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.

**DICHROMAT DECISIONS**

While our three retinal cones allow us to see up to one million different hues, most other mammals make do with two-cone dichromatic colour vision. In horses, the short-wavelength sensitive cone detects blue light, whereas the other cone picks up longer wavelengths – such as long wavelength yellow and medium wavelength green. Comparing the signal from the two cones gives the chromatic space, which is the spectrum of colours a dichromat can see. As Lina Roth from Sweden’s Lund University explains, ‘when both cones are stimulated equally, there is a “neutral” point in the chromatic space’. We would perceive a neutral point as a white or grey colour, but a dichromat could see this point as blue-green. A horse’s grey neutral point sits in the chromatic space between green and blue. One question researchers want to answer is whether dichromats see a continuous spectrum of colours or whether they distinguish two colour categories of long and short light wavelengths divided by the neutral point.

To investigate how horses perceive colours, Roth and her colleagues Anna Balkenius and Almut Kelber trained horses to recognise two ‘positive’ colours and a ‘negative’ colour, which were printed on pieces of paper attached to small doors in a screen (p. 2795). By nudging a door with their noses, the horses gained a tasty carrot reward from positive colours but not negative ones. Once the horses reached a 75% or higher success rate in picking out their positive colours, the team started tests.

In the first test they wanted to find out how horses responded to colours on the same side of the neutral point. They trained the horses with long wavelength yellow and a grey as their positive colours, with short wavelength blue as the negative colour. The horses made choices differently to how humans would make them – rather than choose the positive stimulus if it was presented they chose the colour with the longest wavelength, effectively ‘generalising’ their response to ‘positive equals longer wavelength’. So the horses picked a novel green over shorter wavelength grey and blue, and yellow over grey.

Next the team trained horses with blue and green as the positive colours, and yellow as the negative colour, finding that the horses could also generalise colours either side of the neutral point. The horses chose neutral point grey over yellow, suggesting that they had generalised the grey – in between green and blue – as positive. They preferred blue over both grey and green, showing that the horses were choosing the colour with the shortest wavelength, most different from negative yellow. The results also show that grey is considered a colour in its own right and not as a gap in the chromatic space.

In a final test the team trained the horses with positive green and negative neutral point grey. Given a choice between grey and blue, the horses eventually settled on grey, even though it was the negative stimulus. They chose it because it is closest to green, which was the longer-wavelength positive stimulus.

So horses mostly learn colours in a relative, rather than an absolute manner, but can recognise specific colours too. Because horses have a continuous dichromatic space, the same is likely to be true for other dichromats, and researchers can’t assume that grey is a non-colour. Next, though, Roth is going to turn the lights out, ‘to find out the limits of the horses’ colour vision’.

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**THERMAL VISION?**

Pitvipers quite rightly have a fearsome reputation, injecting their victims with venom from their sharp fangs, causing very painful bites. But there is more to pitvipers than a vicious bite; they are also well known for having heat-detecting organs, called pits, located between their nostrils and mouth, which allow them to sense the temperature of surrounding objects. Conventional wisdom suggests that the pits are highly accurate organs, allowing the snakes to ‘see’ a detailed thermal map of their surroundings with pinpoint accuracy. However as George Bakken and Aaron Krochmal show in their latest paper, the vipers’ pit organs might not give as clear an image as previously suspected (p. 2801).
Pitvipers’ pits are irregular, mushroom shaped cavities with a 1-3 mm long opening. A membrane at the back of the pit, covered in sensory receptors, responds to temperature changes. In order to understand what information is reaching the pit membrane, indicating what the snakes can ‘see’ thermally, Bakken and Krochmal modelled the pit as an optical system. Thermal radiation travelling through the pit’s opening and onto the membrane is much like light travelling through the opening in a simple pinhole camera.

First the model assumed that the pit would work like a perfect optical system, where all light (or heat) entering the pit would form a highly focussed point on the membrane. The second part of the model calculated how the thermal radiation entering the pit would heat up the membrane, stimulating the membrane’s receptors. Because pits have no lenses, the thermal radiation entering the pit forms fuzzy, un-focussed points on the membrane. Therefore the third part of the model took into account the fact that each point is fuzzy, and overlaps with the points around it, and calculated the effect this had on the resulting image.

Having developed their model, the team recorded thermograms – essentially heat maps – of natural situations to show the temperatures and contrasts that the snakes are likely to encounter. By putting the temperature information from the thermograms into their model, they could calculate maps of what the snakes could ‘see’.

They found that the size of the pit opening influenced both the resolution – or the ‘fuzziness’ – of the image, and signal strength, or brightness. With a bigger opening, images are fuzzier, but brighter. ‘It looks like you lose more by making the image fuzzy than you gain by making it brighter’, says Bakken. So on balance, smaller apertures appear to form a better image on the pit membrane. The team also found that prey such as a mouse was hard to detect even if they assumed that the membrane could pick up temperature differences of 0.001°C, because the image ‘smears’ over a large area of membrane. This meant that the mouse didn’t show up as a temperature ‘hotspot’, indicating that latching onto the strongest thermal signal is not a reliable way for a pitviper to be sure of getting a meal.

Scenes with larger targets and a strong thermal contrast, such as the cool opening to a rodent burrow, fared much better, supporting the team’s idea that pits probably evolved to help the snakes regulate their body temperatures by seeking out warm or cool spots. Given that the model suggests that pitvipers’ temperature sensing is not as accurate as previously thought, the team suspect that the snakes’ brains might be sharpening up the thermal image formed on the pit membrane. Next they plan to find out whether the snakes do this, and if so, how they do it.


LIZARDS TRADE-OFF ENERGY SUPPLIES

Life is about compromises, and energy-hungry processes such as reproduction and immunity compete for an organism’s sometimes limited energy supplies. A female tree lizard’s energy demands go up when she is reproductive and producing lots of eggs, but if there isn’t enough energy to go around, then another process suffers. The availability of energy is probably partially regulated by corticosterone (CORT), which mobilises energy stores and regulates the stress response. Susannah French from Arizona State University and her colleagues tested the idea that CORT regulates the distribution of limited energy between the reproductive and immune systems by manipulating CORT levels in reproductive and non-reproductive female tree lizards under different stresses (p. 2859).

However, as French explains, ‘one major problem with trying to manipulate hormone levels is measuring the response’. Handling animals is stressful for them, and so is giving them regular injections. ‘The best thing to do is leave them alone’, she says. So when French’s colleague Dale DeNardo saw a talk by bioengineer Brent Vernon on an injectable gel implant which releases substances into the body at a controlled rate, French knew that they had arrived at a solution. Collaborating with Vernon, the team developed a lizard implant consisting of a mixture of polymers, plus CORT, which they mixed up and injected into the animals under sedation such that the implant set in the lizards’ bodies just after the injection.

For their experiments, the team captured non-reproductive and reproductive female lizards in their Sonoran desert home before transporting them back to the lab in cloth bags. They placed half of both groups on a restricted diet of two crickets a week, while the others could gorge themselves on as many crickets as they could eat. Within each dietary group, half the animals received CORT implants.

Two days after receiving their slow-releasing CORT implants and starting their new diets, the team gave each of the lizards a small circular wound in the top layer of their skin. They were surprised by what they found when they measured the area of the wounds five days later to see how CORT affected wound healing in the different groups. ‘We expected that all the CORT animals would have a suppressed [immune] response’, French says. Instead, the team found that CORT had an effect only when the lizards were energy limited.

They found that the non-reproductive females on a restricted diet had slower wound healing when they received a CORT implant than untreated animals. This suggests that CORT is effective in compromising non-reproductive females’ immune response only when they are in energy debt.

Turning their attention to the reproductive females, the team found that all those on restricted and unrestricted diets who had also received a CORT implant had restricted wound healing. So the added stress of reproduction, which uses up a lot of energy, makes a bad energetic situation worse. Having unlimited access to crickets isn’t enough to redress the energy deficit. ‘CORT is involved in mobilising resources, but not directly involved in the immune response’, says French, who is working next on tracking down what other factors affect the wound healing response.

Crocodilians – alligators and crocodiles – deserve their reputation as fearsome predators, taking on prey that most other animals would leave alone. Once they have a victim grasped in their jaws, however, they have a problem. Although their conical teeth are perfect for grabbing food, they’re pretty useless for tearing it up. So crocodilians developed the ‘death-roll’, a fearsome spinning manoeuvre which they use to subdue and dismember their prey. Interested to know how alligators perform the death-roll, Frank Fish and his colleagues filmed juvenile alligators spinning in a tank holding onto tasty meat morsels with their jaws (p. 2811). To spin, an alligator presses its limbs against its body and bends its head and tail relative to its body, forming a C-shape. After the turn, it straightens itself out again and splays its legs to stop turning. By mathematically modelling the death roll, the team calculated that the baby alligators generate shear forces of 0.015 N, but these forces disproportionately increase to 138 N when scaled up to a ferocious 3 m alligator, allowing them to tear up and dine on ever larger prey.


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