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# CO<sub>2</sub> Detection and Processing in Insects and Higher Mammals – a Comparative Study

Master's thesis in Neuroscience  
Supervisor: Bente Gunnveig Berg  
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Master's thesis in Neuroscience

Trondheim, June 2019

Supervisor: Bente G. Berg

Co-supervisor: Pramod KC

Norwegian University of Science and Technology

Faculty of Medicine and Health sciences

Kavli Institute for Systems Neuroscience

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## **Abstract**

Carbon dioxide (CO<sub>2</sub>) is a gas abundant in Earth's atmosphere, and being a product of cellular respiration, it is present in concentration gradients around respiring organisms and organic decay. The atmospheric CO<sub>2</sub> concentration has risen steadily since the industrial revolution, and its present level is historically unsurpassed. Carbon dioxide also constitutes a powerful chemosensory signal to animal systems that have developed the ability to detect it. Fluctuations in external CO<sub>2</sub> levels can prompt disparate behaviours in different organisms, dependent on the species, ecological niche, and context. Modern molecular biology has offered new insight into how CO<sub>2</sub> is detected by sensory organs, yet it is not known how the general rise in CO<sub>2</sub> will affect animals that use this gas as an environmental cue. The present text explores the putative vulnerability of these organisms to the rising CO<sub>2</sub> concentration by comparatively reviewing the chemosensory mechanisms of CO<sub>2</sub> detection in insects and higher mammals, from the detection organs, via central nervous pathways, to the possible behavioural implications. Recent reports about several insect species declining in numbers, has brought about a sense of urgency in learning more about them, as they are vastly relevant to all terrestrial ecosystems due to their role as pollinators, decomposers, and as food to other animals. It is therefore of unreserved importance to investigate the effects of external CO<sub>2</sub> levels on insects.

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## Background

The atmospheric composition of the planet has varied greatly since its inception. The gaseous compounds present in the terrestrial atmosphere has fluctuated in ratio and has consequently shaped the evolutionary diversity in terrestrial life. One of these compounds is carbon dioxide (CO<sub>2</sub>). Set in a geological time scale, its atmospheric concentration has decreased continuously. However, as a result of human activity globally, since the industrial revolution, the atmospheric CO<sub>2</sub> level has risen steadily with about 2 parts per million (ppm) per year, from 280 ppm (µl/l) to around 385 ppm in 2007, and thereby surpasses the natural range over the past 650 000 years (180-300 ppm), with a 22% increase since 1960 alone (International Panel on Climate Change, 2007). This is measured as compared to background levels in the atmosphere; today, the concentration is approximately 0.038% (Cummins et al., 2014). Carbon dioxide is a product of cellular metabolism, which makes the CO<sub>2</sub> concentration higher inside respiring organisms than in the external atmosphere. Inside the body, CO<sub>2</sub> can serve as an indicator of significant conditions in order for the organism to monitor its internal homeostasis, assuring optimal tissue levels of CO<sub>2</sub> (Cummins et al., 2014; Luo, Sun, & Hu, 2009). The gas is then expired and is therefore often concentrated in confined sub-environments in proximate distance to respiring organisms and decomposing organic material, in gradient concentrations around the source (Cummins et al., 2014; Hu et al., 2007).

This omnipresence of CO<sub>2</sub> in the external environment has led to the assumption that it might simply constitute a by-product of cellular metabolism with little relevance to animals (Stange, 1996). However, many organisms use CO<sub>2</sub> as a cue for species-relevant events in the external environment. Gradients of external CO<sub>2</sub> in the environment make up complex and contextual environmental signals aiding the adjustment of appropriate physiological responses and behaviours. The signal elicits responses needed in order to locate food and to avoid stressful environments, including predators. The signalling effect of a specific or fluctuating CO<sub>2</sub> concentration holds different relevance to different organisms, and produces distinct behaviours in these, depending on their ecological niche (Jones, 2013). Hence, local CO<sub>2</sub> concentration is a physiologically important sensory stimulus.

The rising atmospheric CO<sub>2</sub> level makes this issue particularly relevant, proving it important to explore the mechanisms by which each group of organism processes and utilises input about this gas. The CO<sub>2</sub>-related responses of animals are less studied than those of plants, yet animals

may be more vulnerable to CO<sub>2</sub> changes (Sage, 2002). Focus should to be directed at CO<sub>2</sub> as a sensory signal. Exploring how and why CO<sub>2</sub> detection happens, and which behaviours are affected by it, will tell us something about which organisms are most vulnerable to a rise in global CO<sub>2</sub> and in which way they may be affected. The aim of this review is thusly to gather relevant literature on CO<sub>2</sub> signalling in insects and mammals, and then describe CO<sub>2</sub> processing in organisms within each group. This work will summarise, compare, and contrast mechanisms and functionalities of CO<sub>2</sub> detection across distinct animal systems. Finally, the putative vulnerability of the organisms to changes in atmospheric CO<sub>2</sub> concentration will be briefly discussed based on their CO<sub>2</sub>-evoked behaviours.

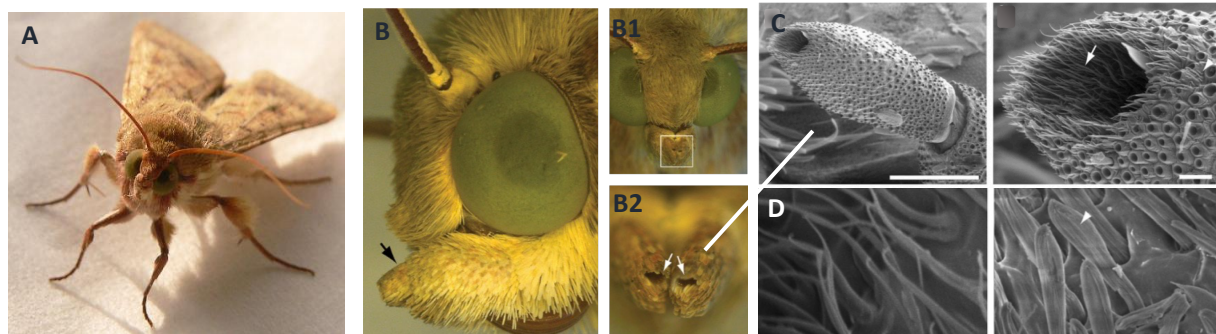
### **Detection and Processing of CO<sub>2</sub> in Insects**

Most insect have the ability to sense external CO<sub>2</sub> concentrations. This capacity is provided by a distinct sensory pathway that is closely integrated with the olfactory system. Being a ubiquitous atmospheric compound, the external CO<sub>2</sub> concentration is well above the concentration of most other olfactory stimuli detected by insects (Stange & Stowe, 1999). In that sense, the CO<sub>2</sub> signal is markedly different from other volatile chemicals detected by these organisms. In several insect species, sensilla with CO<sub>2</sub>-sensitive olfactory sensory neurons are clustered together, forming specialised detection organs. These include the labial palp pit organ (LPO) in Lepidoptera and the Lutz's organ on the maxillary palps of mosquitos (Stange & Stowe, 1999). The subsequent paragraphs describe the morphology and function of the detection organs for CO<sub>2</sub> in moths, mosquitos, and flies.

#### **Detection organ, sensilla, and cells**

In Lepidoptera, CO<sub>2</sub> detection is pervasive and adult moths detect CO<sub>2</sub> via a distinct sensory organ located on the mouthparts (Bogner et al., 1986; Kent, Harrow, Quartararo, & Hildebrand, 1986). Laterally protruding on both sides of the proboscis are the labial palps (Figure 1A, B). These extremities are covered in scales and have three segments (indicated by the arrow in Figure 1B), where the third contains the CO<sub>2</sub>-sensing organ. It consists of a bottle-shaped pit, narrow at the top and wider by the base, forming an inner cavity (Figure 1B2, B3, and C). This is the labial palp pit organ (LPO), which is the sensory organ for CO<sub>2</sub> detection in Lepidoptera (Kent et al., 1986). This pit, located distally in the labial palp, contains sensilla with sensory neurons

extending their axons through the epidermis below. Homologous LPO structures are found in several Lepidoptera, including *Manduca sexta* (Kent et al., 1986), *Helicoverpa armigera* (Zhao et al., 2013), and *Cactoblastis cactorum* (Stange et al., 1995). Two morphologically different groups of sensilla have been described in the LPO of *H. armigera*, a hair-shaped and a club-shaped type (Figure 1D). The hair-shaped sensilla have a smooth cuticle surface and are found from the top to the middle part of the pit, whereas the club-shaped sensilla are corrugated and housed more proximally in the pit (Zhao et al., 2013). Each sensillum typically contains one sensory neuron and two at the most (Figure 4A). These CO<sub>2</sub>-associated sensory neurons, particularly those in club-shaped sensilla, are found to have branches of lamellated dendrites in several species (Bogner et al., 1986; Stange & Stowe, 1999).

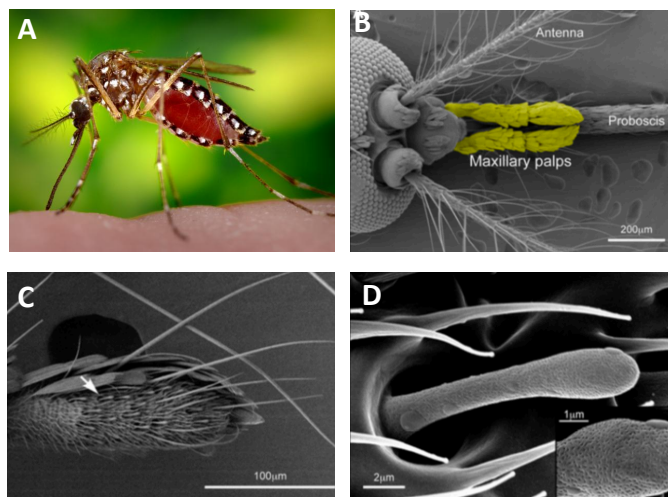


**Figure 1.** Carbon-dioxide sensing sensilla in the labial-palp pit organ (LPO) in *Helicoverpa armigera*. **A)** The image of an *H. armigera* male. Reprinted from eurekaalert.org **B)** Location of the LPO in *H. armigera*. The black arrow points to the third terminal segment of the LPO. **B1)** Anterior view of the two labial palps. **B2)** Highly magnified image of labial palp opening (square box in B1). **C)** Left: The third segment of the labial palp with the scales removed. Right: The pit of the LPO with numerous sensilla (arrow). **D)** Hair-shaped sensilla (left) and club-shaped sensilla (right). The club-shaped sensilla have grooves on the cuticle (arrowhead). Figures B, C, and D, are adapted from Zhao et al. (2013). Scale bars: C, left: 100  $\mu$ m; C, right: 20  $\mu$ m; D, left: 5  $\mu$ m; D, right: 10  $\mu$ m.

Studies on the sphinx moth, *M. sexta*, have demonstrated that the CO<sub>2</sub>-detecting neurons housed inside the LPO are highly sensitive, and specific to ambient CO<sub>2</sub> concentrations. Thus, these sensory neurons detect CO<sub>2</sub> only, and do not respond to any other tested volatile compound (Guerenstein, Christensen, & Hildebrand, 2004). The fact that LPO sensilla of adult Lepidoptera seem to house CO<sub>2</sub>-specific neurons only, points to the importance of this signal (Guerenstein, Christensen, et al., 2004). The uniqueness of the CO<sub>2</sub> signal, including compound lipophilicity and insensitivity to wind, could explain the special morphology of the detection organ, constituting an almost enclosed space. In some moth species, like *C. cactorum*, the LPO is

morphologically different across the sexes (vestigial vs. prominent; Stange, 1997; Stange et al., 1995), the implications of which will be explored later in this text.

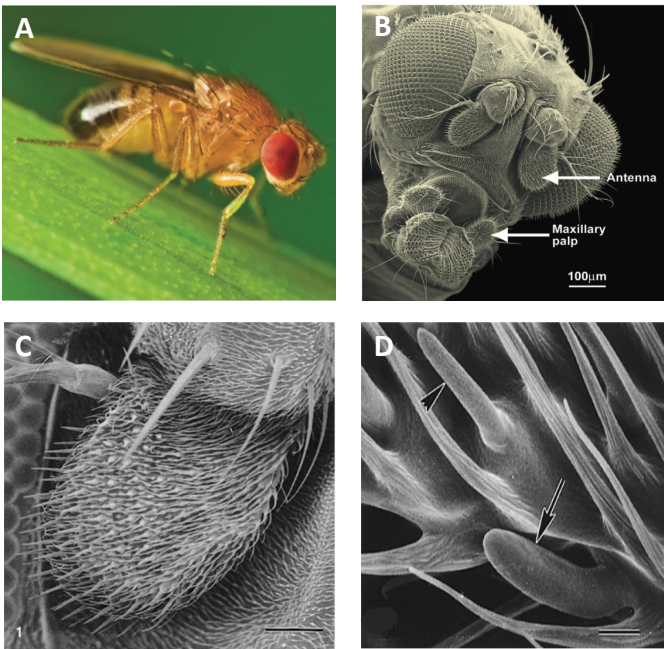
Mosquitos have three chemosensory extremities, the antennae, the proboscis, and the maxillary palps (Figure 2A, B), of which the maxillary palps are essential for CO<sub>2</sub> detection. The maxillary palps are paired appendages in five segments located in the mouthparts of the mosquito and are the principle location for CO<sub>2</sub>-sensitive neurons. Distally in the fourth segment (fifth segment often not shown or discussed, as it falls off during preparation) of each maxillary palp in the female yellow-fever vector mosquito, *Aedes aegypti*, there are approximately thirty peg-shaped, porous basiconic sensilla (Figure 2C, D), each housing a sensory neuron responsive to low concentrations (150-300ppm) of CO<sub>2</sub> (Distler & Boeckh, 1997; Grant, Wigton, Aghajanian, & Connell, 1995). Each of these CO<sub>2</sub> neurons are clustered in a triad together with two other chemosensory neurons (Figure 4B). The last-mentioned neurons are sensitive to octenol, a chemical present in the breath and sweat of humans, and to other human skin odorants, respectively (Bohbot, Sparks, & Dickens, 2014). This is the general organisation across mosquito species, though some variation exists in sensillar distribution and in the external morphology (Grant & Kline, 2003). Sensilla are of the same type across the sexes but exist in fewer numbers in male anopheline mosquitos (McIver & Siemicki, 1975). *Ae. aegypti* can not only detect very low levels of CO<sub>2</sub> but are also sensitive to very small changes in ambient CO<sub>2</sub> concentrations, suggested by both behavioural and electrophysiological data (Distler & Boeckh, 1997; Grant et al., 1995).



**Figure 2.** Olfactory organs of the female mosquito (*Aedes aegypti*). **A)** Lateral view of a female feeding on the host species. The image is reprinted from eurekaalert.org. **B)** Dorsal view of *A. aegypti* head showing the three olfactory appendages: the antenna, proboscis, and maxillary palps (highlighted in yellow). **C)** Basiconic sensilla on the fourth segment of the maxillary palps (arrow). **D)** High-magnified image of one single club-shaped sensillum with small pores on the cuticle (inserted image). Figure B, C and D are adapted from Bohbot et al. (2014). Scale bars are mentioned in the figures.



In contrast to the LPO and the maxillary palps which have rather specialized olfactory functions, the antennae are the primary olfactory organs of insects (de Bruyne, Foster, & Carlson, 2001). In the Fruit fly, *Drosophila melanogaster*, the antenna (Figure 3B, C) is the organ where the CO<sub>2</sub>-sensitive neurons are situated, meaning that their CO<sub>2</sub> detection takes place in the classical olfactory organ, not in a separate structure. This type of sensory neuron has been named antennal basiconic type 1c (ab1c) and exists in basiconic ab1 sensilla (Figure 3D) together with three other neurons sensitive to other odorants (Figure 4C; de Bruyne et al., 2001). The ab1c neuron represents an extreme case in stimulus response activity among *D. melanogaster* antennal neurons, in that it responds very strongly to its stimulus, i.e. CO<sub>2</sub>, and that it responds to that compound only.



**Figure 3.** Olfactory detection system in *Drosophila melanogaster*. **A)** Full image of a genetically modified *D. melanogaster*. The figure is adapted from Science. **B)** Anterior view of the head of *D. melanogaster* showing the antenna and maxillary palp. The figure is adapted from Laissue & Vosshall, (2008). **C)** The third antennal segment showing numerous sensilla. **D)** Magnified image of two types of basiconic sensilla on the antennae. A large basiconic sensillum (*arrow*) and a small basiconic sensillum (*arrowhead*). Figures C and D are adapted from Riesgo & Escovar, (1997). Scale bars: C: 25 μm; D: 2 μm.

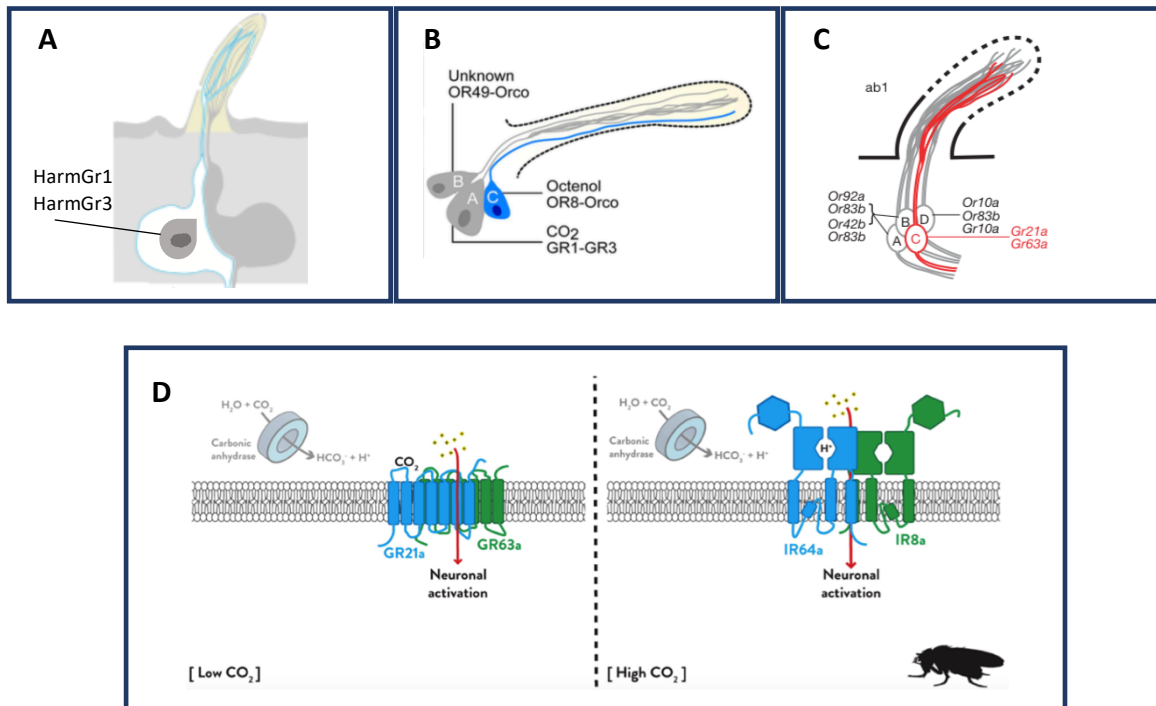
## Receptors

Though their sense for picking up the CO<sub>2</sub> signal is closely integrated with smell, it is reported that several insect species possess CO<sub>2</sub> receptors consisting of members of a gustatory receptor (Gr) subfamily, making up a heterodimeric complex (Figure 4; Jones, 2013). Three CO<sub>2</sub> receptor genes, HarmGr1, HarmGr2 and HarmGr3, have been identified in Lepidoptera, and all are expressed specifically in the labial palps of *H. armigera* (Xu & Anderson, 2015). Testing their putative ligands, it was first discovered that HarmGr3 is activated by sodium bicarbonate

(NaHCO<sub>3</sub>) when expressed in insect Sf9 cells. All three receptors were later found to be co-expressed within the same labial palp cells. Only when all three receptor genes were co-expressed, or when HarmGr1 and HarmGr3 were expressed together, did the cells respond to NaHCO<sub>3</sub> (Ning et al., 2016). It was then determined that the co-expression of HarmGr1 and HarmGr3 together forms a heterodimeric structure that functions as the CO<sub>2</sub> receptor in *H. armigera* (Figure 4A). Due to similarity in genetic sequence, it is believed that the components of this heterodimer have orthologues in other insect species (Ning et al., 2016; Robertson & Kent, 2009).

These orthologue receptor genes include AgGr22 and AgGr24, identified in the malaria mosquito *Anopheles gambiae*. These receptors are co-expressed in a subset of neurons, in the maxillary palp only, and are not found in either the proboscis or the antennae (Jones et al., 2007). Similarly conserved orthologues, AaGr1 and AaGr3, were found in *Ae. aegypti* (Kent, Walden, & Robertson, 2008). Both pairs are seen to function as heterodimeric CO<sub>2</sub>-receptors, and their inactivation leads to insensitivity to CO<sub>2</sub> (Erdelyan, Mahood, Bader, & Whyard, 2012; Lu et al., 2007).

In the fruit fly, *D. melanogaster*, the CO<sub>2</sub>-sensitive ab1c neurons co-express the Gr genes, Gr21a and Gr63a (Figure 4C), that together form a complex similar to that described for moths and mosquitos. Jones et al. (2007) have concluded that the two together are sufficient and necessary to form the complex serving as the CO<sub>2</sub> receptor in the fruit fly (Figure 4C, D). Neurons innervating the V glomerulus expressing the GR21a/GR63a complex in the fruit fly respond to CO<sub>2</sub> concentrations lower than around 2%. Interestingly, CO<sub>2</sub>-related avoidance behaviour is still seen in flies with inactivated GR21a/GR63a receptors when the concentration is above 5% (Ai et al., 2010). Studies on the fruit fly have demonstrated that the ionotropic receptors IR64a and IR8a, which are members of a chemosensory ionotropic glutamate receptor family, combine to signal a pH decrease, serving as a putative proxy for high CO<sub>2</sub> levels (Figure 4D; Ai et al., 2010; Jones, 2013). So, perhaps, the fruit fly employs two different systems or receptor complexes for low and high concentrations, respectively. The signal transduction of these insect receptor complexes has not been determined (Figure 4D introduces the concept of enzymatic activity in order for CO<sub>2</sub> to activate the receptor complex, and this topic will be examined in the discussion section).

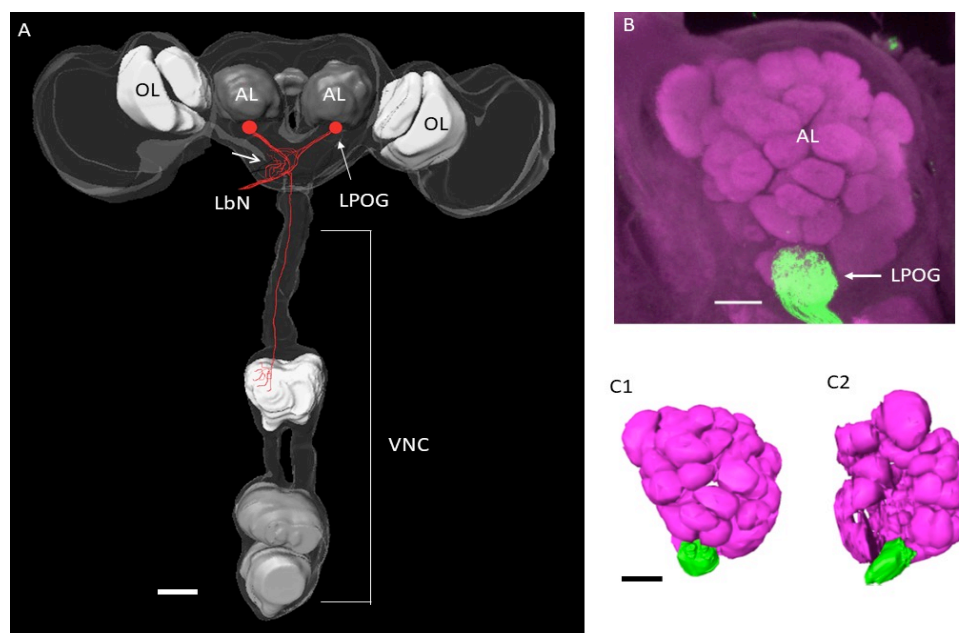


**Figure 4.** A, B, C: Schematic illustrations demonstrating CO<sub>2</sub> neurons within single sensilla in three insect species, *Helicoverpa armigera* (A), *Aedes Aegypti* (B), and *Drosophila melanogaster* (C) adapted from Kwon et al. (2007), Bohbot et al. (2014); Jones et al. (2007), respectively. D) Schematic representation of the CO<sub>2</sub> receptor complexes, Gr63a/Gr21a and IR64a/IR8a, in *D. melanogaster*. Adapted from Jones, (2013).

## Central projections

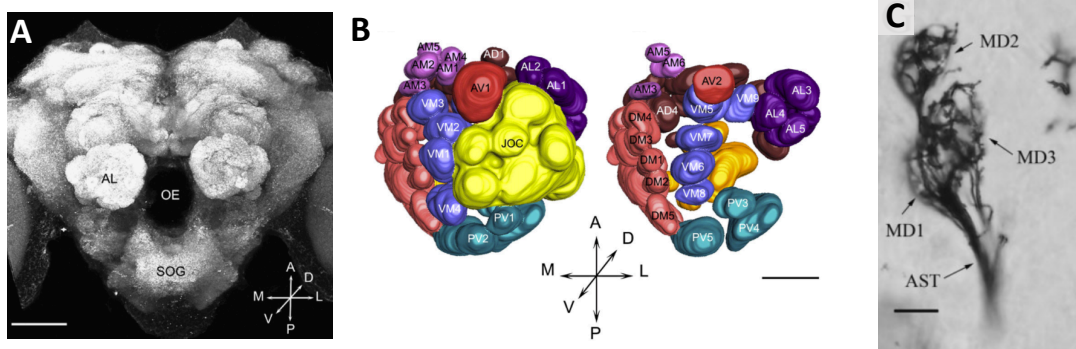
Tracing experiments in different lepidopteran species have shown that sensory axons from the LPO project to three main areas in the central nervous system: 1) to a particular glomerulus in the primary olfactory centre of the brain, 2) to a part of the brain called the gnathal ganglion (GNG), and 3) to the ventral nerve cord (Figure 5A). Tracings of this axonal path with consecutive confocal imagery in *H. armigera*, show that the CO<sub>2</sub>-associated axons bundle as they project through the labial palp nerve and enter the ipsilateral GNG. Here, they divide into three separate sub-tracts (Zhao et al., 2013). One of them further splits, projecting ipsi- and contralaterally to the labial pit organ glomerulus (LPOG; Figure 5B, C1, C2) situated bilaterally in the postero-ventral antennal lobe (AL; figure 5A; Kent et al., 1986; Zhao et al., 2013). The AL constitutes the primary olfactory region in the insect brain and is the first-order processing site. The LPOG is the

target of LPO axons only, though its location hints to the assumption that CO<sub>2</sub> input is integrated with classical odour input originating from the antenna (Zhao et al., 2013). This is further substantiated by the finding of multiglomerular interneurons in the AL of *M. sexta* (Christensen, Waldrop, Harrow, & Hildebrand, 1993). The two remaining sub-tracts target the GNG, which includes the primary processing centre for taste, and the ventral nerve cord corresponding to the mammalian spinal cord, respectively. From the LPOG in the AL, the CO<sub>2</sub> information is carried, via second order projection neurons (PNs) to higher centres in the insect brain. The dendritic arborisations of these PNs are confined to the LPOG, and their axons extend through one of the antennal-lobe tracts (ALTs) towards the protocerebral target regions. In a previous electrophysiological study performed on *M. sexta*, PNs were found to pass along the prominent medial ALT, targeting the ipsilateral calyces of the mushroom body (CMB), and the protocerebral lateral horn (LH), ipsilaterally (Guerenstein, Christensen, et al., 2004). A rise in CO<sub>2</sub> level leads to excitation of these PNs, which show very little spontaneous activity otherwise (Guerenstein, Christensen, et al., 2004).



**Figure 5.** Schematic representation of projection pathway of the Labial palp pit organ (LPO) sensory neurons in the central nervous system of *Helicoverpa armigera*. **A)** Overview of the LPO sensory neurons projecting to three areas in the central nervous system: 1) LPO glomerulus (LPOG) in each antennal lobe (AL), 2) gnathal ganglion (*arrow*), and 3) ventral nerve cord (VNC). The figure is provided by Pramod KC. **B)** Confocal image of the LPO axon terminals targeting the LPOG in the AL. Provided by Mari Reitstøen Arnesen. **C1 and C2)** Amira reconstructions of the LPO terminal axons targeting the LPOG in frontal and lateral view, respectively. The image is provided by Pramod KC. LbN: Labial pit Nerve. OL: Optic lobe. Scale bars: 50  $\mu$ m.

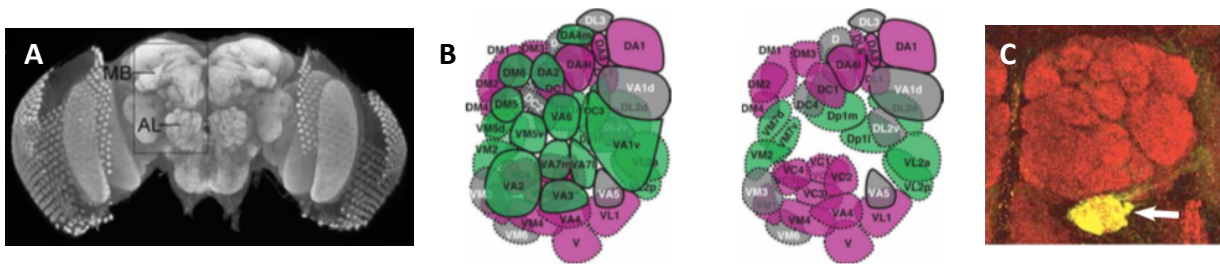
In a previous study on the mosquito, *An. gambiae*, Anton et al. (2003) stained one sensory neuron arising from the maxillary palp. This neuron branched and projected bilaterally to a dorso-medially situated glomerulus in the ALs. Like in moths, these symmetrically situated AL regions were not innervated by processes from antennal sensilla. In *Ae. aegypti*, the projection target was larger than in *An. gambiae*, but similarly located within the AL, though arborisations ran ipsilaterally only, from the maxillary palps, via the GNG and into the AL (Figure 6A-C; Anton et al., 2003; Distler & Boeckh, 1997). Three glomeruli have later been identified in both species as receiving maxillary palp afferents, ipsilaterally in *Ae. aegypti* and contralaterally in *An. gambiae* (Figure 5C; Ignell, Dekker, Ghaninia, & Hansson, 2005). One of these AL glomeruli however, the MD1 glomerulus in *Ae. aegypti* and glomerulus 1 in *An. gambiae*, is the supposed target of CO<sub>2</sub> associated sensory neurons only (Anton & Rospars, 2004; Distler & Boeckh, 1997; Ignell et al., 2005).



**Figure 6.** Central projection of the CO<sub>2</sub> sensory neuron in *Anopheles gambiae* and *Aedes aegypti*. **A)** Confocal image of an *An. gambiae* brain with antennal lobes (ALs). **B)** AMIRA three-dimensional reconstruction of AL glomeruli in *An. Gambiae*. **C)** The axonal terminals of sensory neurons from the maxillary palp to three glomeruli (MD1, MD2, and MD3) in the AL of the female *Ae. aegypti*. Figure A and B are adapted from Ghaninia et al. (2007); Figure C from Ignell et al. (2005). OE: Esophagus. SOG: Subesophageal ganglia. AST: Antenno-subesophageal tract. A: Anterior; P: Posterior; M: Medial; L: Lateral; D: Dorsal; V: Ventral. Scale bars: A: 50  $\mu$ m; B: 25  $\mu$ m; C: 10  $\mu$ m.

In *D. melanogaster*, the target of the CO<sub>2</sub> signal arising from the CO<sub>2</sub> sensory neurons, is a single glomerulus in the AL, the V glomerulus (Figure 7A-C). This most ventral glomerulus in the AL is innervated by ipsilateral input fibres only (Figure 7C; Stocker et al., 1983). This is in contrast to most other glomerular input from the antennae, where each glomerulus most often receives input from both antennae. The V glomerulus is activated by CO<sub>2</sub> concentrations ranging

from 0.05-10% above ambient and is not the target of any other tested odorant signals, suggesting it is the dedicated first order processing site for CO<sub>2</sub> (Suh et al., 2004). The V glomerulus is innervated by second-order PNs, carrying CO<sub>2</sub> information to higher centres of the brain via one of the three main antennal-lobe tracts (ALTs). These are the medial ALT, the medio-lateral ALT, and the lateral ALT. Lin et al. (2013) have described four different CO<sub>2</sub>-responsive PN types connecting the V glomerulus with higher brain centres in *D. melanogaster*, of which two seems fundamental to CO<sub>2</sub>-evoked behaviours. The first of these two neuron types, PN<sub>v</sub>-1, runs from the V glomerulus bilaterally via the lateral ALT to the LH and the calyces of the mushroom body (Figure 7A). The second PN, the likewise bilateral PN<sub>v</sub>-2 neuron, projects along the medial ALT to the protocerebrum in both brain hemispheres (Lin et al., 2013). The avoidance behaviour normally seen to CO<sub>2</sub> concentrations of 0.5%, is eliminated when PN<sub>v</sub>-1 neurotransmission activity is blocked. Inactivating PN<sub>v</sub>-2, on the other hand, results in the avoidance behaviour being impaired at 2% CO<sub>2</sub>. These results suggest that key aspects of these PNs functionalities are the appropriate CO<sub>2</sub> avoidance responses to low and high concentrations, respectively (Lin et al., 2013).



**Figure 7.** Central projections of the CO<sub>2</sub> sensitive neurons in *Drosophila melanogaster*. **A)** Confocal image of the brain of the fruit fly. Adapted from Butcher et al. (2012). **B)** Three-dimensional reconstruction of the whole antennal lobe (AL) in anterior and posterior view, respectively. Adapted from Endo et al. (2007). **C)** Confocal image showing CO<sub>2</sub> responding sensory neurons targeting the V glomerulus in the AL (arrow). Adapted from Suh et al. (2004). MB: Mushroom body calyces.

## Physiology

Moth CO<sub>2</sub> sensory neurons have a relatively phasic response, meaning that the course of the spiking frequency coincides proportionally with the rise and fall of the CO<sub>2</sub> level (Guerenstein,



Christensen, et al., 2004). The responses mirror the stimulus concentration bi-directionally, i.e. the sensory neurons excite in response to increases, and cease firing in response to decreases. These high frequency responses could point to the possible role of tracking CO<sub>2</sub> accumulations during long-range orientation (Guerenstein, Christensen, et al., 2004; Stange, 1992). Carbon dioxide-sensitive neurons in moths also signal background concentration continuously. This tonic component, also found in mosquitos and ants, does not adapt to stimulation over time, and thusly allows for high-resolution monitoring of CO<sub>2</sub> fluctuations in the environment (Bogner et al., 1986; Grant et al., 1995; Guerenstein, Christensen, et al., 2004; Stange, 1992). The two parts of CO<sub>2</sub> signal monitoring are performed simultaneously, and mirrors well CO<sub>2</sub> stimuli occurrence in the environment. These phenomena make the CO<sub>2</sub> sensory neurons different from typical olfactory cells as they seem to be concentration detectors, rather than flux detectors (Guerenstein & Hildebrand, 2008). Concentration detecting sensory receptors are, unlike those deemed flux detectors, directly exposed to the actual stimulus concentration of the external environment (Kaissling, 1998). Carbon dioxide absorbs and desorbs in and out of the sensory cell membrane according to the inside/outside concentration, attaining equilibrium with the outside world. This means that the insect can distinguish between the accumulation of stimulus molecules in and the speed at which the wind directs the stimulus toward the sensory organ (Guerenstein & Hildebrand, 2008). In this sense, CO<sub>2</sub> receptive cells have more in common with gustatory receptor cells and those for other volatile stimuli like humidity and temperature, than with olfactory receptor cells.

Electrophysiological research suggests that mosquitos can discriminate very small differences in CO<sub>2</sub>. In the female *Ae. aegypti*, the basiconic sensilla house sensory neurons that respond to low CO<sub>2</sub> concentrations with action potentials of large amplitudes. The response thresholds of most neurons, which are silent in CO<sub>2</sub>-free environments, lie at approximately 300ppm (sample range 150-600ppm), which is at or just below normal levels in ambient air (Grant et al., 1995). Increases in measurable activity can be seen as a response to increments as small as 50ppm; hence, the function of response, as a result of increasing concentration, is steep. These neurons monitor steady-state CO<sub>2</sub> concentration without response desensitisation, seemingly unaffected by varying degrees of background levels of CO<sub>2</sub>, providing mosquitos with the ability to detect absolute CO<sub>2</sub> levels (Grant et al., 1995). The same neurons even activate bi-directionally, according to rate and direction of CO<sub>2</sub> concentration change, excited by increases and inhibited

by decreases (Grant et al., 1995). These phasic/tonic response mechanisms are also present in the CO<sub>2</sub> neurons of other Diptera (Grant & Kline, 2003). The range of detected levels fits well with the expected concentrations present in the environment around potential hosts for a feeding mosquito (Grant et al., 1995).

*D. melanogaster* ab1c neurons has been classified electrophysiologically, as having a spontaneous firing rate of roughly 15 spikes per second (de Bruyne, Foster, & Carlson 2001). When stimulated with 5% CO<sub>2</sub>, the response activity increases significantly above spontaneous activity to approximately 180-200 spikes per second. Faucher, Hilker & de Bruyne (2013) showed that ab1c neurons increase their firing rate in relation to the CO<sub>2</sub> concentration, in a dose-dependent manner. The rate of firing rises from 10 to 70 spikes per second when the concentration is increased from 0.01% to 0.1%, suggesting that flies should be able to correctly discriminate very small differences. In contrast to mosquitos and the Lepidopteran species described, the spontaneous spiking rate of *D. melanogaster* CO<sub>2</sub> neurons is unchanged by different background concentrations, meaning they cannot monitor constant background levels. Furthermore, this also means that the ab1c neurons can detect changes in increments as small as 0.03%, even when background level is around 0.07%, and are exceedingly good at representing stimulus onset and offset (Fauchner et al., 2013). As mentioned previously, *D. melanogaster* avoids CO<sub>2</sub>, and these avoidance responses are elicited during exposure to concentrations below 1% (Suh et al., 2004).

## CO<sub>2</sub>-Related Behaviours

Several insect behaviours are the result of sensory input from CO<sub>2</sub> in the environment. Organisms have their own olfactory CO<sub>2</sub> signature, being either CO<sub>2</sub> source or sink (Stange, 1997). Generally, olfaction is an important sensory modality in environment-specific insect-plant interactions, where even low concentrations of volatiles released from host plants are indicative of a preferred host. Both spatial and temporal gradients of CO<sub>2</sub> can be informative of organic matter and breathing organisms in the vicinity. For instance, CO<sub>2</sub> can indicate to a female where to lay her eggs, presumably to ensure the best environment for them, as in the case of the cactus moth *C. cactorum*. This moth is active at night, when the prickly pear cactus, *Opuntia stricta*, assimilates CO<sub>2</sub>, creating CO<sub>2</sub> gradients and serving as a CO<sub>2</sub> sink (Stange et al., 1995). A distinct sexual dimorphism has been identified in the *C. cactorum* labial palps, where those of



female moths are facing more frontward as compared to the male. The female LPO is also proportionally longer than that of the male. This has prompted the idea that that the *C. cactorum* LPO is somehow used, not only for detecting CO<sub>2</sub> gradients in the air as seen in both sexes, but also for behaviour specific to the female moth. Indeed, when preparing to oviposit, the female *C. cactorum* walks on the plant surface while using her labial palps to prod the plant exterior (Stange et al., 1995). This is presumably done to investigate the CO<sub>2</sub> gradients near the potential host plant surface, examining its appropriateness for oviposition. The female then positions her eggs. Electrophysiological tests show that the tapping movements of the labial palps correlates with changed rate of firing of single CO<sub>2</sub> receptor neurons. The metabolic activity in the form of nightly CO<sub>2</sub> assimilation of the plant may serve as an indicator of plant health or its local metabolic activity, i.e. whether it is suitable for oviposition (Stange et al., 1995; Stange, 1997). This is an example of a short-range CO<sub>2</sub> signal having an unequivocal effect on animal behaviour. Interestingly, some trees lessen their degree of local photosynthesis as a consequence of oviposited insect eggs, resulting in a reduction in CO<sub>2</sub> assimilation (Schröder, Forstreuter, & Hilker, 2005). Trees with eggs give off less of a ‘CO<sub>2</sub> sink-signal’, which could change the effect of CO<sub>2</sub> gradients on the insect olfactory system (Guerenstein & Hildebrand, 2008).

A slightly more indirect, but similarly short-range CO<sub>2</sub> signal can affect other insect females to oviposit. The female tephritid fly, *Bactrocera tryoni*, oviposits in lesioned skin of fruit, and prefers to do so in already existing lesions as opposed to creating a new hole in which to oviposit (Stange, 1999). This behaviour implicates the fact that the existing lesion emits respiratory CO<sub>2</sub>. Furthermore, experimentally created sources of CO<sub>2</sub>, at naturally occurring concentrations, with no other sensory stimuli, similarly attract female individuals of the tephritid fly which then proceeds to oviposit. It is therefore believed that CO<sub>2</sub> is the basis for the behaviour, as opposed to other volatile chemicals released by the lesioned fruit (Stange, 1999). The response is elicited at local concentrations over 350 ppm, which is 100 ppm over ambient.

The CO<sub>2</sub> signal can also be a cue for feeding behaviour. Contrary to some other moth species, *M. sexta* LPO is found to be sexually monomorphic (Kent et al., 1986). Thus, it is likely that CO<sub>2</sub> information in this species is equally important to male and female individuals alike. *M. sexta* feeds on nectar from the *Datura wrightii* flower and researchers have explored the probable link between *Datura* nectar secretion and its CO<sub>2</sub> release, to examine whether it could inform moths about food quality or abundance (Guerenstein, Yopez, van Haren, Williams, & Hildebrand,

2004). The rate at which the *Datura* flower produces nectar seems to coincide with their emittance of CO<sub>2</sub>, both increasing over time during the hours after dusk, and decreasing by midnight (Guerenstein, Yopez, et al., 2004). With CO<sub>2</sub> being the by-product of plant metabolic activity, the levels of emitted CO<sub>2</sub> may indicate to the moth a healthy plant, an abundance of nectar, and/or a flower not recently fed on. It is known that *M. sexta* does detect CO<sub>2</sub>, and because the LPO of both sexes are similar and not used for prodding the plant surface, CO<sub>2</sub> gradients are likely detected from a distance. Hence, this may provide a signal informing about which flowers to approach for feeding in order to avoid spending energy on empty or unhealthy flowers with less nectar. In fact, experimentally naïve moths first approach to feed on flowers with higher CO<sub>2</sub> discharge (Thom el al., 2004).

CO<sub>2</sub> as an external signal is also important to hematophagous insects. Mosquitos, that feed on the blood of other organisms are attracted by CO<sub>2</sub>, which guides them towards a potential host (Gillies, 1980). Zoophilic mosquitos are mostly attracted to expired CO<sub>2</sub> in the breath of a potential vertebrate host (Takken & Knols, 1999). Mosquitos that feed specifically on human blood, like the adult *An. gambiae* female, which is the principal afrotropical malaria vector (Lu et al., 2007), orient towards the mixture of the compounds present in human breath together with other human body volatiles (Takken & Knols, 1999). Mosquitos with specific host preferences are less influenced by CO<sub>2</sub> signalling than those that feed on many different hosts (Guerenstein & Hildebrand, 2008).

Carbon dioxide is not always an attractant. The fruit fly *D. melanogaster* normally avoids ambient CO<sub>2</sub>, even at levels as low as 0.1% (Suh et al., 2004). When tested, this avoidance behaviour is eliminated when the synaptic activity of the implicated *ab1c* sensory neurons is inhibited genetically, while activating them with light causes facilitation of the behaviour (Suh et al., 2004; Suh et al., 2007). This is also taken as evidence that these neurons enable the behaviour. Carbon dioxide is among the chemicals released by stressed flies (Suh et al., 2004), making this signal relevant in order to avoid environments that have been stressful to other conspecific individuals. Though the olfactory CO<sub>2</sub> signal is an avoidance cue for the fruit fly, the taste of CO<sub>2</sub> in water, mediated by the taste organ, the proboscis, seems to facilitate acceptance behaviour rather than avoidance (Fischler, Kong, Marella, & Scott, 2007). Carbon dioxide detection by *D. melanogaster* via the sensory neurons in the two detection organs, in the proboscis for taste and in the antennae for olfaction, can therefore be independently processed to

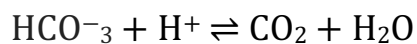
produce opposing behaviours (Fischler et al., 2007). This occurrence is one way in which the same stimulatory compound can produce different behaviours, depending on the context, or in this case, depending on the detection organ. The fly can then detect and approach nutrient-rich food, like yeast growth or other microorganisms on fruit, via proboscis-mediated CO<sub>2</sub> sensing. For example, *D. melanogaster* is attracted to apple cider vinegar which may emit olfactory signals similar to that of fermenting fruit, which would be a preferred food of the fly in a natural setting (Faucher et al., 2013). CO<sub>2</sub> detected by the antennae, can assist in the avoidance of overly rotten fruit or adverse environments (Fischler et al., 2007). The result is a finely tuned behavioural regulation, integrating two sensory modalities and context.

The nests and hives of social insect societies can quickly accumulate CO<sub>2</sub>, because of the many individuals residing within a small space. Thus, these insects often experience CO<sub>2</sub> levels well above atmospheric concentration of 0.038% (390 ppm; Jones, 2013). Honeybees are social insects living together with many other individuals of the same species in hives. When the CO<sub>2</sub> level within their hive increases, the bees fan their wings as to increase circulation of air in and out of the hive opening. A rise in CO<sub>2</sub> normally occurs with a concurrent depletion of oxygen. Testing experimentally if oxygen could be a factor in wing fanning, Seeley (1974) showed that this behaviour was not initiated when oxygen was displaced with nitrogen, only when CO<sub>2</sub> was also increased. The rising of CO<sub>2</sub> concentration co-varied with the number of bees being recruited to fan. Fanning behaviour was initiated at a 1-3% CO<sub>2</sub> increase, which corresponds to the CO<sub>2</sub> detection threshold of the bees (Seeley, 1974). This is presumably to keep an optimal environment in terms of air quality, in nests with many inhabitants in an enclosed space. Wing fanning is also seen in bumblebees. Consequences of fanning include changes in temperature, humidity and CO<sub>2</sub> levels, but when exposed to these changes respectively, only CO<sub>2</sub> and temperature rising, prompts wing fanning (Weidenmüller, Kleineidam, & Tautz, 2002). A final example of CO<sub>2</sub>-dependent air quality adjustments performed by insects is exemplified by that the fungus-rearing leaf cutting ant *Acromyrmex lundii* re-locate their fungus according to the CO<sub>2</sub> concentration in different parts of the nest. It is believed that they do so in order to assure optimal growing conditions for their symbiotic partner (Römer, Bollazzi, & Roces, 2017).

## Mammalian CO<sub>2</sub> Detection

CO<sub>2</sub> can be detected by different physiological systems in nature. Its detection in vertebrates is varied and several sensory systems are employed (Wang, Chang, & Liman, 2010). Mammalian CO<sub>2</sub> detection systems include the olfactory system (GC-D-expressing cells; Luo et al., 2009; Hu et al., 2007), the gustatory system (sour sensing PKD2L1-expressing taste cells; Chandrashekar et al., 2009), and the trigeminal system (TRPA1; Wang, Chang, & Liman, 2010, nociception; Simons et al., 1999). Additionally, at least two chemosensory systems detect elevated CO<sub>2</sub> in the bloodstream, including that of the brainstem (ventilatory; Lahiri & Forster, 2003; Trapp et al., 2008) and that of the amygdala (fear; Ziemann et al., 2009). Further, CO<sub>2</sub> has also been implicated in mammalian cell proliferation, lung epithelial function, inflammation, innate immunity, and muscle function (Cummins et al., 2014). In the present text however, focus will be put on a few relevant systems; the trigeminal, olfactory, gustatory, and central nervous systems. Carbon dioxide is a small volatile molecule produced in respiring cells and though there are some channels in biological membranes transporting water, oxygen and carbon dioxide collectively, CO<sub>2</sub> is mostly transported by passive diffusion (Cummins et al., 2014; Stange & Stowe, 1999).

Most living organisms produce CO<sub>2</sub> as a consequence of the citric acid cycle, which is an integral part of aerobic cellular respiration (Cummins et al., 2014). This occurrence naturally concerns insects as well as mammals, but because this process is more elucidated in mammalian physiology, it will be discussed in relation to mammals first. Important for this mechanism are the carbonic anhydrases (CAs), which are enzymes that maintain homeostatic intracellular pH in the blood and tissues of all aerobic organisms, i.e. in bacteria, algae, plants, and animals (Lahiri & Forster 2003; Tashian, 1989). The function of the CAs is to reversibly catalyse the conversion of CO<sub>2</sub> and water into carbonic acid (H<sub>2</sub>CO<sub>3</sub>), which is then ultimately dissociates into bicarbonate ions and free protons.



Internally, these catalytic products can then be the messengers of signals critical to the internal state, e.g. acid-base homeostasis in capillary blood promoting accelerated transport of CO<sub>2</sub> from the lungs (Lahiri & Forster, 2003). Carbonic anhydrases are also assumed to play a critical role when animals detect CO<sub>2</sub> present in the external air, when CO<sub>2</sub> diffuses onto the sensory cell

membrane. The CAs are highly efficient enzymes; the speed of the chemical reaction ensured by the CAs, include the conversion of millions of CO<sub>2</sub> molecules per second (Chandrashekar et al., 2009; Frommer, 2010; Lahiri & Forster 2003; Tashian, 1989).

### **Trigeminal system**

External carbon dioxide holds no odour to humans, even at high concentrations. High levels (above 30%) of inhaled CO<sub>2</sub> however, does elicit in a painful sensation in the mucous membranes (Wang, Chang, & Liman, 2010). This nociceptive response is not mediated by the olfactory system, but by the trigeminal nerve fibres that innervates the nasal and oral cavity (Bensafi et al., 2008; Wang et al., 2010). Tests on cells expressing the transient receptor potential cation channel A1 (TRPA1), also called the ‘wasabi receptor’, in mouse trigeminal ganglion neurons, show that TRPA1 is the component needed for CO<sub>2</sub> to cause trigeminal sensory neuron responses (Wang et al., 2010). TRPA1 activation happens downstream to intracellular acidification, meaning it is not directly gated by CO<sub>2</sub> or extracellular acidification, but by intracellular protons. This mechanism suggests a role of a carbonic anhydrase also in trigeminal CO<sub>2</sub> responses, and single unit responses has been shown to cease in conjunction with the administration of CA inhibitor acetazolamide (Komai & Bryant, 1993).

### **Olfactory system**

Small volatile chemicals in the air can be detected by the olfactory systems of vertebrates and invertebrates alike; insects, worms and mammals (Luo, Sun, & Hu, 2009). It is useful in recognising food or predators, and to mediate behaviour related to social structures within a species (Cummins et al., 2014). The mammalian olfactory systems include distinct but parallel subsystems that seemingly have specialised functions.

#### *The necklace olfactory subsystem*

Rodents detect CO<sub>2</sub> via olfactory sensory neurons (OSNs) within the necklace olfactory subsystem. Carbonic anhydrase enzymes have been found in the olfactory epithelia of rats (Coates, 2001) and guinea pigs (Okamura, Sugai, & Ohtani, 1996), and these locations served as pointers as to where to find CO<sub>2</sub> chemoreceptive cells. Understanding the role of the CAs in olfactory detection of CO<sub>2</sub>, researchers conducted electrophysiological recordings from the cells

in the rat nasal cavity where CA were most abundant (Coates, 2001). Cells located in the most caudal parts of the rat nasal cavity were found to initiate response at 2% CO<sub>2</sub>, and the maximum response was generated at 14% CO<sub>2</sub>. These findings speak to the olfactory CO<sub>2</sub> receptor tuning being different from that of trigeminal receptors, which seems to be tuned to concentrations from 45% to 100% (Coates, 2001). The olfactory CO<sub>2</sub> receptor function in many organisms, like mammals, is unidentified but no studies have evidenced a role in breathing patterns using olfactory nerve transection experiments (Coates, 2001). It is therefore probable, that the major role of CO<sub>2</sub>-sensitive olfactory cells in mammals, is other than ventilatory. The necklace olfactory system involves neurons in the main olfactory epithelium (MOE) with axons projecting to the necklace glomeruli, located caudally in the olfactory bulb (OB; Luo, 2008). It is now known that carbonic anhydrase-2 (CAII) specifically, is especially abundant in the dendrites, soma, and occasionally in the axon of this small group of neurons, while this enzyme is absent from vomeronasal and respiratory epithelium cells (Coates, 2001).

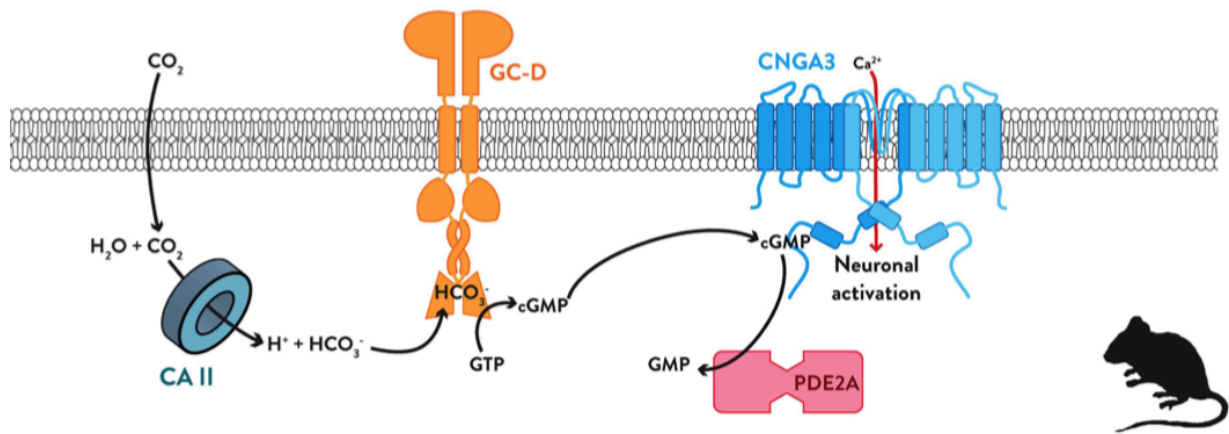
These CO<sub>2</sub> sensitive cells also express both guanylyl cyclase-D (GC-D) and phosphodiesterase 2A (PDE2A; Coates, 2001; Hu et al., 2007). Calcium imaging and targeted electrophysiological recordings from GC-D neurons in vitro show that low concentrations of external CO<sub>2</sub> activate bulbar neurons associated with the necklace glomeruli (Hu et al., 2007). This is suggestive of the GC-D gene being the principle olfactory CO<sub>2</sub> receptor. Though present in lower mammals like rodents, primate evolution has reduced GC-D to a pseudogene, explaining the lack of olfactory perception of CO<sub>2</sub> in humans and other apes (Young, Waters, Dong, Fülle, & Liman, 2007). Hu et al. (2007) determined that the necklace olfactory system detects atmospheric CO<sub>2</sub> specifically, and with distinct sensitivity. These neurons in mice respond to CO<sub>2</sub> when typical OSNs does not, and at CO<sub>2</sub> levels as low as 0.1% (atmospheric level being approximately 0.04%; Hu et al., 2007).

Behaviourally, CO<sub>2</sub> is an avoidance cue. As tested in a T-maze, mice can discriminate concentrations around 0.068%, which is just over normal levels in the air, when put to the test with a go/no-go paradigm after training (Hu et al., 2007). Hence, the detection sensitivity of the neurons is in line with the behavioural threshold of the animal. The avoidance response is eradicated in mice with a mutagen-induced nonsense mutation of CAII, yet the sense for other odorants stay intact (Hu et al., 2007). Odorant detection by canonical OSNs incorporates the activation of cyclic nucleotide-gated (CNG) channels that include the CNGA2 subunit. CNGA2

knock-out mice still detect CO<sub>2</sub> and show expected learning curves in CO<sub>2</sub>-associated assays. This knock-out procedure would normally lead to anosmia, implying that the necklace olfactory neurons do not express CNGA2, but instead CNGA3. Olfactory epithelium lesions also eliminate the CO<sub>2</sub> avoidance behaviour in both trained and untrained mice (Hu et al., 2007). The sensory nature of necklace olfactory neurons was long unknown, and so also their potential behavioural implications. As mentioned above, mice avoid CO<sub>2</sub> levels as low as around 0.2%, and the avoidance effect is heightened with increasing concentrations. This relationship could mean that the implicated cells facilitate an innate avoidance behaviour in these animals (Hu et al., 2007; Luo, 2008). Speculatively, environmental proximity to factors like other animals (predatory or conspecific) may raise the local CO<sub>2</sub> concentration, and its detection could together with other olfactory stimuli, mediate expedient behaviour.

### *Signal transduction*

A somewhat unique intracellular signal-transduction cascade takes place in necklace olfactory neurons, as opposed to other olfactory signalling cascades (Luo, 2008). No G-protein-coupled odorant receptors have been found. Instead, it seems probable that the necklace olfactory neurons use the intracellular messenger cGMP, and cGMP-gated channels opening to initiate cellular activation (Luo, 2008). As CO<sub>2</sub> diffuses onto the sensory neuron, CAII converts CO<sub>2</sub> to bicarbonate that activates guanylyl cyclase (GC), producing cGMP. Cyclic GMP-sensitive CNG channels open initiating an influx of cations, activating the cell and facilitates the action potential travelling to the necklace glomeruli in the olfactory bulb (Figure 8). Bicarbonate acts on GC-D intracellular cyclase domain, making it the compound that stimulates cGMP production (Sun et al., 2009).



**Figure 8.** Schematic representation of type 2 carbonic anhydrase (CAII) mediating CO<sub>2</sub> detection in the mouse. As CO<sub>2</sub> diffuses, bicarbonate (from CAII catalysation) activates receptor guanylate cyclase (GC-D) resulting in cGMP binding to cyclic nucleotide gated channel CNGA3, calcium ions enter, and the cell depolarises. Adapted from Jones, (2013).

## Gustatory system

The taste, or ‘oral sensation’, of CO<sub>2</sub> is, both chemosensory and somatosensory. Regarding the taste of CO<sub>2</sub>, or carbonation, it seems to be tapping into gustation for acidity, i.e. recruiting the sour-sensing taste receptors (Lyall et al., 2001). If these cells are lacking, measurable electrical activity in the nerves serving them is eliminated. Gustatory cells in mice express a gene that encodes the enzyme CA, also determined to be essential to the olfactory detection of CO<sub>2</sub> (Chandrashekar et al., 2009). Specifically, the *Car4* gene encodes the  $\alpha$ -carbonic anhydrase isoform 4 (CA4), which is present on the gustatory cell surface on the mammalian tongue and generates acidifying protons locally (Figure 9). Chandrashekar et al. (2009) tested the electrophysiological response of mouse taste receptor cells (TRCs) to CO<sub>2</sub>, by recording from nerves that innervate them. They showed that the TRCs respond robustly to CO<sub>2</sub> in all tested forms (being carbonated drinks, CO<sub>2</sub> dissolved in buffer, and gaseous CO<sub>2</sub> directly applied to the tongue), in a dose-dependent manner. Further, ablating the ion channel PKD2L1-expressing sour-sensing cells, selectively eliminates the gustatory response to CO<sub>2</sub>. This was evident with all tested acidic stimuli and shows that PKD2L1-expressing cells are important for gustatory CO<sub>2</sub> detection. This notion is further supported when broad acting CA blockers were administered, resulting in no gustatory responses to even the highest concentration of CO<sub>2</sub> (Chandrashekar et al., 2009). The described study points to CA being the principal CO<sub>2</sub> ‘detector’, via *Car4* activity



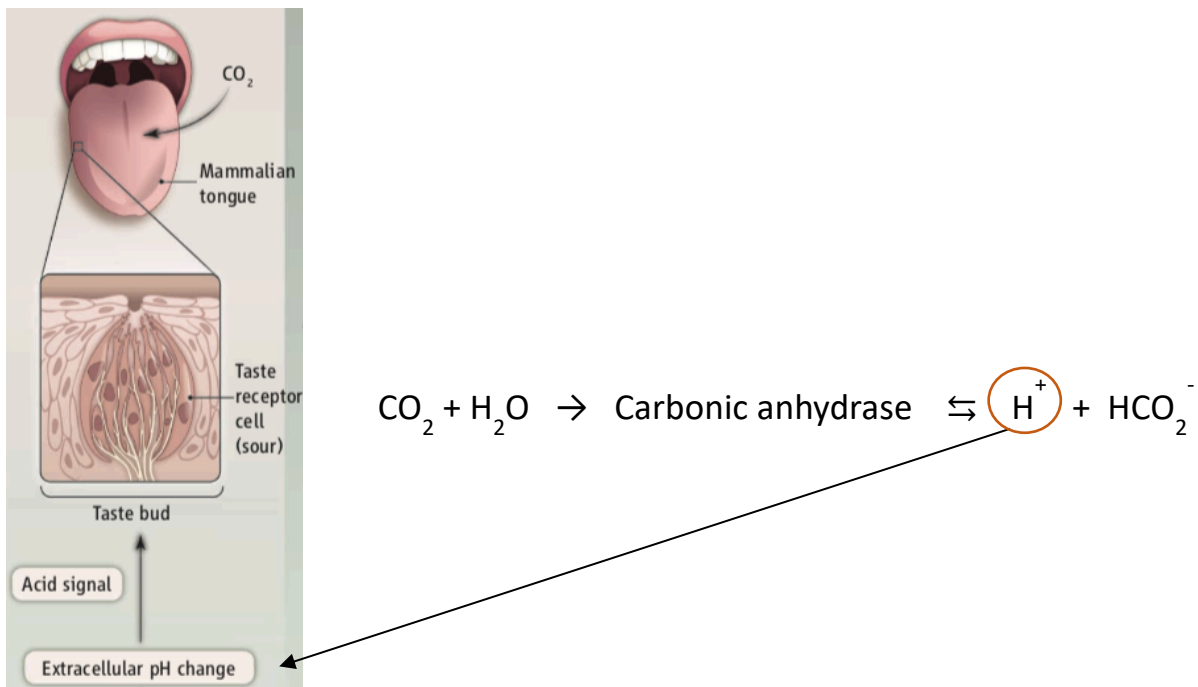
products at the TRC surface. Protons would then be the result of carbonation stimuli, as bicarbonate does not stimulate the TRCs.

Extracellular protons, not intracellular acidification, is then the CO<sub>2</sub> gustatory signal. On this basis, the authors considered this the basis for the relationship between CO<sub>2</sub> and sour taste, via the change in pH (acidification), where the CA-initiated conversion of CO<sub>2</sub> and water into molecules signal the presence of CO<sub>2</sub>, as opposed to the enzyme being the sensing agent in itself (Chandrashekar et al., 2009). Indeed, some individuals would describe the taste, or experience, of carbonated water as somewhat sour. Gustatory nerve responses to CO<sub>2</sub> are voltage sensitive and do correlate with the acidification of the polarised TRCs, but are not governed by stimulus acidity and similarly, the taste nervous response can be prevented when blocking CA (Lyall et al., 2001).

CA inhibitors also change the sensation of carbonation in humans. With this inhibition, the taste of carbonic acid is actually sensed, as opposed to in the normal workings of carbonic anhydrase, lessening the concentration in the local environment around the taste buds (Graber & Kelleher, 1988). Because CA inhibitors change the taste perception of carbonation, it is probable that we actually taste the protons (or the bicarbonate), i.e. the CO<sub>2</sub> metabolites, rather than CO<sub>2</sub> as a whole compound (Jones, 2013). The reason why taste for CO<sub>2</sub> is a preserved function in humans, when the smell of CO<sub>2</sub> is not, is not known. Perhaps, it is to aid the identification of decomposing organic matter, in order to avoid rotten food, a notion in line with the evidence from insects (Fischler et al., 2007). Some put an alternative idea forth, namely that the function is the accidental by-product of Car4 operating to preserve taste bud pH (Chandrashekar et al., 2009).

Consequently, one could argue that the tingling sensation of carbonated drinks is part of the taste sensation. The CO<sub>2</sub> bubbles are also however, detected by somatosensory tongue receptors, generating the tingling sensation (Chandrashekar et al., 2009; Simons et al., 1999). The CA-initiated conversion of CO<sub>2</sub> and water to carbonic acid excites chemosensitive nociceptors on the tongue, projecting to the trigeminal nuclei. Testing this hypothesis, Simons et al. (1999) applied carbonated water to the tongues of anaesthetised rats and conducted single unit recordings in trigeminal nuclei. When a CA inhibitor was co-applied, the neuronal response was weakened. Differences were also seen between the conditions (carbonated water vs. control) in the activated brainstem neurons, and CA inhibitors eliminated this pattern. They also tested the perception of carbonated water in human subjects when half the tongue was pre-treated with a CA inhibitor.

The reported tingling sensation was lessened in the treated side. In sum, carbonated water seems to elicit neural activity and the perception of bubbles on the tongue, both of which are weakened by the CA inhibitor (Simons et al., 1999). These studies point to the taste of CO<sub>2</sub> being mediated by both somatosensation and gustation, since carbonated water activates both the lingual nociceptive system and TRCs.



**Figure 9.** Taste of carbonation: Schematic illustration of the  $\alpha$ -carbonic anhydrase mechanism in the mammalian gustatory cells. Protons, converted by carbonic anhydrase (CA) from CO<sub>2</sub> and H<sub>2</sub>O, are assumed to serve as the acid signal. Adapted from Frommer, (2010).

### Peripheral and central chemoreception: Blood & Brain

Higher mammals can detect the CO<sub>2</sub> concentration in their blood to facilitate respiratory and ventilatory regulation. In fact, the respiratory responses to inspired CO<sub>2</sub> by different animals are more studied than the responses originating from the nasal cavity (Coates, 2001). Chemosensitive cells exist peripherally in the carotid body (CB) glomus cells, and centrally in the brain stem respiratory network in mammals (Putnam, Filosa, & Ritucci, 2004). The latter receives input from the CBs and from central chemoreceptors within the brain (Åstrand & Rodahl, 1986). A rise in CO<sub>2</sub>/H<sup>+</sup> is sensed by specialised chemosensitive brainstem neurons spread among several brainstem regions. The state of hypercapnia, an increased CO<sub>2</sub>/H<sup>+</sup> level in the arterial blood,

activates the brainstem neurons, stimulating increased pulmonary ventilation (Åstrand & Rodahl, 1986). In order to control respiratory regulation in response to the external or internal environment or condition, respiratory motor output is initiated because the brainstem receives regular chemoafferent input about the arterial levels of pO<sub>2</sub>, pCO<sub>2</sub> and pH (Trapp et al., 2008).

Carbonic anhydrase mediates CO<sub>2</sub>/H<sup>+</sup> sensing in the carotid bodies and the CNS due to its presence in many tissues. Its most important function in this context includes the hydration and dehydration of CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> in capillary blood in the periphery and in the lungs, allowing for fast riddance of CO<sub>2</sub> (Lahiri & Forster, 2003). In sum, the Glomus cells of CB are the principal peripheral chemosensors. Carbonic anhydrase in the CB glomus cells catalyses the hydration and dehydration of CO<sub>2</sub> in order to approach equilibrium, increasing H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> production. Carbonic anhydrase function assures the chemosensory activity of the CB, aiding the rapid physiological reflexes needed for respiratory regulation and sympathetic nerve activation (Iturriaga, Mokashi, & Lahiri, 1993).

The amygdala is an important structure of the brain that detects and mediates fear (noxious) stimuli. This structure is also involved in the processing of fear (innate and acquired) and helps to produce behaviour in response to fear. While humans do not detect a rising CO<sub>2</sub> concentration via the olfactory system, it is important for the body to respond. A rise in CO<sub>2</sub> is a threatening stimulus as it alerts to impending suffocation (Wemmie, Taugher, & Kreple, 2013). In most cases, amygdala does not itself, sense noxious stimuli. The acid-sensing ion channel-1a (ASIC1a) is necessary for normal fear responses and is highly expressed in the fear circuit including the amygdalar region (Ziemann et al., 2009). Ziemann et al. (2009) discovered a principal chemosensory role for amygdala. The ASIC1a channels sense increased CO<sub>2</sub> mediated by the consequential dip in pH. Interestingly, the amygdala seems to both detect the threat, and evoke the behaviour needed to avoid it. These phenomena are also important in understanding the molecular workings of CO<sub>2</sub>-evoked panic and fear responses. Freezing behaviour in mice is a fear response, and this behaviour is used as an indication of evoked fear in the mouse. Mice freeze as a result of breathing air that contains 10% CO<sub>2</sub>, a response that is diminished in mice with disrupted ASIC1a gene (Ziemann et al., 2009). Additionally, wild-type mice chose to spend less time in chambers with higher CO<sub>2</sub> concentration, showing clear avoidance behaviour (Ziemann et al., 2009).

## Discussion

### Summary

Gaseous carbon dioxide is a compound relevant as a sensory signal to several animal species. This gas is omnipresent in the atmosphere but concentrated in smaller sub-environments close to breathing animals and decaying organic material. It is not known how the rising atmospheric CO<sub>2</sub> concentration will affect terrestrial life as the putative vulnerability of animals to this phenomenon is less studied than that of plants. Common to the reviewed insect species that sense CO<sub>2</sub> is that they have sensory neurons dedicated to this detection. These sensory neurons are located inside sensilla, either in specialised detection organs, like the labial palps in Lepidoptera and the maxillary palps in mosquitos, or in the classical olfactory organ, the antennae, as in the case of the fruit fly. Carbon dioxide-sensitive receptors work in pairs to mediate cell activation. These receptors are parts of a superfamily of gustatory receptors with “family member” receptors in the three groups of insects mentioned above. The axons of the CO<sub>2</sub>-activated neurons project to a single glomerulus devoted to this signal, in the primary olfactory centre in the insect brain, the antennal lobes, constituting the location for first order signal processing. From here, projection neurons carry the signal to the higher order brain regions. The CO<sub>2</sub>-associated sensory neurons are different to canonical olfactory receptor cells in that they seem to detect stimulus concentration, not only stimulus flux. They are in constant equilibrium with the outside concentration as CO<sub>2</sub> diffuses into the cell membrane, and they detect background levels and concentration increases and decreases. To insects, CO<sub>2</sub> in can be a powerful cue to oviposit or feed on healthy plants, it can assist in locating a host organism, or aide the avoidance of a stressful environment. Further, a high CO<sub>2</sub> level in hives indicates poor air quality and expedites behaviours in social animals to counteract this.

Some mammalian species can detect external CO<sub>2</sub>, in the air by the sense of smell and carbonation of liquids by taste. The smell of CO<sub>2</sub> in rodents is mediated by the necklace olfactory subsystem, with olfactory sensory neurons located in the nasal cavity, responsive to low levels of CO<sub>2</sub>. Exposure to the gas is thought to mediate an innate avoidance response. Regarding the mammalian taste for carbonation, it appears to be both gustatory and somatosensory. Carbon dioxide inhaled in high concentration is sensed via receptors in trigeminal nerve cells. Peripheral chemoreceptive cells in the carotid bodies and central chemoreceptive cells in the respiratory brain network of mammals, will alert to CO<sub>2</sub> mediated drops in pH in the blood and increase

ventilation. Finally, the action of acid-sensing ion channel 1a in the amygdala, fear circuits in the brain can be activated in response to increased CO<sub>2</sub>.

### **Similarities between mammals and insects in the CO<sub>2</sub> detection**

Numerous insect species, but only a few mammalian species have been shown detect volatile carbon dioxide via their chemosensory systems (Jones, 2013). However, common to all reviewed species was the presence of apparently specialised sensory neurons detecting CO<sub>2</sub>. These neurons are situated in specialized organs with direct access to the outside ‘chemical’ world, but likewise sufficiently protected; in encapsulated scale clad organs in insects and inside the nasal cavity neuroepithelium in mammals. The signal processing in the two groups of organisms shares fundamental features in that the first synaptic relays are the AL and the OB, respectively, both unambiguously dedicated to odour information. Moreover, the sensory neurons converge on one distinct glomerulus, in a stereotyped fashion, in both groups of species. The information is then processed by higher centres of the insect and mammal brain and can mediate apt behaviours in both.

### *Regulation of acid-base balance via carbonic anhydrases - a proposed receptor mechanism*

Specific enzymes catalysing the interconversion between CO<sub>2</sub> and water, CAs, have been found in the olfactory epithelia of mammals, suggesting that the mammalian olfactory signal transduction mechanism includes a product of carbonic anhydrase (Coates, 2001; Okamura, Sugai, & Ohtani, 1996). The products of CO<sub>2</sub> conversion by the CAs are proposed to act as second messengers in a metabotropic signalling cascade. It is thought that bicarbonate ions activate mammalian olfactory receptor guanylate cyclase, producing cGMP, which in turn opens CNG channels, permitting an influx of calcium ions that depolarises the cell (Jones, 2013; Scott, 2011). Molecular mechanisms underlying carbon dioxide sensing in insects are less defined. Normally, sensory receptors in insects have an intuitive organisation, with gustatory receptors in gustatory organs and olfactory receptors in olfactory organs (Jones, 2013). As described here however, the CO<sub>2</sub>-sensing mechanism is somewhat different. For example, in *D. melanogaster*, CO<sub>2</sub>-detecting gustatory receptors are situated in the antennae, normally dedicated for olfaction (de Bruyne et al., 2001; Jones et al., 2007). This occurrence raises two problems. Firstly, that of

determining what actually binds to the receptor, warranted by the previously discussed fact that the first found ligand for the Lepidoptera CO<sub>2</sub> receptor was NaHCO<sub>3</sub>. Secondly, if there are additionally shared mechanistic features with other gustatory receptors, bicarbonate could stimulate guanylate cyclases, as demonstrated in rodents. The notion that volatile CO<sub>2</sub> binds directly to the insect receptor complex Gr21a/Gr63a (and its orthologues) is not an impossibility, because the receptor workings is simply not known (Jones, 2013; Scott, 2011). However, if the ligand is the more soluble CO<sub>2</sub> metabolite, bicarbonate, it would need a CA enzyme for the function of catalysing the conversion of CO<sub>2</sub> and water into bicarbonate and protons, for either of these to activate the receptor complex (See Figure 4D, 8 and 9). If bicarbonate is, indeed, the ligand of the Gr21a/Gr63a receptor complex, the mechanism of CA-mediated conversion of CO<sub>2</sub> then crosses the invertebrate-vertebrate line. Alternatively, CA could function upstream to the GR complex, which in this scenario would be directly activated by one of the CO<sub>2</sub> metabolites, similar to processes in mammalian gustation.

Though not discussed in detail here, the honeybee can also detect CO<sub>2</sub>, for which the electrophysiological responses diminish after administration of the CA blocker acetazolamide (Stange, 1974). This occurrence suggests that a CA is involved in the workings of the honeybee CO<sub>2</sub> detection. However, the gene lineage affiliated with the heterodimeric Gr21a/Gr63a receptor complex, though highly conserved and present in several mosquitos, Lepidoptera, and beetles, is absent in honeybees and some wasps, lice, fleas and ticks (Robertson & Kent, 2009; Xu & Anderson, 2015). It is therefore unsure, whether it would be relevant to draw upon knowledge about the honeybee as a valid reference for comparison to other insect species.

Carbon dioxide as a gustatory signal, and its behavioural significance, is less intuitive to untangle in terms of mammals vs. insects. While the taste of carbonated water is attractive to both humans and fruit flies, the gas is also associated with rotten food sources, avoided by both species.

### **Differences between mammals and insects in CO<sub>2</sub> detection**

The receptors that mediate CO<sub>2</sub> detection in mammals are, in some sense, canonical ORs, though the intracellular signalling transduction cascades is atypical, in that the second messenger is believed to be cGMP, not cAMP (Luo, Sun, & Hu, 2009). In insects, gustatory receptors are seen “masquerading” as olfactory detectors of CO<sub>2</sub> inside olfactory detection organs. Moreover,

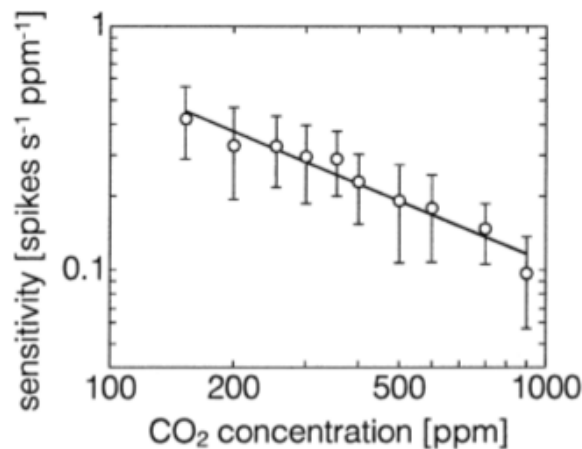
detection appears to require the coupling of two receptors, a concept similarly confirmed for insect antennal ORs detecting typical odorants (Larsson et al., 2004). The exact activation and processing mechanisms of the canonical insect ORs are nonetheless still debated (Nakagawa & Vosshall, 2009).

Mammals generally avoid increased CO<sub>2</sub> in the air while insects show mixed behaviours in response to the gas, approach or avoidance depending on the context (Faucher et al., 2013; Hu et al., 2007). In general, terrestrial vertebrates are poorly equipped in terms of detecting atmospheric CO<sub>2</sub> as compared to invertebrates, which may be a consequence of being exposed mostly to concentrations above the atmospheric background i.e. exhaled air (Stange, 1996). Invertebrates on the other hand, are seen to both respond to and behave as a consequence of small fluctuations of CO<sub>2</sub> in the environment.

### **Possible implications and vulnerability of organisms to changes in atmospheric CO<sub>2</sub>**

Some insects use diminutive CO<sub>2</sub> gradients in their food-seeking efforts and in locating an egg-laying site (Sage, 2002). These species may have difficulty discriminating the signal if the background concentration is increased. For instance, when atmospheric CO<sub>2</sub> is elevated experimentally, *C. cactorum* females are less inclined to oviposit, and also lay fewer eggs in total, suggesting that the artificial concentration increase is masking the CO<sub>2</sub>-sink signal (Stange, 1997). The *C. cactorum* CO<sub>2</sub> detection apparatus is so sensitive that a man-made increase in background CO<sub>2</sub> levels, will likely affect this function in the moth unfavourably (Stange et al., 1995). Moths like *M. sexta*, being dependent on a host that constitutes a strong CO<sub>2</sub> source, on the other hand, may have better adaptability because they naturally evolved in higher-CO<sub>2</sub> environments with larger fluctuations (Abrell et al., 2005). Hence, species like *C. cactorum* and *B. tryoni*, being influenced by external CO<sub>2</sub> for oviposition behaviour and thus ultimately, reproduction, seem to be most vulnerable to atmospheric changes in CO<sub>2</sub>. For *H. armigera*, it has been shown that external CO<sub>2</sub> concentrations over the range of 150-1000ppm induce a drop in the sensitivity to the gas by around 75% (Figure 10; Stange, 1997). Furthermore, above the pre-industrial CO<sub>2</sub> level (270ppm), these CO<sub>2</sub> sensory neurons begin to respond to temperature, meaning that the higher the background levels the more CO<sub>2</sub>-detection becomes temperature-detection, essentially jumbling the signal (Stange, 1997). To be able to detect and discriminate

gradients efficiently in an increasing concentration, these species will have to adapt to the altered environmental condition over time (Sage, 2002).



**Figure 10.** Graph demonstrating the effect of background concentration of CO<sub>2</sub> on the sensitivity (spikes s<sup>-1</sup> ppm<sup>-1</sup>) of the CO<sub>2</sub> sensory neuron in *Helicoverpa armigera*. Adapted from Stange, (1997).

Elevated levels of CO<sub>2</sub> will also include the secondary effect of an overall temperature increase, and likely lead to phenotypic changes in plants that insects feed on, which requires additional adaptability of the part of the herbivorous organism (Hunter, 2001). If the CO<sub>2</sub> detection mechanism is used as a way to locate healthy plants, the finding of quality foods may decline. Consequently, the diet-dependent fitness of the insects will decline, affecting their ability to fight off natural enemies like parasites. The result can be a marked reduction in insect populations with unknown consequences to the ecosystem (Sage, 2002).

Considering the fact that the CO<sub>2</sub> detection threshold of mice is close to the current atmospheric level, an increase in CO<sub>2</sub> may influence mammalian behaviour (Hu et al., 2007). The possible effects are not established but could have an ethological bearing on the animals and associated ecology, as mammals respond acutely to rising CO<sub>2</sub> with avoidance, thought to be innate. Though CO<sub>2</sub> signalling can cue, mediate or produce behaviour in both mammals and insects, it seems that the extent and breadth of related behaviours are more pronounced in insects. It is possible that this notion is exaggerated or misguided, based on the volume of evidence for CO<sub>2</sub>-signalled behaviour in insects, as compared to mammals. If accurate however, this would implicate insect groups as exceedingly more vulnerable to changes in the atmospheric CO<sub>2</sub> concentration. The effects will not be self-contained within certain groups of organisms, rather,



they will pose a threat to terrestrial life as a whole. Some insect species are agricultural pests and vectors of serious diseases, but the absolute majority constitute pollinators for blooming plants, including many that are relevant for our food. It is therefore important to study their chemosensory system in order to be prepared for the changes that will come. Carbon dioxide is an important chemosensory signal and has an observable relevance in the innate behaviours of several animal groups. The current reports on rising CO<sub>2</sub> levels are mainly focussed on temperature and weather at an 1.5% increase (IPCC), whereas this text has outlined that even small increases in atmospheric concentrations may have adverse effects on a variety of species. Understanding the mechanisms by which each organism detect CO<sub>2</sub> will help us understand the direct effect that the man-made rise in CO<sub>2</sub> will have on them.

### **Conclusion**

- There are close similarities between insects and higher mammals in the biological systems devoted to detection and processing of CO<sub>2</sub>, both as an environmentally relevant signal, and as an internal signal indicating and controlling acid homeostasis.
- As a sensory cue, CO<sub>2</sub> produces remarkably complex behaviours. Although both insects and some higher mammals can detect miniscule levels of CO<sub>2</sub>, the range of evoked behaviours and the relevance of the signal seems greater in insects than in mammals. For instance, CO<sub>2</sub> gradients have a direct effect on reproduction in moths, which locate oviposition sites according to these gradients.
- It is likely that carbonic anhydrases play a role in the receptor mechanism catalysing the conversion of CO<sub>2</sub>, in both mammals and insects, though the mechanism in insects is more challenging to substantiate.
- It should also be noted that making predictions about potential vulnerability to rising CO<sub>2</sub> might be difficult since various factors can play a role; as seen in the fruit fly, CO<sub>2</sub> can evoke different responses depending on the concentration and context. This notion further points to the importance of studying CO<sub>2</sub>-related olfaction in insect in detail.
- Considering the effects of external CO<sub>2</sub> levels in insects holds great relevance to humans and all terrestrial life since this huge group of organisms include pollinators of plants we need for food as well as agricultural pests and vectors of serious diseases.

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