Inside JEB highlights the key developments in *The Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

CLOWNFISH SUPPLEMENT ANEMONE OXYGEN BY FANNING

Setting up home in the stinging tentacles of a sea anemone might seem like a risky option, but anemonefish – popularly known as clownfish – are perfectly content in their unlikely abode. Fending off peckish anemone predators in return for refuge, plucky clownfish have achieved a satisfactory arrangement with their deadly partners. Yet Joe Szczebak from Auburn University, USA, wondered whether there might be more to the unconventional collaboration than met the eye. According to Szczeback, coral reefs are awash with oxygen during the day, but levels can plummet overnight when photosynthesis has ceased. Adding that some damselfish waft oxygen-rich water over corals at night to supplement their oxygen supply, Szczebak wondered whether clownfish might have struck a similar deal with their anemone hosts. 'There had been almost no research done on the clownfish–anemone mutualism at night', explains Szczebak, so he and his Master's thesis advisor, Nanette Chadwick, decided to find out whether clownfish fan their anemone hosts to supplement their meagre nocturnal oxygen supply (p. 970).

Szczebak and Chadwick travelled to Fuad Al-Horani's physiology lab at the Marine Science Station in Aqaba, Jordan, and went SCUBA diving in the Red Sea to find the diminutive fish and their anemone partners. Then the team isolated each fish from its anemone and measured their individual oxygen consumption rates before reuniting the partners. They discovered that the fish and anemone consumed 1.4 times more oxygen when they were together than when they were apart. Something was happening when the fish and its anemone were together to increase their oxygen consumption, but Szczebak wasn't sure what.

Having successfully returned the fish to their Red Sea home before flying back to the United States, Szczebak repeated the experiments with Ray Henry's help in Chadwick's Auburn lab. However, this time he tried an additional test. Separating the clownfish from its anemone with plastic mesh – so that the clownfish could still see its partner and they could smell each other – Szczebak remeasured their oxygen consumption, but it was still lower than when they were in contact. 'There was something about the physical contact between them that was the source of the increase', says Szczebak.

Spending long nights filming the clownfish as they nestled in amongst their anemone's tentacles, Szczebak realised that the fish were much more active than had been thought previously. He frequently saw the fish fanning the anemone with their rapidly weaving fins and the fish often burrowed deep into their host, sometimes making a 180 deg turn deep within the mass of tentacles to open up the collapsed anemone and apparently circulate water through it. However, when Szczebak measured the oxygen consumption of isolated anemones as he flowed water through them at speeds ranging from 0.5 to 8.0 $\mathrm{cm\,s^{-1}}$, their oxygen consumption never increased by as much as it did when paired with a clownfish, suggesting that the clownfish also contribute the partnership's increased oxygen consumption.

'I think that I have found foundational evidence that, like similar symbioses on coral reefs, anemonefish may actively modulate flow conditions surrounding their host to benefit them under low oxygen scenarios', says Szczebak. He adds that Chadwick's group is continuing to investigate whether the fish indulge in their nocturnal antics purely to supplement the anemone's oxygen supply or for an as-yet-undetermined reason with the additional benefit of improved circulation.

10.1242/jeb.086678

Szczebak, J. T., Henry, R. P., Al-Horani, F. A. and Chadwick, N. E. (2013). Anemonefish oxygenate their anemone hosts at night. *J. Exp. Biol.* **216**, 970-976.

Kathryn Knight

A BAT NEVER FORGETS: INTERNAL MAPS AID FLIGHT

Although driving home from work or walking around a supermarket without bumping into things may seem simple, these tasks require constant processing of sensory updates from our environment, as well as memory. If we become disorientated, we can just stop, have a look around and adjust our bearings. For bats faced with similar tasks, such as flying around foraging or flying home to roost, life is a little more complex. Many bats have poor eyesight and use echolocation to navigate, sending out calls and then interpreting the returning echoes as 'views'. 'A bat updates its view of the surrounding world five to 20 times per second during normal flight', says Jonathan Barchi, a PhD student from Brown University, USA. 'This


Jonathan Barchi

may not seem low, but when combined with the range of view echolocation provides [up to 5 m] and flight speed of these bats [up to $3\,\mathrm{m\,s^{-1}}$], it means that they are only getting a few views of any given region of a space before leaving it behind.' As bats don't have the luxury of hovering to re-orientate, Barchi wondered whether bats rely on an especially well-developed internal map to enable them to quickly navigate and manoeuvre. Working with his supervisor James Simmons and an undergraduate student, Jeffrey Knowles, he turned to the big brown bat, *Eptesicus fuscus*, to begin his investigation (p. 1053).

Over a period of 6 days six bats were repeatedly released from the same spot into a dark room cluttered with chains hanging from the ceiling in defined locations. The team mapped the movement of the bats as they flew about this obstacle course using highly sensitive microphones embedded in the room's walls; based on the timing with which these microphones picked up the echolocation calls, the researchers could then calculate exactly where in the room the bats were.

After just 2 days most of the bats had already started to adopt stereotyped but individual and unique flight paths. 'The bats seemed to learn how to fly a nice smooth path through the field of obstacles, without needing to make sharp turns or other abrupt changes in course', remembers Barchi. Having remarkably quickly sussed out where all the obstacles lay in the room, they rarely deviated from their chosen aeronautical trajectory. Next, the team wondered how well the bats would cope if they were released from different points in the room. Unperturbed, the bats were soon back to their established paths. When tested again after a month's break, the bats still remembered the room, and flew in their precise characteristic flight paths as if no time had passed at all.

In short, these bats learnt very quickly how to efficiently avoid collisions, and they developed an internal map of the room that they remembered for future guidance. Barchi suggests that their maps might allow them to devote more attention to foraging for prey by making navigation a background process. The map may also help them adjust to changes in their flight environment; when the team changed the location of the chains to be a mirror image of the original setup, the bats had to deviate from their previous preferred flight paths or risk crashing into the chains. However, they devised a new flight path more quickly than when they first experienced the original layout, suggesting that their maps helped in some way.

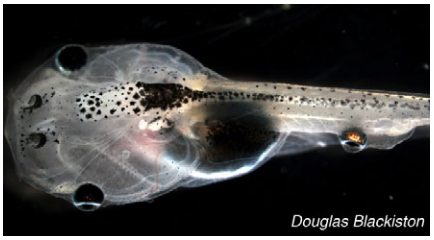
So, it seems that these airborne mammals overcome the disadvantages speedy flight poses for navigation by developing precise and stable internal maps.

10.1242/jeb.085373

Barchi, J. R., Knowles, J. M. and Simmons, J. A. (2013). Spatial memory and stereotypy of flight paths by big brown bats in cluttered surroundings. *J. Exp. Biol.* **216**, 1053-1063.

Nicola Stead

PLUGGING INTO THE SPINE GIVES THE GIFT OF SIGHT


Douglas Blackiston

For most, the idea of loosing one's eyesight is unbearable, and despite many technical breakthroughs, blindness still remains largely untreatable. Douglas Blackiston from Tufts University, USA, explains why: 'Implanting an artificial [retinal implant] or biological replacement eye would require connecting it to the nervous system in some way. For those with damaged optic nerves, or those missing the eye completely, retinal implants are not possible treatment options.' If connection through the optic nerve is not possible, could replacement eyes connect elsewhere? And just how hardwired are our nervous systems to expect data from specific organs in pre-determined locations? This question has intrigued Blackiston's supervisor, Michael Levin, for many years. He realised that they could use blind *Xenopus* tadpoles to investigate how adaptable the brain and central nervous system is to receiving information from abnormally located eyes (p. 1031).

To begin, Blackiston induced blindness in the tadpoles by surgically removing their eyes, with some of the blinded amphibian patients also receiving donor eyes, which were transplanted onto unusual positions along their torsos and tails. Whilst grafting over 200 tiny donor eyes was painstaking work, it was the next step that proved most challenging, recalls Levin. The investigating duo needed to develop a test to determine whether these transplanted ectopic eyes allowed blind tadpoles to see. 'While physiology can show that an eye sends action potentials [electrical signals] in response to light, a behavioural regime is necessary to show that the brain is receiving such data and processing the information in a meaningful way', Levin points out.

After nearly a year of hard work the pair came up with a suitable eye test for blind tadpoles. They placed their amphibious subjects in a well where half of the dish was illuminated with red light and the other half with blue light, which they inverted at regular intervals. During training sessions, whenever the tadpoles ventured into areas bathed in red light they received a little warning zap of electricity. After a break the tadpoles were tested to see whether they had learnt to associate the red light with electrical punishment and whether they would stick to the blue side of the dish. While blind tadpoles never showed a preference for blue light, six tadpoles with donor eyes behaved like their full-sighted relatives and showed a learnt desire to remain in the safe, blue-illuminated areas. These fortunate six tadpoles obviously were able to see through their new ectopic eyes.

However, the team had tested 134 tadpoles endowed with transplanted eyes, so what made these six tadpoles different? The answer lies in the nerve patterns extended by the donor eye after transplantation. Levin and Blackiston had cleverly used tadpoles expressing a red fluorescent protein as donors, and so they were able to see the pattern of red neurons extending from the new ectopic eye using a microscope. Half of the transplant patients showed no innervation. Of the remaining half, 26% showed neurons projecting from the donor eye towards the gut and 24% had neurons extending towards the spine. It was within this latter group that the six lucky tadpoles with colour vision fell.

'The [tadpole's] ability to see when ectopic eyes are connected to spinal cord and not directly to the brain was stunning', remembers Levin. 'We believe that future biomedical treatments for sensory or motor disorders may not need to target the original brain locations to restore function', he adds. It is clear that his finding could radically change our future approach to regenerative medicine for a wide range of disorders.

10.1242/jeb.084921

Blackiston, B. J. and Levin, M. (2013). Ectopic eyes outside the head in *Xenopus* tadpoles provide sensory data for light-mediated learning. *J. Exp. Biol.* **216**, 1031-1040.

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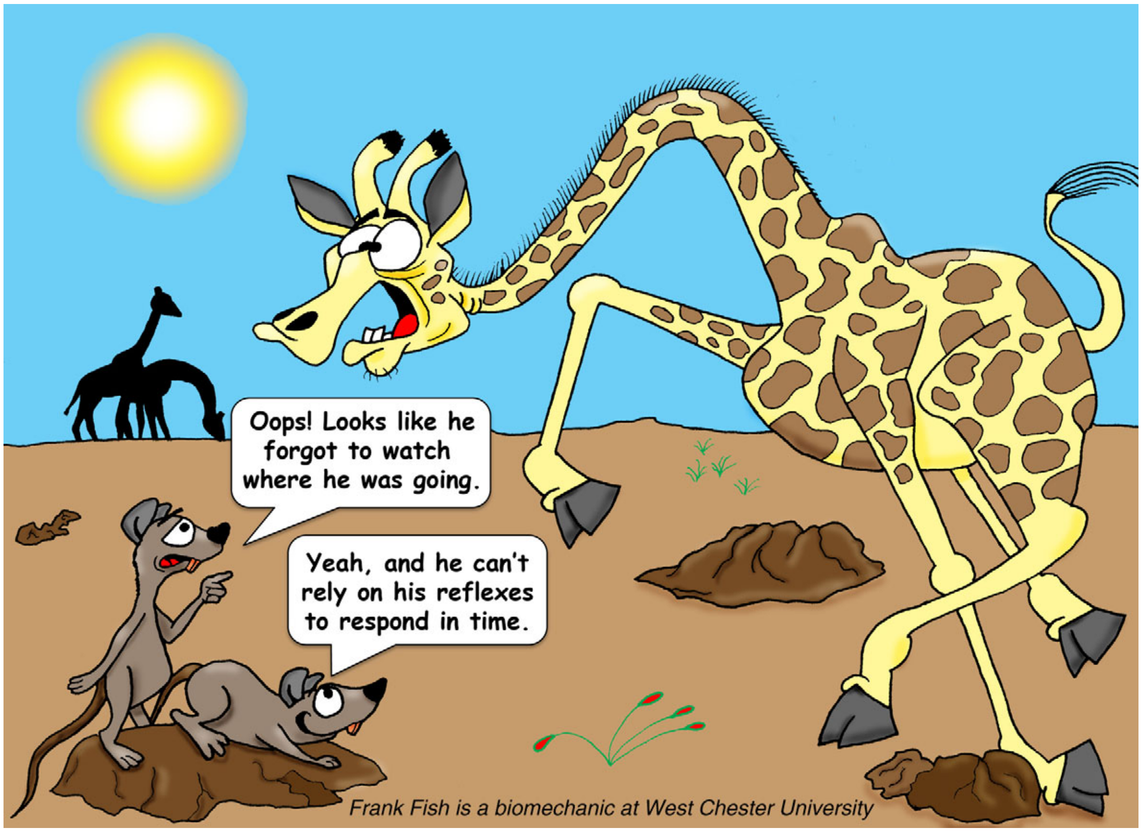
TREADING CAREFULLY: SLOW SENSING AND RESPONDING IN GIRAFFES



When you're stretching for something that is just out of reach do you ever envy the world's tallest land mammal – the giraffe? Although its size might seem like the perfect solution for reaching the highest leaves, being tall does have drawbacks. As animals grow bigger, electric impulses have to travel farther along nerve fibres to deliver their commands. This could have important consequences for how quickly – and appropriately – these towering animals sense and respond to the world around them. Heather More, Shawn O'Connor (both from Simon Fraser University, Canada) and their colleagues travelled to South Africa to investigate just how much of a sensing and responding delay the giraffes' colossal dimensions caused (p. 1003).

The team captured eight giraffes and administered gentle electric jolts to the sciatic nerve and part of the muscle in the hind leg. Carefully placed electrodes showed that the impulses travelled at a nifty velocity of 50.4 m s^{-1} and that it took 13.4 ms for muscle contraction to begin and 45.9 ms to reach full force. Although this may seem speedy, the team points out that in rats, muscle contraction begins after 4.5 ms and nerve impulses travel at 59.4 m s^{-1}. These delays mean that responses to stimuli can take at least 100 ms, and as this is about half the time that a galloping giraffe's foot is on the ground, it doesn't leave much room for error.

Although giraffes are not as quick at sensing and responding to stimuli as their rodent compatriots, perhaps they sense and respond more precisely? To investigate, the team measured how many nerve fibres made up the giraffe's sciatic nerve. As the number of fibres increases, so does the ability to fine-tune muscle movements and precisely detect stimuli. They found that giraffes have, on average, an impressive 106,697 fibres per sciatic nerve. However, to match the precision of the nimble rat they would need 5.6 million fibres per nerve. So, again it seems that rats may be more accurate at sensing and responding than their tall friends. However, as giraffes don't often stumble, they must have developed ways to overcome these disadvantages.

10.1242/jeb.085415

More, H. L., O'Connor, S. M., Brøndrum, E., Wang, T., Bertelsen, M. F., Grøndahl, C., Kastberg, K., Hørlyck, A., Funder, J. and Donelan, J. M. (2013). Sensorimotor responsiveness and resolution in the giraffe. *J. Exp. Biol.* **216**, 1003-1011.

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