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Inside JEB

HIBERNATING HAMSTERS REVERSE LUNG CHANGES

Ate Boerema

Robert Henning, a pharmacologist from the University of Groningen, The Netherlands, is fascinated by how our bodies react to low temperatures. He and his colleagues explain that human lungs are particularly susceptible to damage: even running in cold air can cause injury. However, hibernating animals routinely experience low body temperatures, in a process called torpor, that would prove fatal to humans. They repeatedly allow their bodies to cool to temperatures barely above ambient, before rewarming briefly and then recooling. Henning and his colleagues decided to find out how hibernating Syrian hamster lungs respond to these low body temperatures (p. 1276).

Creating the perfect hibernation conditions for the hamsters, Henning's colleagues, Ate Boerema and Arjen Strijkstra, collected lung tissue from the animals when they had low body temperatures (were in torpor) and as they raised their body temperatures. Then Henning teamed up with Fatemeh Talaei, Hjalmar Bouma, Martina Schmidt and Machteld Hylkema to begin looking for changes in the animals' lungs.

Henning explains that lungs are made up of smooth muscle around the airways – which allows them to expand and constrict – and collagen, the connective tissue between lung cells that collapses the lungs during exhalation. Monitoring the levels of one of the smooth muscle components – actin – in the hamsters' lungs, the team found that the actin levels began to rise slowly after the animals had dropped their body temperature and continued rising steadily, peaking just before the animals raised their body temperature at the end of torpor. However, within 2 h of rewarming, the animals had completely reversed the actin change and the actin levels had returned to normal.

The team also analysed the hamsters' lung collagen levels, and found that they increased dramatically in the first 24 h of cooling, but then gradually decreased, returning to prehibernation levels when the animals' body temperature rose. The hibernating hamsters had modified their lung structure in response to the low temperatures and the levels of key molecules that regulate tissue changes had altered too.

Henning admits that he was surprised that the response was rapid and continued while the hamsters were in torpor. 'Most people think that once you are cold all the molecular process come to a standstill and nothing happens until you rewarm, yet we show that there is on-going change in the collagen and the smooth muscle actin,' says Henning.

Even more remarkably, the team realised that the changes in the hibernating hamsters' lungs looked similar to some of the changes seen in asthma patients. However, instead of being permanent, the hamsters' lungs recovered rapidly when their body temperature returned to normal.

Henning explains that the airways (lumen) of asthma patients constrict during an asthma attack so that sufferers have problems exhaling and their lungs overinflate. The team suspects that the hamsters alter their lung structure so that they overinflate slightly to prevent them from collapsing during torpor when their breathing rate falls to 2–3 breaths min^{-1}.

However, more importantly, hibernators may be able to show us how to reverse the changes that asthma patients endure. 'Hamsters do not prevent lung remodelling, but they are good at reversing it,' says Henning, who is optimistic that we may be able to develop new therapies that reverse some of the structural changes found in the lungs of asthma patients in the same way that hibernating hamsters return their lungs to normal when they warm up.

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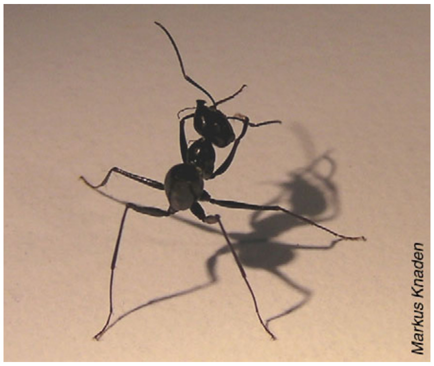
Talaei, F., Hylkema, M. N., Bouma, H. R., Boerema, A. S., Strijkstra, A. M., Henning, R. H. and Schmidt, M. (2011). Reversible remodeling of lung tissue during hibernation in the Syrian hamster. *J. Exp. Biol.* **214**, 1276-1282.

ANTS COMBINE CUES TO INCREASE ACCURACY

Scurrying under the baking sun is a hazardous business for *Cataglyphis fortis* desert ants. They must return swiftly to the nest or risk death in the blistering heat. But these ants have a suite of navigational tricks on hand to help them locate home quickly. Keeping track of every twist and turn on the outbound journey, they speedily calculate the most direct path home when they are ready to return. Yet, tiny errors in the ant's tallying system mean that their



return route can miss the nest by centimetres, so the tiny explorers use local landmarks to navigate visually once they know they are near the nest. However, Markus Knaden from the Max Planck Institute for Chemical Ecology, Germany, noticed that the desert is a very aromatic place. 'When walking through the salt pan I realised that it smells quite different in some places from others, even though visually it looks the same,' says Knaden. Having already found that the ants use their sense of smell to get their bearings when close to the nest, Knaden and his colleagues Kathrin Steck and Bill Hansson began testing whether the ants combine visual and scent cues to find home faster (p. 1307).

'We use long aluminium channels to train and test the ants,' says Knaden. 'We use the channels because the desert is smelly and we do not want them to have contact with the smell of the ground. We wanted them to learn the odours that we provided,' he explains. Connecting the nest entrance to a long channel before the ants were active, Knaden and Steck placed two pieces of black cardboard either side of the nest's entrance in the channel for the ants to use as visual landmarks. Then the ants scampered to a well-stocked feeder before returning to the nest. Allowing some ants to visit the feeder only once, and others 5, 10 and 15 times, one of the scientists then collected each ant and transplanted it to an identical channel – complete with cardboard landmark but lacking the familiar nest entrance – to see how long it took the ants to become confident that the landmarks indicated the nest's entrance.

According to Knaden, it took the ants 15 training journeys to the feeder to be convinced that the cardboard marked the nest entrance. And when the duo replaced the cardboard landmarks with a familiar odour from the insects' surroundings, it took the ants a similar number of training trips with the odour marking the nest for them to trust the cue.

However, when Steck repeated the experiments with both cues at the nest entrance, the duo was in for a surprise. The ant scurried directly to the landmarks and stood still. 'It didn't want to go away. Usually they walk continuously but there it really stopped,' says Knaden. Somehow the combination of the odour and visual landmark was enough for the ant to learn the nest's location in a single foraging trip. And when Steck tested the insect's reactions to the individual landmarks after training with both cues, the association was still as strong: 'They would never have learned this fast from the single cues alone,' says Knaden.

Having shown that the combination of visual and odour landmarks is a more powerful navigation aid for desert ants than each cue individually, Knaden is keen to find out how nest odours waft through the desert to guide ants home.

10.1242/jeb.057687

Steck, K., Hansson, B. S. and Knaden, M. (2011). Desert ants benefit from combining visual and olfactory landmarks. *J. Exp. Biol.* **214**, 1307-1312.

BAD TASTE PROTECTS SEA CUCUMBERS

Sea cucumbers might seem to be sitting targets waiting to be attacked, but they are far from defenceless. Equipped with Cuvierian tubules – sticky threads that the animal expels to ensnare hapless predators – and mutable collagenous tissue, which allows them to stiffen or soften their bodies for protection, sea cucumbers are reasonably well prepared for most attacks. But these echinoderms also have another deterrent mechanism: they taste bad. Patrick Flammang from the University of Mons, Belgium, explains that sea cucumbers produce unpleasant tasting detergent molecules, called saponins. Knowing that saponins can prove fatal for fish in high doses, Flammang and his colleagues decided to find out how sea cucumbers use these noxious chemicals to defend themselves (p. 1347).

Travelling to the south of France, Flammang's students, Séverine Van Dyck and Maïté Todesco, went diving in the Mediterranean to collect *Holothuria forskali* sea cucumbers. Returning to Belgium, Van Dyck took samples of the sea cucumber's body wall and extracted and purified their saponins. Then she and Pascal Gerbaux analysed the saponins with mass spectrometry and found eight in the animal's body wall, the so-called holothurinosides (A, C, E, F, G, H and I) and desholothurin A. But how were these compounds distributed through the animal's body wall?

Teaming up with Isabelle Fournier and Maxence Wisztorski, mass spectrometrists at the University of Lille, France, Van Dyck and Flammang used an innovative mass spectrometry imaging technique to identify the location of each saponin in the sea cucumber's body wall. Together they found six of the saponins (holothurinosides C, F, G, H and I and desholothurin A) in the body wall's outer layer (epidermis) and two (holothurinosides A and E) in the inner layer (mesothelium).

The team also analysed the saponin distribution in the body walls of mildly stressed sea cucumbers after they had been poked and found that it had changed. Flammang suspects that the smaller saponins in the epidermis of the relaxed sea cucumbers are converted into the larger, more soluble, saponins for release into the water when the animals are stressed.

Next, the team looked at the animal's body wall structure with transmission electron microscopy and saw that cells that had been packed with material in the relaxed sea cucumbers were empty in the stressed animals. However, Flammang says, 'We cannot be sure where the saponins are located so this difference could come from other phenomena, but it is an indication that something is changing in the epidermis during stress'.

Having analysed the sea cucumber's saponin distribution, Flammang decided to find out how the animals use the compounds for defence. Collecting seawater surrounding relaxed and stressed sea cucumbers, Guillaume Caulier extracted saponins from the water. He found that the relaxed animals released one saponin, while the stressed sea cucumbers released six, two of which were new and could not have come from the sea cucumber's body wall.

Finally, Flammang decided to find out what effects the saponins have on fish. Adding small quantities of the compounds – similar to the amounts released by the sea cucumbers – to aquaria housing Mediterranean rainbow wrasse and ocellated wrasse, Todesco saw that the fish began breathing heavily and raced around the tank; however, they soon settled and none died. Flammang suspects that fish can smell the unpleasant tasting compounds and give sea cucumbers a wide berth to avoid getting a bad taste in the mouth.

10.1242/jeb.057646

Van Dyck, S., Caulier, G., Todesco, M., Gerbaux, P., Fournier, I., Wisztorski, M. and Flammang, P. (2011). The triterpene glycosides of *Holothuria forskali*: usefulness and efficiency as a chemical defense mechanism against predatory fish. *J. Exp. Biol.* **214**, 1347-1356.

OXIDATIVE STRESS IS TRANSIENT IN COLD STICKLEBACKS

Oxygen is our friend and our enemy. Without it there would be no life on earth, but the damaging effects of oxygen lead to ageing and disease. Kristin O'Brien, from the University of Alaska, Fairbanks, explains that oxidative damage can increase when temperatures drop and this is a risk for cold-blooded species such as fish. But no one knew how much of a risk. 'Although several studies have measured oxidative stress during cold acclimation of fishes, the results have been equivocal,' says O'Brien. Curious to find out how sticklebacks respond to cold acclimation, O'Brien and her colleagues Aaron Kammer and Julieanna Orczewska measured the levels of oxidative damage and antioxidants including superoxide dismutase (SOD) – which detoxifies damaging superoxide – in warm and cold threespine sticklebacks (p. 1248).

The team looked for oxidative damage in liver, oxidative and glycolytic muscles, and initially found damaged proteins in the cold fish's livers; however, the damage levels began to decrease after 4 weeks and there was no evidence of oxidative damage in either muscle type. The team also found an increase in the amount of the antioxidant glutathione in liver within 3 days of dropping the fish's temperature. When they looked at the activity of SOD at 14°C, they found it had increased on the second day of cooling in both muscle types and by day 3 in the fish's liver. However, when the team analysed SOD's activity at the fish's physiological temperature, They found it only rose in the oxidative muscles of fish that had been cooled for 9 weeks and remained constant in the cold fish's liver and glycolytic muscle. O'Brien and her colleagues suspect that the increase in SOD activity is due to modification of the protein after transcription, as the levels of SOD enzyme activity increase prior to an increase in mRNA levels.

O'Brien says, 'Our results demonstrate that oxidative stress is tissue specific and transient during cold acclimation of sticklebacks.' She also suggests that SOD activity levels may not rise in all tissues to combat elevated levels of superoxide production in cold conditions, but may rise instead to counteract the decreased catalytic rate of SOD in the cold.

10.1242/jeb.057653

Kammer, A. R., Orczewska, J. I. and O'Brien, K. M. (2011). Oxidative stress is transient and tissue specific during cold acclimation of threespine stickleback. *J. Exp. Biol.* **214**, 1248-1256.

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