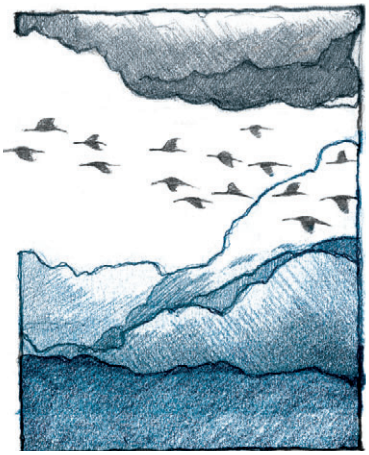
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

COMMUNICATION



WING WHISTLES WARN AND WORRY

What cues can a bird use to indicate the threat of an approaching predator? The most direct, of course, is to keep the head up and keep a look out. However, this clearly limits the time available for bending down and pecking. Similarly, keeping an eye out for movements of other members of the flock could be informative – if you see a neighbour taking off without the usual warm-up, or if you see everyone else flying away, you begin to wonder why – but can also be costly in terms of time spent feeding. Alarm calls from other members of the flock can be an effective means of warning, and need not limit the time spent feeding. However, these can also be very untrustworthy 'cry wolf' signals, benefitting the caller to the detriment of the listener, who might be persuaded to unnecessarily fly away from the food source. An alternative that bridges these extremes is listening to the sounds of take-off of other flock members. Might aspects of these sounds be used as indicators of threat? What are these aspects? And can fearful flights be distinguished from normal take-offs?

These questions were recently approached by Mae Hingee and Robert Magrath (Australian National University, Canberra), studying the crested pigeon. Also known as the 'whistle-winged' pigeon, these birds produce a characteristic sound when they flap, probably due to vibrations of the 4th primary, which is only half the width of neighbouring flight feathers. The sounds of flights were recorded for pigeons in both normal, relaxed take-offs, and ascents (presumably panicked) induced by throwing a gliding model of a predatory hawk over the flock. Alarmed take-offs were both louder (at a similar distance) and had higher 'element cycle rates' indicating higher wingbeat frequencies. This is relatively unsurprising: worried birds flap harder and faster. But do members of a flock take any notice of this acoustic information?

To determine this, Mae and Rob performed playback experiments on flocks of unsuspecting pigeons using the sounds of alarmed and non-alarmed take-off flight, and observed how nearby members of the flock responded. When faced with the sounds of an unfamiliar pigeon in alarmed flight at full volume, the majority of flocks fled. When exposed to non-alarm flight sounds, very few birds responded. When non-alarm flight sounds were amplified to equate to alarmed sounds, there was still little response; when alarm sounds were quietened to equate to non-alarmed noises, some flocks fled, while some birds stayed. However, those staying showed a significant increase in 'vigilance' (they spent more time with their heads up, looking around), indicating that the wingbeat frequency, quite apart from the sound volume, worried the flock.

If a signal as simple as wingbeat frequency is the key to indicating alarm, why have these pigeons evolved special flight feathers and a 'wing whistle' sound? Details of the flight sound that are presumably related to the wing whistle mechanism (for instance the fundamental frequencies) were statistically poor at differentiating alarmed flights from un-alarmed flights. So why evolve the special wing sounds, and not stick to the atonal 'whooshing' sounds that are an inevitable consequence of flapping? One possibility is simply volume – whistles can be considerably louder than whooshing – to catch a neighbour's attention; another, presumably, is some degree of species specificity. There are obvious disadvantages to taking off in fright whenever a bird of a slightly smaller species, and consequently higher, panic-inducing flap frequency, flies nearby.

10.1242/jeb.036335

Hingee, M. and Magrath, R. D. (2009). Flights of fear: a mechanical wing whistle sounds the alarm in a flocking bird. *Proc. R. Soc. B* **276**, 4173-4179.

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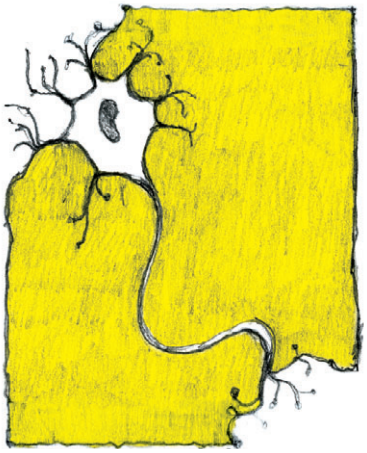
SPINAL CORD RHYTHMS



(RE)DEFINING THE NEURONS THAT CONTROL LOCOMOTION IN VERTEBRATES

The vertebrate spinal cord generates oscillations that ultimately produce the alternating left–right body movements that characterize running, walking and swimming. It has long been hypothesized that the circuitry that produces these spinal cord rhythms resides in the spinal cord itself. The idea is that the circuit lays dormant until higher brain regions send tonic unpatterned excitation down to the spinal cord. This generalized excitatory signal (start moving!) awakens separate oscillatory networks within the spinal circuitry. This hypothesis was first proposed by Graham Brown, and has been widely accepted for ~100 years. But is it actually true? In a recent paper published in the *Journal of Physiology*, Stephen Soffe, Alan Roberts and Wen-Chang Li tested this long-standing idea by studying locomotor circuits in *Xenopus* tadpoles.

One population of neurons (reticulospinal neurons) in the hindbrain (a higher brain region just forward of the spinal cord) have been thought to provide the primary excitatory drive to spinal networks in other systems. The authors reasoned that if Brown's model were true, then these neurons would excite spinal neurons, but not show any kind of rhythmic patterns during locomotor rhythms. To test this, the authors simultaneously recorded from reticulospinal neurons while also recording from motor neurons in the tadpole spinal cord. They found that there are indeed direct excitatory connections descending from reticulospinal neurons in the hindbrain onto motor neurons. However, when they looked at reticulospinal and motor neuron activity during bouts of swimming motor patterns they found something that didn't fit Brown's model. Instead of just tonic (i.e. continuous, unpatterned) excitation to the motor neurons, the reticulospinal neurons were firing in discrete bursts just before motor neurons on every cycle of the swimming rhythm. This suggests that reticulospinal neurons do more than just generally excite spinal circuits – the neurons appear to actively drive every cycle of the rhythm.

But perhaps the tonic excitation comes from even higher order brain regions? Soffe and colleagues decided to test this with a brute force approach. They systematically recorded from hundreds of individual cells in more anterior brain regions and measured when these cells were active relative to reticulospinal neurons during swimming rhythms. The authors saw no evidence for any tonically active descending inputs to the spinal cord in any of these recordings. They found that the reticulospinal neurons were indeed the first neurons in the brain active during every cycle. This suggests that oscillations in these neurons are really what's driving the tadpole spinal cord.

If reticulospinal neurons are driving every cycle of the swimming rhythm, then how do these cells themselves oscillate? The authors show that an interaction between the natural electrical properties of reticulospinal neurons and rhythmic inhibitory inputs onto the neurons could plausibly generate the appropriate rhythmic output. This suggests that the key circuitry for generating spinal cord rhythms does not actually reside in the spinal cord itself.

The work of Soffe and colleagues is important because it challenges a generally accepted hypothesis that has become entrenched. Through a series of careful experiments, they show that in their particular vertebrate (tadpole), the predictions of the entrenched model do not hold true. The authors' then provide an alternative model, one that (even if it's not true in every other vertebrate) will doubtless influence the direction that spinal cord research takes in future years.

10.1242/jeb.036368

Soffe, S. R., Roberts, A. and Li, W. C. (2009). Defining the excitatory neurons that drive the locomotor rhythms in a simple vertebrate: insights into the origin of reticulospinal control. *J. Physiol.* **587**, 4829-4844.

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THERMAL TOLERANCE

HEAT TOLERANCE NOT RELATED TO HSP70 EXPRESSION IN ADULT FLIES

The central importance of the heat shock response to survival of heat stress has become an almost universally accepted concept in physiology. Heat shock proteins of the 70 kDa class (Hsp70) are some of the best studied proteins involved in the stress response and levels of this protein are often used as an indicator of resistance to heat stress. For many years it has been recognized that individuals within a population of organisms express a wide range of Hsp70 levels. Louise Jensen and her colleagues in Denmark and Australia explored the role that this genetically based individual variation in Hsp70 levels plays in determining the heat tolerance of adult fruit flies *Drosophila melanogaster*.

The team conducted three separate experiments to test the importance of Hsp70 expression levels to heat tolerance. Each experiment was conducted on 20–50 inbred lines of flies. Inbred lines of flies have reduced genetic variation making it easier to identify genetic differences in gene expression. To create the inbred lines the team collected wild female flies from locations in Australia and bred their progeny in the lab for at least 3 generations, and up to 45 generations for some experiments.

The team then measured the levels of Hsp70 expression induced by exposing the flies to temperatures of 35°C followed by 1 h of recovery at 25°C. Then they determined each line's heat tolerance using two different methods: in the first they recorded how many of the insects survived a severe 1 h heat shock at 38.3–38.8°C; and in the second they measured the time required for the flies to lose activity at 38.5–39°C. Finally, Jensen and her colleagues evaluated the long-term effects of heat shock protein expression on the life history of female flies from the various

inbred lines by measuring the number of progeny produced per female, the proportion of eggs that successfully developed to become adults, and the total development time for eggs produced by each female.

The team found that Hsp70 expression levels in response to their heat shock regime were different in all the inbred lines produced for all of their experiments. However, these differences in Hsp70 expression level did not correlate significantly with the insects' heat tolerances. Thus, the authors concluded that variation in Hsp70 expression is relatively unimportant in determining the heat tolerance of adult flies.

This result is in opposition to studies conducted on larval flies and suggests that Hsp70 may play different roles in heat tolerance in different life history stages of an organism. In addition, there were no strong correlations with any of the life history characters that were measured in this study, which suggests there is no 'cost' of increased Hsp70 expression as has been suggested by previous studies.

The results of this study suggest that we should seriously rethink the central importance of Hsp70 in determining the heat tolerance of all organisms. The study also provides evidence that measuring levels of Hsp70 in wild organisms may not be a good indicator of thermal tolerance. It is also a warning that we should not make assumptions about the importance of Hsp70 expression levels, or any other protein for that matter, no matter how much we know about that protein. Future studies should confirm a role for this protein in heat tolerance for individual species, or even a different life history stage within a single species, before we draw conclusions about the heat shock response. Finally, the study confirms what many have observed in recent years: there is much more to the heat shock response than Hsp70 and it is time to explore other options.

10.1242/jeb.036350

Jensen, L. T., Cockerell, F. E., Kristensen, T. N., Rako, L., Loeschcke, V., McKechnie, S. W. and Hoffmann, A. A. (2009). Adult heat tolerance variation in *Drosophila melanogaster* is not related to Hsp70 expression. *J. Exp. Zool. A* **311A**, doi:10.1002/jez.573.

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UNDER PRESSURE: GAS GILLS EXTEND DIVE TIMES WHILE LOSING HEIGHT

The solutions aquatic insects have evolved for underwater air breathing range from simple siphons or snorkels to tracheal gills and plastrons. An in-between solution adopted mostly by beetles and bugs is to collect and hold an air bubble over their spiracles while diving. At depth the increased hydrostatic pressure will increase the bubble's oxygen and nitrogen partial pressures (P_{O_2} and P_{N_2}) and O_2 and N_2 will diffuse into the water. Simultaneously, insect O_2 consumption ($\dot{V}_{O_2}$) causes the bubble P_{O_2} to drop below that of the water. Now O_2 diffuses from the water back into the bubble. At this point the bubble becomes a gill. However, bubble P_{N_2} will remain high and N_2 dissolves continually into the water, reducing the bubble's volume. This system is called a compressible gas gill. The gas gill continues to shrink and eventually must be 'refilled' at the water surface.

Two competing models aimed to theoretically describe compressible gas gill function. First, the 'shrinking area' gas gill model states that decreasing volume would also cause gill surface area to decrease. The shrinking area reduces the ratio of gill O_2 uptake to insect oxygen consumption until increased hypoxia in the gill ends the dive.

The 'constant area' gas gill model states that while gas gill volume decreases, the gill surface area remains constant. When a bug collects a bubble, which forms the compressible gill, the air adheres to the entire lower surface of the bug, from below the head to the outer edges and tip of the abdomen. The bubble forms a thin layer over the entire surface of the insect's underside, and as the bubble's volume decreases the air layer becomes thinner, but the surface area remains essentially unchanged. This sustains constant O_2 diffusion into the gill, stabilizing internal gill oxygen partial pressure until the declining gill height reaches a minimal limit and the insects must return to the surface for more air. This results in a theoretical oxygen gain of 8: that is, 7 times the original bubble's O_2 content diffused inward, extending dive time 8-fold.

Both models are mathematically consistent but which holds true? An empirical test was required to resolve the stalemate.

Philip Matthews and Roger Seymour from the University of Adelaide did just that. Using the water boatman, *Agraptacorixa eurynome*, in a series of elegantly conceived experiments, they checked that the bubble adhered to the underside of the bug and measured the P_{O_2}, volume and area of the bug's gas gill, the bug's oxygen consumption and critical P_{O_2}, and gill ventilation through leg movements.

Seeing that the bubble did adhere to the bug's underside, the team found that the initial gill P_{O_2} dropped rapidly from 21 kPa (atmospheric P_{O_2}). However, the interaction between bug oxygen consumption and O_2 diffusion into the gill allowed gill P_{O_2} to stabilize at ~3.23 kPa as long as gill ventilation was maintained. With minimal leg movement gill P_{O_2} reached ~1 kPa, while it stabilized at 5 kPa during swimming. The bug's critical P_{O_2} (the P_{O_2} required to maintain resting oxygen consumption against tracheal resistance) was 2.1 kPa. Finally, the authors calculated that the gill factor (gill oxygen gain) is 7.5, allowing a dive time of 42–78 min before the flattened gill needs 'refilling'.

The authors' empirical test results very closely approximated the 'constant area' gas gill model. An interesting aside to the understanding of the functioning of compressible gas gills is that the bugs can regulate gill P_{O_2} through increased or decreased leg gill ventilation, thus manipulating gill P_{O_2} to supply oxygen at physiologically useful rates.

10.1242/jeb.036343

Matthews, P. G. D. and Seymour, R. S. (2009). Compressible gas gills of diving insects: Measurements and models. *J. Insect Physiol.* doi:10.1016/j.jinsphys.2009.07.011.

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