

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

EMBRYOS SENSE SEISMIC EVENTS



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Karen Warkentin knew she'd have some interesting questions to answer when she saw a hungry snake attacking a clutch of near-term red-eyed treefrog eggs in the lab. As the predator started tucking in to the eggs, tiny frog embryos began tumbling from the clutch, even though they should have waited another 2 days before hatching. Warkentin eventually discovered that the tadpoles were making a tough decision: to escape the snake by fleeing to the water, even though they are much more vulnerable to aquatic predators at such an early age. Intrigued by the youngster's decision, Warkentin was curious to find out which cues had triggered their evacuation. Warkentin began to suspect that vibrations, generated by the snake's assault, prompted the treefrog's bid for freedom, but why didn't other less sinister vibrations send the youngsters tumbling free too? Curious to know how the embryos distinguished a life-threatening attack from vibrations caused by rain or rustling leaves, Warkentin and her student Michael Caldwell decided to see what makes vibration sequences scary for red-eyed treefrog embryos (p. 1376).

Travelling to the Smithsonian Tropical Research Institute at Gamboa, Panama, Warkentin and Caldwell collected frogspawn from trees growing over a local pond. Back in the lab, the team waited until the eggs were 5 days old before attaching a vibrating probe to the clutch to shake the embryos up. Teaming up with Gregory McDaniel, a vibrations engineer, Warkentin designed 32 white noise vibration patterns, with bursts of vibration ranging from 0.5–20 s interspersed with

gaps ranging from 0.5–100 s. Exposing egg clutches to the vibrations, the team recorded how many embryos were scared enough to hatch during the following 10 minutes.

Analysing the embryo's escapology, Warkentin realised that the frogs weren't responding to the percentage of time filled with vibration or the length of the time cycle that the pattern repeated over. However, the vibration duration and gap between vibration bursts had a profound effect on the embryo's desire to hatch; 0.5 s bursts combined with 1.5–2.5 s gaps were very scary, with three quarters of the embryos deciding to take their chances in the water, but combining a scary 0.5 s burst with a lengthy gap wasn't at all scary. 'Vibration duration and interval appear to function as two necessary elements of a composite cue' says Warkentin. The team also realised that the embryos sometimes waited for up to a minute after the vibrations started before beginning to hatch. Warkentin explains that the treefrog eggs are secured with jelly and so are quite tough for the snake to tear loose, giving the embryos enough time to sample several vibration cycles before making their life or death decision.

So how do the embryos sense these seismic events? Warkentin isn't sure. She explains that it is possible that the embryo's lateral line neuromasts pick up the vibrations, or that the embryos simply sense the signal by sloshing around within their capsules. But that is one of the unresolved questions that will keep her returning to Panama for years to come.

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Warkentin, K. M., Caldwell, M. S. and McDaniel, J. G. (2006). Temporal pattern cues in vibrational risk assessment by embryos of the red-eyed treefrog, *Agalychnis callidryas*. *J. Exp. Biol.* **209**, 1376–1384.

STICKY WEBS SUFFER FROM STARVATION

Spiders are a byword for industry. These diligent little engineers constantly tear down their webs and rebuild them in an effort to snare a snack. However, despite their ingenuity some spiders go hungry for days while waiting, so what effect does starvation have on a web's composition? Mark Townley explains that although a web's structure is largely derived from silk, 40–70% of an orb-web's mass is composed of the low-molecular-mass compounds (LMM) that contribute to the web's adhesive coating. Edward Tillinghast and Townley decided to analyse the LMM

components in orb-web glue to find out which glue compounds the spiders synthesize from scratch and what happens to the adhesive when spiders go without (p. 1463).

Knowing that labelled carbon from radioactive glucose would be incorporated in LMM compounds that the spiders synthesized, Townley offered two species of *Argiope* spiders a sip of radioactive glucose solution before collecting their webs to find out which LMMs were home made and which supplied by the diet. Having washed the adhesive from the webs, Townley isolated individual LMM components by electrophoresis before identifying them with NMR and found that the spiders were producing radioactive GABamide, glycine and alanine; the spiders were synthesising these compounds. But choline and glycine betaine remained unaffected by the arachnid's hot glucose drink; the spiders derived these compounds directly from their diet.

Keen to discover which adhesive components were most affected when the spiders went hungry, Townley and Tillinghast collected *Argiope* and *Araneus* spiders and divided them into two groups, fed and unfed, to compare the effects of starvation on the web's adhesive composition. But spider physiology seemed intent on confounding Townley's analysis. Townley explains that the fed spiders continued with normal physiological functions such as moulting and egg production, but most of these activities were severely reduced or even abolished in the starving spiders, making it tricky to isolate the effects of starvation from these routine physiological demands.

Collecting webs from both groups of spiders over the course of several weeks, Townley patiently analysed the adhesive's components from the vanishingly small samples. Teaming up with plant statistician Christopher Neefus, to identify consistent trends in the glue's changing composition, Townley found that the proportion of synthesised compounds in the adhesive, such as GABamide and glycine, increased, and the proportion of diet-derived compounds decreased, as the spiders became hungrier. And when Townley compared the fed spiders' adhesive composition with that of the starved spiders, both sets of spiders produced similar trends; the fed spiders also rapidly lost components derived from their diet while enriching the self-synthesised materials. Why was the glue composition varying in similar ways, even though half

of the spiders were going hungry while the rest were well fed?

Townley suspects that several factors account for the similarity. He suggests that the fed spiders invested the surplus from their diet in activities that starved spiders avoid. One other factor also affected both groups equally; all of the spiders suffered from losing their webs. He explains that spiders constantly recycle their webs, devouring the old before constructing new ones. By taking away the resources invested in the web, Townley suspects that he was depriving the spiders of essential adhesive components that the arachnids normally recycle.

10.1242/jeb.02214

Townley, M. A., Tillinghast, E. K. and Neefus, C. D. (2006). Changes in composition of spider orb web sticky droplets with starvation and web removal, and synthesis of sticky droplet compounds. *J. Exp. Biol.* **209**, 1463-1486.

LEARNING FROM PIG BRAINS



As far as we know, the human brain is one of the most complex structures in the universe. Capable of astounding feats, our brain has fascinated us for centuries but its function has proved difficult to unravel. Given the ethical issues associated with brain research, the search has been on for the last few decades to find a brain model that could teach us about human brain development, and recent interest has focused on the pig. Jacob Jelsing explains that pig brains are similar to human brains in several respects; they have many of the same morphological features, are quite large and all of the cortical neurons appear to be fully developed at birth. But other aspects of the pig brain are less well characterised. Jelsing, working with Ralf Hemmingsen and Bente Pakkenberg, decided to characterise the pig cortex, the region of brain responsible for processing most of our conscious behaviour, by counting the number of neurons in this fundamental structure (p. 1454).

But rather than looking at just one breed of pig, the team decided to investigate two; a

domestic Danish Landrace, Yorkshire crossbreed, and an experimental pig breed, the diminutive Göttingen minipig. Jelsing explains that although the domestic breed is more numerous than the minipig, the minipig's smaller stature and freedom from disruptive pathogens makes them a more attractive breed to work with from the neurobiologists perspective. Aage Olsen and Nanna Grand supplied Jelsing with brains from pigs of both species ready for the team to prepare wafer-thin brain slices before beginning the painstaking task of counting cortex neurons.

Fortunately, the team didn't have to count every single neuron in each cortical sample. Jelsing knew that if he systematically selected brain sections from randomly selected pigs he could calculate the total number of neurons in the cortex, despite having only counted a tiny fraction of the total neurons in the tissue. First Jelsing systematically chose brain slices and then Rune Nielsen counted the number of neurons in a few systematically chosen areas of each section. So long as Jelsing and Nielsen had chosen regions from all of the cortical tissue at random, but then sampled them in a systematic way, they could calculate the total number of neurones in both cortices.

After Nielsen had spent several days peering through a microscope at the delicately stained samples, the team were able to calculate the number of cortical neurons that each breed had at birth: 425 million in the domestic pig and 253 million in the smaller minipig. But when the team calculated the number of neurons in the adults' brains, they were in for a surprise; while the domestic pig's neuron count had hardly changed, the minipig's had increased significantly to 324 million. Unlike the neurons in the human cortex, which do not develop postnatally, the minipig's neurons had continued developing after birth. Jelsing does not know how long it takes the minipig's brain to complete development but it could be anything from weeks to several months. Given the shock finding that the Göttingen minipig's brain continues developing after birth, the team suggest that the domestic pig's brain may be a better model for human brain development than the smaller minipig's.

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Jelsing, J., Nielsen, R., Olsen, A. K., Grand, N., Hemmingsen, R. and Pakkenberg, B. (2006). The postnatal development of neocortical neurons and glial cells in the Göttingen minipig and the domestic pig brain. *J. Exp. Biol.* **209**, 1454-1462.

PROLACTIN PROMOTES PENGUIN KIDNAPS



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It's probably an emperor penguin parent's worse nightmare: having to defend their chick from a kidnapper's attack. Sadly, on the occasions when a kidnap bid has succeeded, the kidnapper often abandons their victim several hours later. But what drives the kidnapper to such a fruitless act? Frédéric Angelier and colleagues wondered whether kidnapping behaviour might be caused by unusually high levels of the parenting hormone, prolactin, in penguin parents who have lost their own chick (p. 1413).

The team injected failed penguin parents with bromocriptine to artificially reduce the

birds' prolactin levels and waited to see if the incidence of kidnapping declined too. Amazingly, the probability that a failed parent would stage an abduction fell 4.5 fold when their hormone levels were reduced. Although lowering the birds' prolactin levels hadn't abolished the behaviour, it had modified it.

But why do the failed parents maintain such high levels of prolactin when prolactin levels fall in other species that have lost their chicks, especially when the hormone has such drastic consequences? Angelier and colleagues suspect that the emperor penguins sustain high levels of

prolactin to encourage them to return to their chick after a lengthy separation. Sadly, this incentive to come home after a long foraging trip seems to have a nasty side effect when parents return to find their chick gone.

10.1242/jeb.02217

Angelier, F., Barbraud, C., Lormée, H., Prud'homme, F. and Chastel, O. (2006). Kidnapping of chicks in emperor penguins: a hormonal by-product? *J. Exp. Biol.* **209**, 1413-1420.

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