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INSIDE JEB

Being smart makes stressed snails drop a grade

White Sand Lake snail that had probably been attacked by a crayfish. Photo credit: Ken Lukowiak.

Sometimes life is just too stressful. Whether you're a freshwater snail struggling to breathe in a dwindling puddle or a student facing an assessment that could change your future, it can be hard to form memories when the pressure is on; even the smartest can struggle. 'We have been exploring how stress alters both memory formation and the ability to recall memory for a number of years', says Ken Lukowiak from the University of Calgary, Canada, who is intrigued by how the humble pond snail (*Lymnaea stagnalis*) forms memories. Having recently discovered a 'smart' colony of pond snails at White Sand Lake in Saskatchewan that learn quickly (after just one training session) to keep their breathing tubes closed when immersed in deoxygenated water – compared with 'average' snails, which require multiple training sessions before they get the point – Lukowiak wondered how resilient the 'smart' Saskatchewan snails' memories would be when they experienced a snail-scale shock.

Lukowiak and a large team of assistants trained 'smart' and 'average' snails to keep their breathing tubes closed when immersed in deoxygenated water by gently tapping the snails on the breathing tube whenever they tried to extend it above the surface to breathe. While the unstressed 'smart' snails got to grips with the idea of keeping their breathing tubes closed after a single training session, the 'average' unstressed snails – which had been found in a pond outside Calgary – required two training sessions, 1 h apart to catch on. Then, considering a range of scenarios that are stressful for pond snails – including experiencing a heat wave, starvation, an approaching predator and incurring damage to the shell – the team wondered how the memories of the 'smart' snails would fare after experiencing the stress and whether the 'average' snails might receive a memory boost and recall the tube-tapping lesson after just one training session.

Impressively, the stressful sensations seemed to sharpen the 'average' Calgary snails' memories, allowing them to recall that they must keep their breathing tubes closed after the reduced training regime. However, the A-grade snails seemed to be struggling. While a sniff of the scary odour of the aggressive crayfish that share their Saskatchewan home did not affect the snails' memories, a heat wave, the smell of food when hungry and receiving a small amount of damage to their shells all impaired the 'smart' snails' ability to form memories.

'Being smart made them less resilient and thus they could not handle stress as well as the more average individuals', says Lukowiak, who admits that he was surprised that the Saskatchewan snails were so badly affected. 'Even though both the 'smart' and 'average' snails were freshly collected from ponds that experience temperature spikes in the summer, the 'smart' snails have their ability to form memory obstructed by a thermal shock', he says. Adding that many of the snails that he encountered in the wild had also incurred damage to their shells – which should affect their memories – Lukowiak says, 'How that alters their ability to survive in the wild is not clear to us'.

In short, the 'smart' snails' superior intellect is a clear disadvantage for their memories in stressful situations and Lukowiak is now eager to understand why the snails are so impaired. 'One idea is that maybe the 'smart' snails do in fact make memory, but they can't retrieve it because of the stress, much like a smart student taking an exam', he suggests. Maybe exam-brain-freeze has a longer history than any of us knew.

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Hughes, E., Shymansky, T., Swinton, E., Lukowiak, K. S., Swinton, C., Sunada, H., Protheroe, A., Phillips I. and Lukowiak, K. (2017). Strain-specific differences of the effects of stress on memory in *Lymnaea*. *J. Exp. Biol.* **220**, 891-899.

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Young rats' calls assembled like human speech

A young male rate that produced hybrid calls. Photo credit: Tobias Riede.

Rats are far more vocal than our blunt hearing will ever perceive: 'The most interesting aspect of rat vocal communication is that it mainly occurs in the ultrasonic realm', says Tobias Riede from Midwestern University, USA. Expressing fear and discomfort with shrieks around 22 kHz, and squeals of pleasure around 50 kHz, rats have an extensive repertoire of ultrasonic cries for most occasions. Riede is fascinated by how the animals assemble combinations of squeaks and peeps to learn more about vocal communication. So, when Christine Hernandez discovered that young inexperienced males seem to combine the two differently pitched ultrasonic cries when they first encounter a female, Riede was intrigued. Could the unexpected hybrid call have something in common with human speech? 'Speech is based on our ability to concatenate syllables into words and words into sentences … during a single extended breath', says Riede. Were

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the young males controlling their breathing to produce both components of the combined call during one breath, instead of inserting a swift minibreath between the two as they transition seamlessly between the differently pitched ultrasonic calls? Puzzled, Hernandez played Cupid, introducing young inexperienced male rats to females, while recording the novices' cries and breathing patterns to learn more about how rats articulate their high-pitched cries.

'Recording lung pressure and electromyographic [muscle] activity of laryngeal muscles in an awake and spontaneously behaving animal was a major challenge', says Riede, recalling how difficult it was to implant the electrodes in the minute vocal muscles to record muscle activity while a young male serenaded females during his first encounter. Investigating the frequency structure of the unusual hybrid cries, Reide's colleague Mark Sabin was convinced that the lower pitched portion of the hybrid serenade was identical to the 22 kHz calls that the animals produce when they are fearful. Meanwhile, the 50 kHz trill, which forms an overture or finale to the curious call, matched the mating calls produced by more experienced males. However, it was only when Hernandez and Sabin analysed the lung pressure traces alongside the recordings of laryngeal muscle activity that they were sure that the two-stage call was emitted during a single breath. And when they checked the air pressure traces in the seconds leading up to the excited male's utterances, they could see evidence of the signature deep breaths also taken by humans as they prepare to speak.

'At least during the initial encounter with a female, young and sexually mature male rats concatenate the two calls. They not only produce the calls in close succession but also adjust their breathing in order to produce the two calls in a single breath', says Riede. As the animals are able to coordinate the two vastly different movements when merging the calls, Riede observes, 'Non-human mammals and humans seem to share a number of features of how they control vocal movements'. He also suggests that we may be able to learn more about the neurophysiology underpinning breath control in human speech by studying how amorous young rat males coordinate their serenades during their first 'teenage' crush.

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Hernandez, C., Sabin, M. and Riede, T. (2017). Rats concatenate 22 kHz and 50 kHz calls into a single utterance. *J. Exp. Biol.* **220**, 814-821.

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High and dry oysters at most risk from climate change

Oysters on the shore at Port Stephens, Australia. Photo credit: Elliot Scanes.

While it may be fashionable in some circles to doubt the provenance of climate change, there is no doubt that our oceans are warming and becoming more acidic. And even though a pH drop of 0.1 units may not sound that drastic, the reality is that ocean acidity has increased by 26% since the beginning of the Industrial Revolution, and the change is only going to accelerate. Although many fish appear to be able to tolerate the acidity shift physically, it is not clear how molluscs that cling to life on the wave-battered shoreline will cope. Elliot Scanes from the University of Sydney, Australia, explains that oysters that are routinely exposed to the air as the tide recedes may be better prepared for more acidic conditions than shore-mates that live beneath the tide: when the tide goes out, bivalves exposed above the waves close their shells, which restricts water flow over the gills and leads to a natural accumulation of CO_2 – and acidification – of their tissues. Could this regular exposure to more acidic conditions leave high-and-dry Sydney rock oysters better prepared for future climate change than their perpetually immersed cousins, or could the additional stress tip them over the edge?

Scanes, Laura Parker and Laura Stapp headed by boat along the shore at Port Stephens, Australia, to prise free clumps of oysters before testing their resilience in Wayne O'Connor's laboratory at the Port Stephens Fisheries Institute. 'It was difficult to set up the experimental system; we needed to try and recreate a natural tidal cycle in the laboratory', says Scanes, who teamed up with Pauline Ross to design a system of pumps and timers to pump seawater into the laboratory tanks twice a day over 3 weeks to simulate the natural tidal cycle that oysters high up on the shore would experience in current (390 µatm CO_2) and future climate scenarios (1000 µatm CO_2). Then, the pressure was on to measure the effects of the different tidal and CO_2 regimes on the molluscs' ability to maintain their delicate pH balance, their metabolic rate and growth: 'We had to make sure that oysters weren't out of the water for longer than necessary, because this would have affected the results', says Scanes.`

Worryingly, oysters that had been exposed to the air each day for two 9 h periods in the high CO_2 conditions (to simulate the experience of molluscs at the upper end of the tidal range at the end of the century) had significantly higher metabolic rates than the molluscs that had remained immersed. Meanwhile, the concentration of CO_2 in their tissues was higher and the pH of their tissues had fallen an enormous 0.35 pH units. In addition, the molluscs that lived higher up the shore were in poorer shape and had grown more slowly than the oysters that were living below the tideline.

'Ocean acidification is likely to make life more difficult for oysters high on the shore and in the future there are likely to be fewer oysters on the rocky shore', says Scanes. And the loss could have implications for other species clinging to life in the turbulent tidal zone. 'Oysters are one of the most important habitat-forming animals on the rocky shores of Australia', says Scanes, explaining that the layers of shell built up by subsequent generations of the filter feeders provide a rich environment for other coastal species, in addition to the molluscs cleansing the water. 'Fewer oysters means less habitat for other organisms', warns Scanes.

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Scanes, E., Parker, L. M., O'Connor, W. A., Stapp, L. S. and Ross, P. M. (2017). Intertidal oysters reach their physiological limit in a future high-CO_2 world. *J. Exp. Biol.* **220**, 765-774.

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