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Inside JEB

## ALPINE NEWTS' MULTIPLE DINING OPTIONS


If you've ever been apple bobbing, you'll know just how hard aquatic feeding can be; every time you get close to grasping that tantalising apple, it is pushed away again by the bow wave created by your jaw entering the water. Underwater diners have overcome this problem, as Egon Heiss, a post-doc at the University of Antwerp, Belgium, explains: 'They use a technique called suction feeding where they very rapidly expand their mouth and throat cavity through a series of very coordinated and fast movements. This then drives the prey and the surrounding water to flow into the mouth. But the whole thing changes on land, you cannot use suction feeding on land because the air is not dense enough, so most animals use a prey-capture mode based on jaw movements or tongue movements.' This is all well and good if you decide to stick to living on land or solely underwater, but what about animals that regularly transition between the two? For example, Alpine newts spend half of the year as aquatic animals before transitioning to a terrestrial phase during the autumn and winter months. To find out whether, and how quickly, these newts changed their feeding techniques between phases, Heiss teamed up with Peter Aerts and Sam Van Wassenbergh (p. 4426).

Heiss began by capturing several newts during their aquatic phase before settling them into an aquarium in the lab. Tempting them with maggots, he could then use high-speed cameras to capture their underwater feeding dynamics. As expected, during their aquatic phase they used suction feeding to capture the tasty meal. When the newts switched to their terrestrial phase, the team found they had also switched feeding tactics, using their tongues to capture the maggots.

But how quickly could they switch between the two feeding modes? During their aquatic phase, Alpine newts are known to wander from pond to pond and might decide to have a mid-trip meal on land. Similarly, during their terrestrial phase, these newts can temporarily enter and feed underwater. 'I thought that the neuromotor control that controls these movement patterns also changes when the animals change their phase and so I thought that when they're in the terrestrial phase they would use lingual prehension on land and underwater, because they would not be able to change that fast', says Heiss. 'And *vice versa* I thought that when they're in the aquatic phase they would use suction feeding movements on land.' However, after patiently luring his newts to feed on land during the aquatic phase and in water during the terrestrial phase, Heiss and his colleagues found the exact opposite. These newts have learnt that using terrestrial feeding mechanisms underwater is not an effective way to ensure hunting success, and they had easily switched to suction feeding. Feeding on land during the aquatic phase was a little harder, with newts adopting an intermediate approach between suction feeding and terrestrial feeding.

When the team went back and re-analysed the tongue-dependent terrestrial feeding method used during the terrestrial phase, the team could also see elements of aquatic feeding. 'We're pretty convinced that aquatic feeding is the more ancestral form of feeding, and that terrestrial prey capture has been achieved by step-wise modifications of this ancestral pattern', concludes Heiss. What's more, this bit-by-bit modulation of the aquatic feeding behaviour not only has helped newts successfully transition between land and water but it may also represent what happened in our evolutionary history in the transition from water to land.

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**Heiss, E., Aerts, P. and Van Wassenbergh, S.** (2013). Masters of change: seasonal plasticity in the prey-capture behaviour of the Alpine newt *Icthyosaura alpestris* (Salamandridae). *J. Exp. Biol.* **216**, 4426-4434.

**Nicola Stead**

## MITOCHONDRIA TO THE RESCUE IN TURTLE NEURONS

When neurons are deprived of oxygen, NMDA receptors become activated, allowing an influx of calcium ions into the neuron. As the mitochondria can't efficiently produce ATP in the absence of oxygen (anoxia), ATP-fuelled pumps can't pump out the ions and calcium accumulation eventually leads to cell death. However, in anoxia-tolerant animals, such as the western painted turtle, calcium flow through the NMDA receptors is rapidly reduced during anoxic periods and neuronal death is avoided. So how do they do it? It's a question that intrigues Leslie Buck, from the University of Toronto, Canada, and over the last few years, Buck and his team have


found that a small increase in intracellular calcium (below the levels that induce cell death) is necessary to reduce the NMDA receptor's activity. Buck suspected that mitochondria might be the source of this calcium and that the mitochondrial permeability transition pore (mPTP) was involved; to find out, he recruited postgraduate student Peter Hawrysh to the project (p. 4375).

To test the mPTP's involvement in regulating NMDA receptor activity, the duo surgically removed small flaps of cerebral cortex from their turtles. 'It's a very nice system because it functions like an intact brain, all the connections in that particular area are present', explains Buck. 'We'd put this piece of tissue in a recording chamber where we could control the flow of the saline into the chamber. We could then gas that saline flow with nitrogen or oxygen and $CO_2$ to give us what we thought was a fairly good representation of a transition from a normoxic to an anoxic situation. We could then put recording electrodes into the tissue and record various ion channel activities.' To begin with they added NMDA during normoxia to measure NMDA currents, and when the tissue was made anoxic, the level of NMDA receptor activity decreased as expected. When the duo blocked the mPTP with cyclosporine A during anoxia, the NMDA receptor activity returned to normoxic levels and when the pair added atractyloside (which activates the mPTP) during normoxic conditions, they saw NMDA receptor activity was decreased. By using dyes that reacted to calcium, Buck and Hawrysh could also show that activation of the mPTP corresponded to an increase in intracellular calcium.

Next, the duo moved on to working out how the mPTP was activated. 'We measured membrane potential with rhodamine, which is a mitochondrial membrane potential-sensitive indicator, and we saw it change and that connected up with changes in the NMDA receptor activity', recalls Buck. In addition, when they pharmacologically activated potassium channels to depolarise the membrane, they also saw mPTP activation.

However, the duo found that membrane depolarisation was carefully controlled: 'It doesn't fully depolarise', explains Buck. 'If we use an uncoupling compound like FCCP, we get a much larger decrease in membrane potential, which likely represents the full depolarised state. Under normoxic conditions it's [the membrane's] polarised and now we have a new intermediate state during the anoxic phase.' To maintain this intermediate state, the duo found that the $F_1/F_0$ ATP synthase, which usually uses a gradient of hydrogen ions to produce ATP, switches instead to using ATP to pump hydrogen ions across the mitochondrial membrane to maintain this intermediate potential. On the surface, this might seem to use up valuable ATP when reserves are running low, but by carefully controlling the activation of the mPTP channel, and thus facilitating a small intracellular change in calcium, it seems that the turtles have found a way to prevent unregulated calcium entry *via* the NMDA receptor.

10.1242/jeb.097204

**Hawrysh, P. J. and Buck, L. T.** (2013). Anoxia-mediated calcium release through the mitochondrial permeability transition pore silences NMDA receptor currents in turtle neurons. *J. Exp. Biol.* **216**, 4375-4387.

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## HOW BIG IS BIG ENOUGH? $O_2$'S ROLE IN SENSING BODY SIZE

To become an adult fruit fly, a *Drosophila* larva will have to undergo complete metamorphosis. However, before they are able to begin this process they first need to reach a critical mass during their final larval instar. Once attained, a well-studied hormonal cascade is initiated, culminating in a peak of the hormone ecdysone just prior to the transition to the pupal stage where they undergo metamorphosis. Although the hormones' roles are well characterised, Viviane Callier wondered: 'What are the physiological cues that an insect uses to initiate metamorphosis? How do they physiologically know that they are big enough to become adults?' Callier, a post-doc in Jon Harrison's lab at Arizona State University, USA, suspected oxygen might be involved, as she explains: 'Larvae do not have lungs; instead they breathe through tubes called tracheae. During the growth phase, oxygen demand is going to increase but the supply structures are largely fixed. So, the hypothesis was that as larvae are growing they start to sense that they're running out of oxygen, and that might be the physiological cue that tells them: okay, I'm going to be running out of oxygen soon, I need to moult and make a bigger tracheal system to continue growing.' So, Callier decided to investigate with help of other lab members and collaborators (p. 4334).

To test the theory, the team reared larvae just up until their transition to the final larval instar under normal oxygen conditions. The larvae were then divided into three groups: one group remained in a chamber with normal oxygen conditions (about 21% oxygen), another group was put in hypoxic conditions (10% oxygen) and the third group was housed in a hyperoxic environment (30% oxygen). The team then measured the critical weight for each group as well as growth rates and time taken to form the pupa after reaching critical weight. 'The idea was, again, very simple; if larvae are running out of air as they grow through their instar, then if you give them less oxygen they should run out of air at a smaller size and therefore moult [into a pupa] when they're smaller. If you give them more oxygen, that should relieve a constraint on the oxygen supply and they should therefore moult larger and later', explains Callier.

As expected, the team did indeed find that the critical weight had decreased in hypoxic larvae: 'They initiate metamorphosis at a smaller size, so they do pupate smaller', says Callier. 'But they actually take much longer to pupate, so their third instar is actually extended.' What's more, the team found that hyperoxic larvae didn't grow bigger as anticipated, and in fact, they showed slower growth rates at the beginning of the instar. When the team measured ecdysone levels they saw basal levels were raised in both hypoxic and hyperoxic larvae, which they suspect might be responsible for slowing growth rates down.

On the whole, Callier concludes, 'We found that oxygen is important for determining the timing and the size at which metamorphosis is initiated, but it's not a single cue and there's probably no single physiological cue for body size regulation. There are multiple inputs, multiple cues and those are going to differ from one species to another.'

10.1242/jeb.097592

**Callier, V., Shingleton, A. W., Brent, C. S., Ghosh, S. M., Kim, J. and Harrison, J. F.** (2013). The role of reduced oxygen in the developmental physiology of growth and metamorphosis initiation in *Drosophila*. *J. Exp. Biol.* **216**, 4334-4340.

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## ALL THE BETTER TO SEE YOU WITH: PIGMENT VARIATIONS IN PARROTS


Birds around the world rely on colourful plumage to identify and assess their kinsmen. Birds see colour using four colour visual pigments in their cone cells in addition to a rod pigment that is used in dim light vision. Each of these visual pigments is made up of one of five opsin proteins (LWS, SW1, SWS2, RH2 and RH1) and a chromophore, where each pigment absorbs a different range of light wavelengths. In some non-avian species the spectral sensitivities of these visual pigments have co-evolved with body colour and differ between individuals of the same species. Curiously, however, to date all studies suggest that the visual pigments vary little within the avian class, and even less within a particular species. To investigate further, Ben Knott, a researcher at Deakin University, and Wayne Davies, from the University of Western Australia, both in Australia, and their colleagues turned to the unusual parrot *Platycercus elegans* whose plumage can range from red to yellow (p. 4454).

To begin with, the team characterised the wavelength sensitivity of the different visual pigments, by isolating rod and cone cells expressing the five different pigments. As expected, the five different pigments, absorbed light from the ultraviolet to the red ends of the visual light spectrum.

Next, the team moved on to characterising the opsin proteins in more detail and sequenced the mRNA transcripts of the five opsins. As expected, three out of the five (*LWS*, *SWS1* and *SWS2*) did not vary very much and were similar to opsins found in other bird species. However, the *RH1* and *RH2* opsins were noticeably different from the equivalent opsins in other birds, with proteins that would have extended carboxyl termini. What's more, the RH2 protein varied considerably, with mRNA transcripts encoding short, medium and long variants. The team suspect that by controlling the size of the RH2 opsin protein, parrots may be able to tweak the spectral sensitivity of the cone cell containing the RH2 visual pigment, thereby aiding them to see better in the yellow and red parts of the spectrum. Although the team still need to test this theory, it seems that there is more variation in avian colour vision than hitherto thought.

10.1242/jeb.098418

**Knott, B., Davies, W. I. L., Carvalho, L. S., Berg, M. L., Buchanan, K. L., Bowmaker, J. K., Bennett, A. T. D. and Hunt, D. M.** (2013). How parrots see their colours: novelty in the visual pigments of *Playcercus elegans*. *J. Exp. Biol.* **216**, 4454-4461.

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