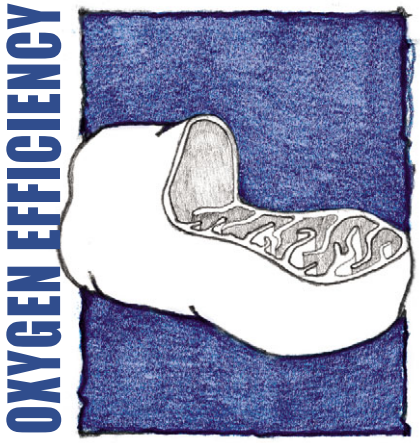
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB



GETTING EFFICIENT IN LOW OXYGEN

With every breath, we inhale the oxygen our cells need to sustain metabolism. But whenever we hold our breath, climb a mountain or sprint on a track, the amount of oxygen getting into our cells can fall below normal. When it does, numerous responses ensure that cells keep respiring so that the intracellular machinery keeps working. Biologists know that the hypoxia inducible factor, HIF, initiates many of these responses, including increasing oxygen-free anaerobic metabolism. How oxygen dependent aerobic metabolism is regulated by HIF during hypoxia is poorly understood, however, so Ryo Fukuda and colleagues from Johns Hopkins University decided to explore this area in cultured mammalian cells.

Cytochrome oxidase (COX) is a multi-subunit enzyme in the electron transport system of mitochondria. During aerobic metabolism, COX catalyzes the reaction with oxygen that helps to generate cellular energy in the form of ATP. Subunit 4 of the enzyme, COX4, contains multiple 'varieties', or isoforms, and takes part in regulating mitochondrial respiration. The authors suspected that COX4 might somehow be altered in low oxygen conditions, or hypoxia, to help maintain normal cell function. To test this hypothesis, the authors did a series of experiments measuring the expression and regulation of COX4 isoforms 1 and 2, in both normal and low levels of oxygen. The authors saw a switch in the COX4 isoform used during hypoxia: COX4-2 expression increased during low oxygen, replacing the normally predominant COX4-1 subunit which was simultaneously broken down. The team found that activation of HIF directly increased expression of the gene coding for COX4-2, and also increased expression of a mitochondrial protease called LON, which degraded COX4-1. This showed that aerobic metabolism was being regulated specifically by hypoxia.

But what was the purpose of this COX isoform switching? The authors addressed this question with a series of experiments where they added or removed either isoform to cultured cells, and then measured the cells' rates of oxygen consumption. They found that in hypoxia, cells consumed more oxygen with COX4-2 present than when COX4-1 was present, making sense of why this isoform was induced in hypoxia. At normal oxygen levels cells with COX4-1 or COX4-2 consumed oxygen at similar rates, so why then is COX4-1 ever expressed?

The benefit of COX4-1 at normal oxygen levels instead appeared to be that it reduced the production of reactive oxygen species. These are formed when the mitochondrial electron transport system is inefficient, and they damage cellular proteins, lipids and nucleic acids. The COX4-1 subunit therefore helps protect the cell from damage under normal conditions. Conversely, fewer reactive oxygen species were generated with COX4-2 than with COX4-1 in hypoxia, in addition to COX4-2 increasing the cells' rate of oxygen consumption.

The authors concluded from these studies that the COX4 subunit switch induced by HIF in hypoxia is critical to cell function and survival when oxygen levels change. Different isoforms are probably specialized for different cellular oxygen levels, optimizing the efficient balance between aerobic ATP production and reactive oxygen species generation. By initiating this and many other responses in hypoxia, HIF protects our cells so they can function properly; it's enough to make one gasp!

10.1242/jeb.001263

Fukuda, R., Zhang, H., Kim, J. W., Shimoda, L., Dang, C. V. and Semenza, G. L. (2007). HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell* **129**, 111-122.

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SQUIRREL SURVIVAL

PRENATAL GOODIES IMPROVE SURVIVAL

For mammals, a good start in life predominantly depends on the quality of a mother's milk, which in turn is strongly influenced by maternal nutrition and energy allocation. Consequently, nutritional supplements for nursing mothers may improve the survival of juveniles in the long term. While this effect has already been reported in birds, only few data related to this question have been collected in wild mammals. In addition, it is questionable whether effects of maternal nutrition on offspring traits and fitness act either short term, in the first stage of life, or persistently, with consequences on succeeding stages in life and later survival.

To address these questions in wild, free-living mammals, Tricia Kerr from McGill University in Canada and her colleagues in both Canada and the United States studied North American red squirrels near Kluane National Park, in south-western Yukon, Canada, for nearly 2 years. While all of the squirrels involved both in the 'Kluane Red Squirrel Project' and in Kerr's study were marked and monitored for survival and reproduction, some of them were also provided with peanut butter and sunflower seeds over winter and during reproduction, from autumn to the following spring when their babies were born. When the offspring appeared above ground for the first time later in the spring, all offered food items were removed because the aim of the study was to exclusively manipulate maternal nutrition without altering the food supply of the adolescent, weaned offspring. Kerr and her team took advantage of the squirrels' territoriality, which allowed them to target specific adult mothers with food supplements while ensuring that other squirrels didn't get any extra food.

The team found that maternal food provisioning in red squirrels greatly and persistently improved juvenile survival from their birth to the onset of reproduction, which happens a year after they are born. On average, 78% of young from supplemented mothers survived from birth to when they first emerged from the nest, whereas only 54% of juveniles from unprovided squirrels survived the same period of time. After removal of the food delicacies, total survival of young between first coming out of the nest and territory settlement continued to be higher in privileged offspring descended from food-supplemented mothers. As the young grew up, the difference in survival magnified over time: 94% of privileged young survived the first winter to the following spring, whereas only 62% of the offspring from control squirrels survived the first winter.

When further analyzing the differences between supplemented and non-supplemented mothers, the squirrel research group observed that the two groups of females produced litters of a similar size, with offspring of similar weight. However, females provided with goodies gave birth 18 days earlier. The potential benefits to the supplemented mothers and offspring could include improved immunocompetence, metabolic benefits and reduced stress hormone levels, but these have yet to be uncovered. Despite the unknowns, the authors highlight the fact that because prenatally food-privileged squirrels were born 3 weeks earlier in the year they were ahead by a nose in all respects, giving them a long-term advantage over their less well-nourished peers.

10.1242/jeb.001255

Kerr, T. D., Boutin, S., LaMontagne, J. M., McAdam, A. G. and Humphries, M. M. (2007). Persistent maternal effects on juvenile survival in North American red squirrels. *Biol. Lett.* doi: 10.1098/rsbl.2006.0615

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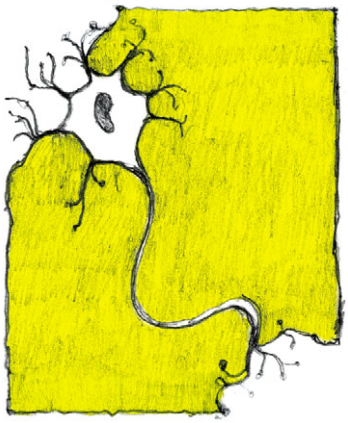
MEMORY LOSS



ERASING MEMORIES

It was first thought that when memories are stored, or consolidated into a long-term stable form, they are resistant to disruption. Much of the focus in memory research has been on identifying the molecules involved in this storage process. But, it then became clear that when one of these long-term stable memories is reactivated, it can return to an unstable state and again be vulnerable to disruption. A wave of studies then focused on how to re-stabilize a memory after it had been reactivated. Now, a recent study in *Science* by Reut Shema and colleagues from the Weizmann Institute of Science in Israel has shown that by inhibiting one very specific enzyme, PKMζ, a memory can still be disrupted long after it has been formed, even when it is not reactivated first. PKMζ has been previously implicated in maintaining memories in the brain area involved in forming memories, the hippocampus, but its role in the brain area involved in storing memories, the neocortex, was not known.

The team trained rats to dislike a certain taste by pairing the taste with a lithium chloride injection, which results in the rats being sick. This procedure, conditioned taste aversion, is similar to the effect experienced by those unfortunate individuals who have had food poisoning. The learned 'dislike' is rapidly learned and long remembered. After aversion training, the authors injected an inhibitor of PKMζ, ZIP, into the insular cortex, an area believed to store taste memories. Rats demonstrated no memory for the learned dislike 1 week or 1 month later.

Having shown that ZIP inhibits memory directly after learning, the authors wanted to know whether ZIP can disrupt the memory after it has been formed and stored. They repeated the experiment but now injected ZIP 3, 7 or 25 days after aversion training. Regardless of when ZIP

was injected, rats again demonstrated no memory for the aversion training. Thus, despite what is traditionally thought in learning theory, there was no closure of the consolidation window, which is the time frame when memories are stored, making them long-term and stable. In addition, the memory did not need to be reactivated first to make it vulnerable. Persistence of memory is thus dependent on the ongoing activity of this enzyme long after the memory is considered to have consolidated into a long-term stable form.

The effects of these experiments seem irreversible suggesting that PKMζ permanently maintains long-term taste memories in the insular cortex. The role of PKMζ in maintaining other types of memories in other areas of the neocortex remains to be determined. Even though it is difficult to prove the absence of memory it seems that the authors managed to permanently erase the memory for the learned dislike. This offers hope for those who would like unwanted memories erased. But, as an unfortunate sufferer of food poisoning, I think I would like to keep the memory of that particular restaurant that ruined my holiday weekend.

10.1242/jeb.001222

Shema, R., Sacktor, T. C. and Dudai, Y. (2007). Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKMζ. *Science* **317**, 951-953.

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STRESSED LIZARDS RUN FURTHER

'Stress has serious consequences.' These are words you might expect to hear from a psychiatrist, family physician or even your mom, but what about a zoologist? Absolutely. The study of how animals respond to and cope with stressful social situations or environments has blossomed in the last 10–20 years. While many of us may be more familiar with work of this nature in primates and other mammals, it turns out that reptiles, particularly lizards, are an important model for understanding the effects of stress on animal behavior and physiology. Stress commonly leads to elevated levels of the hormone corticosterone in the blood, and in lizards this can have detrimental effects on a variety of behaviors that have consequences for reproductive success. It is less clear what effects corticosterone has on whole-organism physiology, but recent work by Donald Miles at Ohio University, Ryan Calsbeek at Dartmouth College and Barry Sinervo at the University of California, Santa Cruz, sheds more light on how elevated stress hormones impact traits like locomotor performance and metabolic rate.

Miles and colleagues traveled to the coastal mountains of California and collected male blue-throated side-blotched lizards (*Uta stansburiana*), known from previous work to get stressed by interactions with both more dominant and sneakier conspecifics. Once back in the laboratory, the team tested the lizards' endurance, by twice measuring how long they maintained a 0.5 km h^{-1} pace on a motorized treadmill. They then implanted either a corticosterone treatment or a saline sham into the lizards to find out how this affected their locomotor performance; the corticosterone implant is known to elevate hormone levels approximately 2- to 5-fold for several months. Testing the lizards' endurance again every 2–7 days for 1 month following treatment, the team found that in sham animals endurance remained steady before and for a month after treatment at about 200–250 s. In contrast, males with hormone implants lasted longer on the treadmill from approximately 2 weeks after the implantation surgery, peaking at day 29 where they ran for around 350 s.

To find out what impact the hormone implants might be having on metabolic rates, the team measured resting and maximum metabolic rates for 3–4 weeks in a second group of lizards with identical sham or hormone implants. They measured the resting rates at 15 min intervals during a 6 h period in a dark chamber, noting in which 15 min interval the lowest rate occurred. The single highest value obtained from each individual during treadmill exercise to exhaustion at 1 km h^{-1} gave the maximum rate. Metabolic tests revealed that males implanted with corticosterone had significantly lower resting rates than animals with saline shams and although maximum rates were not significantly different, hormone-implanted animals also exhibited values about 15% lower, on average, than the sham lizards.

Thus, stress hormones appear to help lizards save metabolic energy and also improve their locomotor endurance. While the authors can only speculate as to what the mechanism underpinning these effects might be, it is clear that increased circulating corticosterone is not necessarily all bad news. Perhaps I should look on the bright side the next time I'm stressing out; better yet, maybe I should go for a run – a long run.

10.1242/jeb.001248

Miles, D. B., Calsbeek, R. and Sinervo, B. (2007). Corticosterone, locomotor performance, and metabolism in side-blotched lizards (*Uta stansburiana*). *Hormones and Behavior* **51,** 548-554.

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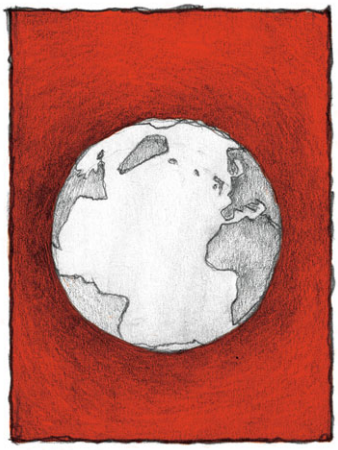
STARVATION STRATEGY



STARVING SNAKES

All animals face the risk of periods of food deprivation, which can lead to starvation and ultimately death. Most animals, especially mammals, are not well adapted to withstand food deprivation and extended periods of starvation. But some animals, such as penguins and ground squirrels, have developed strategies that enable them to survive multiple months without food. Snakes, however, are in a league of their own in their ability to deal with food limitation and can endure multiple *years* of starvation. Although this has been known for a long time, very little is known about the underlying biological mechanisms. To investigate this stunning phenomenon, Marshall D. McCue from the University of Arkansas, USA, examined changes in physiology, morphology and body composition in response to 168 days of starvation in three species of snakes: the ball python (*Python regius*), the ratsnake (*Elaphe obsolete*) and the western diamondback rattlesnake (*Crotalus atrox*).

It is not a simple task to define when fasting turns into starvation, especially in infrequently eating animals. In this study, McCue defined the starvation period as starting when animals were deprived of a meal they would otherwise voluntarily have eaten, which is around 2 weeks after a meal. With this in mind, the 62 snakes were subdivided into four groups: fasting, and 56, 112 and 168 days of starvation. All animals had access to fresh water throughout the experiment. McCue then measured the effects of starvation on body composition, mass and length, and resting metabolic rate over a 24 h period.

Following 168 days of starvation, all snakes had lost a percentage of their initial body mass: ratsnakes 9.3%, pythons 18.3% and rattlesnakes 24.4%. Despite this serious weight loss, and in contrast to previous investigations on reptiles and fish, all three species increased in length by around 4%. This indicates that there is a rather high selection pressure on length in these sub-adult snakes – size apparently does matter. Starvation also induced a highly significant decrease in resting metabolic rate in all three species, especially in rattlesnakes, which had a metabolic depression of an astounding 72%. This is surprising, since snakes have a very low resting rate even before the onset of starvation, and it was not expected that they could reduce this much further.

To find out how starvation affected body composition, McCue measured the water content of dead snakes by freeze-drying and subsequently measured the amount of lipid, carbohydrate and protein in their bodies. Because the snakes had access to water during the experiment, relative water content increased in all the species by an average of 6%, despite their weight loss. The relative protein content increased in all species during starvation, whereas lipid and carbohydrate content decreased. This shows that all snakes preferably use fat stores over protein as an energy source during starvation. Comparing body composition between the species, McCue found that ratsnakes began to break down proteins faster than pythons and rattlesnakes. This is probably because ratsnakes generally have an abundant food supply in their natural habitat and are maybe not as adapted to starvation as the other species.

The results show that starving snakes reduce their resting metabolic rate and change to metabolising lipids while sparing their protein stores. This was done to a degree where all snakes were able to increase in length despite a significant weight loss. Further investigations are needed to determine whether the observed metabolic depression is achieved through reductions in protein synthesis, reducing nerve activity or by something else entirely. Nevertheless, this paper very elegantly demonstrates one of the reasons why snakes are such an evolutionary success – they are well adapted to survive in areas with a low density of prey.

10.1242/jeb.001271

McCue, M. D. (2007). Snakes survive starvation by employing supply- and demand-side economic strategies. *Zoology* **110**, 318-327.

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