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 highlights the papers that JEB readers can't afford to miss.

Outside JEB

RHYTHM GENEERATION

INSPIRATION FROM THE LEGS

How does the nervous system coordinate the control of different behaviors? For example, different rhythmic behaviors that normally function independently may need to work together under certain circumstances. One example of this phenomenon is the way that our respiratory rate goes up when we start running. Vertebrate breathing frequency increases immediately with locomotor activity, so it's not just the lack of oxygen that makes us breathe faster. Respiratory rates and limb movements can also be coupled in a one-to-one fashion during fast gaits, presumably both to maintain sufficient oxygen levels and to avoid mechanical interference.

Several mechanisms have been proposed to underlie this coordination, including mechanical coupling through whole body movements and common drive from higher brain centers to the separate neuronal networks controlling breathing and locomotion. In the article by Didier Morin and Denise Viala, an elegantly simple experimental approach was used to investigate possible mechanisms of coupling between these networks.

The pattern-generating neurons for breathing are found in the brain stem and the neurons that control hindleg movements are found in the lumbar spinal cord. By removing the spinal cord and brain stem from newborn rats and putting it into a dish, the authors could test how the pattern generating system for respiration can be influenced by the locomotor system in the absence of other inputs. They bathed the lower part of the spinal cord in the neurochemical NMDA to induce rhythmic activity in the networks that control the leg movements, and then increased the dose while monitoring the resulting activity in both the respiratory and leg motoneurons to look for coupling between the pattern generators. As they increased the level of NMDA, the frequency of the locomotor rhythm in the lumbar spinal cord rose, and once above a certain threshold, the respiratory rhythm sped up too. However, the authors saw no apparent phase coupling between "walking" and "breathing" rhythms, suggesting that no specific timing information is contained in signals from the local locomotor centers to the respiratory networks.

In contrast, mimicking sensory feedback from leg movements by electrically stimulating nerves that contain the axons of leg proprioceptors had a dramatic effect. A brief stimulation elicited a burst in the respiratory motoneurons, and reset the respiratory rhythm. Rhythmic stimulation of the leg proprioceptors could also force the respiratory rhythm to follow a wide range of stimulation frequencies in a one-to-one fashion.

The authors go on to show that these effects are directly mediated by sensory pathways from the leg to the respiratory-rhythm-generating networks and even found reflex-like connections to the phrenic motoneurons innervating the diaphragm. Therefore, sensory feedback from the legs during walking appears to play a key role in providing timing information for the respiratory system to couple the breathing frequency to the locomotor rhythm.

10.1242/jeb.00044

Morin, D. and Viala, D. (2002). Coordination of locomotor and respiratory rhythms *in vitro* are critically dependent on hindlimb sensory inputs. *J. Neurosci.* **22**, 4756-4765.

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AT LAST: AN '-OMICS' THAT DELIVERS!

A cynic might suggest that there's an 'in' game among the apostles of post-genomic technology to invent the most obscure 'ome'. By now, we're all aware of the transcriptome, the proteome and, perhaps, the metabolome. But how about the peptidome? At present, there are only six papers on this topic in the whole of PubMed, yet the latest delivers information of real utility to comparative physiology.

The peptidome is defined as all the peptides in a cell or tissue, together with their post-translational modifications. It's thus a cut-price proteome, a snapshot of all peptides small enough to extract and load onto a mass spectrometer, without prior tryptic digestion. This sounds dry, until you see it in the context in which it will invariably be unleashed; the tissue is the central nervous system, and the peptidome is all the neuropeptides. So in a single experiment, involving (in this case) surprisingly small batches of 50 *Drosophila* larval brains, we get a view of all the neuropeptides that interest the animal, rather than the peptides that we (as experimenters) deduce by staring at the genome.

How well did it work? Surprisingly, considering that it's something of a sport to pick off *Drosophila* brain-related genes, only seven neuropeptides have been purified traditionally, and 18 have been identified from other routes. Here, the authors identified 28 neuropeptides, including eight that had not been identified or predicted in any way previously. That's a pretty impressive hit rate. Of course, the approach was not perfect, as low abundance peptides, or those with restricted temporal patterns of expression, might not be picked up in a single assay. The authors flag this limitation with a list of known or plausible peptides that do not show up on their assay. Among them, for example, is a CRF-like diuretic peptide that our group has just shown to be expressed in only six cells, so this may provide a lower estimate for the sensitivity of the system.

Could this approach be extended to other species? Very quickly and easily: *Drosophila* is famous for having a sequenced genome and a whole battery of genetic resources. However, only the former quality is significant here. It is easiest to deduce peptide identities by comparing mass fragmentation patterns against all possible peptides encoded by the genome (there are computer programs like MASCOT to do exactly this). There are several other insect genomes coming onstream in the next few months (*Anopheles gambiae Aedes aegypti* and, perhaps, *Apis*). So relatively few experiments could provide us with a database of most insect neuropeptides of species of interest, and modest scaling up could do the same for fish, rodents…

The learning curve and capital cost for some of the new '-omics' technologies can baffle or frustrate the physiologist: but here is one that delivers real results now. Given that *J. Exp. Biol.* has published 53 papers on neuropeptides in the last few years, peptidomics is likely to become important to many of us.

10.1242/jeb.00046

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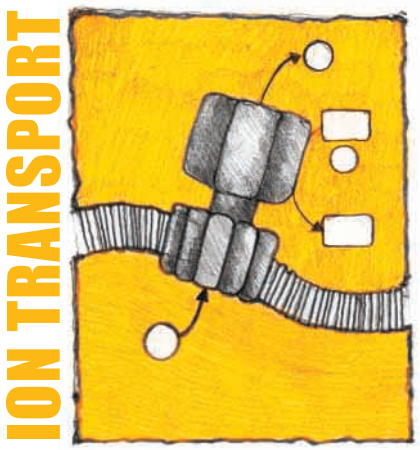

MITOCHONDRIA-RICH CELL SUBTYPES IN FISH GILL

The roles of different cell types involved in ion transport in the fish gill have been widely debated but it is commonly accepted that mitochondria-rich chloride cells and pavement cells play key roles in ion and acid–base transport in freshwater fish. Mitochondria-rich cells function in acid–base regulation by altering chloride and carbonate exchange, while pavement cells are currently believed to be the site of sodium uptake at the gill.

Galvez and colleagues focus on the mitochondria-rich chloride cells in the freshwater trout gill. They use a novel magnetic bead separation technique to isolate different mitochondria-rich cell subtypes. The existence of these cell subtypes in fish gill had been proposed but evidence to support their existence was lacking.

Different sub-types of mitochondria-rich cells in other animals have been identified by looking at the differential binding of peanut lectin agglutinin (PNA) to the apical surfaces of mitochondria-rich cell types. Galvez and colleagues adopted this technique to distinguish between subtypes of mitochondria-rich cells in the fish gill.

Research on the fish gill from their lab indicates that PNA binds to mitochondria-rich chloride cells on the apical surface of the gill epithelium. Here, they use PNA binding to separate mitochondria-rich gill cells into PNA^+ and PNA^- populations. They identify the existence of at least two mitochondria-rich cell subtypes. The ultrastructure of the PNA^+ cells was characteristic of mitochondria-rich chloride

cells, whereas the PNA$^-$ cell morphology was reminiscent of pavement cells.

The functional properties of these subtypes in acid–base regulation were examined by the expression of two proteins important in epithelial transport. The Na^+-K^+-ATPase is involved in energising both sodium and chloride uptake across the gill and the H^+-ATPase provides an electrochemical gradient that drives sodium movement. The expression of these proteins under normal, acidosis and alkalosis conditions was examined.

Both ATPases were expressed in the PNA$^+$ and PNA$^-$ cell types. However, both of these proteins were expressed differently in the two subtypes during acid–base disturbances. Most notably, only the PNA$^-$ cell types responded during acidosis, by increasing expression of the H^+-ATPase.

The authors suggest that the site of proton excretion in gill tissue is the PNA$^-$ cells and that the PNA$^+$ cells are analogous in function to the β-mitochondria-rich chloride cells of the mammalian kidney collecting ducts, which are responsible for base-secretion. In the mammalian kidney, there is a functional separation of mitochondria-rich cell types into acid-secreting and base-secreting cells. Separating and identifying different subtypes in the fish gill is both important and interesting to determine whether the functional separation also occurs in gill tissue.

Galvez and colleagues are now hoping to clone the apical anion exchanger in the PNA$^+$ mitochondria-rich chloride cells of the fish gill. The use of magnetic bead separation to enrich for PNA$^+$ mitochondria-rich chloride cells should help improve the ability of cloning rare transport proteins on this cell type.

10.1242/jeb.00047

Galvez, F., Reid, S. D., Hawkings, G. and Goss, G. G. (2002). Isolation and characterisation of mitochondria-rich cell types from the gill of freshwater rainbow trout. *Am. J. Physiol.* **282**, R658-R668.

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SPRINGS IN LEGS AND RUNNING SURFACES

Its been nearly a quarter of a century since McMahon and Greene suggested that the stiffness of running tracks could be adjusted to enhance a runner's performance. They demonstrated that tracks made to be compliant (deformed by the force of the runner's landing foot) could result in greater running speeds. Indeed, subsequent installation of several "tuned tracks" have resulted in increased running speeds as well as reduced running injuries. This early work was based on a surprisingly simple model of a running animal behaving like a single mass and spring. However, details of how surface stiffness relates to an animal's energetics and biomechanics have remained sketchy.

In this study, the authors set up a treadmill at Harvard that had one of five different platforms beneath the running area. These platforms resulted in a 12-fold range in track stiffness, from very stiff (≈950 kN m^{-1}) to very compliant (≈75 kN m^{-1}), including the range of track stiffnesses used in tuned tracks. By simultaneously measuring forces, kinematics (limb and body movements and angles) and oxygen uptake, the authors could test several hypotheses, including predictions that runners would make mechanical changes (in the knee in particular) to become "stiffer springs" while running on more compliant tracks, resulting in a lower cost of running.

What they found is that as the vertical displacement of the track increases (on increasingly compliant tracks), displacements in the runner's center of mass change very little, as predicted. However, sweep angles, stride frequencies, stride lengths and duty factor (time the foot is on the ground) are also nearly constant, independent of track stiffness. They had specifically postulated that these measures of leg posture would change to adjust leg stiffness inversely with track stiffness. Their results were somewhat surprising as the overall stiffness of the leg did indeed increase (by 29%), presumably by increasing the muscle stiffness. Further, as the track compliance increased, the metabolic cost of running decreased a great deal and did so nearly linearly, as elastic strain energy could be returned to the runner with each stride as the track deformation was restored. Further, because the "muscle machine" is not 100% efficient, for every watt of mechanical power returned by the track, the runner saves 1.8 W of metabolic power. The result is an overall reduction of 12% in the runner's metabolic rate on the most compliant track.

This paper is notable for at least two reasons. The first is the reminder that statistical significance is not sufficient to demonstrate biological significance. The authors rejected their "knee stiffness" hypothesis despite most results being statistically significant, as they argued convincingly that these results lacked the magnitude necessary not to reject the hypothesis. The second is that simple models should be retained as long as possible. While it is obvious that running is a complex behavior, the greatest insights often result from the simplest models.

What this study failed to do was find the "optimally compliant" running surface. As long as the resonant period of track plus runner is close to the surface contact times of the runners, an ideal track stiffness could be achieved. This experiment will remain for another day. In the meantime, those of us with dreams of setting our personal records for running distances best look to the most springy running surfaces we can find.

10.1242/jeb.00048

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HEARING

ALLIGATORS TUNED INTO WATER

We all admire animals that can move with ease between air and water, but how do these creatures cope with the challenge of interpreting sounds transmitted through different media? Sound is a key sense in many animals, yet relatively little is known about how hearing sensitivity differs between environments. D. Higgs and his colleagues at the University of Maryland wondered how sensitive alligators are to sounds in water and air, and discovered that alligators hear well in both environments.

The ultimate response to all sound, whether detected in air or water, is driven by the stimulation of sensory hair cells in the inner ear, and creatures that are sensitive to airborne sounds process the sensory signals in the brain stem region of the brain. But how the sound is transmitted to the inner ear depends on the animal's situation. For example, humans are acutely sensitive to airborne sounds, but when submerged, noises that are carried through the water are deadened as they are transmitted to the inner ear through the skull.

However, alligators are perfectly happy lounging on riverbanks or skulking in water. Higgs explains that the alligator's ear and brain structures are perfectly adapted for hearing airborne sounds. But they spend significant amounts of time immersed in murky waters. Does this mean that their senses are equally sharp in both environments?

The scientists tested the hearing of eight young alligators, above and below the surface. They played the reptiles a series of tone pips that ranged in pitch from 100 to 8000 Hz, and tested the animal's sensitivity at each pitch by increasing the volume. The team measured the animal's responses to the sounds they heard by recording the neurological signals from the inner ear through electrodes placed beneath the reptile's skin.

Not surprisingly, the alligator's hearing in air was as good as most air-adapted species, including their close relatives, birds. But how well did the animal's respond to water-borne sounds? Amazingly the alligator's hearing under water was as good as that of goldfish, who are real hearing specialists amongst fish! Although the reptiles heard over a greater range in air than in water, peak sensitivities were around 800 Hz in both environments – these peaks correspond quite well to the range of "chirping" sounds made by hatchling alligators. Since these animals have no obvious specialisation for channelling underwater sound, it's likely that they hear underwater by conducting sound information to their ears through their skull bones. Whilst this possibility remains untested, Higgs' study successfully shows that alligators have managed to overcome the problems associated with hearing in two different media. This success may be worth remembering if you ever holiday in the Everglades!

10.1242/jeb.00045

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