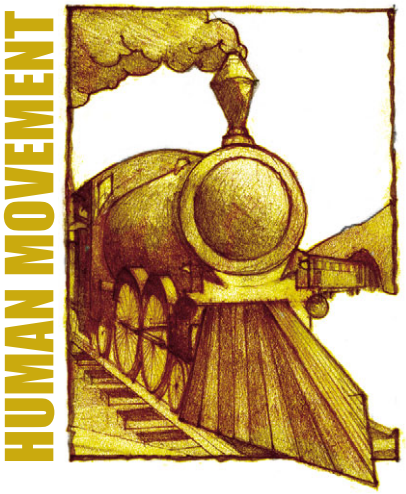
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB



WE GOT THE BEAT

My various, albeit thankfully limited, experiences with dancing, both as an observer and participant, always left me with the impression that people typically have their own rhythm and move to their own beat. Some folks tend to move faster than others, some slower and some… well let's just say that the movements of some of us might be harder to characterize. By contrast, such variation appears not to be characteristic of the rhythm or tempo of another, more common, type of human movement: walking. In fact, laboratory studies have shown that the cadence of human over-ground walking is quite consistent and that a stepping frequency of approximately 2 Hz is generally adopted. If you're like me, several questions come to mind upon learning this: (1) why 2 Hz and (2) are such results simply some artefact of studying walking in a laboratory? In a recent paper, Hamish MacDougall and Steven Moore of Mount Sinai School of Medicine provide possible answers to both of these questions by studying locomotor tempo under natural conditions.

Ten men and 10 women of varying age (22–62 years) and body mass (40–100 kg) were outfitted with a device that monitored linear accelerations of the head in three orthogonal directions (forward–backward, side-to-side and up–down). Previous studies have shown that vertical accelerations of the head map well onto patterns of stepping frequency during walking, thus the device could essentially measure a subject's movement tempo when used. The 'activity monitor' was mounted onto a baseball hat worn by each of the subjects for 10 hours during his/her everyday activities. A subject could flag periods of particular types of activity *via* a button on the device and recorded information about activity types and times in a logbook.

Using fast Fourier transformation, power spectra of head accelerations from each subject could be calculated and analyzed. Despite the varied forms of activity within and among the subjects (e.g. walking, climbing stairs, riding a bike, attending dance class), there was an obvious dominant peak of vertical head movements at 2.0 Hz. Moreover, this result was always present, regardless of gender, age or body size. This value corresponds well with the previously published stepping frequency of 1.95 Hz observed during laboratory studies and illustrates that, in natural circumstances as well as in the lab, adult humans consistently adopt a specific locomotor cadence.

This is quite interesting in itself, but the questions of why an inherent locomotor tempo might exist, and why at 2 Hz, remain open to some speculation. The authors refer to the fact that locomotor energy cost is lowest at a stepping frequency near 2 Hz, and this may be part of the answer. This frequency might also reflect some inherent rhythmicity generated by our central nervous system (e.g. a spinal central pattern generator) – humans adopt a spontaneous frequency of finger tapping at 2 Hz as well. Finally, the tempo may be related to the coordination of head and eye movements during locomotion as the reflexes underlying such coordination display their optimal response at ~2 Hz. Regardless, this work shows that there may well exist a 'common frequency' to human movement; which reminds me, the next time I go dancing I'm going to bring a metronome and set it to 120 beats per minute to keep up with everyone else.

10.1242/jeb.02072

MacDougall, H. G. and Moore, S. T. (2005). Marching to the beat of the same drummer: the spontaneous tempo of human locomotion. *J. Appl. Physiol.* **99**, 1164-1173.

Gary B. Gillis
Mount Holyoke College
ggillis@mtholyoke.edu

GET IN TOUCH AND CALM DOWN

Within the animal kingdom there are numerous examples where many individuals of the same species aggregate. Fish school to avoid predation and to minimise cost of transport, penguins huddle to keep warm in the harsh Antarctic winter, and snakes aggregate to save heat and ensure reproduction in early spring. Many insect species also aggregate and the reasons seem to be diverse. A recent study looked further into one such example and found that the provisionary shield bug (*Parastrachia japonensis*) relaxes when it gets in touch with its own kind.

The provisionary shield bug feeds only on the fruit of the plant *Shoepfia jasminodora*, consequently experiencing prolonged periods without food. When starving, the bugs enter diapause and form aggregations that hang suspended from the leaves and branches of a variety of evergreen plants. Aggregating behaviour has been shown to conserve water and energy in other insect species. To investigate whether this is also the case for the provisionary shield bug, Sumio Tojo and colleagues measured the bug's oxygen consumption, under a variety of experimental conditions, including altered humidity, where they manipulated the number of individuals in the respirometry chamber.

The team's study revealed that the metabolic rate of diapausing bugs halves when they are allowed to aggregate. This effect seems to be attributed to a reduction in physical activity, as the bugs calm down when they touch each other. By contrast, the reduced metabolism did not seem to be related to water conservation, as the metabolic rate of aggregations remained low irrespective of the relative humidity in the respiratory chamber. When the researchers looked further into the cues for the soothing effect of company, they found that physical contact was needed. Thus, if five bugs were in the same respirometry chamber but were prevented from direct physical contact, they did not reduce their metabolic rate. If a bug was allowed to aggregate with dead specimens of its own species, only some of the metabolic reduction occurred, but if these dead specimens were washed in an organic solvent or replaced by specimens of a different species, the effect was totally obliterated. Thus, physical stimulation of a kindred species is needed for the bugs to relax.

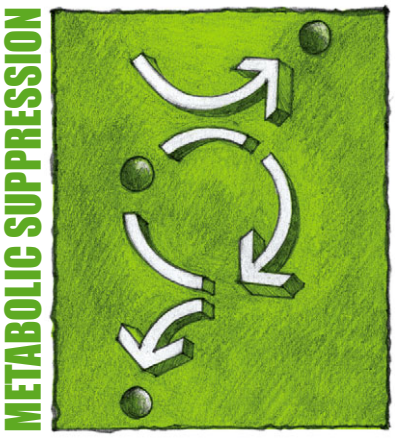
The metabolic reduction associated with aggregation may be of great importance for the provisionary shield bug, as it must survive nine months of the year without an appropriate food source. Indeed, Tojo and colleagues showed that the reduction in metabolism was primarily associated with diapause and that the low metabolic rate of aggregating bugs persisted throughout the diapause season. This low metabolic rate allowed the bugs to survive more than 20 weeks at 25°C with access only to water, and more than 80% survived for a whole year.

Besides being an interesting study of the ecology of the provisionary shield bug, the present study underlines the importance of the conditions under which test animals are investigated. Measurements of resting metabolic rate must be conducted in an optimal environment, even for a simple bug. Curiously, this particular bug species might not be that simple, as it seems to be highly socially evolved in other respects; the females are known for their provisionary behaviour, transporting fresh deliveries of food to nests containing newly hatched juveniles, suggesting that provisionary shield bugs really do seem to get in touch quite often.

10.1242/jeb.02074

Tojo, S., Nagase, Y. and Filippi, L. (2005). Reduction of respiration rates by forming aggregations in diapausing adults of the shield bug, *Parastrachia japonensis. J. Insect Physiol.* **51**, 1075-1082.

Johannes Overgaard
National Environmental Research Institute
jov@dmu.dk



EELS HOLDING THEIR BREATH: NO ROLE FOR PROTEIN KINASES

We all breathe air to supply our organs and cells with sufficient oxygen, but what happens when oxygen becomes scarce? To get an answer to this question we could learn a thing or two from eels that experience hypoxic and anoxic periods routinely when buried in mud. Eels seem to get over anoxia without any difficulties, as they profit from a mechanism that instantaneously and reversibly downregulates their metabolic rate. This process extends the duration of stored energy reserves and, in turn, postpones ATP fuel depletion, lengthening an eel's survival time. Yet relatively little is known about the cellular oxygen-sensing mechanisms that induce this so-called 'metabolic arrest'. To fill this gap, Morten Busk from Aarhus University, Denmark and Bob Boutilier from The University of Cambridge, UK studied liver cells from European eels.

Firstly, they exposed isolated hepatocytes to anoxia by turning off the cellular oxygen supply. Doing so, they found that anoxia caused an amazing 85-fold decrease in ATP production. Accordingly, they quantified the accumulation of cellular lactate, produced when ATP is generated anaerobically from intracellular glycogen, and found that lactate production peaked when ATP levels were at their lowest. Reoxygenating the same hepatocytes 4 hours later, they were surprised to find how reversible anoxic hypometabolism is; the cells quickly returned to their previous cellular ATP levels by metabolizing lactate. The eels survive anoxia by rapidly dropping their metabolism to conserve ATP and switching to glycolytic ATP synthesis to regenerate ATP as soon as the episode has ended.

To discover the nature of the signal that

induces metabolic suppression during anoxia, the team compared cell survivability and energy metabolism in cells experiencing physiological anoxia with oxygenated cells that had been treated with respiratory blockers to stimulate pharmacological anoxia.

Busk and Boutilier quickly concluded that the presence of oxygen itself does not regulate anaerobic ATP generation when they showed that lactate levels were similar in cells experiencing both pharmacological and physiological anoxia. However, the team found that oxygen appears to coordinate ATP consumption rates with the reduced mitochondrial ATP production caused by metabolic arrest. Busk and Boutilier explain that protein kinases have been suggested to be responsible for the control of anoxic downregulation of metabolism in turtles. But when the team specifically tested the role of protein kinase A and C in eel hepatocytes, it turned out that both kinases had no effect on survivability, metabolic rate or energy equilibrium during anoxia, suggesting that kinases play no role in metabolic suppression in hepatocytes.

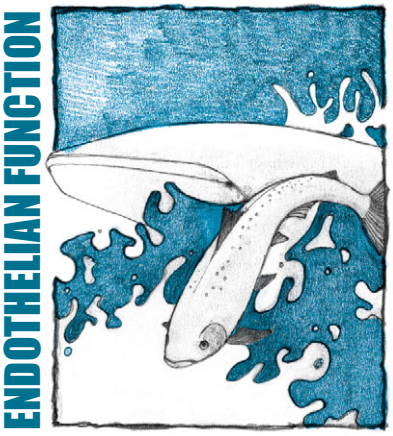
Next, the two scientists wondered whether anoxic cell survival could depend on stress hormones released from distant oxygen-sensing cells. To test this idea, they treated the isolated hepatocytes with adrenaline just before exposure to anoxia. They discovered that adrenaline in fact elevated glycolytic ATP production during anoxia while simultaneously dropping total cellular ATP availability, probably due to increased glucose synthesis and release, or unidentified metabolic costs arising from adrenergic stimulation. Thus, after treatment with adrenaline, total cellular ATP supply during experimental anoxia was substantially lower than in unstressed hepatocytes.

Whilst the entire mechanism of fully reversible anoxic downregulation of metabolic rate still remains to be resolved, Busk and Boutilier have successfully shown the importance of certain stimuli that elicit metabolic arrest in eel hepatocytes. Finally, from the eel perspective, surviving anoxia will always be a capability envied by us humans.

10.1242/jeb.02076

Busk, M. and Boutilier, R. G. (2005). Metabolic arrest and its regulation in anoxic eel hepatocytes. *Physiol. Biochem. Zool.* **78**, 926-936.

Teresa Valencak
Veterinary University Vienna
Teresa.Valencak@vu-wien.ac.at



ET_B PUTS THE SQUEEZE ON PILLAR CELLS

Endothelians are a family of three peptide hormones (ET-1, ET-2, ET-3) with cardiovascular functions that are mediated by two main receptor subtypes, ET_A and ET_B. In mammals, ET_A is responsible for vasoconstriction in blood vessels while ET_B is believed to facilitate their relaxation. Endothelians have also been described in some fish species and play a role in gill vascular resistance, dorsal aortic blood pressure and the contraction of pillar cells, which are interspersed throughout the gill lamellae and credited with keeping the 'roof' and 'floor' of the lamellae together. Ultimately, these vascular effects of endothelians have direct implications on gill blood flow and gas exchange. Furthermore, endothelian-like immunoreactivity has been observed in the gill neuroepithelial cells. Although the function and cellular localization of endothelians are relatively well described in fish, information on the receptors that mediate its response is contradictory. And so, Kåre-Olav Stensløkken, Lena Sundin and Göran Nilsson set out to clarify the nature of endothelian receptors in fish gills in an onslaught of experimentation using a combination of cardiovascular physiology, receptor pharmacology and microscopy.

Atlantic cod, mackerel and sculpin were anesthetized and surgically implanted with a variety of catheters to measure different cardiovascular parameters and to permit the injection of various pharmacological agents. To observe gill blood circulation, a digital video camcorder was hooked up to an epi-illumination microscope and placed in close proximity to the fish gill. Using this setup, the team could observe 'real-time' changes in the diameter of arteries and pillar cells and make direct measurements of their contraction in response to various compounds.

Stensløkken and the group found that intra-arterial injection of ET-1 resulted in the contraction of gill pillar cells, and a dose-dependent increase in ventral aortic blood pressure and gill vascular resistance of all three fish species, with the cod being the most sensitive. Wondering which type of endothelian receptor mediates pillar cell contraction, they injected the fish with the specific ET_B agonist BQ-3020 and found that it mimicked the contractile effect of ET-1 on pillar cells. By contrast, injection of the ET_A antagonist BQ-610 did not block the pillar cell contraction induced by ET-1, suggesting that it is an ET_B-like receptor and not an ET_A receptor that mediates pillar cell contraction. Using antibodies specific for both receptor subtypes, the team revealed ET_B-like immunoreactivity in the gill lamellae adjacent to the pillar cells, consistent with the receptor's role in pillar cell contractility in neuroepithelial cells and blood vessels. By contrast, very little ET_A-like immunoreactivity was detected in the lamellar region, although very faint ET_A-like staining was found adjacent to pillar cells in some lamellae, suggesting that ET_A's role in pillar cell contraction is minor at best. Instead, ET_A was clearly detected in gill nerve fibers, suggesting that endothelian and ET_A might be involved with the transmission of sensory signals and not vasoconstriction.

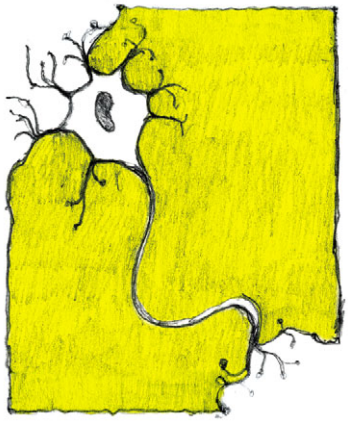
With an arsenal of techniques and a thorough approach, Stensløkken, Sundin and Nilsson have been successful in their quest to further characterize endothelian mechanisms in fish and relieve some of the confusion about the receptors that mediate the hormones' effects. There are still many questions left unanswered; however, we now know that, in general, fish pillar cells are sensitive to ET-1. Furthermore, at least in the Atlantic cod, the contractile effects of ET-1 are mediated by ET_B-like receptors, while ET_A-like receptors may serve another, as yet unknown, function.

10.1242/jeb.02073

Stensløkken, K.-O., Sundin, L. and Nilsson, G. E. (2005). Endothelian receptors in teleost fishes: cardiovascular effects and branchial distribution. *Am. J. Physiol.* doi:10.1152/ajpregu.00618.2004.

M. Danielle McDonald
RSMAS, University of Miami
dmcdonald@rsmas.miami.edu

MEMORY



LESS STUDYING, BETTER MEMORY?

Protein synthesis initiation can be regulated, either positively or negatively, by protein kinases. One such kinase, GCN2, results in general inhibition of protein synthesis, but there is one exception; it stimulates the synthesis of the protein ATF4. ATF4 in turn inhibits the CREB protein, which is responsible for synaptic plasticity and behavioural learning, so GCN2 indirectly leads to the inhibition of CREB. As GCN2 is an evolutionarily conserved kinase and is normally expressed in the hippocampal areas of mammalian brains, a major site for memory formation, Costa-Mattioli and co-workers investigated the effect of GCN2 inactivation upon synaptic plasticity and behavioural learning.

Wondering what effect the loss of GCN2 would have on synapse formation, the team decided to look at mice that had been modified to lack GCN2. They knew that GCN2 regulates expression of the CREB-inhibitor ATF4 and wondered if levels of the inhibitor would fall in the knockout mouse with a resulting rise in the levels of active CREB. Accordingly, the authors saw both a decrease in ATF4 levels and a 25–35% increase in genes regulated by CREB, suggesting that CREB activity had increased.

Next, the authors explored how long-term potentiation (LTP) induction, a model for memory formation, was affected in the hippocampus of knockout mice. LTP has long been used as an experimental tool to study the cellular mechanisms of memory formation. Electrical stimulation of neurons can result in an artificial increase, or potentiation, in synaptic strength. When the team tested a protocol that typically elicits only early-LTP (i.e. lasting 2–3 hours) in normal mice, they found that the protocol resulted in late-LTP (i.e. lasting longer than 2–3 hours) in the knockout mice. Amazingly, a protocol that normally produces early-LTP in normal mice had produced a completely different form of LTP in mice that lacked GCN2. Thus, less stimulation in the knockout mice resulted in longer potentiation of synaptic strength compared with normal mice. But what effect would it have on real memory formation?

The authors behaviourally tested the knockout mice to see if hippocampal-dependent memory was, in fact, similarly affected by the lack of GCN2 and subsequent rise in CREB levels. The popular Morris water maze was used to test for hippocampal-dependent spatial memory. In this task, mice are placed in a pool of opaque water and expected to swim to a hidden platform positioned in a particular spot. After mice learn where the platform is they will swim directly to it when put back in the maze. In the course of normal training (three times a day for five days) the performance of both normal and knockout mice improved, with the normal mice learning faster than the knockout mice. However, when mice were trained only once a day, knockout mice showed better spatial memory than normal mice during training and three days later. Enhanced spatial memory in knockout mice was only seen after weaker training.

Overall, the authors saw a decrease in the threshold for late-LTP in the hippocampus, which was associated with improved spatial memory of weaker conditioning in knockout mice. The authors propose that, in mice lacking the *GCN2* gene, there is enhanced CREB function after weak stimulation, but with stronger stimulation an unknown inhibitory pathway may be activated. The results indicate that neurons might not only have a threshold for activating gene expression but also a second threshold at which too much gene expression blocks synaptic plasticity. So, in the end, the idea of everything in moderation still holds true; from exercise to red wine and now to CREB.

10.1242/jeb.02075

Costa-Mattioli, M., Gobert, D., Harding, H., Herdy, B., Azzi, M., Bruno, M., Bidinosti, M., Mamou, C. B., Marcinkiewicz, E., Yoshida, M. et al. (2005). Translational control of hippocampal synaptic plasticity and memory by the eIF2α kinase GCN2. *Nature* **436**, 1166-1170.

Susan Sangha
Otto-von-Guericke Universitaet Magdeburg
susan.sangha@medizin.uni-magdeburg.de