Inside JEB is a twice monthly feature, which 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.

Inside JEB

TADPOLE TRANSITIONS


Photo by Richard Kinkead

Metamorphosis is a stressful time in a frog's life; not only does the tadpole develop legs and lose its tail, but it also has to cope with the replacement of its gills with lungs. This transition from water-breathing to air-breathing coincides with a 'switch' in the animal's response to plummeting oxygen levels: while tadpoles respond vigorously and start breathing faster, frogs are relatively insensitive and tend to breathe more slowly when oxygen levels drop. How is this developmental switch regulated? To find out, neurobiologist Richard Kinkead and his students at Université Laval in Québec, Canada, took a closer look at the neurons in a frog's developing brain (p. 3015).

The team suspected that the neurotransmitter noradrenaline, which is known to influence breathing rhythms in mammals, might play a role in the different responses of frogs and tadpoles to falling oxygen levels. To see if noradrenaline mimics the developing frog brain's response to low oxygen levels, Kinkead's student Stéphanie Fournier carefully removed the brainstems of tadpoles and adult bullfrogs and bathed the brainstems in noradrenaline. Using suction electrodes, the team recorded the bursts of brainstem activity that would drive breathing movements in a live animal. As the team had suspected, brainstems bathed in noradrenaline responded just like brains exposed to oxygen-depleted bathing solution; tadpoles' brainstems showed increased bursts of activity, while those of adult frogs decreased their activity.

Noradrenaline binds to different kinds of receptors, called α-adrenergic receptors, in the brain. 'We wanted to examine which specific receptors are involved in the switch,' Kinkead says. When the team added blockers of α_1 and α_2 adrenergic receptors to the solution bathing the brainstems, they saw that blocking either of these receptor types prevented the normal response to low oxygen levels in tadpole brains, but only α_1 blockage prevented the normal response of frog brains to oxygen depletion. This suggests that although activation of any α-adrenergic receptor mediates tadpoles' responses to falling oxygen levels, only α_1 receptor activation mediates the breathing responses of adult frogs in oxygen-depleted conditions.

'But these findings still didn't explain why the response to low oxygen levels changes during development,' Kinkead says. His attention was drawn to the neurotransmitter γ-aminobutyric acid (GABA). He knew that, in mammals, activation of GABA-releasing (GABAergic) neurons has excitatory effects early in development, but exerts inhibitory effects later on. So Kinkead reasoned that 'GABAergic neurons are a good candidate to explain the developmental switch seen in frogs.' To determine whether GABAergic neurons act as the 'middle man' in frogs' developmental changes, the team added chemical blockers of GABA to the brainstem bathing solutions to see whether this would prevent the typical responses of frogs and tadpoles to low oxygen levels. They saw that blocking this neurotransmitter before adding noradrenaline to the bathing solution prevented the traditional response to low oxygen in both tadpole and frog brains. The team concluded that activation of GABA-producing pathways is necessary for noradrenaline to exert its effects on the brain when oxygen levels fall. 'These results allowed us to pinpoint a mechanism for the developmental switch,' Kinkead says, reasoning that maturation of these pathways during development accounts for the switch.

Taken together, the findings shed light on the transition from water- to air-breathing. 'Bits and pieces of information were already there on the table,' Kinkead says. 'What's interesting about this study is that it puts various parts of the puzzle together.'

10.1242/jeb.010900

Fournier, S., Allard, M., Roussin, S. and Kinkead, R. (2007). Developmental changes in central O_2 chemoreflex in *Rana catesbeiana*: the role of noradrenergic modulation. *J. Exp. Biol.* **210**, 3015-3026.

Yfke Hager

ONE PEAK OR TWO?

As anyone who has ever heard a frog chorus can testify, the cacophony of trills, peeps, croaks and whistles make it difficult for us to distinguish individual frogs from the background din. Female frogs, though, have two inner ear organs that are relatively more sensitive to the range of frequencies that their males produce, which helps the females find them. Because different frog calls have different sound properties, Carl Gerhardt and colleagues wanted to know how this affected females' preferences for different calls in four closely related species of American treefrog (p. 2990).

Three of the treefrog species – two species of grey treefrog and the canyon treefrog – all produce calls with two distinct sound peaks at different frequencies. The fourth species, the bird-voiced treefrog, has a single high-frequency peak in its song. The team wanted to test two competing hypotheses for how frogs recognise sounds. The matched-filter hypothesis predicts that the nervous system is more sensitive to the specific frequencies in a male's call, so if a call has two sound peaks, a signal with both peaks is preferred over a call with just one of the peaks. The pre-existing-bias hypothesis takes into account that there is sometimes a mismatch between female preference and the calls that males produce, which could lead to sexual selection on male calls.

Using simplified electronic signals which accurately mimicked a natural call, the team tested females' responses in sound-proof rooms to calls played out of one of two speakers 2 m apart. If a female preferred a call, she would move towards the speaker producing it. Comparing the preferences of the four species to the number of sound peaks in a call, they found that grey and canyon treefrogs with double-peaked calls preferred this type of call over a call with only one of the frequency peaks. The bird-voiced treefrog preferred the calls with single high-frequency peaks, not two, just like the males' natural calls. This finding was consistent with the matched-filter hypothesis, and not the pre-existing-bias hypothesis.

Knowing that the higher-frequency peak in the call stimulated one inner ear organ, while the lower frequency peak stimulated the other, the team tested which peak the frogs preferred when given a choice between a two- or one-peaked call. The bird-voiced treefrogs, as expected, overwhelmingly chose the single high-frequency peak. They found that the other frogs responded differently, however: some populations of grey treefrogs, for example, preferred the high-frequency peak much more than grey treefrogs from another population in another part of the country. This suggests that there is probably a bias towards certain frequencies in the nervous system of different frogs, and that different populations could evolve to prefer and produce different calls.

So did female grey treefrogs prefer high- or low-frequency peaks? The team found that preference depended on sound intensity: both species of grey treefrog preferred lower frequencies, which stimulate one ear organ, at 65–75 dB. At 85–90 dB, however, one species preferred higher frequencies, which stimulate the other ear organ, while the other had no preference. This means that females' frequency preference differs depending on how intense the sound is: in other words, how close she is to a male. With this in mind, researchers will have to be very careful when designing future experiments.

10.1242/jeb.010918

Gerhardt, H. C., Martínez-Rivera, C. C., Schwartz, J. J., Marshall, V. T. and Murphy, C. G. (2007). Preferences based on spectral differences in acoustic signals in four species of treefrogs (Anura: Hylidae). *J. Exp. Biol.* **210**, 2990-2998.

BIG CHEEKS HOARD FOOD


Photo by Carolyn Buckley

Anyone who has ever owned a pet hamster can't fail to be impressed by the amount of food that these little creatures can stuff into their cheek pouches: at times, up to 20% of their body weight. When hamsters pouch food, they are behaving in a so-called 'appetitive' way, meaning that they are driven by hunger to find and store food, rather than to immediately eat what they find. Because hamster cheek pouches are only involved in hoarding, but not eating, 'they are a beautiful model system for studying appetitive behaviour', says Carolyn Buckley of Lehigh University, who investigated pouch filling behaviour in the Syrian hamster (*Mesocricetus auratus*) with her colleagues Jill Schneider and David Cundall (p. 3096).

The team wanted to know how hamsters could get so much food into their pouches, so they filmed hamsters at 60 frames per second as they pouched food pieces and measured their forepaw and jaw movements. They found that the movements differed slightly depending on whether the hamsters were pouching large 2.5 g pellets from their feed or smaller items such as sweetcorn kernels or sunflower seeds. The hamsters tended to use their forepaws to help manipulate large food pieces into their mouths, while they simply grabbed smaller food items with their tongues. Studying the jaw movements, the team found that the hamsters used repeated patterns of jaw movements to get food into their pouches, regardless of the size of the food item. These cyclical movements were very similar to chewing, except that they would pause regularly with their mouths open. 'This is probably to allow the tongue to push food into the pouch', says Buckley.

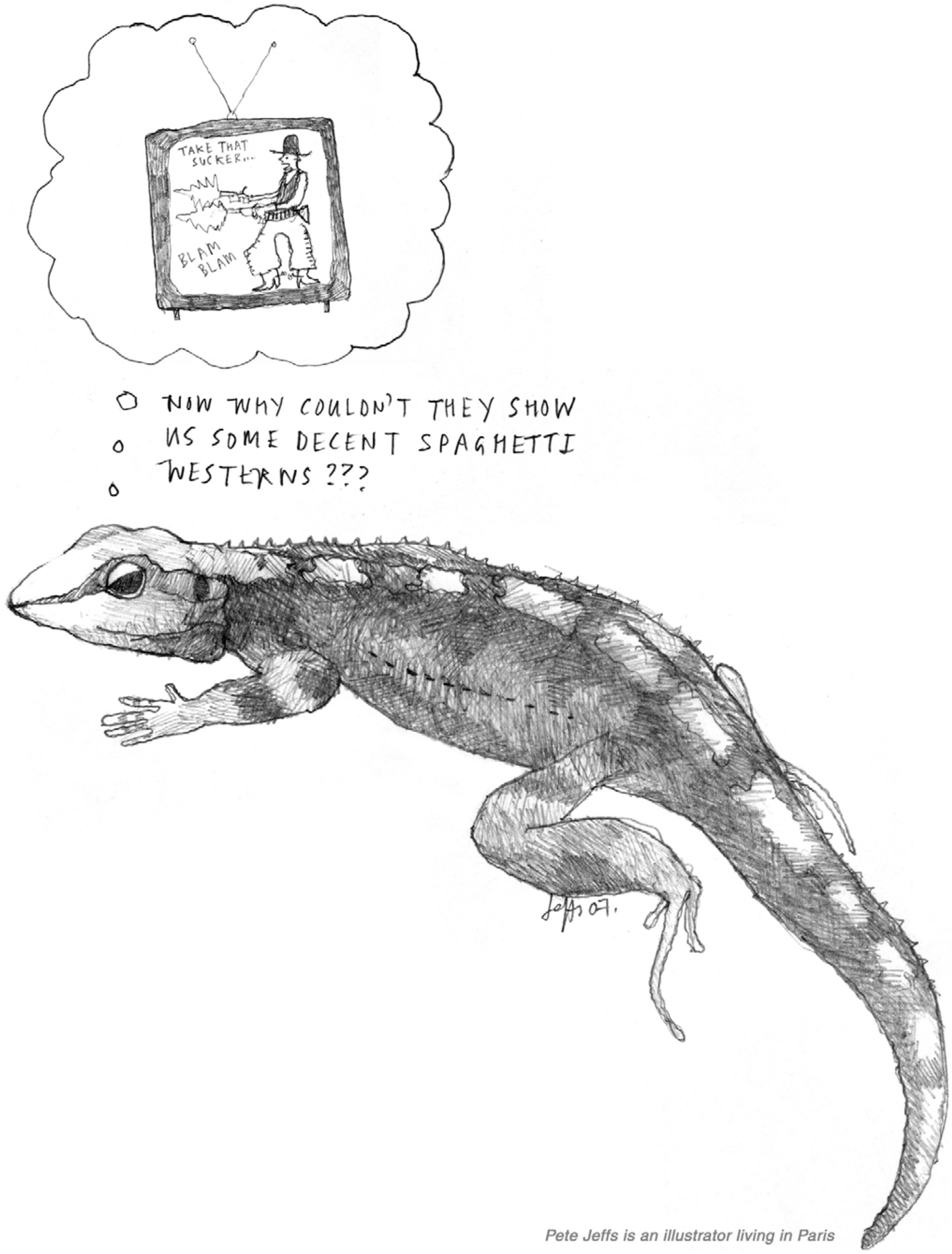
Previously researchers had suggested that the retractor muscle, a large muscle which travels from the mid-spine to each side, over and under the pouch, merely helped support full rodent pouches. But the team's videos showed that the muscle was very active during pouch-filling, so to investigate further, the team operated on some hamsters and cut the nerve supplying the muscle to one pouch while leaving the nerve on the other side intact. If the muscle played a vital role in pouching, 'we thought that they might not be able to pouch on the denervated side', Buckley explains. However, they found that hamsters could still pouch the same amount of food into denervated pouches, although they pouched slightly fewer larger items. They also noticed that hamsters filled their denervated pouches more to the side, not pushing food that far into the pouch.

This led the team to suspect that the retractor muscle might play a role in moving food down the pouch, so they stimulated the retractor muscle in one anaesthetised hamster while pushing a blunt probe into a pouch containing a single corn kernel to simulate a second piece of food entering the pouch. They found that retractor muscle contraction straightens folds in the pouch walls, allowing food to slide further into the pouch. Examining their videos once more, the team found that it took hamsters 40% longer to put a second food piece into their denervated pouch than into their unaffected pouch, showing that retractor activity helps move food out of the way as more is added. 'The retractor helps make the pouch more efficient', says Buckley.

10.1242/jeb.010926

Buckley, C. A., Schneider, J. E. and Cundall, D. (2007). Kinematic analysis of an appetitive food-handling behavior: the functional morphology of Syrian hamster cheek pouches. *J.*

JACKY DRAGONS ASSESS AGGRESSION



Pete Jeffs is an illustrator living in Paris

Males often fight over resources, but to avoid costly fights they display to each other instead to size each other up. If they decide it's not worth fighting an opponent, they back out. Interested to know which features of a signal male Jacky dragon lizards rely on to assess their opponents, Daniel Van Dyk, Alan Taylor and Chris Evans from Macquarie University, Australia, filmed male Jacky dragon displays (p. 3027). The lizards' displays involve rapid arm waves followed by sequences of push-ups and body rocks, along with tail flicking and expansion of their gular pouches in the throat. The team used a film editing program to make up sequences of Jacky dragon displays, each containing the same number of push-up/body rocks, but with groups of push-up/body-rocks divided into bouts, separated by different time intervals. Playing these sequences to test lizards to see how they responded, they found that the test lizards were less aggressive towards their virtual opponent when the displays were arranged into fewer bouts, that is, when push-up/body-rocks were more concentrated. This suggests that lizards rapidly assess their opponent's prowess by averaging the number of push-up/body-rocks they complete per bout, and the results will help researchers work out the rules that govern aggressive interactions between animals.

10.1242/jeb.010934

Van Dyk, D. A., Taylor, A. J. and Evans, C. S. (2007). Assessment of repeated displays: a test of possible mechanisms. *J. Exp. Biol.* **210**, 3027-3035.

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