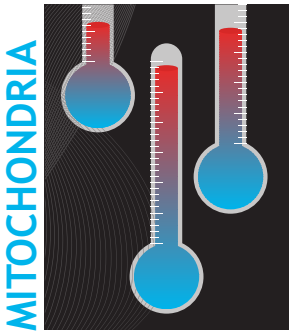
OUTSIDE JEB

Mitochondria are too hot to handle!



Back in 1957, in his article published in Scientific American (volume 197, pp. 131–144), biologist Philip Siekevitz called the mitochondrion the 'powerhouse of the cell', a term very much used today when we talk about this complex organelle. Mitochondria are organelles that use oxygen and nutrients to generate energy and heat to maintain a stable body temperature. But did you ever wonder how hot they actually get when they 'work' to heat us up? A group of researchers did, and their findings are extraordinary: mitochondria can heat up to close to 50°C, even though our core temperature is closely maintained at 37.5°C.

Dominique Crétien, a researcher working with Pierre Rustin at the Université Paris 7 in France, along with a team of international researchers, decided to investigate how hot mitochondria actually get when they generate heat in healthy human cells. To do so, the authors used a brand-new dye that targets the mitochondria and changes its fluorescence with temperature, called MTY. When the researchers monitored the fluorescence of the dye during cellular respiration in a human kidney cell line, they found that mitochondria are able to function at temperatures that are approximately 10°C higher than the core body temperature. The group recorded this result multiple times and determined that, as long as the mitochondria are functional and have optimal conditions, they indeed increase their temperature during normal functioning.

Because of the controversial nature of their findings, Crétien and his colleagues decided to use various chemicals to impair mitochondrial function, while monitoring heat generation using the same fluorescent dye, in order to ensure that their results were real and not due to a faulty organelle or artifacts caused by the dye. To eliminate the possibility that the changes in MTY fluorescence were due to changes in pH and membrane potential of the mitochondria during respiration, the authors treated the human kidney cells with cyanide, oligomycin or respiratory enzyme inhibitors such as rotenone and antimycin – which are known to affect the mitochondria by altering membrane potential, changing the mitochondrial pH, disrupting membrane structure and affecting the function of the mitochondrial respiratory enzymes. Indeed, the authors found that MTY fluorescence correlated with mitochondrial respiration and that it was not due to faulty mitochondria or artifacts in the fluorescence. The researchers further validated their findings when the response of the dye was enhanced in the presence of proteins involved in heat generation. Therefore, MTY seems to work, and work well, when it comes to monitoring mitochondrial heat generation and mitochondria really do seem to run hot when they are generating energy.

Through their work, Crétien and his colleagues have raised even more questions about heat regulation in vertebrates. How can the mitochondrial enzymes perform so efficiently at such high temperatures? What implications do these findings have for mitochondrial structure and function? How do they affect (if at all) how we approach mitochondrial disorders and how we treat them? One thing is certain, though: the term 'powerhouse' just got a whole new meaning.

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Crétien, D., Bénit, P., Ha, H.-H., Keipert, S., El-Khoury, R., Chang, Y.-T., Jastroch, M., Jacob, H. T., Rustin, P. and Rak, M. (2018). Mitochondria are physiologically maintained at close to 50°C. *PLOS Biology* **16**, e2003992.

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Sick ants save themselves by acid-spraying their sisters

Nurseries are notorious hotbeds of disease and contagion. Place one snotty child in a room with 10 healthy kids and you're on the cusp of an epidemic. Now imagine this nursery multiplied by hundreds and you'll have an idea of life inside an ant colony, where sick ants can easily transmit pathogens to their sisters. To reduce this risk, ants use different strategies to detect and disinfect diseased nestmates, effectively acting as nurses, executioners or morticians. But what if the nurse herself is infected? Will she fall victim to disease or can she act to reduce her risks of further decline? The answer, as shown in an exciting new paper in PNAS by Matthias Konrad and his colleagues from the Institute of Science and Technology in Austria, is that it depends on what her patient is infected with.

Konrad and his colleagues began by exposing ants to one of two different fungal pathogens. The first, called *Metarhizium*, kills ants rapidly, while the second, called *Beauveria*, causes only minimal mortality. When ants infected with one pathogen were subsequently co-infected with the other, known as a heterologous infection, things got much worse and mortality rates shot up. This, in itself, is not surprising. Co-infection often elevates mortality because it forces animals to simultaneously solve distinct immunological problems. But what was surprising is that the singly infected ants behaved as if they were aware of the added

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dangers of a heterologous infection – and acted to reduce it.

To examine the possibility that singly infected ants are risk adverse, the team confronted them with a nestmate carrying the other pathogen. What they found was both amusing and sensible. In short, singly infected ants are cranky, just like any other sick animal. In contrast to their relatively docile uninfected counterparts, singly infected ants bite, grab and drag their nestmates about. Worse still, they spray their infected nestmates with an antimicrobial poison, especially if their nestmates carry heterologous infections. Both types of response are sophisticated and effective.

Konrad and his team argue that becoming ornery is part of a suite of adaptive responses called 'sickness behaviors' that involve things like crankiness, lethargy and a diminished appetite. By reducing activity and keeping others at bay, 'sickness behaviors' are thought to help animals fight infection. At the same time, acid spraying serves two crucial functions. First, ants that spray have fewer fungal spores transmitted to them. This lowers their risk of heterologous co-infection more than twofold, thereby forestalling a premature death. Second, because sprayed ants are disinfected, they become less dangerous to the rest of the colony.

People have a natural aversion to sickness. Watch the horror in passengers' eyes when someone sneezes on the subway without covering their nose and mouth! But we generally don't distinguish between specific types of contagion, simply because we don't know which of the myriad of diseases our fellow travelers are carrying. Did the sneeze indicate flu, the common cold or something as benign as a snuffle of dust? We can't tell. But remarkably, infected ants can tell, and moreover they then use this recognition to dictate their behavior. At present, the authors don't understand the mechanisms that regulate this complex feedback, but finding out promises to be an exciting journey.

10.1242/jeb.169946

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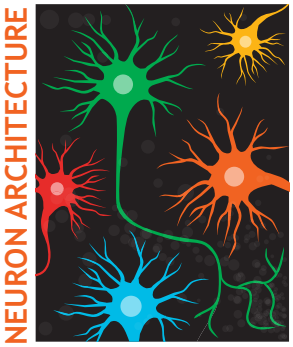
Birds and mammals share smartly designed neurons



Birds can do most things just as well as mammals. Crows use tools to solve complex puzzles. Lyrebirds are expert vocal mimics, putting polyglots to shame. And even the humble pigeon is a discerning enough art critique to distinguish between paintings by Picasso and Monet. Nevertheless, birds still get a bad rap. Calling someone a 'bird brain' insults their intelligence due in part to bird brains not having the fanciful folds and layers found in mammal brains.

The neocortex, the outer layer of mammalian brains, is often touted as being the hallmark of a mammal's advanced cognition. It's the largest part of human brains, and is thought to give rise to primates' advanced intellectual abilities. As bird brains are smooth and are without layers, it was long presumed they were behind the times and that their neurons didn't play a similar function or come from a similar evolutionary origin to mammals.

However, scientists have begun to peck away at this ancient notion of 'bird brain' being an insult. In a recent paper published in Current Biology, Steven Briscoe, working in the lab of Clifton Ragsdale at the University of Chicago, USA, demonstrated that birds, mammals and reptiles all share similar brain cells in the cognitive cortex, challenging old ideas about avian aptitude, anatomy and ancestry, and suggesting that 'bird brain' should really be a compliment.

To start, Briscoe and his team first evaluated whether any genes were specially enriched in the brain of an evolutionarily ancient bird: the chicken. Specifically, they focused on uniquely expressed genes in the mesopallium – a region of the brain involved in higher-order processing. Briscoe found a whopping 78 genes that were uniquely and highly expressed in the mesopallium. The team then focused on five of the more highly expressed unique genes and mapped out their expression visually to gain a bird's eye view of how well it distinguished the mesopallium from surrounding brain areas. The stained brains revealed that the top five genes cleanly delineated the mesopallium from other surrounding brain regions, suggesting the genes are important genetic markers for the special cell types that comprise mesopallium.

One caveat is that chickens are lower in the cognitive pecking order compared to the linguistic abilities of vocal learning avians, such as songbirds. As a follow-up, the team confirmed that expression of the same genes delineated mesopallium in the European starling, a songbird with an extensive vocal repertoire, suggesting these genes are conserved across birds.

While the avian mesopallium and mammalian cortex originate from the same brain region early in life (telencephalon), they take on different shapes across development: the cortex becomes layered in mammals, whereas clusters of neurons assemble in birds (referred to as 'nuclei'). Therefore, Briscoe and colleagues examined whether mouse cortex expressed a similar genetic signature to that which characterized the mesopallium. Lo and behold, expression of the same five genes that distinguished the mesopallium outlined the mouse cortex as well, suggesting the neurons are cut from the same evolutionary cloth.

The research is fascinating as it suggests that birds and possibly primates evolved intelligence independently and that cortical neurons may be as ancient as the last common ancestor shared by birds and mammals. In short, neurons just took on different architecture for bird brains, but it doesn't make them any less brilliant.

10.1242/jeb.170001

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Mantises see in 3D, but not like you and me

Praying mantises are inarguably one of the coolest insects: lurking about, still as statues, waiting to snatch their oblivious prey unawares. Researchers at Newcastle University, UK, have managed to dial-up the mantis coolness factor one notch further. They outfitted these tiny assassins with miniscule 3D glasses to uncover that mantises use a completely novel form of 3D vision when capturing their prey.

Many visual cues can be exploited to help judge how close or far an object is in the world. One way that depth is processed is through stereopsis: each eye sees a slightly different view of the world and the brain can identify these differences. Human stereoscopic vision specifically uses differences in luminance or brightness to help judge depth in the world. Stereopsis isn't an easy task and it is computationally expensive, which might help explain why numerous vertebrates like humans and macaques use stereopsis, but mantises are the only insects known to use it. This unique ability captured the attention of Vivek Nityananda and his colleagues at Newcastle University's Institute of Neuroscience, who were amazed that mantises, with only about a million neurons (compared with our ∼100 billion), could do such computations.

The researchers set out to test whether mantis stereovision was similar to that of humans, or whether they had evolved a new solution to seeing in 3D. Using some of the smallest 3D glasses imaginable, the researchers presented mantises with special stereopsis movies (which control for and eliminate other depth cues) in a custom-made insect cinema. The 3D glasses allowed the researchers to show a slightly different image to each eye, creating the illusion of a 3D object in their visual field – exactly the way we experience 3D movies. In Nityananda's experiments, both humans and mantises saw complex dot patterns where a camouflaged and moving target spiralled into view. The patterns could be adjusted so the target appeared far away or close up, and the human participants would say whether it was close or far, while the mantises expressed their response by trying to catch the tasty, but fake, 'prey' target when it came within their striking range (∼2.5 cm).

The researchers showed that both humans and mantises could see the 3D targets when the dot patterns that were seen by the two eyes were similar. However, when Nityananda inverted the brightness in one eye, making it look like a photo negative, the humans were completely derailed. Their trusty luminance cues were wrong and they couldn't judge depth. But, the mantises kept attacking their prey. Clearly, they were exploiting another cue. Next, the researchers showed both groups two completely different dot images. For humans, this was very confusing, as the brain couldn't make comparisons between different images in each eye. Human performance was abysmal, but the mantises remained unfazed by the researchers' attempts to thwart their vision. The team concluded that mantises might not compare the details of the entire image in each eye; what seemed to matter, instead, was motion. Mantises were picking up on changes in light patterns linked to object motion and not the picture details behind the motion –something that humans are simply unable to do.

It is important to accurately judge depth when you are an ambush predator like a praying mantis. Fumbling your prey might mean you go hungry or alert other, bigger, predators waiting nearby. Mantis hunting style, 'sit-and-wait', might have selected for this novel, motion-based form of stereoscopic vision. Great for mantises, this new 3D vision is less computationally costly. The research team thinks the simpler form of vision will have exciting applications for streamlining the visual depth algorithms used in robotics.

10.1242/jeb.170019

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Highways and byways into thin air for songbirds

The harsh environment and thin air of mountain landscapes and highland plateaus is a fertile testing ground for broad evolutionary questions about how animals scrape by in hostile environments. Evolution comes with a lot of baggage and different species may hit upon unique solutions to common problems simply because they had different ancestral starting points. For small, active songbirds, a perennial problem at high altitude is supplying enough oxygen to fuel metabolism.

Haemoglobin is critical to oxygen transport. It is responsible for shuttling oxygen collected in the lungs to the rest of the body and is implicated in adaptation to low oxygen (hypoxia). Many animals that are well adapted to hypoxia have haemoglobins with high oxygen binding affinities. An international team of researchers led by Jay Storz from the University of Nebraska, and Fumin Lei, from the Chinese Academy of Sciences and the University of Chinese Academy of Sciences, compared haemoglobin function in high- and low-altitude birds to find out whether changes in haemoglobin function had similar molecular bases in different evolutionary lineages.

The team collected muscle and blood samples from 17 species of tit from high-altitude sites on the Qinghai-Tibet plateau, in the mountains of southwestern China, and from lowlands throughout eastern China. They extracted haemoglobin from the blood samples and subjected it to a battery of tests to characterize its oxygen binding properties. As haemoglobin function is sensitive to subtle differences in the chain of amino acids that comprise the protein, the team also sequenced the haemoglobin genes for each species from portions of

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muscle. Next, they constructed a haemoglobin family tree to suss out the evolutionary relationships between the haemoglobins. Finally, the team manufactured their own haemoglobin with custom sequence mutations to match specific changes in the amino acid sequence with effects on oxygen binding affinity.

The haemoglobin of the species that live at high altitude had a higher affinity for oxygen than that of their lowland relatives, meaning that their blood was better at grabbing onto oxygen, a useful feature for life in hypoxia. The high-altitude haemoglobins also displayed a slew of amino acid substitutions absent from those of the low-altitude species. Different branches of the family tree generally had unique sets of substitutions, suggesting that there are multiple, independent molecular pathways by which the affinity of haemoglobin for oxygen can be increased. However, the team identified one case where a pair of species found the same solution for living the high life. Both the grey-crested tit, *Lophophanes dichrous*, and the ground tit, *Parus humilis*, swapped a threonine residue for an alanine residue at position 34 in one of the two chains of amino acids that comprise haemoglobin, but this substitution was absent in their lowland cousins. Intrigued, the researchers tested whether this modification had any consequences for oxygen transport by reconstructing the haemoglobin of the last common ancestor shared by the grey-crested tit and ground tit and comparing its functional properties with those of the tits' haemoglobins. Both of the living species had haemoglobin with higher affinity for oxygen than that of their ancestor, but the differences were attributable to other amino acid alterations elsewhere in the amino acid chains. While the substitution at position 34 alone improved the affinity of haemoglobin for oxygen, it wasn't enough to make a fully fledged, modern highland haemoglobin: additional substitutions were essential for high-altitude adaptation.

High-altitude birds take quite a few liberties with the biochemical properties of their haemoglobin, considering it's so integral to oxygen transport and survival. Even in the rare cases where elements are shared, different families still rely on subtle twists to get it just right. In solving the riddle of life in thin air, birds of a feather do not flock together.

10.1242/jeb.170324

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