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Neurobiology: Crowdsourcing CO_2 to Conserve Brain Energy

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Naked mole-rats are adapted to living in a low-oxygen and high-CO_2 environment. Elevated environmental CO_2 inhibits brain activity and has acted as a selection pressure to reduce GABAergic tone, which in turn reduces energetic needs in a hypoxic habitat.

The naked mole-rat (*Heterocephalus glaber*) is a poikilothermic (cold-blooded), exceptionally long-lived rodent — they can reach over 35 years of age. They are of interest to biomedical researchers owing to several special traits: cancer resistance, healthy ageing, unusual pain biology and adaptations to hypoxia and hypercapnia (high CO_2) [1–4]. Sequencing of the naked mole-rat genome [5] has enabled researchers to identify genes of interest to a variety of physiological phenomena exhibited by naked mole-rats, but it is also important to determine the evolutionary pressures that have driven these genetic changes. Naked mole-rats are eusocial, living in subterranean colonies of up to 300 individuals, headed by a single breeding female, who rules over tunnel-digging workers and is the sole female to suckle pups [6]. Owing to colony size and limited gas exchange in complex tunnel systems, it might be expected that naked mole-rats are exposed to a hypoxic and hypercapnic environment. Indeed, naked mole-rats display extreme resistance to hypoxia and hypercapnia — they can survive 18-minutes of anoxia — and display no response to subcutaneous injection of acid [2,3,7]. In a new study in this issue of *Current Biology,* Michael Zions, Daniel McCloskey and colleagues [8] demonstrate that naked mole-rat nests have elevated CO_2 levels, which suppress neuronal activity as an energy-saving adaptation to their environment: tonic CO_2-mediated neuronal inhibition relieves the necessity for a highly active GABAergic system, enabling the naked mole-rat brain to require less oxygen-fuelled energy.

Various mammals show adaptations to a life underground, from the mechanosensory star's tactile fovea of the star-nosed mole (*Condylura cristata*) that replaces sight with touch for finding prey [9], to the evolution of a hypertrophied malleus in many extant golden moles (Chrysochloridae) that likely supports detection of ground vibrations [10]. Naked mole-rats have several adaptations to subterranean life: lack of external ears enables smooth traversing of tunnels, five longitudinally oriented rows of vibrissae run down each side of the body and mediate orientation to mechanical stimuli (Figure 1) [11], and there are numerous factors suggesting adaptation to a hypoxic and hypercapnic environment, such as high O_2 affinity haemoglobin [12], reduced CO_2 affinity of the connexin26 gap junction hemichannel protein [13], ability to withstand prolonged hypoxia and hypercapnia [2,14], and absence of somatic, but not visceral, acid-sensitivity (CO_2 induces tissue acidosis) [3,7,15]. Generally speaking, CO_2 has an inhibitory effect on brain activity [16], and it has been known for almost 100 years that inhalation of

Figure 1. Naked mole-rats.
Top: A dark background highlights one row of sensory vibrissae; every naked mole-rat has 10 rows of body vibrissae in total (photo: Emily Vice). Bottom: Naked mole-rats peer out of a tunnel in a simulated subterranean burrow system at the University of Illinois at Chicago (note the absence of external ears). In their native East Africa, they live in crowded, unventilated tunnels and nest chambers where they likely experience chronic low levels of O_2/high levels of CO_2 (photo: Thomas Park).

raised CO_2 suppresses seizures [17]. Zions and colleagues [8] thus hypothesised that living in a chronic hypercapnic (and thus inhibitory) state may have resulted in positive selection for less energy-greedy gene variants relating to other mechanisms of inhibitory tone within the brain.

The first key finding by Zions and colleagues [8] is that naked mole-rats spend a vast proportion of their time in the nest and that the highest CO_2 levels occur in the nest chamber (maximum >2%, concentrations that other mammals avoid). Do naked mole-rats just put up with hypercapnia as a result of their need to sleep huddled together to conserve heat, or do they actually prefer a hypercapnic environment? When test chambers were infused with differing CO_2 levels, naked mole-rats actively preferred a hypercapnic environment. Indeed, prior data have shown that in a 2-choice test naked mole-rats only show preference for a room air chamber over a CO_2 chamber once 10% CO_2 is reached [2]. If naked mole-rats are adapted to hypercapnia, the authors next investigated how adult animals respond to a surface atmosphere. Remarkably, normal air induced seizures.

Measurement of blood gas parameters suggested that the surface environment-induced seizures were the result of respiratory alkalosis (the blood pH becomes too basic). Naked mole-rats are paedomorphs, maintaining juvenile traits throughout life [4], and neonatal laboratory rats also show susceptibility to alkalosis-induced seizures, which are prevented by inhalation of 5% CO_2, but this phenomenon of alkalosis-induced seizure activity disappears by the third week of life [18]. What then is responsible for this developmental change in rats and can that inform how naked mole-rats behave? The key inhibitory neurotransmitter in the mammalian brain is gamma aminobutyric acid (GABA), which hyperpolarises neurones by binding to the $GABA_A$ receptor, leading to a Cl^- influx. For $GABA_A$ receptors to mediate Cl^- influx, an electrochemical gradient is required to enable entry of Cl^- into the cell. Maintaining ionic gradients in the brain is an energy consuming process, and in the case of Cl^-, the potassium-chloride transporter member 5 (KCC2/SLC12A5) is key, an indirectly active transporter that extrudes Cl^- off the back of the K^+ gradient maintained by the Na^+-K^+-ATPase. In rats and mice, during embryonic development, KCC2 expression increases, resulting in a decrease in the neuronal Cl^- concentration, which enables GABA to exert its hyperpolarising effects, i.e. maturation of GABAergic signalling is what protects adult rats from

alkalosis-induced seizures. Indeed, aberrant KCC2 function is implicated in a wide variety of neurological disorders from epilepsy to neuropathic pain [19]. Could then altered KCC2 and GABAergic signalling underlie the susceptibility of naked mole-rats to alkalosis-induced seizures?

Zions and colleagues [8] found that a KCC2 loss-of-function variant known from humans (R952H) that results in lower KCC2 plasma membrane expression [20] is also present in the naked mole-rat genome. From this, it would be predicted that naked mole-rats would have a diminished ability to extrude Cl^-, which would result in a shift in the reversal potential (the membrane potential where no net Cl^- flow from one side of the membrane to the other occurs) for GABA in the positive direction. When recording responses to GABA in cortical pyramidal neurons, the authors did indeed observe a substantial difference in the reversal potential for GABA in naked mole-rat neurons compared to mice. Moreover, the anticonvulsant drug diazepam, a positive allosteric modulator at certain $GABA_A$ receptors that potentiates GABAergic function, was actually proconvulsant in naked mole-rats in room air, but not in the presence of 5% CO_2.

Zions and colleagues [8] thus propose that the naked mole-rat's hypercapnic environment has acted as selection pressure to reduce GABAergic inhibition and thus save energy in a hypoxic environment where there is less O_2 to make energy. To support this notion, the authors note that the Damaraland mole-rat (*Fukomys damarensis*), the only other known eusocial mammal, also has a KCC2 substitution of R to C, suggestive of impaired Cl^- extrusion.

The study of Zions and colleagues [8] describes a further adaptation to the hypercapnic nesting environment in naked mole-rats, whereby the inhibitory action of CO_2 on brain function has resulted in changes to GABAergic function that reduce energy consumption of the naked mole-rat brain. Furthermore, this study provides the basis of several future investigations: is naked mole-rat KCC2 responsible for the altered GABA reversal potential, i.e. does it show low plasma membrane expression, or is the R952H variant compensated for by other factors? Considering their smaller colony size (10 to 40 animals/colony and homeothermic (warm-blooded) nature, do Damaraland mole-rats show similar susceptibility to simulated surface-induced convulsions? Is the altered GABAergic function common to the entire naked mole-rat nervous system, i.e. do naked mole-rats have an unusual neuropathic pain phenotype considering the correlation of reduced KCC2 with neuropathic pain and therapeutic agents that target KCC2 reversing the pain phenotype [19]? Using CO_2 that naked-mole rats produce themselves to conserve brain energy: what a clever evolutionary trick!

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