forms of eCB-mediated synaptic plasticity are induced by CB1s, DSI can be initiated in less than 1 s, whereas induction of long-term depression requires CB1 activation persisting for several minutes[10]. PKA and cAMP are involved in the transition from short-term to long-term eCB-dependent plasticity, although what factors actually control the transition are still obscure. Exploration of the role of mtCB1s in this process might prove rewarding.

Cannabis derivatives and the ECS are being investigated for their physiological effects and therapeutic potential in a variety of phenomena. An important tactic is to enhance the levels of eCBs, which are physiologically produced when and where needed, by interfering with their degradation[11]. How will regulation of mitochondrial respiration by cannabinoids alter our interpretations of the problems and answers arising from these studies? It was recently shown that neurotoxic damage inflicted by kainic acid is greater in mice lacking CB1 than in wild-type mice[12]; evidently, kainic acid induces eCB synthesis and release, and the resulting CB1 activation is neuroprotective. However, mitochondrial functional insufficiency contributes to the extent of stroke-induced neurological damage[2]. It seems that eCBs released during neurocytotoxic insult could also, by suppressing mitochondria, oppose the neuroprotective effect and exacerbate the lesion, with the ultimate outcome depending on a balance of the two tendencies. Therapeutic approaches that increase eCB availability by decreasing eCB degradation may not avoid such complexities.

Finally, certain diseases or other maladies are accompanied by upregulation of the ECS; examples include behavioral stress[13], fragile X syndrome[14,15] and Parkinson's disease[2]. Mitochondrial dysfunction has been suspected as an etiopathological cofactor in many of these cases[2]. If upregulation of eCBs downregulates mitochondrial respiration, then eCBs and mtCB1s could link certain disease states with mitochondrial dysfunction. These and other implications of the work by Bénard *et al.* will surely energize future investigations of the ECS.

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Sensing the long and the short of it

Richard Benton

How do sensory systems encode prolonged stimuli? A study reveals molecular and circuit mechanisms by which *C. elegans* interprets oxygen concentration to produce both transient and long-lasting behaviors.

Sights, sounds and smells flood our senses. Whether punctual or persistent, most of these signals are rapidly ignored or cause us to react only briefly. We need to keep pace with new information in the environment rather than hold onto the old. Our sensory systems achieve this through adaptation mechanisms that turn off (or at least reduce) the activity of individual proteins, signaling pathways and neural networks.

Certain stimuli hold our attention, however, such as those causing us harm; consider the consequences of pain subsiding even as your hand remains on a hotplate. Internal sensory cues, such as body temperature, must also be constantly monitored. Neurons that exhibit sustained, or tonic, responses to sensory stimuli have been identified electrophysiologically throughout the nervous system; they include peripheral nociceptors and mechanoreceptors and internal, homeostatic temperature or pH sensors. How do these neurons generate constant activity over many minutes or hours? How can this prolonged activity produce meaningful behavioral responses?

In this issue of *Nature Neuroscience*, Busch *et al.*[1] answer these questions through their characterization of tonic receptors, the O_2-sensing neurons in the nematode worm *Caenorhabditis elegans*. They identify the molecular components in sensory neurons that are required to generate tonic signals to downstream circuitry, and they characterize how these sustained signals are transformed in different types of interneurons to elicit both short- and long-term behavioral changes (**Fig. 1**).

Sliding through microbe-rich rotting fruit or hitchhiking on snails[2,3], *C. elegans* is likely to be exposed to O_2 concentrations ranging from near-anoxia to atmospheric values (21%). In the laboratory, this tiny worm prefers 5–10% O_2 (ref. 4), probably because this range optimizes internal O_2 concentrations to be sufficient for cellular respiration while avoiding excess oxidative stress[5]. When exposed to higher or lower O_2 concentrations, *C. elegans* displays several behavioral responses, including changes in speed, turns and reversals, which together may help the worm to navigate through O_2 gradients[4,6]. High O_2 concentration also promotes aggregation, as worms can collectively reduce the concentration of O_2 when clustered[4,7].

Although ambient conditions may vary markedly over short timescales, *C. elegans* probably often finds itself in fairly stable O_2 conditions. Busch *et al.*[1] began by asking whether the worms' behavior can be persistently set by the level of O_2 in their environment. Under optimal conditions (7% O_2), worms moved slowly and dwelt locally, but when exposed to undesirably high concentrations (21% O_2), they immediately started to move faster (perhaps in their vain attempt to seek more comfortable O_2 levels within the assay arena). Notably, the worms never slowed down in high O_2, even after 2 h, and only decelerated once their preferred 7% O_2 was restored.

Two mechanisms might explain this behavior: O_2 sensors could be transiently activated but induce persistent physiological changes in downstream interneurons to cause long-lasting (but reversible) changes in locomotion, or the

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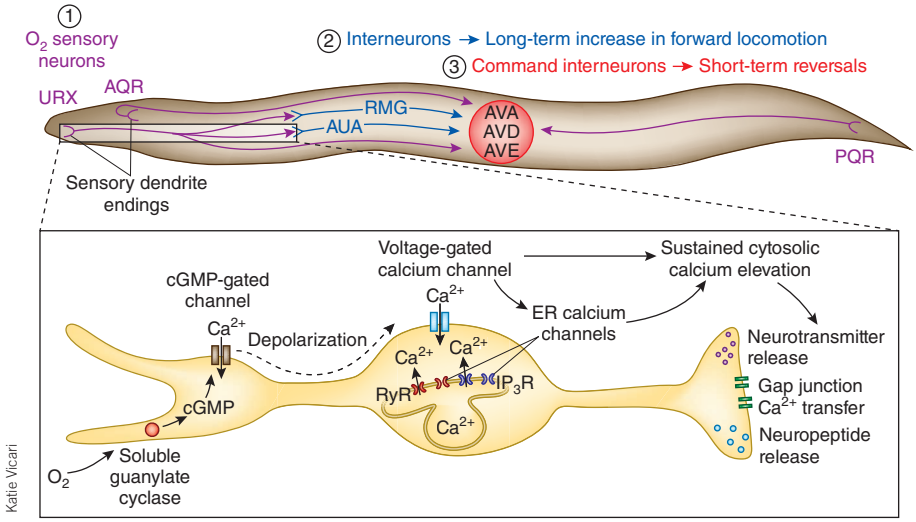

Figure 1 Tonic O_2 sensing circuitry of *C. elegans*. (1) Three types of O_2-sensory neurons (purple), URX and AQR in the head and PQR in the tail, respond tonically to high ambient O_2 (top) through a relay of Ca^{2+}-permeable ion channels (bottom, adapted from Busch *et al.*[1] with permission). (2) Tonic URX signals are maintained in two types of downstream interneuron (blue), RMG and AUA, which may signal prolonged increased forward locomotion in high-O_2 conditions. (3) The O_2-sensory neurons, and the RMG and AUA interneurons, synapse onto three command interneurons (red), AVA, AVD and AVE. Tonic signals from sensory neurons are transformed into transient activity in the command neurons, which triggers short-term reversal behavior. ER, endoplasmic reticulum; IP_3R, IP_3 receptor; RyR, ryanodine receptor.

O_2 sensors could signal tonically to downstream circuits, continuously representing the external O_2 levels. To distinguish between these, Busch *et al.*[1] measured O_2-evoked neuronal activity by calcium imaging in three previously identified O_2-sensing neurons: AQR, PQR and URX[4]. All three exhibited sustained activity (elevated intracellular Ca^{2+}) in high O_2, consistent with the second mechanism. Both these tonic neuronal responses and prolonged behavioral responses to 21% O_2 required the atypical soluble guanylate cyclase GCY-35, which is thought to function, with its partner GCY-36 (ref. 8), as a sensory receptor for O_2 in these three neurons[4,8].

How does activation of GCY-35–GCY-36 produce prolonged neuronal signaling? Some downstream signal transduction components were already known, such as the TAX-2–TAX-4 sensory ion channel, which is probably gated directly by cyclic GMP produced by GCY-35 (ref. 4). However, tonic signaling is likely to require special mechanisms to maintain neural activity, as well as sufficient reserves of second messengers. Through some intelligent guesswork and exploitation of the genetic resources of *C. elegans*, the authors provide evidence that the sustained Ca^{2+} evoked by high O_2 levels relies on a series of calcium-permeable ion channels (**Fig. 1**): TAX-2–TAX-4 and a voltage-gated calcium channel, which may generate and amplify sensory potential, respectively, and the intracellular inositol-1,4,5-trisphosphate (IP_3) receptor and Ca^{2+}-gated ryanodine receptors, which control release of Ca^{2+} from internal stores in the endoplasmic reticulum. Although some gaps in the pathway remain, such as the source of the IP_3 second messenger, this 'relay' mechanism, and use of intracellular Ca^{2+} reserves, is a very plausible way to maintain high cytosolic Ca^{2+} for a long time.

Busch *et al.*[1] went on to examine how tonic activation of these sensory neurons is communicated to downstream interneurons. Given that AQR, PQR and URX are peptidergic[9], the authors monitored neuropeptide secretion in these cells under low and high O_2 conditions with a fluorescently tagged neuropeptide reporter. This reporter continued to be secreted during at least an hour of exposure to 21% O_2, implying tonic release. Although the relevant endogenous neuropeptide(s) is not known, Busch *et al.*[1] found that selective downregulation of neuropeptide processing in the three O_2 sensory neurons strongly reduced high O_2–induced behaviors.

Long-term exposure to high O_2 induces prolonged increases not only in forward speed, but also other behaviors of shorter duration, including a bout of turns and reversals that lasts just 1–2 min after stimulus exposure[7]. How can the tonically active O_2 sensors evoke different behaviors? The AQR, PQR and URX O_2 sensors are unlikely to be functionally equivalent in the worm's highly compact nervous system[4,7,8]. The URX neuron extends its sensory dendrites to the tip of the worm's nose (**Fig. 1**), presumably to detect external stimuli. In contrast, the PQR neuron is located in the tail, with sensory endings that are exposed to body fluid and may sense internal O_2 (ref. 4). By stimulating these distinct sensory neuron types optogenetically or using localized O_2, Busch *et al.*[1] found that activation of URX was sufficient to elicit reversal behaviors, whereas activation of PQR promoted only forward acceleration (the role of AQR was not investigated).

What are the neural mechanisms underlying the differences in the duration of the forward locomotion (long term) and reorientation (short term) behaviors? More detailed analysis of the activation of URX by changing O_2 concentration revealed a transient (phasic) Ca^{2+} response. This short-lived signaling depended on the voltage-gated calcium channel, but, unlike tonic responses, not on the IP_3 or ryanodine receptors. Nevertheless, phasic Ca^{2+} responses also ultimately appear to lead to neuropeptide release. The URX O_2 sensor (and perhaps also AQR and PQR) therefore has the capacity to encode exposure to high O_2 in both short- and long-term neural activity patterns.

URX has several postsynaptic partners, including the AUA and RMG interneurons, to which it is connected by means of chemical and electrical (gap junction) synapses. Busch *et al.*[1] found that exposure to high O_2 promotes tonic Ca^{2+} increases in both these interneurons. For AUA (RMG was not examined), this was dependent, in part, on peptidergic input. Although Busch *et al.*[1] did not explore neural communication by means of gap junctions, it is possible that the distinct temporal signaling properties of URX may feed into the interneuron network differentially through neuropeptide signals and electrical coupling.

To understand why high O_2–evoked reversal behavior is only transient, the authors looked at the network of command interneurons, AVA-AVD-AVE, that direct reversal behavior[10]. These neurons receive synaptic input from all three O_2 sensory neurons, as well as from the AUA and RMG interneurons (**Fig. 1**). Using Ca^{2+} imaging in AVA neurons, Busch *et al.*[1] found only transient Ca^{2+} increases on exposure of worms to high O_2. These observations indicate the occurrence of a transformation from a sustained tonic response (albeit with a phasic component) in the sensory input to a phasic response in a command neuron. The phasic response in AVA correlated well with reversals of the worms, suggesting that this transient activity is the trigger for this

short-lived behavior. The precise locus of this transformation—for example, at synapses between URX and command neurons or between the interneuron RMG and the command neurons—is unknown.

As a pervasive and vital environmental stimulus, O_2 is sensed by *C. elegans* using a suite of guanylate cyclases that are expressed in several different sensory neurons[4,8,11,12]. Some of these receptors, such as those in the BAG neurons that respond to decreases in O_2 concentration, may induce only transient physiological and behavioral responses[8]. Others, as characterized by Busch *et al.*[1], can evoke tonic responses and long-lived behaviors. This latter class defines a powerful *in vivo* model for exploring the physiology, molecular and cellular biology of tonic signaling. Through the course of evolution, most neurons have acquired many feedback mechanisms that tend to 'turn things off' after stimulation, to conserve energy and signaling resources, to avoid damage, and to provide clarity of communication to downstream circuitry. Neurons that are constantly, but reversibly, activated by particular stimuli are therefore likely to use special signaling mechanisms, such as the Ca^{2+} channel relay uncovered by Busch *et al.*[1].

In the vertebrate visual and auditory systems, tonically active photoreceptors and hair cells, respectively, both bear specialized ribbon synapses, which appear to be morphologically specialized for rapid and constant synaptic vesicle release[13]. It should be informative to examine the ultrastructure of the axon terminals in these *C. elegans* O_2-sensing neurons for similar adaptations.

Busch *et al.*[1] also go a long way towards disentangling the transformation of sustained O_2 signals downstream of these tonic sensors to yield temporally separable behavioral responses. Although the mechanisms of these transformations remain to be fully elucidated, their observations highlight how distinct interneurons may independently interpret a common sensory message to produce distinct behaviors. The demonstration that the RMG interneuron is important for persistent behavioral responses to O_2 levels is particularly intriguing. This neuron is one of the 'Chicago O'Hare–like' gap junction hubs of the *C. elegans* nervous system and receives input from pheromone sensors to control sexual and aggregation behaviors[14]. O_2 levels, as continuously reported by RMG activity, may therefore indirectly influence other sensory responses[11]. It will be interesting to explore whether tonic sensors in other animals not only maintain conscious awareness of a particular environmental stimulus—or unconscious homeostatic control of internal conditions—but also have broader influences on an animal's sensory-evoked behaviors.

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Dendritic architecture: form and function

Robyn M Javier & Anatol C Kreitzer

A study now demonstrates how dendritic architecture and differential synaptic innervation can account for functional heterogeneity of dopaminergic neurons in the substantial nigra.

In the nervous system, structure and function are closely intertwined. The visionary anatomist Santiago Ramón y Cajal spent his life exploring the complex architecture of neural circuits, from which he made remarkably accurate predictions about the way these circuits work. Now, more than a century later, neuroscientists continue to explore the relationship between form and function, applying the ever-expanding toolbox of modern biology to address this fundamental question. One area of intensive inquiry is the brain's dopaminergic network, which is involved in diverse behavioral functions, including learning, movement, reward prediction, motivation and attention[1–3]. Dopamine neurons are known to exhibit heterogeneous activity in response to various stimuli and behavioral states[4–6], but the anatomical and electrophysiological properties that give rise to specific individual firing patterns *in vivo* are poorly understood.

In this issue of *Nature Neuroscience*, Henny *et al.*[7] demonstrate the power of analyzing neuronal architecture, synaptic innervation and *in vivo* activity together at the level of single cells. Their results describe a way in which differential structural organization of dendrites can give rise to heterogeneous patterns of dopaminergic neuronal activity in response to specific stimuli, shedding new light on the relationship between form and function in the substantia nigra pars compacta (SNc).

The midbrain dopaminergic system comprises the retrorubral field, SNc and ventral tegmental area (VTA). Dopaminergic neurons respond to many different kinds of information, including rewards, punishment, conditioned cues and alerting signals[1–3]. Henny *et al.*[7] used a brief tail pinch in rats, a salient aversive stimulus that modulates the activity of midbrain dopaminergic neurons[8,9].

A previous study by this same research group characterized the responses in SNc, revealing profound functional heterogeneity[4]. Most cells are unresponsive, but ~20% exhibit transient depression and a small percentage exhibit complex multiphasic responses.

What accounts for these different patterns of activity in response to a single aversive stimulus? Henny *et al.*[7] hypothesized this might be a result of differences in neuronal structure and synaptic innervation. In the previous study, the authors obtained extracellular recordings *in vivo* and then juxtacellularly labeled the cells with neurobiotin. The new study[7] picks up where the previous one left off, using the subset of nigral neurons that could be completely reconstructed and subjecting these cells to thorough follow-up analyses.

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