African Naked Mole-Rats Demonstrate Extreme Tolerance to Hypoxia and Hypercapnia

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Photo Credit: Thomas Park

Abstract

Naked mole-rats are extremely tolerant to low concentrations of oxygen (hypoxia) and high concentrations of carbon dioxide (hypercapnia), which is consistent with the environment that they inhabit. Naked mole-rats combine subterranean living with living in very densely populated colonies where oxygen becomes depleted and carbon dioxide accumulates. In the laboratory, naked mole-rats fully recover from 5 hours exposure to 5% O_2 and 5 hours exposure to 80% CO_2, whereas both conditions are rapidly lethal to similarly sized laboratory mice. During anoxia (0% O_2) naked mole-rats enter a suspended animation-like state and switch from aerobic metabolism of glucose to anaerobic metabolism of fructose. Additional fascinating characteristics include that naked mole-rats show intrinsic brain tolerance to anoxia; a complete lack of hypoxia-induced and CO_2-induced pulmonary edema; and reduced aversion to high concentrations of CO_2 and acidic fumes. Here we outline a constellation of physiological and molecular adaptations that correlate with the naked mole-rat's hypoxic/hypercapnic tolerance and which offer potential targets for ameliorating pathological conditions in humans, such as the damage caused during cerebral ischemia.

Glossary:

Anoxia: absence of oxygen or extremely little oxygen.

Hypercapnia: excessive carbon dioxide.

Hypoxia: less than adequate oxygen supply to tissues.

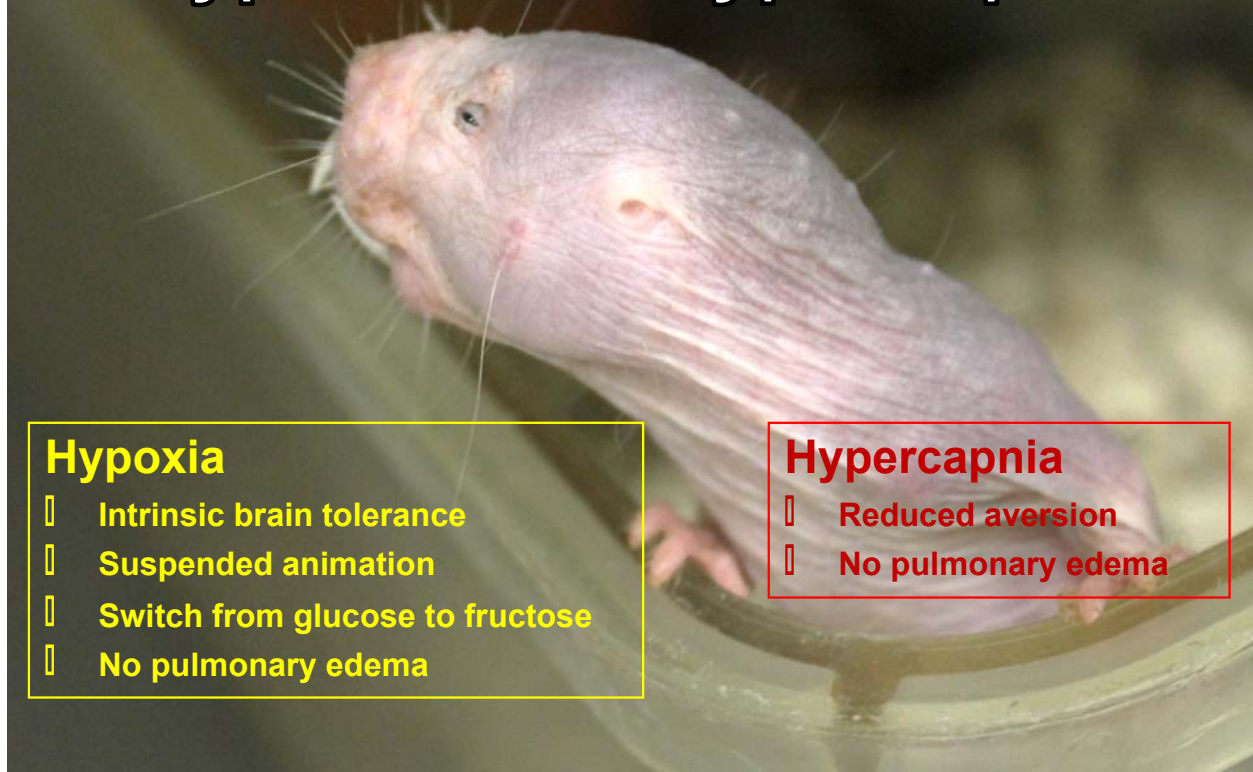Ischaemia / Ischemia: inadequate blood supply.

Figure 1. Summary of the characteristics that make naked mole-rats tolerant to hypoxia and hypercapnia. Photograph by Thomas Park.

Tolerance to hypoxia and anoxia

The naked mole-rat (*Heterocephalus glaber*) shows robust tolerance to both low concentrations of oxygen (O_2) and high concentrations of carbon dioxide (CO_2) (Larson and Park, 2009; Park, et al, 2017). We suggest that these tolerances and their underlying mechanisms, which are detailed below, are adaptations to living in a chronically hypoxic/hypercapnic environment where many individuals share the same limited air supply resulting in low concentrations of O_2 and high concentrations of CO_2 (Bennett and Faulkes, 2000). **Figure 1** provides the highlights of the findings presented in this chapter.

In the laboratory, we found that naked mole-rats tolerate severe hypoxia (5% O_2) for at least 5 hours with no notable effect, whereas 5% O_2 is lethal to similarly sized laboratory mice (*Mus musculus*) in less than 15 minutes (**Figure 2A**). Similarly, We also tested naked mole-rats and mice in an even more extreme challenge: 0% O_2 (anoxia). The procedure we followed was to monitor breathing and terminate the exposure when 60 seconds passed without a breath for the naked mole-rats, and when 20 seconds passed for mice. Both species became unconscious in less than a minute. However, the last breath for naked mole-rats was, on average, 250 seconds after entering 0% O_2 whereas the last breath for mice was, on average, a much shorter 46 seconds (**Figure 2B**). Another striking species difference was that all of the naked mole-rats recovered and were returned to their colonies, whereas none of the mice recovered.
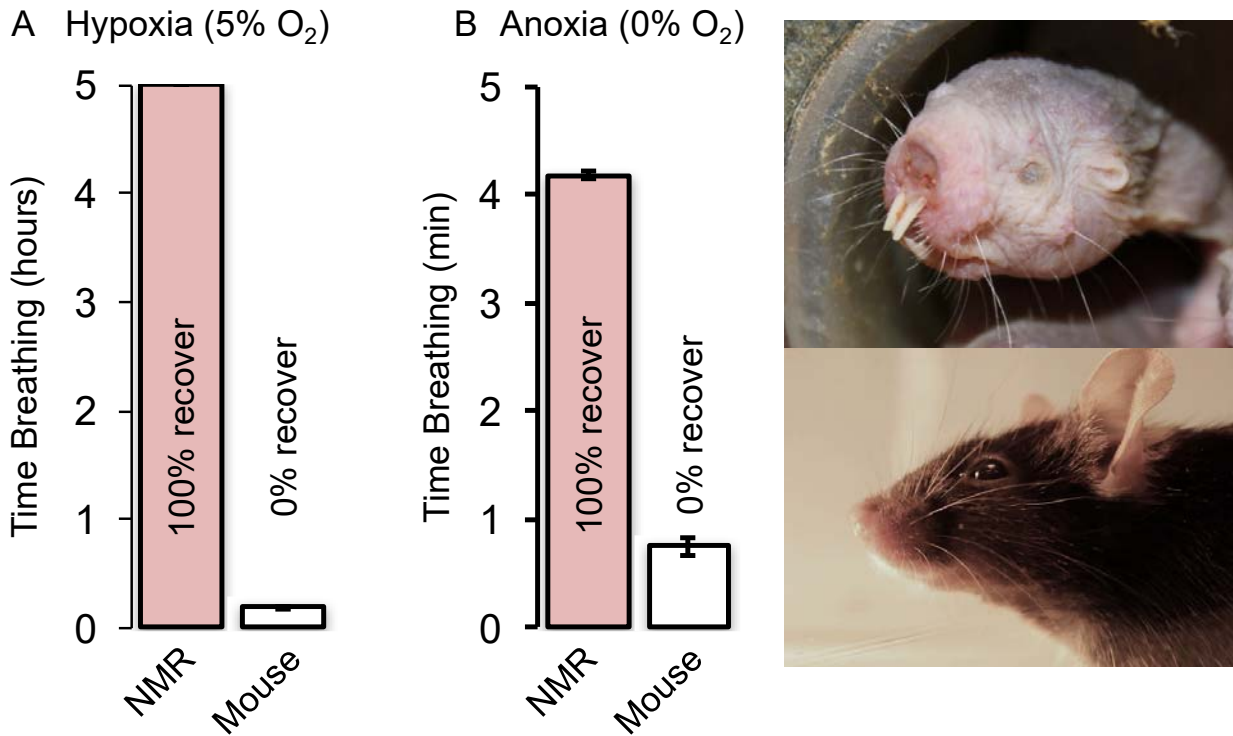
Figure 2. Extreme hypoxia (5% O_2) and anoxia (0% O_2) resistance in naked mole-rats. **A**. Animals were placed into an atmosphere chamber that was pre-filled with 5% O_2 balanced with nitrogen. The bars indicate the average duration in hours until breathing ceased or until the experiment was arbitrarily terminated after 5 hours. N = 4 animals for each group. Time to cessation of breathing was significantly shorter for mice compared to naked mole-rats (t-test, t=563 (df=6), p<.0001). Also, after re-exposure to room air, all naked mole-rats recovered, but none of the mice recovered. **B**. Animals were placed into an atmosphere chamber that was pre-filled with 0% O_2 (100% nitrogen). The bars indicate the average duration in minutes until breathing ceased. N = 4 animals for each group. Time to cessation of breathing was significantly shorter for mice compared to naked mole-rats (t-test, t=112.53 (df=6), p<.0001). Also, after re-exposure to room air, all naked mole-rats recovered, but no mice recovered. Photos on the right depict a naked mole-rat and a mouse. NMR = naked mole-rat. These data were originally published in Park, et al, 2017.

In a different experimental protocol, we exposed naked mole-rats and mice to 0% O_2 for a variety of fixed durations (6, 10, 18, and 30 minutes) while we monitored breathing and heart rates. The groups of naked mole-rats exposed for 6, 10, and 18 minutes recovered, whereas the group exposed for 30 minutes did not. Data from the group exposed for 18 minutes are shown in **Figure 3**. The atmosphere chamber that we used to test the animals is shown in **Figure 3A**. The naked mole-rats showed a rapid and dramatic decrease in both breathing rate (**Figure 3B**) and heart rate (**Figure 3C**), with heart rate remaining remarkably invariant for the duration of the exposure. In contrast, the mice showed a steady decline in heart rate which became undetectable after 6 minutes (black curve in **Figure 3C**) and breathing ceased in less than a minute.
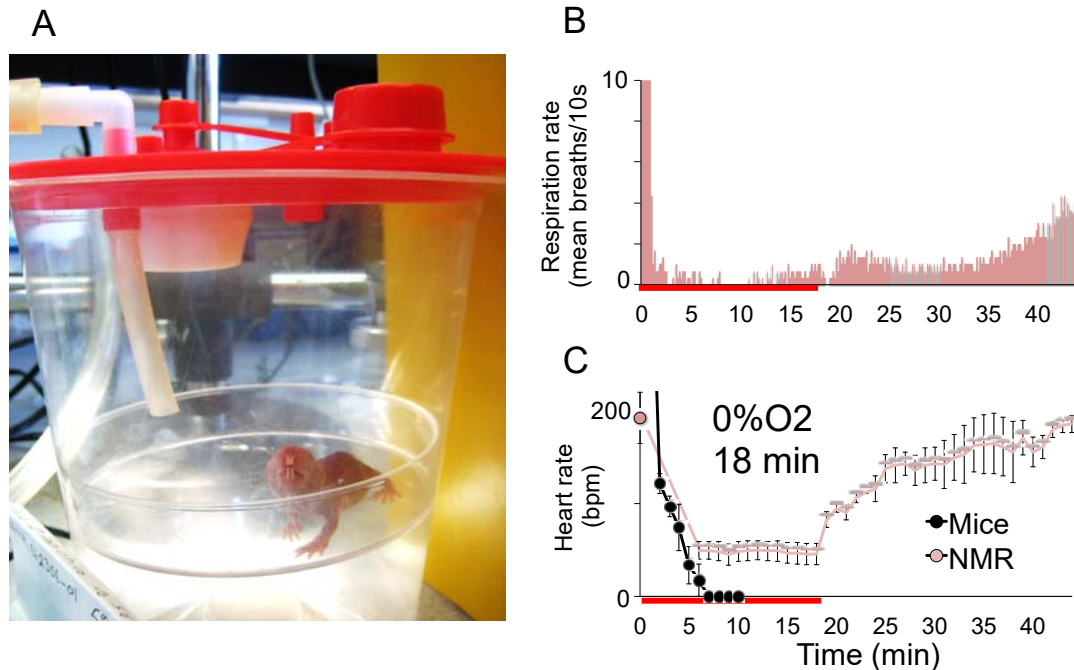
Figure 3. Effects of anoxia on respiration rate and heart rate in mice and naked mole-rats. A. Photograph showing a naked mole-rat standing in an atmosphere chamber where gas mixtures were delivered. B and C. During 18 minutes exposure to anoxia, respiration rate and heart rate were drastically reduced in naked mole-rats. The red bar on the x axis of B and C indicates when the animals were exposed to anoxia. During anoxia, the heart of the naked mole-rats continued to beat (pink curve in C), while the heart rate of the mice (black curve in C) declined rapidly to the level of noise. N=3 naked mole-rats and 4 mice. After re-exposure to room air, all of the naked mole-rats recovered, but none of the mice did. These data were originally published in Park, et al, 2017.

Next, we used electroencephalography (EEG) to measure brain activity before, during, and after a 10-minute bout of anoxia. We found that anoxia resulted in a severe reduction in the average amplitude of the EEG recordings (**Figure 4**). The dramatic reduction in brain activity, in addition to the robust reduction in respiration rate and heart rate (**Figure 3**), suggests that naked mole-rats enter a suspended animation-like state (Blackstone, et al, 2005; Blackstone and Roth, 2007) during anoxia, which would greatly reduce energy demand. This hypothesis is supported by Pamenter, et al (2018) who demonstrated that naked mole-rats can suppress their metabolism by up to 85% in acute severe hypoxia.
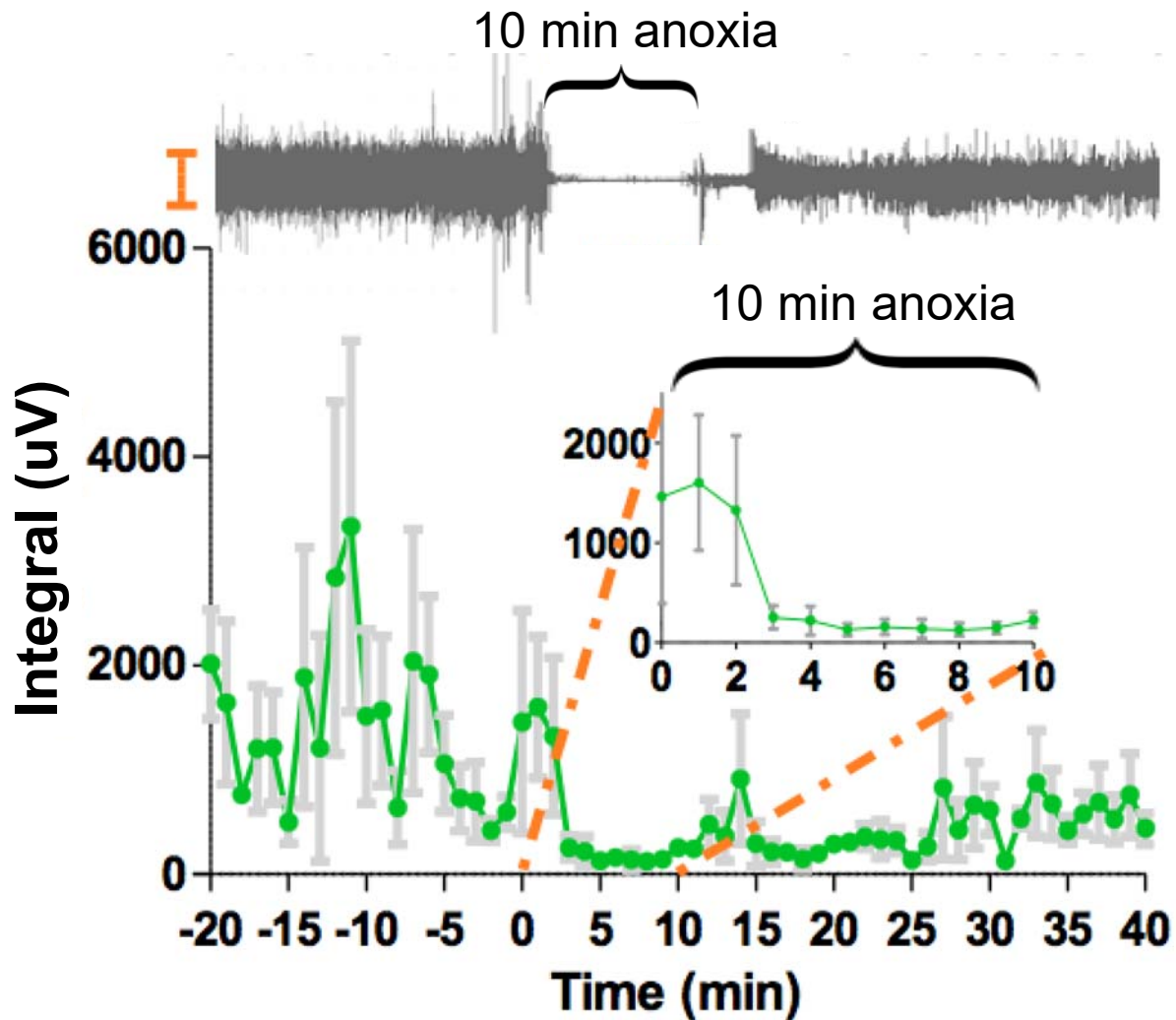
Figure 4. Effects of anoxia on EEG recordings from naked mole-rats. The trace at the top is an example from one animal. The vertical calibration bar corresponds to 600 microvolts. The graph below shows the average integral over time. Anoxia began at time 0. The inset expands the data for the 10 minutes of anoxia. n=3. For each animal, two recording electrodes were stereotaxically positioned into the caudate putamen. Recordings were acquired using a 4 channel Pinnacle Technolog EEG system.

In the *in vivo* experiments described above, the naked mole-rats showed robust tolerance to oxygen deprivation compared to mice. Another hypoxia-related phenomenon displayed by naked mole-rats is intrinsic brain tolerance to hypoxia *in vitro* (Larson and Park, 2009, Larson, et al, 2014). To assess intrinsic brain tolerance, we used the hippocampal brain slice paradigm. We measured time to anoxic depolarization (loss of all electrical activity) after switching from 95% O_2 to nominal 0% O_2 in the slice recording chamber. Slices from naked mole-rats maintained synaptic function during anoxia for significantly longer than slices from mice (**Figure 5**).
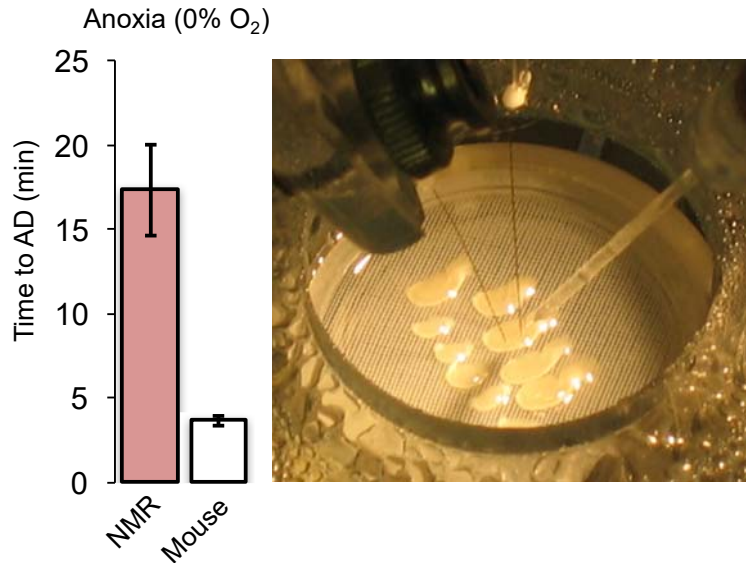
Figure 5. Naked mole-rat hippocampal neurons are resistant to anoxic depolarization. The gas mixture supplying the slice chamber was switched from 95% O_2 / 5% CO_2 to 95% nitrogen / 5% CO_2. The graph indicates the average duration in minutes to anoxic depolarization (AD). N = 11 slices from naked mole-rats and 36 slices from mice. Slices from mice showed AD significantly faster than slices from naked mole-rats (t-test, t=8.85 (DF=45), p<.0001). These data were originally published in Larson and Park, 2009, but this graph includes additional data collected since the original publication. The photograph shows slices in the recording chamber. Photograph by Thomas Park.

Tolerance to hypercapnia

In their home environment, naked mole-rats are not only challenged by hypoxic conditions, but also by hypercapnic conditions. To assess global, *in vivo* tolerance to chronic hypercapnia in naked mole-rats and mice, we exposed them to a mixture of 80% CO_2 and 20% O_2 for 5 hours (hypercapnia without hypoxia). The naked mole-rats tolerated and recovered from this exposure with no overt issue, whereas the mice died, on average, in under 5 minutes (**Figure 6**).
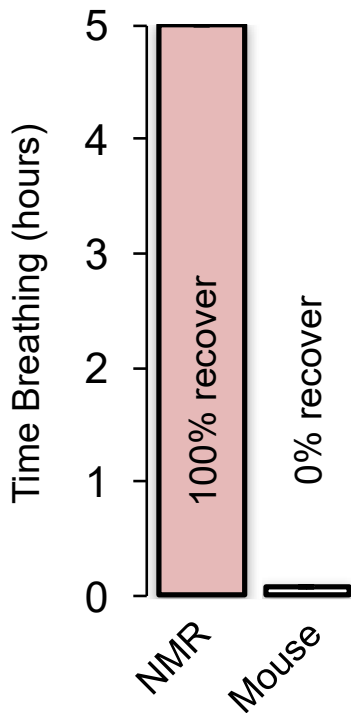
Hypercapnia (80% CO$_2$)

Figure 6. Naked mole-rats are resistant to hypercapnia. Animals were placed into an atmosphere chamber that was pre-filled with 80% CO$_2$ and 20% O$_2$. The bars indicate the average duration in hours until breathing ceased or until the experiment was arbitrarily terminated at 5 hours. N = 4 animals for each group. Time to cessation of breathing was significantly shorter for mice compared to naked mole-rats (t-test, t=980.73 (df=6), p<.0001). Also, after re-exposure to room air, all naked mole-rats recovered, but none of the mice recovered. These data were originally published in Park, et al, 2017.

Lack of pulmonary edema from hypoxia or hypercapnia

   In humans and laboratory mice and rats, both hypoxia and hypercapnia cause pulmonary edema, (Bärtsch, et al, 2005; Sitkovsky, et al, 2004; Guais, 2011; Pritchett, et al, 2005). In contrast, naked mole-rats do not develop either hypoxia- or hypercapnia-induced pulmonary edema (Park, et al, 2017). To assess hypoxia-induced pulmonary edema, we exposed naked mole-rats and mice to low O$_2$ concentrations for 15 minutes and then determined the wet-to-dry ratio of the lungs post-mortem at the end of the experiment. Naked mole-rats showed a complete absence of pulmonary edema following exposure to low O$_2$, whereas the mice showed severe pulmonary edema for O$_2$ concentrations below 10% (**Figure 7A**). To assess CO$_2$-induced pulmonary edema, we measured lung wet-to-dry ratios after exposing animals to a high concentration of CO$_2$ for 15 minutes. As for hypoxia, naked mole-rats showed no pulmonary edema following exposure to any CO$_2$ concentrations they were exposed to, whereas mice showed robust pulmonary edema from exposure to all concentrations of CO$_2$ over 15% (**Figure 7B**).

A Hypoxia

Pulmonary edema (% increase in W/D ratio) vs % O_2 (20, 10, 7.5, 5). Mouse and NMR curves.

B Hypercapnia

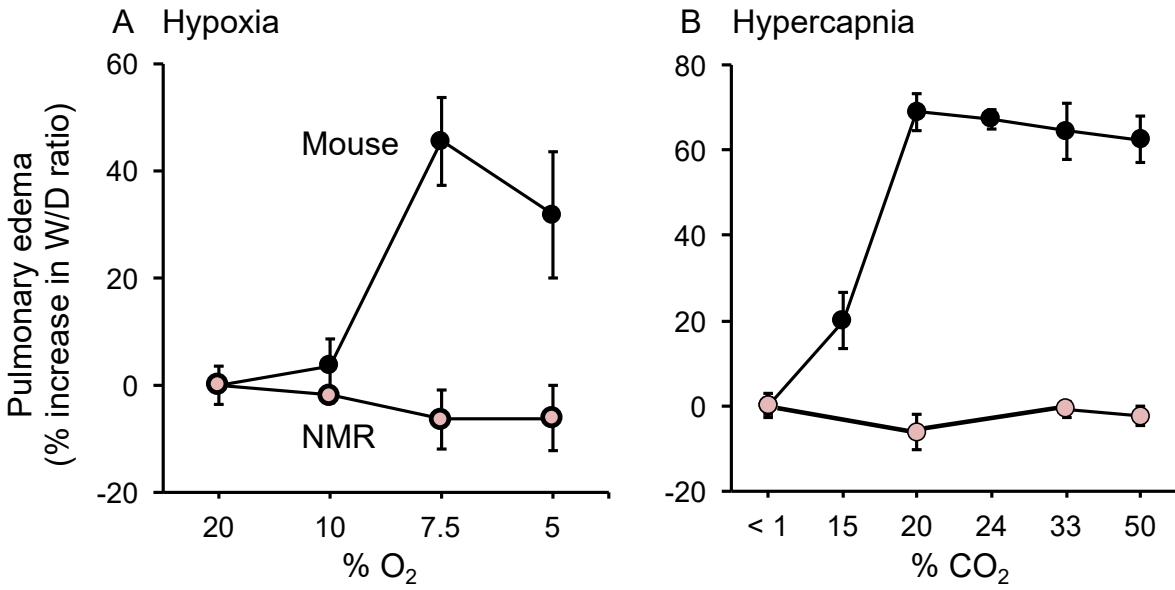Pulmonary edema vs % CO_2 (< 1, 15, 20, 24, 33, 50).

Figure 7. Naked mole-rats do not develop hypoxia-induced or hypercapnia-induced pulmonary edema. Edema was assessed by calculating the wet lung weight-to-dry lung weight ratio. The curves represent percent increase from baseline. N = 3-5 animals for each concentration. A. Hypoxia-induced pulmonary edema. For 7.5% O_2, mouse lung tissue showed significantly higher wet-to-dry ratio values than naked mole-rat lung tissue (t=4.84 (df=5), p<.001). This was also the case for 5% O_2 (t=2.94 (df=6), p<.05). B. CO_2-induced pulmonary edema. For 20% CO_2, mouse lung tissue showed significantly higher wet-to-dry ratio values than that of naked mole-rats (t=12.03 (df=5), P<.0001). This was also the case for 33% CO_2 (t=8.35 (df=5), P<.001) and for 50% CO_2 (t=11.05 (df=4), P<.001). These data were originally published in Park, et al, 2017.

<u>Putative Adaptations for Hypoxia Tolerance</u>

Naked mole-rats display a number of biological features that may be adaptations that contribute to this species' hypoxia tolerance. The resting metabolic rate of naked mole-rats is about two-thirds that of similarly sized mammals (Buffenstein and Yahav; 1991), meaning that they require less oxygen-derived energy to maintain normal function. Much of that saving is due to lack of thermogenesis in this species (Buffenstein and Yahav; 1991). In addition, hemoglobin in the blood of naked mole-rats has an unusually high affinity for oxygen (Johansen, et al, 1976), meaning that naked mole-rat hemoglobin can achieve a higher percentage of saturation in low O_2 concentrations compared to most other mammals.

As shown in **Figure 5**, the brain of the naked mole-rat shows intrinsic hypoxia tolerance (Larson and Park, 2009). Hippocampal brain slices from naked mole-rats maintain synaptic function under hypoxia and anoxia for much longer than other mammals that have been tested (Larson, et al, 2014). Part of the intrinsic tolerance is likely associated with retention of the glutamate N-methyl-D-aspartate (NMDA) receptor subunit GluN2D in adulthood. In contrast to other glutamate receptors, NMDA receptors are highly Ca^{2+} permeable and during periods of hypoxia, a lack of oxygen results in decreased ATP production and a concomitant membrane depolarization causing glutamate release (and impaired glutamate reuptake), as well as relieving NMDA receptors from magnesium ion block. Consequently, NMDA receptors are a key source of neuronal excitotoxicity during hypoxic events, such as during stroke (Wu and Tymianski, 2018). NMDA receptors are tetrameric ion channels and the GluN2D subunit is inhibited by hypoxia (Bickler, et al, 2003; Peterson, Park and Larson, 2012), which can thus suppress NMDA-mediated

excitotoxicity. In most mammals, the GluN2D subunit is prevalent in neonates, but its expression decreases precipitously during maturation (Laurie, et al, 1997). Indeed, we observed that adult mouse brains only retain about 13% of neonatal levels of GluN2D, whereas adult naked mole-rat brains retain about 66% of neonate levels (**Figure 8A**). Therefore, we propose that the maintained high level of GluN2D in naked mole-rat brain provides resistance to hypoxia-mediated, NMDA receptor-induced excitotoxicity.

Consistent with retention of high levels of GluN2D in adult naked mole-rat brain, hippocampal brain slices from adult naked mole-rats show substantially less intracellular calcium accumulation during hypoxia compared to laboratory mice (Peterson, Larson, et al, 2012). Hypoxia-induced intracellular calcium accumulation is associated with neuronal injury and cell death (Deshpande, et al, 1987; Lee, et al, 1991; Bickler, 2004). We observed that slices from both mice and naked mole-rats responded to hypoxia with a decrease in fluorescence, corresponding to an increase in intracellular Ca^{2+} (**Figure 8B**). Also, whereas in both mice and naked mole-rats, more Ca^{2+} accumulated in slices from older animals, slices from naked mole-rats still accumulated significantly less Ca^{2+} than the slices from mice regardless of age.
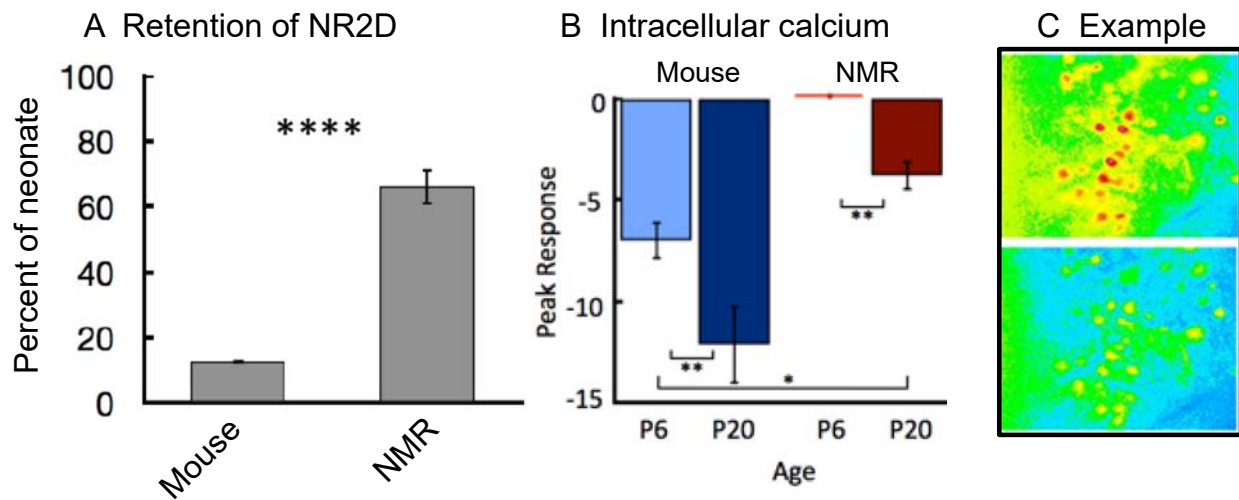

Figure 8. Retention of the GluN2D NMDA receptor subunit in adult naked mole-rat brains. A. Graph showing the percentage of GluN2D retained in adult for mice and naked mole-rats compared to neonatal levels. The raw data was optical density of immunoreactive bands in hippocampal slices from neonates and adults. N=4 neonates and 4 adults for each species. The graph is from Peterson, Park and Larson, 2012. B. Peak intracellular Ca^{2+} accumulation from a 10-minute exposure to hypoxia in hippocampal slices from p6 and p20 mice and naked mole-rats. The raw data were measurements of relative internal cytosolic calcium indicated by fura-2 fluorescence emission. N=10-14 slices from 3-6 animals for each group. C. Example fluorescence images from a mouse before hypoxia (top) and during hypoxia (bottom) Quenching in the bottom image indicates an increase in intracellular Ca^{2+}. B and C are from Peterson, Larson, et al, 2012.

During hypoxia, oxygen depletion forces the brain into generating ATP from anaerobic glycolysis, which produces lactic acid as a by-product. Consequently, alongside glutamate-mediated excitotoxicity, a process called acidotoxicity occurs due to the activation of Ca^{2+}-permeable acid-sensing ion channel 1a (ASICa) (Xiong, et al, 2018). There are 6 different ASIC subunits, which show similar distribution throughout the brain of naked mole-rats and mice (with the exception of a virtual absence of the acid-insensitive ASIC4 subunit) (Schuhmacher and

Smith, 2016). However, hippocampal and cortical neurons from naked mole-rats have much smaller ASIC-mediated currents compared to mice and cortical neurons show resistance to acidotoxicity (Husson and Smith, 2018), i.e. reduced ASIC function provides resistance to the impact of acidosis during periods of hypoxia.

An additional adaptation that contributes to the naked mole-rat's anoxia tolerance involves a switch from aerobic metabolism of glucose to anaerobic metabolism of fructose (Park, et al, 2017; **Reznick, et al, 2021**). The evidence for this remarkable adaptation is as follows. Under anoxia, concentrations of fructose increased dramatically in the blood and organs of naked mole-rats, but not mice (**Figure 9**). Importantly, cells in naked mole-rat organs have higher expression levels of the fructose transporter, GLUT5 (**Figure 10A**) which transports fructose into cells, and significantly higher levels of the enzyme ketohexokinase (**Figure 10B**), which converts fructose into fructose-1-phosphate that can enter glycolysis. Thus, under anoxia, it appears that naked mole-rats release fructose into their blood and organs, and they have the molecular machinery in place to pump the fructose into their cells and convert it into an intermediate that can be used to make energy.
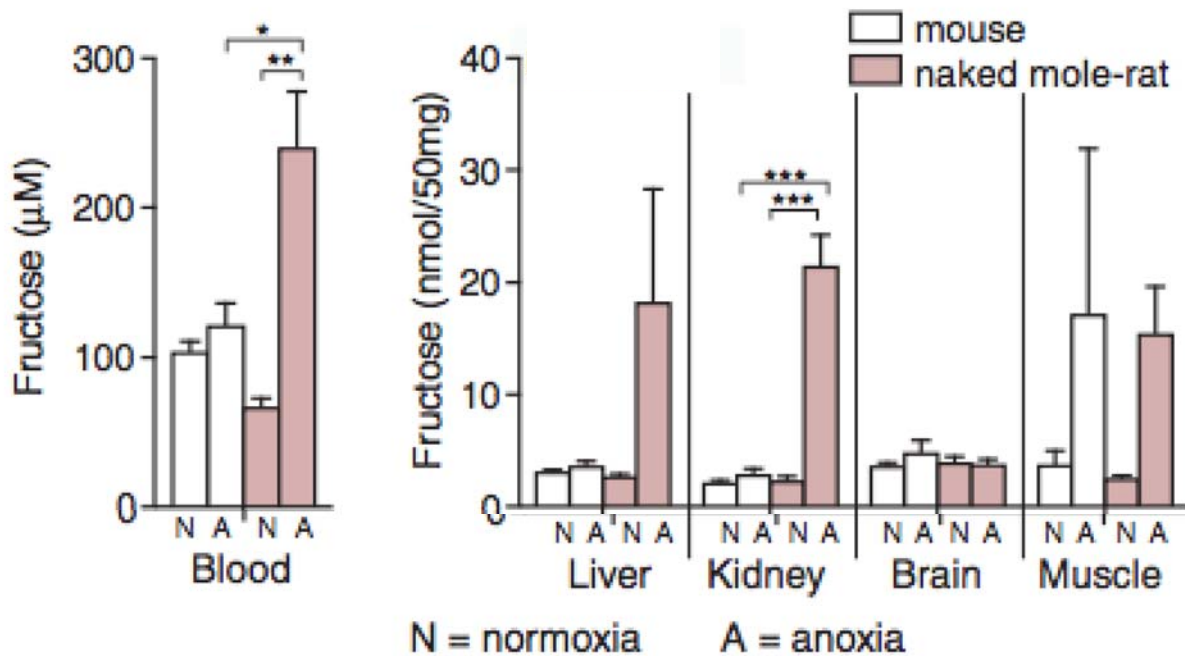

Figure 9. Concentrations of fructose in the blood and organs of mice and naked mole-rats. Exposed to normoxia versus 10 minutes of anoxia. These data were originally published in Park, et al, 2017.
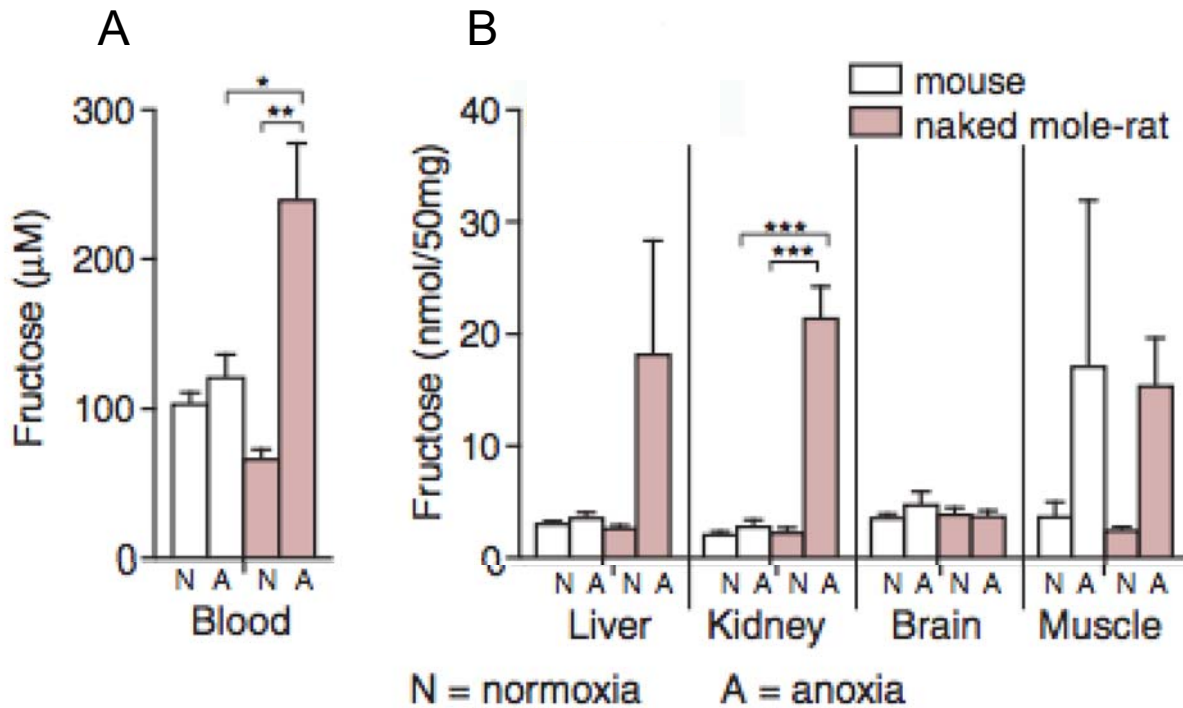
Figure 10. Naked mole-rat cells show multiple adaptations for utilizing fructose. A, the fructose transporter, GLUT5 is expressed at higher levels in all naked mole-rat organs compared to mice, other than in the kidney. B, ketohexokinase (KHK-A) can metabolize fructose into fructose-1-phosphate and is expressed at significantly higher levels in multiple organs of naked mole-rats compared to mice. Mouse data is depicted with white bars and naked mole-rat data is depicted with pink bars. These data were originally published in Park, et al, 2017.

The advantage of the fructose pathway is that it can drive glycolysis in the absence of oxygen. In contrast, the glucose pathway is blocked during anoxia due to the generation of protons as a by-product of glycolysis inhibiting phosphofructokinase, which is essential for converting fructose-6-phosphate to fructose-1,6-bisphosphate (**Figure 11A**). However, fructose metabolism in the absence of oxygen generates much less ATP compared to glucose metabolism in the presence of oxygen. And this may be the driving force behind the naked mole-rat entering a suspended animation-like state during anoxia (**Figures 3&4**): to reduce and match energy use with reduced energy production. Presumably, only a small amount of energy is needed to maintain this suspended animation state under anoxia.

To demonstrate that naked mole-rat brain cells could actually use fructose, we incubated brain slices in a solution that included [13]carbon-labeled fructose. Then we exposed the slices to hypoxia and measured the quantity of [13]carbon in several glycolysis intermediates. Most of the intermediates that we sampled showed significantly larger increases in [13]carbon in slices from naked mole-rats compared to slices from mice (**Figure 11 B,C**).
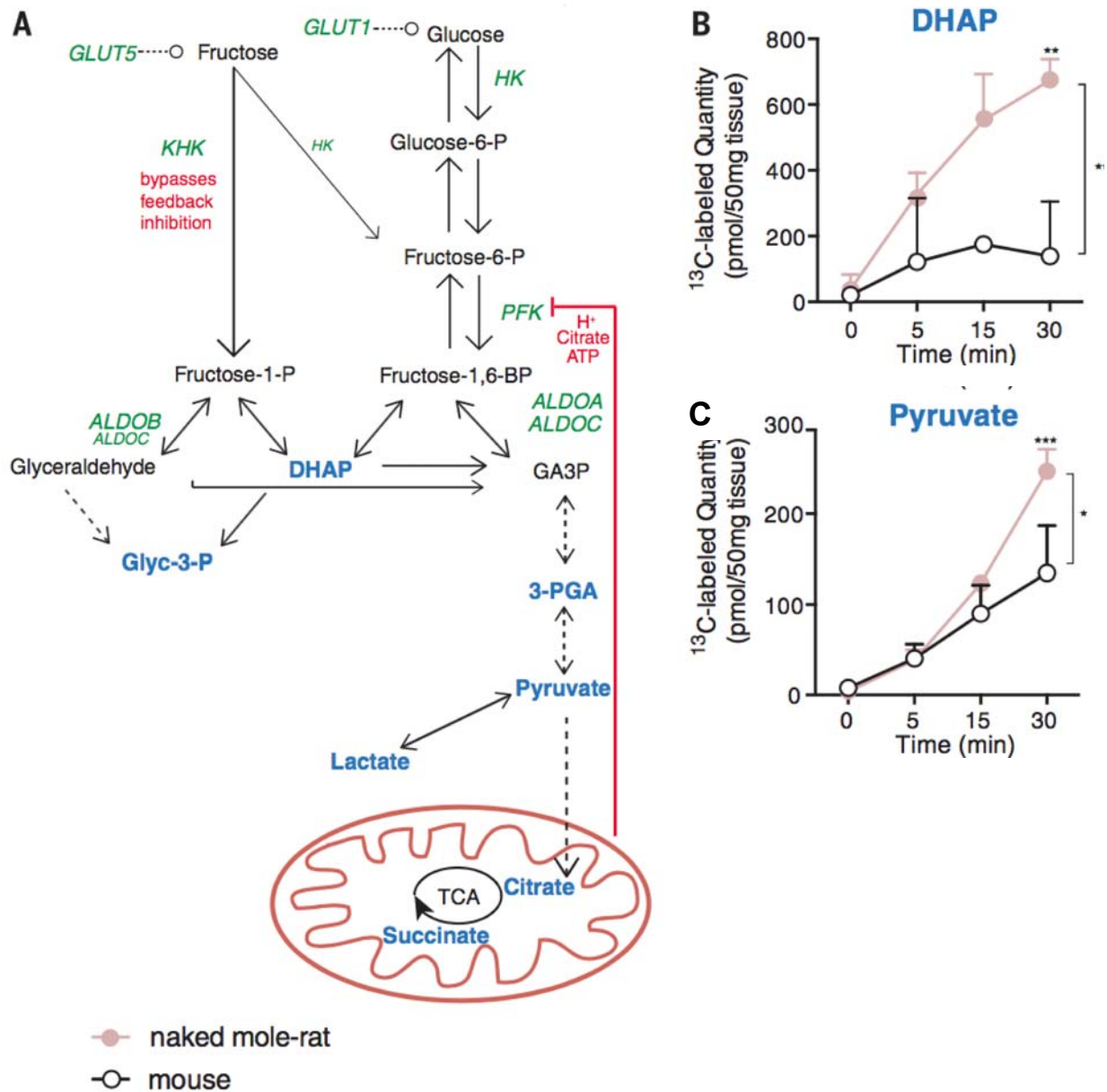
Figure 11. Utilization of fructose by naked mole-rat brains. (A) Model of the glycolysis pathway showing how fructose enters cells via the GLUT5 transporter and is converted into fructose-1-phosphate by ketohexokinase (KHK) which enters the glycolysis pathway; note the end-production-inhibition of phosphofructokinase by protons, which prevents continuous glucose fueled glycolysis. (B) Data from brain slices that were incubated with carbon 13 labeled fructose and then exposed to 5% O2 for 5, 15, or 30 minutes. For the two example intermediates (DHAP and Pyruvate) there was significantly more [13]carbon accumulation during hypoxia in slices from naked mole-rats compared to slices from mice, indicating that naked mole-rat cells can incorporate more fructose-derived carbons compared to mice. These data were originally published in Park, et al, 2017.

Next, we tested the ability of hippocampal brain slices and isolated hearts from naked mole-rats and mice to function when we switch from glucose to fructose in the bath solutions. Brain slices from naked mole-rats showed less decline and better recovery from application of fructose compared to slices from mice (**Figure 12A**). Isolated hearts from naked mole-rats showed virtually no decline in function, whereas hearts from mice showed a substantial decline, particularly during a second application of fructose (**Figure 12B**). These data support the hypothesis that naked mole-rat cells are adapted to utilize fructose as an energy source to maintain cellular function during periods of hypoxia.

Intriguingly, Farhat, et al (2020) showed that, under chronic hypoxia (11% O_2 for 4 weeks), naked mole-rats brain tissue showed a significant and substantial decrease in metabolic enzyme activity and brain sodium/potassium-ATPase activity. These results suggest a strategy for energy conservation during oxygen deprivation: naked mole-rat cells can make adjustments to match metabolic need with energy production.
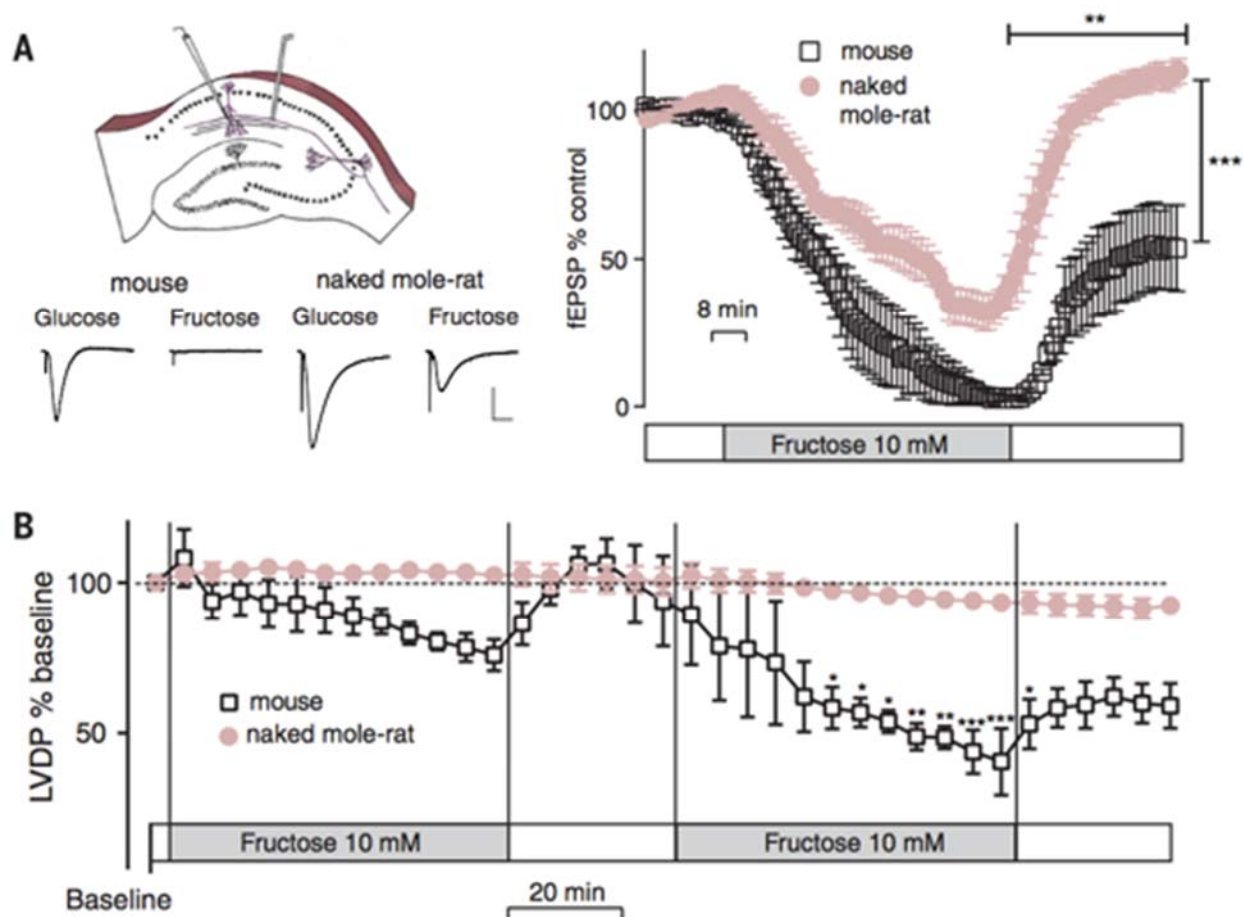

Figure 12. Brain slice and isolated heart function when glucose was replaced with fructose in naked mole-rats, but not in mice. (A) Drawing of the hippocampal slice preparation (left) and evoked potential data from naked mole-rats and mice before, during, and after switching from glucose to fructose in the bath solution. (B) Cardiac function data from isolated hearts from naked mole-rats and mice before, during, and after switching from glucose to fructose in the bath solution twice in series. Abbreviations: fEPSP – field Excitatory Postsynaptic Potentials; LVDP - Left Ventricular Developed Pressure. These data were originally published in Park, et al, 2017.

Putative Adaptations for Hypercapnia Tolerance

There are also several underlying features that have been identified as potential adaptations that contribute to this species' hypercapnia tolerance. A major pathological issue associated with breathing high concentrations of CO_2 is the resulting acidosis that triggers pulmonary edema (Lee and Pisarri, 2001; Russell, et al, 1984; Gourine, 2005; Park, et al, 2017). The blood of naked mole-rats is however exceptionally efficient at buffering acid (Johansen, et al, 1976; Park, et al, 2017).

Also, there are several interesting features of the sensory cells in naked mole-rats that normally detect and respond to acidosis. These unmyelinated peripheral nerve fibers are called C fibers. Firstly, naked mole-rats have far fewer cutaneous C fibers than many other mammals, including several other African mole-rat species (Smith et al, 2012). Secondly, the C fibers of naked mole-rats lack the neuropeptides Substance P and calcitonin gene related peptide (Park, et al, 2003; Park et al, 2008), which, in other mammals are released during acidosis of the lungs and are thought to contribute to pulmonary edema (Germonpré, et al, 1995). Third, the voltage-gated sodium channel $Na_V1.7$ on the C fibers of naked mole-rats has an amino acid variation that results in enhanced $Na_V1.7$ inhibition preventing action potential generation in the presence of acid, even though acid sensors (e.g. TRPV1 and ASICs) are functional, i.e. acid acts like an anesthetic of sensory neurones (Smith, et al, 2011). As a result, naked mole-rats not only show a lack of CO_2-induced pulmonary edema (**Figure 7B**), but they also show reduced avoidance to CO_2 **(Figure 13A)** and air borne acetic acid fumes (**Figure 13B**) and virtually no response to foot injection of acidic saline (**Figure 13C**) compared to mice (Park et al, 2008; Eigenbrod, et al, 2019). See **Lewin, et al (2021)** for details on the pain biology of the naked mole-rat.
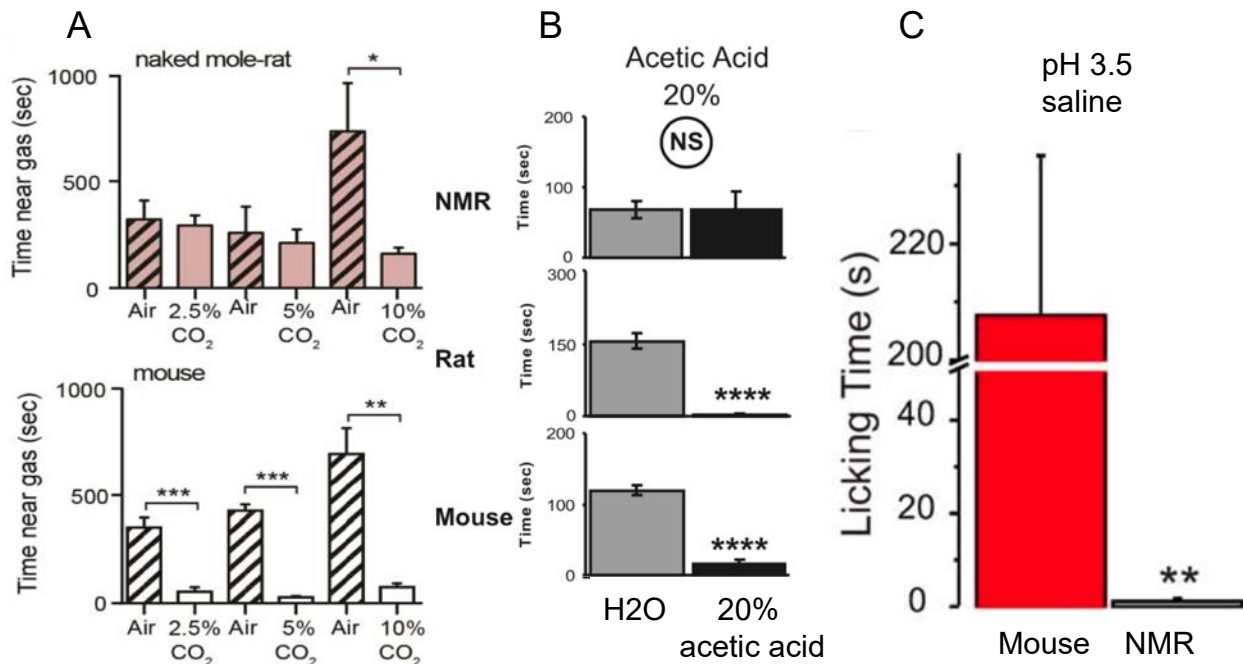

Figure 13. Naked mole-rats show reduced sensitivity to CO_2 and complete insensitivity to acetic acid fumes and injection of acetic saline. (A) Avoidance to CO_2 was tested in a chamber where one end was infused with room air and the other end with CO_2 (either 2.5%, 5%, or 10%). We recorded the time spent near each end as a metric of avoidance. Mice spent relatively little time near the CO_2 end of the chamber compared to the room air end for each concentration of CO_2. Naked mole-rats only avoided the highest concentration. These data were originally published in

Park, et al, 2017. (B) A similar behavioral test using 20% acetic acid fumes showed strong avoidance in laboratory rats and mice but not naked mole-rats. These data were originally published in LaVinka and Park, 2012. (C) Mice injected in the foot with acidic saline show robust licking at the injection site indicative of pain but naked mole-rats show virtually no licking. These data were originally published in Park, et al, 2008.

Discussion

In summary, naked mole-rats have evolved to thrive in an environment that would be deadly to many other mammals: an environment characterized by chronically low concentrations of O_2 and chronically high concentrations of CO_2. In laboratory experiments, naked mole-rats show remarkable tolerance to both hypoxia and hypercapnia. A constellation of putative adaptations has been identified in association with their tolerances. For hypoxia tolerance: intrinsic brain tolerance to hypoxia, the ability to enter a low-energy, suspended animation-like state, and the ability to use anaerobic fructose metabolism. Hypercapnia tolerance is provided for by peripheral nerve fibers that do not respond to acidosis from CO_2, which would usually be associated with pain and pulmonary edema.

We previously proposed that naked mole-rats achieve at least part of their tolerance by retaining some neonatal characteristics (Larson and Park, 2009; Peterson, Larson, et al, 2012; Peterson, Park and Larson, 2012; Penz et al, 2015; Orr, et al, 2016). It has been known for some time that neonate mammals are tolerant to hypoxia and hypercapnia (Bickler, et al, 2003; Kirschbaum and DeHaven, 1968; Pritchett, et al, 2005). Naked mole-rats and neonatal mice share a common brain characteristic associated with intrinsic hypoxia tolerance in the form of elevated levels of the NMDA GluN2D subunit (Peterson, Park and Larson, 2012), which is switched off by hypoxia and thus limits Ca^{2+} entry (Peterson, Larson, et al, 2012) and calcium toxicity (Bickler, et al, 2003). In addition, naked mole-rat hippocampal and cortical neurons also display smaller acid-gated currents mediated by ASICs, which provides resistance to acidotoxicity (Husson Z and Smith, 2018) in response to hypoxia generated acidosis.

Tolerance to CO_2-induced pulmonary edema (**Figure 7**) is likely related to differential functioning of the sensory nerves in the lungs that normally detect acidosis and trigger edema. This population of sensory nerves are C fibers that come from the spinal nerves and the vagus nerve. Normally these nerve fibers respond to acidosis by releasing neuropeptides that trigger vasodilation and bronchoconstriction, resulting in pulmonary edema (Germonpré, et al, 1995; Lee and Pisarri, 2001; Russell, et al, 1984). This process does not happen in the naked mole-rats. Naked mole-rats do not fire action potentials in response to acidosis because they express a variant of $Na_V1.7$ that inhibits the channel (Smith, et al, 2011). Neonatal mice also appear to have a non-functional neurokinin pathway. However, in the neonates this appears to be a developmental issue, not a gene variant (King and Barr, 2003; Pritchett, et al, 2005).

Fructose metabolism in the naked mole rat is particularly interesting as it is an adaptation of preexisting pathways present in other mammalian models. In naked mole-rat cells, the fructose transporter GLUT5 is upregulated compared to the mouse, as is ketohexokinase, a key enzyme that converts fructose to a metabolite that can be utilized in glycolysis to produce energy in the absence of oxygen. This secondary energy source can fuel the brain and heart during anoxic challenges and is important in our understanding of metabolism under stressors as experienced in ischemic stroke, heart attack, metabolic syndrome and cancer.

It seems likely that the extreme tolerance of the naked mole-rat will prove to involve numerous adaptations in addition to those that have been published and discussed in this chapter. One avenue to discovery will undoubtedly involve using the published genome of the naked mole-rat, which is proving to be a tremendously powerful tool for exploring the relationship between genetic sequence and the extreme phenotypic characteristics of this species (Kim, et al, 2011; Fang, et al, 2014). For example, naked mole-rats display a unique point mutation in hypoxia-inducible factor 1α (HIF1α), a master regulator of gene transcription that activates over 40 downstream

genes in response to low oxygen conditions. This mutation in the VHL-binding domain is highly consequential because under normoxic conditions, VHL controls ubiquitin-dependent degradation of HIF1α. The naked mole-rat's T407I mutation is consistent with a relaxation of HIF1α degradation and resultant higher gene expression of this critically important regulator of hypoxic signal transduction (Kim, et al, 2011). Indeed, there is now some evidence for a contribution of HIF1α to hypoxia resistance in the naked mole-rat (Xiao, et al, 2017).

In summary, it is becoming clear that there is great value in the study of animals such as the naked mole-rat, which can complement the traits held by conventional laboratory animal models. The naked mole-rat exhibits a constellation of features that map to unique scenarios similar to human pathologies and provide insights into mechanisms and therapies that could prove translational.

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