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

NEURON MAPPING

NEURONS TO CONTROL MATE-FINDING BEHAVIOR IDENTIFIED

Some problems are so universal that even insects' brains must be able to solve them. Male crickets croon a species-specific song, and female crickets meet up with attractive-sounding males by following a song to its source. A cricket's ears (located on the front legs) connect to just two thoracic interneurons and two more neurons ascending into the brain. Thus, an invertebrate's 'neural parsimony' leads to single neurons performing tasks that require whole neural subsystems in mammals. Merely within the thorax, the biophysics of the ears combine with local comparisons to quickly identify the song's frequency and its direction. How steering maneuvers follow from that information still remains something of a mystery, so Maja Zorović and Berthold Hedwig took up the challenge to find out what kind of processing occurs in the brain to transform these auditory signals into motor commands.

These experiments were technically challenging. In order to show that a given brain cell was involved in phonotaxis, Zorović and Hedwig had to show that the neuron both responded to a calling song and altered its activity during walking. However, recording from and identifying a neuron must be done by impaling its axon with a hollow glass electrode, sharpened until the tip is approximately 10 μm across. Such a tenuous connection is highly sensitive to vibrations or movements – like those occurring in a walking animal! The authors ameliorated this motion by affixing the cricket to a metal pin and suspending it above an air-floated plastic ball, so that the cricket could move the ball with its legs but its body would remain stationary. They further stabilized the brain by immobilizing the head, cutting away some of the surrounding muscles and sandwiching the brain into a tiny metal holder. The motion of the ball was then monitored using the sensor from an optical computer mouse.

In keeping with the theme of neural parsimony, Zorović and Hedwig found just three brain cells processing the auditory information ascending from the thorax. These, in turn, fed a small pool of at least two neurons descending back to the body. The neurons along this pathway successively increased in selectivity to the correct song and in the robustness of their responses during walking, and nearly all the neurons showed a strong correlation with the walking movements of the cricket on the ball. Thus, it appears that a complete behavioral circuit can be mapped onto a chain of as few as five neurons, each uniquely identifiable in every individual cricket.

But why is the brain needed for this behavior in the first place? As sound frequency and direction are computed in the thorax, couldn't motor neurons be activated directly? The ability to integrate relevant information from other neural systems into the control of behaviors provides a clear adaptive advantage to an animal. In the case of crickets, obstacles, predators, hunger or thirst, or having already mated could all make a female cricket decide not to walk toward a calling male. Brains are where such high-level decisions take place – cutting out the brain would be taking the concept of neural parsimony too far.

10.1242/jeb.049783

Zorović, M. and Hedwig, B. (2011). Processing of species-specific auditory patterns in the cricket brain by ascending, local and descending neurons during standing and turning. *J. Neurophysiol.* doi:10.1152/jn.00416.2010

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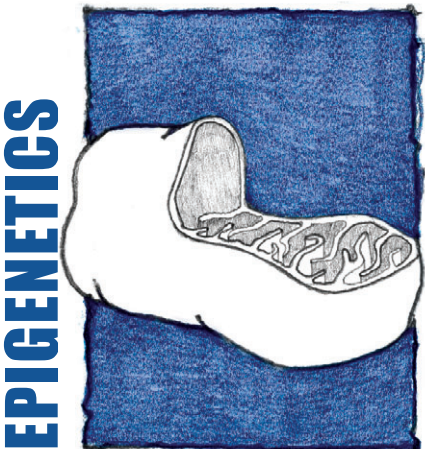

NEW BASES IN THE MITOCHONDRIAL GENOME

Gene expression is a tightly regulated process that allows cells, tissues and ultimately whole organisms to thrive. At the heart of this regulation, transcription factors play a fundamental role in keeping things in check. However, over the last few decades, another level of transcriptional control, termed epigenetic regulation, has garnered extensive attention. In vertebrates, for example, epigenetic modification through cytosine methylation is thought to repress nuclear gene expression, and is implicated in the regulation of both normal and aberrant gene expression. However, most investigations to date have focused on epigenetic modifications of the nuclear genome, with little attention devoted to mitochondrial DNA. In their study, Lisa Shock, Prashant Thakkar, Erica Peterson, Richard Moran and Shirley Taylor, from the Virginia Commonwealth University, USA, specifically investigated the epigenetic control of mitochondrial gene expression. The team hypothesized that cytosine methylation of mitochondrial DNA plays an important role in the regulation of mitochondrial gene expression.

Initially, the authors examined the genomic region neighbouring the established coding sequence of an enzyme capable of methylating cytosine nucleotides in the nuclear genome, DNA methyltransferase 1 (DNMT1), to investigate whether this enzyme could be involved in mitochondrial DNA methylation. They discovered that a region upstream of the 'nuclear form' of DNMT1 encoded a sequence sufficient to import this protein into the mitochondrion, and that mammalian cells express both 'nuclear' and 'mitochondrial' DNMT1 transcripts and proteins.

Then the authors overexpressed two proteins (nuclear respiratory factor 1 and the peroxisome proliferator-activated γ coactivator-1α, which upregulate expression of nuclear encoded mitochondrial genes) in mammalian cells to explore the role of 'mitochondrial' DNMT1 in mitochondrial epigenetic regulation. The team found that when expressed together, these factors increased the abundance of DNMT1 in mitochondria fivefold, suggesting that 'mitochondrial' DNMT1 expression is responsive to endogenous factors regulating mitochondrial function.

In addition, the team looked at cells deficient in p53, a protein known to regulate mitochondrial respiration, to ensure that DNMT1 affected mitochondrial gene expression in a situation where mitochondrial function is altered. They observed a threefold increase in 'mitochondrial' DNMT1, which differentially affected mitochondrial gene expression, providing further evidence of a link between DNMT1 and changes in mitochondrial function. Then, the team used cells expressing DNMT1 with a tag allowing immunoprecipitation of the protein and found that DNMT1 coprecipitated with mitochondrial DNA, indicating their direct interaction in these cells. Finally, the authors established the presence of methylated nucleotides (5-methylcytosine and 5-hydroxymethylcytosine) in mitochondrial DNA, providing further confirmation of epigenetic regulation of the organelle.

Overall, these results confirm epigenetic modification of mitochondrial DNA through the action of DNMT1. In the mitochondria, this enzyme appears to methylate cytosine nucleotides, thereby affecting mitochondrial gene expression. In addition, this work adds to the complexity of the cross-talk between the nuclear and mitochondrial genomes necessary for cellular metabolic homeostasis. Although DNMT1 mitochondrial targeting sequence appears to be conserved across multiple mammalian species, it is not clear whether this conservation extends to other species. However, if this process is evolutionarily conserved, one can wonder how organisms that lack functional DNA methyltransferases (such as fruit flies) compensate for this deficiency.

10.1242/jeb.049775

Shock, L. S., Thakkar, P. V., Peterson, E. J., Moran, R. G. and Taylor, S. M. (2011). DNA methyltransferase 1, cytosine methylation, and cytosine hydroxymethylation in mammalian mitochondria. *Proc. Natl. Acad. Sci. USA* **108**, 3630-3635.

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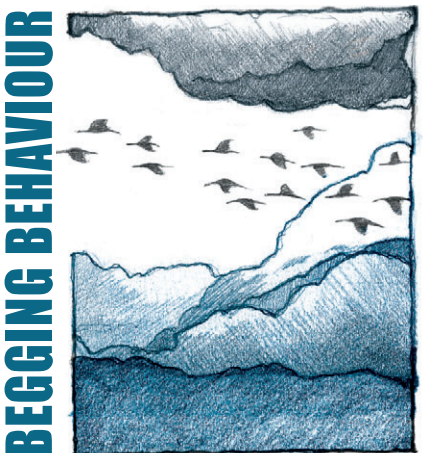

CHICKS KNOW NOT TO BEG

Many avian studies have documented how birds respond – both behaviorally and physiologically – to stress. Researchers most commonly describe predator–prey interactions from the adult birds' perspective. Birds exhibit a range of aggressive and defensive behaviors in response to threat. In many species, such overt displays have been linked directly to hormonal changes. For example, in an attempt to maintain a breeding area, the male bird will continuously patrol his claimed territory, present 'threat displays' such as very rapid wing beats, and sing repetitively when intrusion threats occur. Furthermore, each set of these behavioral responses has been shown to be influenced by testosterone levels.

A recent study by Ibáñez-Álamo, Chastel and Soler published in *General and Comparative Endocrinology* offers a new perspective on direct hormonal responses to stress through their analysis of corticosterone and testosterone levels in young 'nestling' birds.

As has been observed in adults, chicks too modify their behavior when threatened. By suppressing begging behavior, reducing vocalizations and calls for food/parents, young birds reduce their exposure to immediate threat from outside the nest. Ibáñez-Álamo and colleagues asked whether hormonal levels in common blackbird (*Turdus merula*) nestlings also change to reflect this protective behavioral modification.

The team took into account the fact that certain hormones are affected by stress (including handling by researchers) and suggested two possible but opposing scenarios for hormonal changes within nestlings. Either corticosterone levels increase when chicks perceive a potential nest predator (previous studies have suggested that when exposed to an acute

stressor, nestlings have reduced vocalization and locomotion and increased levels of circulating corticosterone) or, alternatively, corticosterone levels decrease (high levels of this hormone have been shown to be linked to begging – a risky behavior for chicks if a predator looms just outside the nest).

As the birds are well known to limit their begging behavior when a predator is nearby, the team played recordings of magpies (*Pica pica*), a known predator, in the nest's surroundings and collected blood samples from the youngsters to check their hormone levels. They also took blood samples from chicks that heard no recording, as they had found in an earlier study that playing no recording and playing recordings of non-predatory birds had similar effects on the nestlings' hormone levels. Finally, they compared the hormone levels of safe birds with the hormone levels of nestlings at risk.

Ibáñez-Álamo and coworkers found that blackbird nestlings in the high-risk situation had lower corticosterone levels than the birds that were not at risk, fitting the prediction that lower corticosterone reduced begging behavior and thus reduced detection by a predator. They also detected higher testosterone levels in the chicks exposed to magpie calls compared with the low-risk condition, fitting the prediction that increased testosterone contributes to a reduction in begging behavior in this species.

While it is still unclear whether the hormonal changes measured here in chicks were triggered directly by predator cues or by the nestlings' parents' alarm calls, these findings offer insight from a novel perspective – nestling physiology – into the physiology of survival.

10.1242/jeb.049791

Ibáñez-Álamo, J. D., Chastel, O. and Soler, M. (2011). Hormonal response of nestlings to predator calls. *Gen. Comp. Endocrinol.* **171**, 232-236.

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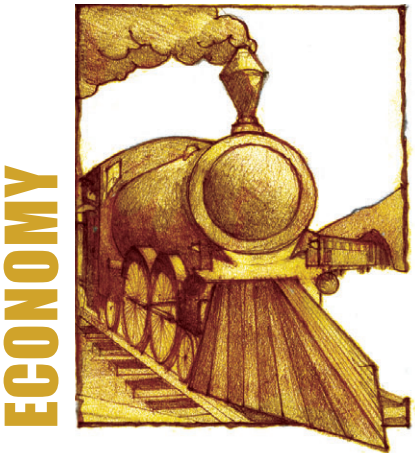

RUNNING FORERUNNERS

Our ancestors were likely endurance runners. To clarify, I'm not talking about my great grandfather running marathons; I'm referring to the notion that natural selection acted on running performance in some early human populations, leading to the development of high endurance. Endurance capacity is thought to have been useful for persistence hunting, in which humans forced animals to run for long periods and at high enough speeds that they couldn't dissipate heat fast enough and would eventually succumb to hyperthermia (and their human hunters). This form of hunting is hypothesized to have worked best in dry, hot environments, where thermoregulating during heavy exercise is most challenging. David Raichlen and colleagues from the University of Arizona and Harvard University, who are interested in the role of running in human evolution, recently developed a way to test the potential importance of climate for the development of endurance running in *Homo*.

Running economy, or the mass-specific energy required to run at a given speed, has an anatomical basis. For example, previous work has shown that human running economy is inversely related to the moment arm of the Achilles tendon, an important site of elastic energy storage and return during running. Dr Raichlen and co-workers recognized that this connection between running economy and anatomy could be put to use in a paleontological framework. If they could demonstrate that the moment arm of the Achilles tendon is directly correlated to some measure of heel bone (calcaneus) size, then they could take measurements of fossil heels and make inferences about running economy in our long-gone ancestors. To address the importance of climate, they could make these measurements in Neandertals, who lived in colder environments and might not have developed high levels of endurance because persistence hunting wouldn't have worked as well.

To explore relationships between heel anatomy and running economy in modern humans, the researchers used eight trained endurance runners to quantify individual variation in mass-specific oxygen consumption rates during running at 16 km h^{-1}. These same individuals subsequently underwent MRI scans of their ankles so that precise measurements of their heel bone (specifically, calcaneal tuber length CTL), could be taken. The measurements demonstrated a tight correlation between CTL and the moment arm of the Achilles tendon (r^2=0.91) and, more importantly, between CTL and the mass-specific energy costs of running (r^2=0.80).

The authors then took CTL values from the literature for seven Neandertals and 13 early *Homo sapiens*. Mean CTL was nearly 62 mm in Neandertals, just over 57 mm in the early humans and only 55 mm in the modern humans. Recall that running economy is inversely related to the size of the Achilles tendon moment arm, so longer calcanei imply less economical runners. In fact, the authors estimate that the metabolic costs of running in Neandertals and early humans would have been about 11.5% and 6.9% greater, respectively, than in modern humans. Such data must be interpreted cautiously, but they suggest that Neandertals were less economical runners and couldn't rely on endurance running for subsistence. Given the colder climates in which Neandertals lived, these data are also consistent with the idea that persistence hunting, running economy and climate were all linked in human evolution.

10.1242/jeb.049809

Raichlen, D. A., Armstrong, H. and Lieberman, D. E. (2011). Calcaneus length determines running economy: implications for endurance running performance in modern humans and Neandertals. *J. Hum. Evol.* **60**, 299-308.

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