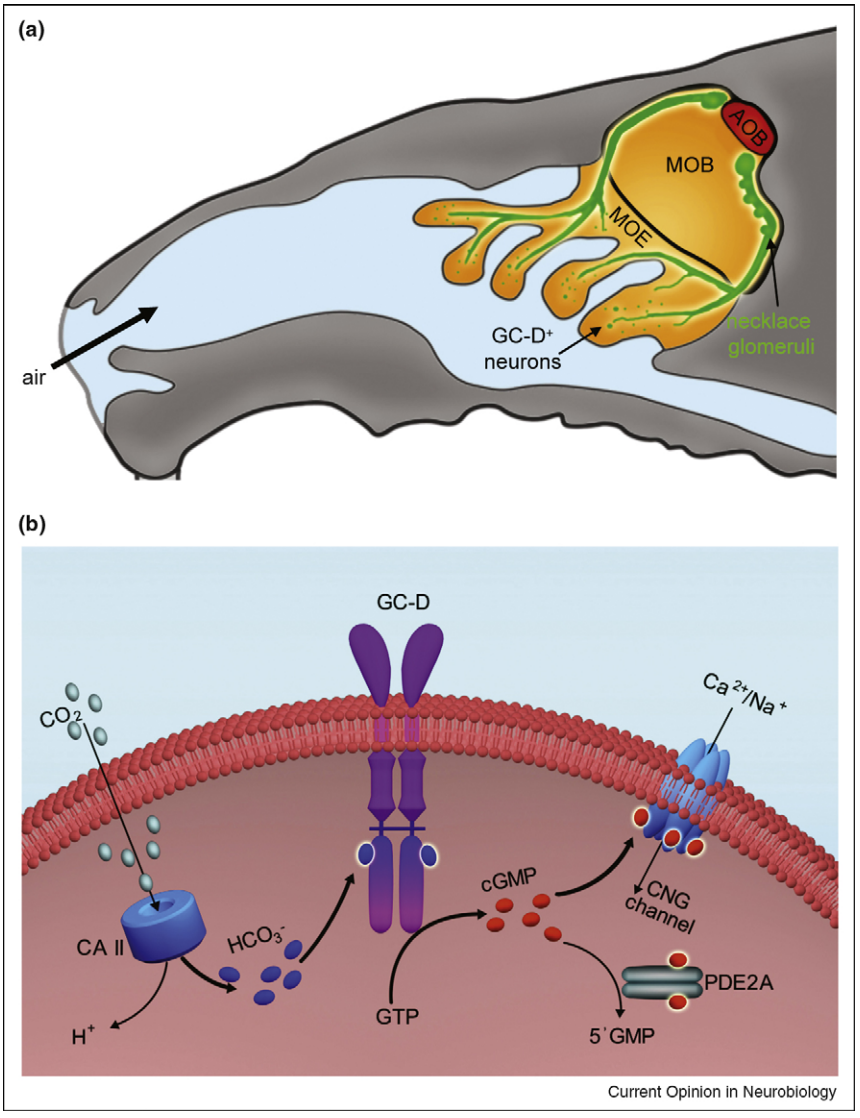
Several questions remain unanswered. First, how are CO_2 signals integrated with typical olfactory signals in the olfactory bulb? Second, what are the projection targets in downstream pathways that receive input from mitral/tufted cells associated with the necklace glomeruli? In addition, what is the exact function of CO_2 sensing in natural environment? One study suggests that GC-D$^+$ neurons may also respond to guanylin and uroguanylin [23], two gut peptides that have been traditionally considered ligands of GC-C [24]. A recent study indicates that the necklace glomeruli also receive input from GC-D$^-$ OSNs [25]. Therefore, it is possible that bulbar neurons associated with the necklace glomeruli may respond to odorants as well. Additionally, through intrabulbar circuitry, CO_2 may modulate the olfactory responses of bulbar neurons that are not directly associated with the necklace glomeruli. Thus, it will be interesting to test whether CO_2 signals are combined with some specific olfactory signals, including peptides, to regulate behavior. In a T-maze assay we found that CO_2 is an avoidance cue [3••]. Local CO_2 levels can be elevated by food, predators, or other conspecific individuals. Thus CO_2 cues may be combined with olfactory cues in these contexts to regulate animal behavior.

What are the molecular and cellular mechanisms of CO_2 sensing in mammals? Typical odorants are detected by a large set of G-protein-coupled odorant receptors (GPCRs), which are encoded by over 1000 genes in rodents [26,27]. The binding of odorous ligands to odorant receptors on the ciliary membrane of OSNs leads to the activation of $G\alpha s_{olf}$ and rapid stimulation of type III adenylyl cyclase (ACIII) to produce cAMP, which in turn opens the cyclic nucleotide-gated (CNG) cation channels and depolarizes OSNs [28]. GC-D$^+$ OSNs, however, lack many of these signaling components found in canonical OSNs. GC-D$^+$ OSNs are not known to express any odorant receptors. In addition, they do not express $G\alpha s_{olf}$, ACIII, and the CNGA2 subunit, all of which are richly expressed in canonical OSNs [29]. In contrast, they express GC-D, cGMP-stimulated PDE2A [18,19]. In addition, the CNG channels in GC-D$^+$ neurons contain CNGA3 subunit and are much more sensitive to cGMP than to cAMP, suggesting that the CO_2 responses of GC-D$^+$ OSNs may involve cGMP as a second messenger [29].

Our findings that CO_2 responses require the enzymatic activity of CAII suggest that CO_2 is converted to bicarbonate (HCO_3^-) and protons (H^+) and that one or both of these metabolites trigger intracellular signal cascades [3••]. Recent biochemical assays from our group and the group led by Xin-Yun Huang revealed that bicarbonate can activate GC-D to produce cGMP [22•,30•]. Finally, our imaging and pharmacological experiments suggest that CO_2 responses require the opening of cGMP-sensitive CNG channels [3••]. These results lead us to propose a model of signal transduction cascades for CO_2 sensing by GC-D$^+$ neurons (Figure 1b). On the basis of this model, CO_2 diffuses across cell membranes and is converted by CAII to bicarbonate and proton. Bicarbonate activates GC-D to produce cGMP, which in turn opens cGMP-sensitive CNG channels to depolarize GC-D$^+$ cells. This model suggests that CO_2 is sensed by mechanisms substantially different from those of typical odorants. The model can be further substantiated in future work by studying the behavioral and physiological effects of both GC-D-knockout mice and CNGA3-knockout mice. Since biochemical studies indicate that GC-D can be activated by uroguanylin [31], the effects of uroguanylin on CO_2 sensing should also be addressed.

Because GC-D is a pseudogene in humans [32], this model provides an attractive explanation for why humans lack the ability to detect low concentrations of CO_2 via the olfactory system. Further, a recent bioinformatic study revealed that the GC-D gene became nonfunctional early in primate evolution [33•], suggesting that CO_2 sensing ability might be lost in most of primates. Nevertheless, the fact that carbonic anhydrase is expressed in the OSNs of

Figure 1



Mechanisms of CO_2 sensing in mammals. **(a)** In mice, CO_2 is detected by a subset of OSNs that express GC-D (GC-D^+ neurons, green) and project their axons to the necklace glomeruli at the caudal end of the olfactory bulb. MOE, main olfactory epithelium; MOB, main olfactory bulb; AOB, accessory olfactory bulb. **(b)** Cellular mechanisms of CO_2 sensing in mammals. CAII, GC-D, PDE2A, and cGMP-sensitive CNG channels are coexpressed in GC-D^+ OSNs. We hypothesize that after CO_2 diffusion into cells, CAII catalyzes the conversion of CO_2 into bicarbonate (HCO_3^-) and H^+. Bicarbonate in turn activates GC-D to produce cGMPs, which then open CNG channels and depolarize the cell.

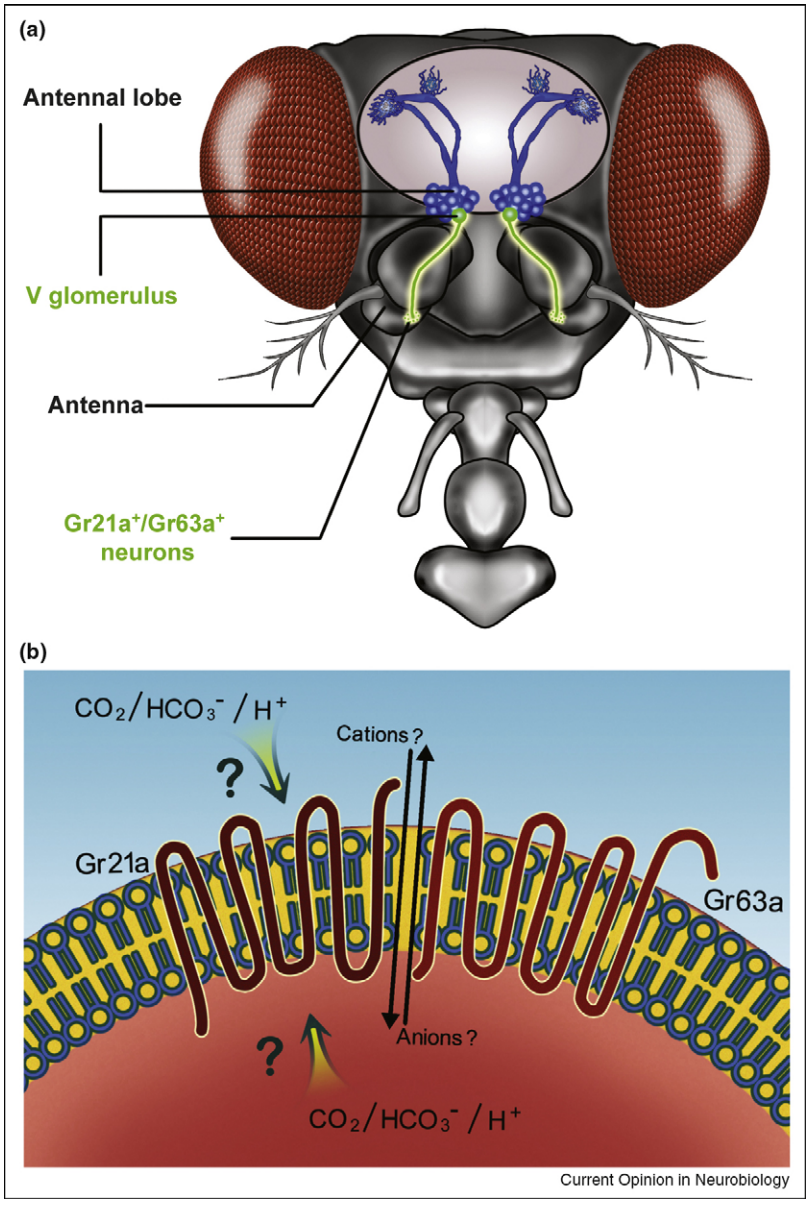
amphibians, reptiles, and many mammals suggests that CO_2 is sensitively detected by many vertebrates [3••,16].

CO_2 sensing by the invertebrate olfactory system

As in mammals, CO_2 is detected by a subset of specialized OSNs in insects. In the moth *M. sexta*, CO_2-responsive OSNs are located in the labial-palp pit organ (LPO) [34]. These neurons extend their axons to the LPO glomerulus in the AL [9,34], the counterpart of the mammalian olfactory bulb. The anatomical position and central projection patterns of CO_2 receptor neurons of mosquitoes share many characteristics with those of the moth [35]. OSNs in the LPO respond to CO_2 at levels as low as 0.03%. They are not activated by typical odorants, suggesting that they are dedicated to sensing atmospheric CO_2 [34]. Unlike to odorants, the responses to CO_2 by both LPO OSNs and AL neurons associated with the LPO glomerulus do not adapt to chronic stimulation by CO_2 [9,34].

CO_2 is a key constituent of chemicals released by stressed fruit flies [2••]. *Drosophila melanogaster* avoids CO_2 at levels as low as 0.1% [2••]. Using two-photon imaging

Figure 2



Mechanisms of CO_2 sensing in insects. **(a)** In *Drosophila*, CO_2 is detected by a small subset of OSNs that express both GR21a and GR63a ($GR21a^+$/$GR63a^+$ neurons, green) and project to the V glomerulus. ANT, antenna. **(b)** In *Drosophila*, Gr21a and Gr63a are critical for CO_2 sensing. These two genes encode membrane proteins that may form CO_2-gated channels, although it remains unclear whether the binding of CO_2 occurs extracellularly or intracellularly or even whether CO_2 or some other metabolite such as bicarbonate or H+ constitutes the true ligand for this receptor complex (question marks).

with the Ca^{2+}-sensitive fluorescent protein G-CaMP, this study further shows that CO_2-responsive OSNs in the antenna and AL neurons associated with their projection targets, the V glomerulus, are activated by CO_2 (Figure 2a) [2••]. Moreover, these neurons are not activated by any other odorants tested, suggesting that they are dedicated to CO_2 detection [2••,36]. When ChannelRhodopsin-2 is expressed in CO_2-responsive neurons, photostimulation produces similar avoidance behavior, strongly suggesting that the CO_2-responsive neurons in *Drosophila* provide a simple and genetically tractable system to dissect the neural circuits that translate a specific sensory signal into an avoidance response [37•]. Interestingly, a subset of fly taste receptors on the proboscis labellum also responds to carbonated water, indicating that CO_2 or bicarbonate might have taste quality in addition to an olfactory quality senses by the antenna [38].

What is the molecular identity of olfactory CO_2 receptors in insects? In *Drosophila*, CO_2 responses of OSNs projecting to the V glomerulus require the coexpression of a pair of membrane receptors, *Gr21a* and *Gr63a* (Figure 2b) [39••,40•]. Ectopic expression of these two receptors in other OSNs confers CO_2-responsiveness to these cells [39••,40•]. Similarly, mosquito CO_2 responses require the coexpression of two homologous receptors, *GPRGR22* (*AgGR22*) and *GPRGR24* (*AgGR24*) [39••,41]. Because many insect pests use CO_2 for host-seeking, the identification of these CO_2 receptors may facilitate efforts of discovering compounds for blocking CO_2 sensing.

Recent studies indicate that the detailed mechanisms of odorant detection in the insect antenna can be quite different from those in the mammalian olfactory epithelium. In mammals, odorants are detected by odorant GPCRs. However, Compelling evidences indicate that the insect olfactory receptors are heteromeric ligand-gated ion channels [42••,43••]. For example, pentyl acetate, a ligand of the *Drosophila* odorant receptor *OR47a*, can directly evoke electric currents from heterologous cells coexpressing *OR47a* and *Or83b* [42••,43••]. The fact that other insect odorant receptors are ionotropic receptors [42••,43••] raises the intriguing possibility that the olfactory CO_2 receptors in insects share the same ionotropic coupling mechanism. However, it remains unclear whether CO_2 acts on the extracellular domains or intracellular domains of these receptors. Additionally, it is unclear whether the responses are the direct results of CO_2 action or indirect action after CO_2 is converted into bicarbonate or H^+ (Figure 2b).

Under natural conditions, the nematode *C. elegans* live in an environment with a broad range of CO_2 concentrations. Two recent studies report that CO_2 is an avoidance sensory cue for well-fed *C. elegans*, which avoid CO_2 levels above 0.5% [4•,6•]. These two studies also show that such avoidance is modulated by metabolism and environmental contexts. Although the exact sensory neurons responding to CO_2 have not been identified, genetic analysis indicates that the BAG neurons are critical for CO_2-mediated avoidance behavior [4•]. In addition, acute CO_2 sensing requires a cGMP-sensitive heteromeric channel TAX-2/TAX-4 [4•,6•]. A membrane guanylyl cyclase, DAF-11, is also essential [4•]. However, DAF-11 is not expressed within the BAG neurons [4•,44]. It remains unclear whether DAF-11 or other membrane GCs in *C. elegans* can be activated by bicarbonate. Nevertheless, the fact that CO_2 responses in worms require membrane guanylyl cyclase and cGMP-gated CNG channels is reminiscent of the signaling transduction pathway in CO_2-responsive OSNs in mammals (Figure 1b). It will be interesting to test whether the mechanisms of CO_2 sensing are conserved between mammals and worms.

O_2 sensing by *C. elegans*

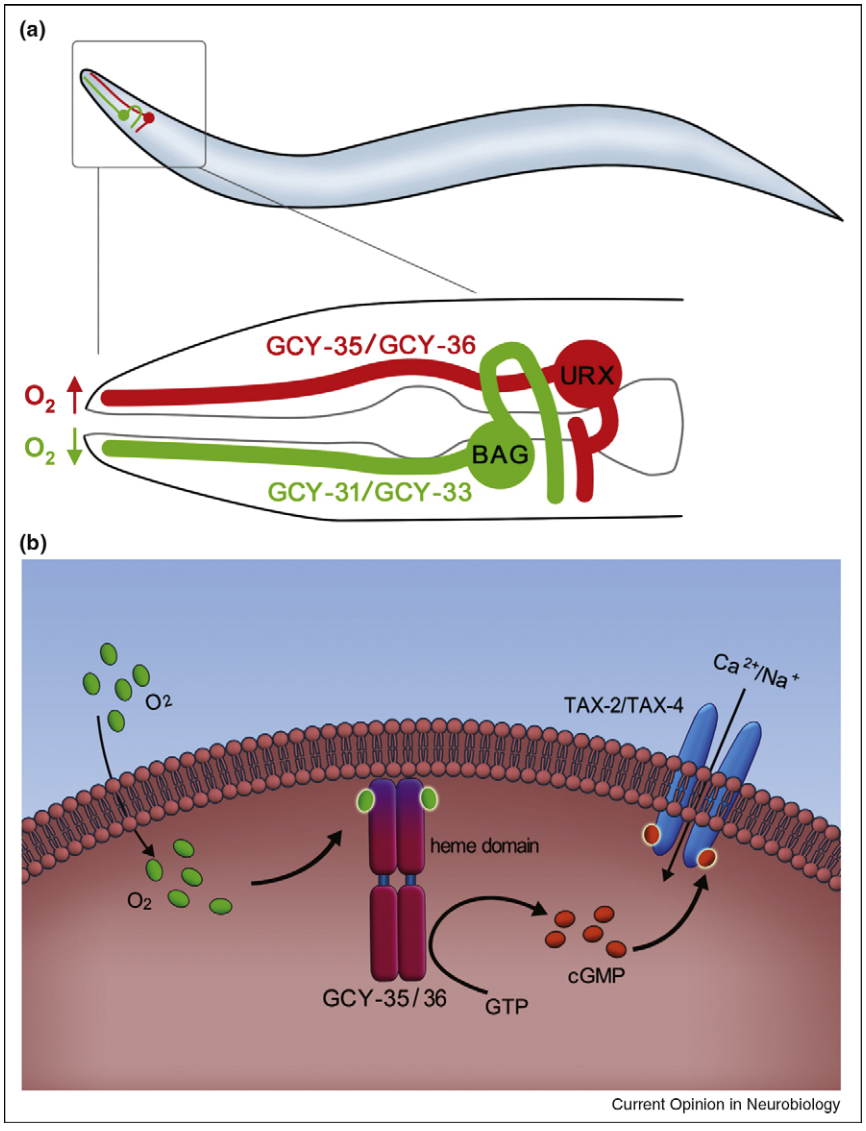
O_2 homeostasis is critical for an animal's survival. For mammals, internal blood O_2 level is believed to be detected by the carotid body [45]. For a vast majority of terrestrial animals, atmospheric O_2 concentrations are rather stable at high concentrations (~21%). However, nematode *C. elegans* live in natural environments that have dramatically variable O_2 levels. They exhibit a strong behavioral preference for 5–10% O_2, avoiding higher and lower O_2 levels [5••]. Several recent studies have identified sensory neurons for O_2 and shed light on the molecular mechanisms underlying rapid O_2 sensation. URX and BAG neurons mediate the behavioral responses to O_2 upshifts and downshifts, respectively [5••,10••] (Figure 3a). Consistently, calcium imaging revealed that URX neurons are rapidly activated by an upshift of O_2 levels to 15–21% [5••,10••,11], whereas BAG neurons are activated by a downshift to 4–10% O_2 [10••]. Wild strains of *C. elegans* respond much more strongly when O_2 upshifts are simultaneously coupled to CO_2 downshifts [46••]. As mentioned earlier, BAG neurons have been found to be critical for CO_2 sensing as well [4•], suggesting that they might be important for integrating CO_2 and O_2 signals to regulate CO_2-triggered avoidance behavior [6•].

Soluble guanylyl cyclases (sGCs) are important for O_2 sensing. Unlike conventional sGCs that are activated by nitric oxide, a separate class of atypical sGCs are rather insensitive to nitric oxide but are activated by O_2 [47]. These O_2-sensitive sGCs include Gyc-88E and Gyc-89D in *Drosophila* [47] and GCY-35, GCY-36, GCY-31, and GCY-33 in *C. elegans* [5••,10••,11,48]. More specifically, it has been shown that GCY-35/36 heterodimers in URX neurons are activated by O_2 upshift [10••,11], whereas GCY-31/33 heterodimers might respond to O_2 downshift [10••]. Supporting this view, it has been found by biochemical assays that the heme domain of GCY-35 binds to O_2 molecules [5••]. Although biochemical assays on the O_2 sensitivity of GCY-35/36 hetereodimers have not yet been performed, current data support the hypothesis that the activation of GCY-35/36 by O_2 increases cGMP levels, which can in turn open the cGMP-gated channel TAX-2/TAX-4 (Figure 3b).

Interestingly, food context and naturally occurring genetic variation regulate the O_2 preference of *C. elegans* through NPY homolog, NPR-1 and the TGF-β homolog DAF-7 [6•,7]. Two recent studies have also revealed that *glb-5*, which encodes a neuronal globin protein, acts in O_2-sensing neurons to modulate O_2-evoked calcium signals as well as behavioral responses [46••,49••]. GLB-5 may be activated by varying O_2 and then modulate the components within the sGC signaling pathway [49••].

In addition to rapid response to O_2, slower O_2 sensation of *C. elegans* is responsible for monitoring internal O_2 levels

Figure 3



Mechanisms of O_2 sensing in *C. elegans*. **(a)** URX neurons (red) and BAG neurons (green) detect O_2 upshift and downshift, respectively. The sGC genes expressed in each sensory neuron are indicated. This image is adopted from pictures kindly provided by M Zimmer. **(b)** A simplified schematic showing the cellular mechanisms of O_2 sensing by sensory neurons in *C. elegans*. O_2 may activate the sGC heterodimer GCY-35/36 to produce cGMPs, which in turn open the cGMP-sensitive channels TAX-2/TAX-4. Other components, such as NPR-1 and GLB-5, have also been implicated in O_2-evoked responses in these neurons.

to regulate metabolism and proliferation. Such slower sensation is mediated by hypoxia-inducible factor-1 (HIF-1) transcriptional pathway [6•,50], which is also conserved in mammals [21].

Conclusion

In this review, we have summarized recent progresses in our understanding of neurobiological mechanisms of CO_2 sensing by the olfactory systems of both vertebrates and invertebrates. We also briefly reviewed recent studies on the mechanisms of O_2 sensing by nematode *C. elegans*. A common feature revealed by these studies is that CO_2 as well as O_2 is detected by specialized subsets of sensory neurons. The intracellular signaling transduction cascades for CO_2 sensing in mammals are substantially different from those for sensing typical odorants. Specifically, cGMP, rather than cAMP, appears to be the critical second messenger. Interestingly, CO_2 and O_2 sensing by *C. elegans* also depends on components of cGMP signaling. In contrast, CO_2 sensing of insects requires the coexpression of two receptors that may form ligand-gated channels. At the systems level, these two gas molecules mediate innate behaviors such as avoidance or attraction. The fact that they are detected by a

small subset of sensory neurons suggests that they may activate discrete neural circuits that mediate some easily quantifiable animal behaviors. Future studies on these neural circuits may have the exciting potential to reveal neural mechanisms of animal behavior at levels from genes to behaviors.

Acknowledgements

We thank Ning Yang for his assistance with figure preparation. ML is supported by China Ministry of Science and Technology, China National Natural Science Foundation, and the Human Frontier Science Program.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Thom C, Guerenstein PG, Mechaber WL, Hildebrand JG: **Floral CO_2 reveals flower profitability to moths**. *J Chem Ecol* 2004, **30**:1285-1288.

2. •• Suh GS, Wong AM, Hergarden AC, Wang JW, Simon AF, Benzer S, Axel R, Anderson DJ: **A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila**. *Nature* 2004, **431**:854-859.
The authors show that a subset of OSNs in *Drosophila* detect CO_2 and mediate avoidance behavior.

3. •• Hu J, Zhong C, Ding C, Chi Q, Walz A, Mombaerts P, Matsunami H, Luo M: **Detection of near-atmospheric concentrations of CO_2 by an olfactory subsystem in the mouse**. *Science* 2007, **317**:953-957.
The authors show that the mouse necklace olfactory system detects CO_2 sensitively.

4. • Hallem EA, Sternberg PW: **Acute carbon dioxide avoidance in *Caenorhabditis elegans***. *Proc Natl Acad Sci U S A* 2008, **105**:8038-8043.
The authors show that well-fed *C. elegans* avoid CO_2. Several components of the cGMP signaling pathway appear to be important for this behavior.

5. •• Gray JM, Karow DS, Lu H, Chang AJ, Chang JS, Ellis RE, Marletta MA, Bargmann CI: **Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue**. *Nature* 2004, **430**:317-322.
The authors show that *C. elegans* prefer O_2 levels of 5–12% and GCY-35, a sGC, is activated by O_2.

6. • Bretscher AJ, Busch KE, de Bono M: **A carbon dioxide avoidance behavior is integrated with responses to ambient oxygen and food in *Caenorhabditis elegans***. *Proc Natl Acad Sci U S A* 2008, **105**:8044-8049.
The authors show that in *C. elegans* CO_2-mediated avoidance behavior is modulated by ambient oxygen and food.

7. Chang AJ, Chronis N, Karow DS, Marletta MA, Bargmann CI: **A distributed chemosensory circuit for oxygen preference in *C. elegans***. *PLoS Biol* 2006, **4**:e274.

8. Luo M: **The necklace olfactory system in mammals**. *J Neurogenet* 2008, **22**:229-238.

9. Guerenstein PG, Christensen TA, Hildebrand JG: **Sensory processing of ambient CO_2 information in the brain of the moth *Manduca sexta***. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2004, **190**:707-725.

10. •• Zimmer M, Gray JM, Pokala N, Chang AJ, Karow DS, Marletta MA, Hudson ML, Morton DB, Chronis N, Bargmann CI: **Neurons detect increases and decreases in oxygen levels using distinct guanylate cyclases**. *Neuron* 2009, **61**:865-879.
The authors have identified URX and BAG neurons for sensing changes of O_2 levels. In addition, they show that different sGCs in these neurons mediate O_2 detection.

11. Cheung BH, Cohen M, Rogers C, Albayram O, de Bono M: **Experience-dependent modulation of *C. elegans* behavior by ambient oxygen**. *Curr Biol* 2005, **15**:905-917.

12. Shusterman D, Avila PC: **Real-time monitoring of nasal mucosal pH during carbon dioxide stimulation: implications for stimulus dynamics**. *Chem Senses* 2003, **28**:595-601.

13. Bensafi M, Iannilli E, Gerber J, Hummel T: **Neural coding of stimulus concentration in the human olfactory and intranasal trigeminal systems**. *Neuroscience* 2008, **154**:832-838.

14. Youngentob SL, Hornung DE, Mozell MM: **Determination of carbon dioxide detection thresholds in trained rats**. *Physiol Behav* 1991, **49**:21-26.

15. Coates EL, Ballam GO: **Olfactory receptor response to CO_2 in bullfrogs**. *Am J Physiol* 1990, **258**:R1207-R1212.

16. Coates EL: **Olfactory CO(2) chemoreceptors**. *Respir Physiol* 2001, **129**:219-229.

17. Coates EL, Wells CM, Smith RP: **Identification of carbonic anhydrase activity in bullfrog olfactory receptor neurons: histochemical localization and role in CO_2 chemoreception**. *J Comp Physiol [A]* 1998, **182**:163-174.

18. Fulle HJ, Vassar R, Foster DC, Yang RB, Axel R, Garbers DL: **A receptor guanylyl cyclase expressed specifically in olfactory sensory neurons**. *Proc Natl Acad Sci U S A* 1995, **92**:3571-3575.

19. Juilfs DM, Fulle HJ, Zhao AZ, Houslay MD, Garbers DL, Beavo JA: **A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway**. *Proc Natl Acad Sci U S A* 1997, **94**:3388-3395.

20. Walz A, Feinstein P, Khan M, Mombaerts P: **Axonal wiring of guanylate cyclase-D-expressing olfactory neurons is dependent on neuropilin 2 and semaphorin 3F**. *Development* 2007, **134**:4063-4072.

21. Lahiri S, Forster RE 2nd: **CO_2/H(+) sensing: peripheral and central chemoreception**. *Int J Biochem Cell Biol* 2003, **35**:1413-1435.

22. • Sun L, Wang H, Hu J, Han J, Matsunami H, Luo M: **Guanylyl cyclase-D in the olfactory CO_2 neurons is activated by bicarbonate**. *Proc Natl Acad Sci U S A* 2009, **106**:2041-2046.
By biochemical assays the authors show that GC-D can be activated by bicarbonate ions, supporting its role in CO_2 sensing.

23. Leinders-Zufall T, Cockerham RE, Michalakis S, Biel M, Garbers DL, Reed RR, Zufall F, Munger SD: **Contribution of the receptor guanylyl cyclase GC-D to chemosensory function in the olfactory epithelium**. *Proc Natl Acad Sci U S A* 2007, **104**:14507-14512.

24. Garbers DL, Lowe DG: **Guanylyl cyclase receptors**. *J Biol Chem* 1994, **269**:30741-30744.

25. Cockerham RE, Puche AC, Munger SD: **Heterogeneous sensory innervation and extensive intrabulbar connections of olfactory necklace glomeruli**. *PLoS ONE* 2009, **4**:e4657.

26. Buck L, Axel R: **A novel multigene family may encode odorant receptors: a molecular basis for odor recognition**. *Cell* 1991, **65**:175-187.

27. Zhang X, Firestein S: **The olfactory receptor gene superfamily of the mouse**. *Nat Neurosci* 2002, **5**:124-133.

28. Prasad BC, Reed RR: **Chemosensation: molecular mechanisms in worms and mammals**. *Trends Genet* 1999, **15**:150-153.

29. Meyer MR, Angele A, Kremmer E, Kaupp UB, Muller F: **A cGMP-signaling pathway in a subset of olfactory sensory neurons**. *Proc Natl Acad Sci U S A* 2000, **97**:10595-10600.

30. • Guo D, Zhang JJ, Huang XY: **Stimulation of guanylyl cyclase-D by bicarbonate**. *Biochemistry* 2009, **48**:4417-4422.
The authors show that GC-D is activated by bicarbonate ions, suggesting that it plays a role in CO_2 sensing.

31. Duda T, Sharma RK: **ONE-GC membrane guanylate cyclase, a trimodal odorant signal transducer**. *Biochem Biophys Res Commun* 2008, **367**:440-445.

32. Torrents D, Suyama M, Zdobnov E, Bork P: **A genome-wide survey of human pseudogenes**. *Genome Res* 2003, **13**:2559-2567.

33. • Young JM, Waters H, Dong C, Fulle HJ, Liman ER: **Degeneration of the olfactory guanylyl cyclase D gene during primate evolution**. *PLoS ONE* 2007, **2**:e884.
By bioinformatics analysis, the authors show that GC-D is a pseudogene in most of primates, suggesting that primates lack the ability to sensitively detect atmospheric CO_2.

34. Bogner F, Boppre M, Ernst KD, Boeckh J: **CO_2 sensitive receptors on labial palps of *Rhodogastria* moths (Lepidoptera: Arctiidae): physiology, fine structure and central projection**. *J Comp Physiol [A]* 1986, **158**:741-749.

35. Kellogg FE: **Water vapour and carbon dioxide receptors in *Aedes aegypti***. *J Insect Physiol* 1970, **16**:99-108.

36. de Bruyne M, Foster K, Carlson JR: **Odor coding in the Drosophila antenna**. *Neuron* 2001, **30**:537-552.

37. • Suh GS, Ben-Tabou de Leon S, Tanimoto H, Fiala A, Benzer S, Anderson DJ: **Light activation of an innate olfactory avoidance response in Drosophila**. *Curr Biol* 2007, **17**:905-908.
Photostimulation of *Drosophila* CO_2 neurons produces avoidance behavior.

38. Fischler W, Kong P, Marella S, Scott K: **The detection of carbonation by the Drosophila gustatory system**. *Nature* 2007, **448**:1054-1057.

39. •• Jones WD, Cayirlioglu P, Kadow IG, Vosshall LB: **Two chemosensory receptors together mediate carbon dioxide detection in Drosophila**. *Nature* 2007, **445**:86-90.
The authors show that two chemoreceptors, *Gr21a* and *Gr63a*, are necessary and sufficient for CO_2 sensing in *Drosophila*. They have also identified the mosquito homologs of these two genes.

40. • Kwon JY, Dahanukar A, Weiss LA, Carlson JR: **The molecular basis of CO_2 reception in Drosophila**. *Proc Natl Acad Sci U S A* 2007, **104**:3574-3578.
This study shows that *Gr21a* and *Gr63a* are Drosophila CO_2 receptors.

41. Lu T, Qiu YT, Wang G, Kwon JY, Rutzler M, Kwon HW, Pitts RJ, van Loon JJ, Takken W, Carlson JR *et al.*: **Odor coding in the maxillary palp of the malaria vector mosquito *Anopheles gambiae***. *Curr Biol* 2007, **17**:1533-1544.

42. •• Sato K, Pellegrino M, Nakagawa T, Vosshall LB, Touhara K: **Insect olfactory receptors are heteromeric ligand-gated ion channels**. *Nature* 2008, **452**:1002-1006.
Mammalian odorant receptors are GPCRs. This study shows that insect olfactory receptors are instead heteromeric ligand-gated ion channels.

43. •• Wicher D, Schafer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann SH, Hansson BS: **Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels**. *Nature* 2008, **452**:1007-1011.
The authors show that insect odorant receptors are heteromeric ligand-gated channels that can also be activated by intracellular cyclic nucleotides.

44. Yu S, Avery L, Baude E, Garbers DL: **Guanylyl cyclase expression in specific sensory neurons: a new family of chemosensory receptors**. *Proc Natl Acad Sci U S A* 1997, **94**:3384-3387.

45. Lopez-Barneo J: **Oxygen and glucose sensing by carotid body glomus cells**. *Curr Opin Neurobiol* 2003, **13**:493-499.

46. •• McGrath PT, Rockman MV, Zimmer M, Jang H, Macosko EZ, Kruglyak L, Bargmann CI: **Quantitative mapping of a digenic behavioral trait implicates globin variation in *C. elegans* sensory behaviors**. *Neuron* 2009, **61**:692-699.
This study shows that a neuronal globin, GLB-5, is expressed in O_2-sensing neurons, increases O_2-evoked calcium signals, and regulates behavioral responses to changes of O_2 levels.

47. Morton DB: **Atypical soluble guanylyl cyclases in Drosophila can function as molecular oxygen sensors**. *J Biol Chem* 2004, **279**:50651-50653.

48. Rogers C, Persson A, Cheung B, de Bono M: **Behavioral motifs and neural pathways coordinating O_2 responses and aggregation in *C. elegans***. *Curr Biol* 2006, **16**:649-659.

49. •• Persson A, Gross E, Laurent P, Busch KE, Bretes H, de Bono M: **Natural variation in a neural globin tunes oxygen sensing in wild *Caenorhabditis elegans***. *Nature* 2009, **458**:1030-1033.
The authors show that GLB-5 is expressed in O_2-sensing neurons, tunes the dynamic response range of O_2, and regulates behavioral responses to changes of O_2 levels. It also suggests that GLB-5 interacts with the signaling pathway of sGCs.

50. Chang AJ, Bargmann CI: **Hypoxia and the HIF-1 transcriptional pathway reorganize a neuronal circuit for oxygen-dependent behavior in *Caenorhabditis elegans***. *Proc Natl Acad Sci U S A* 2008, **105**:7321-7326.