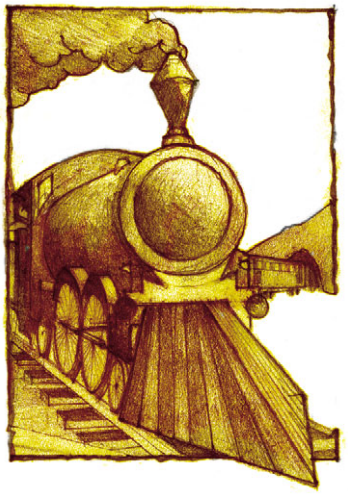
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

SENSORY FEEDBACK



## THE BENEFITS OF POSITIVE FEEDBACK

In both vertebrates and invertebrates, clusters of neurons fire rhythmically to generate the basic pattern for regular movements such as walking, swimming and gut peristalsis. These central pattern generators, or CPGs, need neither sensory input nor higher brain function to produce a more-or-less appropriate rhythm at some baseline frequency. But even though they can operate in isolation, they're also strongly affected by the senses. The walking CPG, for example, can reset its rhythm if one leg catches on something as it swings.

Sensory feedback on CPGs isn't limited to dealing with simple perturbations. It can also help make movements more efficient by taking advantage of mechanical resonance. For example, when a leg swings during walking, it's like a pendulum. And, like a pendulum, it has a natural frequency that it 'prefers'. Pushing it to swing faster or slower than this frequency takes more energy. If a CPG's baseline frequency and the body's mechanical resonant frequency aren't too far apart, often the CPG will burst at the resonant frequency, which helps conserve energy.

But what types of sensory input does a CPG need to achieve this 'resonance tuning?' To probe this question, Carrie Williams and Stephen DeWeerth of the Georgia Institute of Technology started with a simple mathematical model of a CPG called a 'half-centre oscillator', two neurons that are linked so that they fire alternately. Then they linked the neurons to a mathematical model of a classic mechanically resonant system: a pendulum whose swinging is gradually damped out with friction. Each neuron controlled 'muscles' that pushed the pendulum in opposite directions.

Then they tried four different types of feedback from the pendulum to the CPG. The first two were negative feedback – swinging to the left either inhibited the left-side neuron or excited the right side. Either way would tend to stop the pendulum and push it back the other way. The other two ways were positive feedback – swinging to the left now excited the left-side neuron or inhibited the right. These feedback modes would tend to enhance a swing in one direction.

Even though positive feedback loops cause many systems to spiral out of control, in Williams and DeWeerth's model both positive and negative feedback loops settled at the resonant frequency. Negative feedback can speed up the system when the resonant frequency is higher than the CPG's baseline. The stronger the sensory input, the wider the range of resonant frequencies the CPG can use. By contrast, positive feedback slows the system down, but only if the input has just the right strength. With a weak input, the CPG just runs along at its own frequency. With a strong input, the positive feedback causes the system to oscillate unstably. Somewhere in the middle, though, the CPG slows down nicely to a resonant frequency below its baseline.

The researchers' results point towards a way for experimentalists to differentiate between positive and negative feedback. In the model, negative feedback could only speed the CPG up. So if the CPG runs slower without any sensory input at all, as the CPGs in most vertebrates do, then at least some of the feedback is probably negative. By contrast, if the CPG runs faster in isolation, or, better yet, behaves strangely with very high amplitude inputs, then positive feedback probably plays a role.

10.1242/jeb.001016

**Williams, C. A. and DeWeerth, S. P.** (2007). A comparison of resonance tuning with positive versus negative sensory feedback. *Biol. Cybern.* **96**, 603-614.

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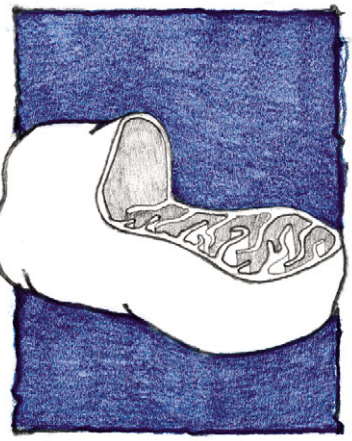
ROS STRESS



## ROS STRESS IN MICE: NOT JUST A GUY THING

It is common knowledge that American and most European women, on average, live longer than men, possibly because women take better care of their health overall and are more likely to see a doctor! But females of other species also live longer, including rats, other primates, and ungulates, suggesting a sex-specific mechanism that increases lifespan. It has been proposed that estrogens might be protective by decreasing the damage caused by reactive oxygen species (ROS) by increasing antioxidant defenses, thus reducing the chance of cellular senescence and death. But in some animals there is no sex-linked difference in longevity despite higher female estrogen levels, and in other species, such as Syrian hamsters, males live longer. Thus, Alberto Sanz from Complutense University, Madrid and collaborators at the University of Florida and University of Wisconsin analyzed a number of parameters of oxidative stress in a strain of mice with no male–female differences in longevity to determine if there were sex-linked differences in oxidative stress.

The team used 10-month-old male and female mice to determine mitochondrial oxygen consumption, ATP content and production of the ROS hydrogen peroxide in liver, skeletal muscle and heart tissue when they inhibited aerobic respiration. These measures would reveal any gender differences in basal metabolism or ROS production. They also measured oxidative damage to mitochondrial DNA (mtDNA) and levels of protein carbonyls that result from oxidative protein damage. To calculate the amount of apoptosis – programmed cell death – in male and female tissues they measured the levels of caspase-3 and caspase-9 enzymes, which break down proteins during apoptosis, and levels of DNA fragmentation that could indicate sex-specific differences in cellular damage.

The group found no significant differences between males and females for nearly every parameter measured, including oxygen consumption, ATP content and ROS production. There were also no differences in oxidative stress markers – cytosolic protein carbonyls or mtDNA damage – nor were there sex differences in apoptotic markers. Since the female mice had twice the levels of estrogen compared with the male mice, this means that high estrogen levels alone do not reduce ROS production, at least in this strain of mice.

The only real differences between the sexes was body mass: males weighed on average 20% more than females. Since male and female mice had similar lifespans and no differences in ROS production or oxidative damage, the group's results agree with several studies in rats that have demonstrated reduced ROS production in longer-living animals. The authors suggest that levels of ROS production may be linked to body mass differences between genders, as the size difference is much smaller in mice at 11–20% than in rats at 70%, and in general there is a negative correlation between body mass and longevity within species. Small animals also have a higher mass-specific oxygen consumption, and other researchers have found that increases in mitochondrial oxygen consumption decrease ROS production. Thus, large male rats would have higher rates of ROS production and ROS damage than female rats, while mice without gender-related size differences would have similar rates of ROS production and equal lifespans.

And so we learn that size *does* matter, and bigger isn't necessarily better!

10.1242/jeb.001024

**Sanz, A., Hiona, A., Kujoth, G. C., Seo, A. Y., Hofer, T., Kouwenhoven, E., Kalani, R., Prolla, T. A., Barja, G. and Leeuwenburgh, C.** (2007). Evaluation of sex differences on mitochondrial bioenergetics and apoptosis in mice. *Exp. Geront.* **42**, 173-182.

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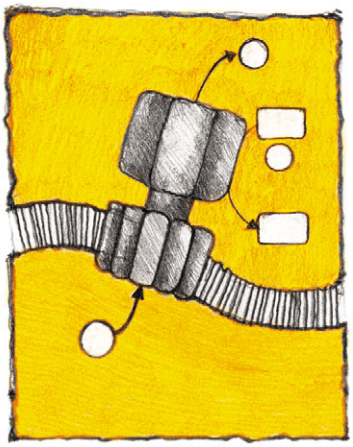
CILIAR MOTION



## CILIA'S COXSWAIN

Millions of hair-like structures called cilia move in unison on the inner surface of the lungs to sweep out mucus and prevent respiratory infections. But cilia cover many more animal epithelia – tissues that cover surfaces in the body – and their versatile functions include moving cerebrospinal fluid in the brain and coaxing an egg down a fallopian tube. To push fluid in one direction, cilia move in time, similar to eight rowers in a boat steered by a coxswain. While the mechanisms behind ciliar motility are reasonably well understood, only little is known about how movements are coordinated. When trying to find the coxswain in charge of ciliar movements, Chris Kintner and his team from the Salk Institute for Biological Studies and the University of California, San Diego recently proposed a positive feedback mechanism that ensures cilia row in the same direction.

Cilia are protrusions of a cell's plasma membrane and are supported by longitudinal filaments called microtubules that are arranged in nine pairs around a central pair. Motor molecules, called dyneins, between the microtubule pairs facilitate sliding of the filaments against each other in a process that is dependent on calcium. As the filaments slide, the cilium eventually bends as the ends of the microtubules are anchored inside the cell by a structure called the basal body, which has a foot at its bottom. This foot is always orientated in the direction of the ciliar stroke.

To analyze the coordination of ciliar movements, the team of Californian scientists needed an appropriate experimental system. They took skin samples from frog larvae and placed them in cell culture because each of the skin's epithelial cells are covered with hundreds of cilia. By scoring the orientation of

countless basal feet using transmission electron microscopy the scientists observed that all of them roughly pointed to the back of the larvae during early development. A few hours later in development, however, the cilia refined their polarity and precisely aligned on the front–back axis. Interestingly, when the team removed the skin before the front–back axis had established and transferred it to the cell culture, the cilia failed to align. The latter finding suggested that the cilia of the prospective skin miss some signal that helps to 'agree' on a common orientation.

To further analyze ciliar orientation, the team took advantage of known genetic defects that affect ciliar motility and cause primary ciliary dyskinesia (PCD), a rare congenital disease that impairs ciliary flow and causes persistent respiratory infections, infertility, ear inflammation or fluid accumulation in the brain. After the team blocked the expression of these genes in the frog embryo, the cilia didn't work properly, which is typical in PCD patients. Although the cilia's basal feet still pointed towards the back of the larvae, they could not generate a detectable flow on the skin's surface and the cilia also didn't reorient during development. The team suspected that the initial fluid flow causes the cilia to refine their alignment and decide on a common direction. To prove this hypothesis the scientists constructed a flow chamber, in which they traced the ciliar orientations in response to different external flow. Indeed, the cilia could actively sense and respond to externally applied fluid flow by changing their orientation.

By showing that the flow itself influences ciliar orientation, Kintner and his team have identified a type of coxswain determining the polarity of the strokes. The cilia use the flow to self-correct the polarity and motion in a positive feedback mechanism. Although it is unclear how the cilia sense and respond to the flow, the finding might yet deliver new insights into the molecular details of PCD.

10.1242/jeb.001032

**Mitchell, B., Jacobs, R., Li, J., Chien, S. and Kintner, C.** (2007). A positive feedback mechanism governs the polarity and motion of motile cilia. *Nature* **447**, 97-101.

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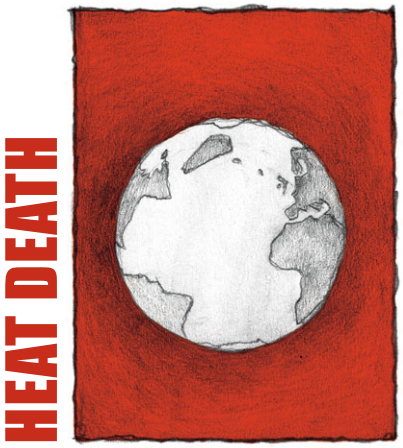


## CAN HEATING SUFFOCATE INSECTS?

As animals heat up, their metabolism accelerates. This is because biochemical reactions speed up, leading to an increased demand for oxygen in cells and tissues. At high temperatures, the ventilatory system of some marine animals is unable to supply mitochondria with enough oxygen to continue producing ATP and there is a harmful switch to anaerobic metabolism, which ultimately leads to death. Essentially, the supply of oxygen by the animal's gas exchange system cannot meet the increased metabolic demand from the cells as the temperature rises, meaning that oxygen limitation is probably the main cause of heat death in organisms. However, insects have a far more efficient and highly developed gas exchange system than marine animals, with tracheae penetrating directly into the flight muscle in some species. Therefore, is oxygen limitation, or hypoxia, the cause of heat death in insects?

John Lighton from the University of Nevada, Las Vegas designed an experiment to test this idea using *Drosophila melanogaster*. He used a technique developed previously with Robbin Turner that allows the simultaneous measurement of the flies' gas exchange and activity under ever increasing temperatures, known as thermolimit respirometry. This technique allows scientists to accurately determine the temperature at which an individual insect suffers what is known as heat death, or critical thermal limit. This is usually associated with the onset of muscle spasms. Because animals can no longer fly, mate or escape predation, it is also considered an ecologically relevant measure of the temperatures that may affect their survival in the wild. Lighton measured individual flies under a variety of different oxygen concentrations ranging from 2.5 to 21%. Using this method, he reasoned that he could observe exactly which oxygen level reduced the animals' critical thermal limit, if such a reduction took place at all.

Under normal conditions, 21% oxygen, the flies could tolerate a toasty 39°C. Lighton found that below 10% oxygen, their thermal tolerance reduced significantly – to 36°C at 2.5% oxygen. The flies had lost 3°C of their heat tolerance, which supported the oxygen limitation hypothesis. Could such low oxygen values have significant effects on *Drosophila* thermal tolerance in the wild? Oxygen levels as low as 5% are only found at very high altitudes such as the peak of Mount Everest, and the flies obviously do not live at such altitudes or low oxygen levels under normal conditions in nature. But Lighton notes that, particularly during high activity levels such as flight, when metabolic rates are higher, oxygen levels in the tracheae may drop below 10% and could limit performance. So when flies are buzzing around frantically, oxygen limitation may become important, leaving them in danger if it's also very hot.

Because researchers have investigated how oxygen availability limits thermal tolerance in so few insect species, Lighton is careful to conclude that while some insects may not become oxygen limited, it is clear that *Drosophila melanogaster* is affected to some degree. Scientists now need to unravel whether or not different insect species have evolved alternative ways of dealing with oxygen limitation during extreme temperature exposure, which will help them understand how hypoxia might set insect temperature tolerance.

10.1242/jeb.001057

**Lighton, J. R. B.** (2007). Hot hypoxic flies: whole-organism interactions between hypoxic and thermal stressors in *Drosophila melanogaster*. *J. Therm. Biol.* **32**, 134-143.

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## VISION COSTS

Ecologists and physiologists assume that biological systems show a balance between energy costs and performance benefits, with natural selection acting to maximise performance and minimise costs. To see how performance varies with energy cost, Jeremy Niven from the University of Cambridge and two co-workers studied the activity of photoreceptor cells in four species of flies: the large, big-eyed blowfly *Calliphora*, the fleshfly *Sarcophaga*, and two much smaller, small-eyed species of *Drosophila*, including the smallest fly tested, *D. melanogaster*. Because photoreceptors in the four fly species are very similar, and their biochemistry and physiology are well understood, the team could reliably compare their performance.

By stimulating fly photoreceptors with a range of light intensities and by recording photoreceptor spike rate, Niven and his colleagues calculated that each species processed information at the same rate in dim light levels, 1000 photons per second, but that differences soon appeared as light intensity increased.

At the kind of intensity seen in broad daylight, $10^6$ photons per second, the four species showed highly significant differences in processing rate, which were positively correlated with the size of the fly and of its eyes. *Sarcophaga* showed the highest processing rates, while *D. melanogaster* showed the lowest. Because *Drosophila* species fly slowly and tend to be most active at dawn and dusk, while *Calliphora* and *Sarcophaga* are active in full daylight and fly fast, the authors suggest that these differences enable the larger, faster-flying species to process more information, more rapidly.

Using a simple electrical model of a photoreceptor, the team then indirectly calculated the rate of ATP consumption. They found that the energetic cost of maintaining these different coding capabilities was greatest in the species with the largest eyes and lowest in *D. melanogaster*, suggesting that as processing power increases in more intense light, so too does the energetic cost of producing that response.

However, the four species also differed in how much ATP they needed to keep their photoreceptors functioning at rest, in the dark. This substantial fixed cost contributes to the total processing cost, so to determine exactly how much it cost each species to signal a response, the authors subtracted the resting costs from the total energetic costs. The authors found that the cost of processing each bit declined with increasing light intensity for each species. But when they looked at the maximum processing power and compared it to cost per bit, they found that costs per bit were higher in the large-eyed *Sarcophaga* and *Calliphora*, which could process the highest amount of information, than in *Drosophila*, which could process the least. As a result, the higher the maximum rate of information that could be processed, the greater the costs of processing and signalling that information.

Although the large flies could process five times as much visual information as the smaller species, this increase in performance was coupled with a 25-fold increase in energy consumption, suggesting that there is a law of diminishing returns affecting processing power in the insect retina.

This elegant study not only confirms a key assumption of neurophysiology, that performance is balanced by cost, but also shows how relatively simple measures can be used to investigate complex aspects of the evolutionary and physiological forces that shape nervous systems. This approach might be usefully applied to studying processing power in other sensory systems, such as olfaction, and as a tool for investigating how an organism's environment can influence its nervous system's structure.

10.1242/jeb.001040

**Niven, J. E., Anderson, J. C. and Laughlin, S. B.** (2007). Fly photoreceptors demonstrate energy-information trade-offs in neural coding. *PLoS Biol.* **5(4)**: e116 doi:10.1371/journal.pbio.0050116

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