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Inside JEB

MUSCLES HOOD COBRAS

The image of a hooded cobra with its neck flared is one of the most iconic from the animal kingdom. But how do they do it? Bruce Young from the University of Massachusetts Lowell and Kenneth Kardong from Washington State University explain that P. Russell and E. Home suggested in 1804 that muscles were entirely responsible for the hood's erection. Since then most people had suggested that the reptiles use their ribs and some had suggested that the reptile's skin might be involved in pulling the ribs into place, but no one had tried to find the mechanism. Unfazed by the snake's reputation, Young and Kardong decided to find out exactly how cobras hood (p. 1521).

The duo decided to measure the electrical activity of all of the muscles in the neck region as the animals erected their hoods, to find out exactly which ones were involved. 'Doing the surgery was the riskiest part of the study because you have to work around the head but the snakes are prone to waking, which can be disconcerting,' says Young. Having successfully inserted as many as 20 minute EMG electrodes into the tiny neck muscles, Kardong and Young waited for the snake to recover before filming and recording the muscle activity as the animals flared their necks. But it soon became clear that no two hooding events were the same. Sometimes the animals would hold a 70% hood for minutes, while on other occasions they could flare the hood completely and retract within 45 s, making it almost impossible to map the muscle activity to specific phases of the hood's erection.

However, after months of patience, Kardong and Young could see that eight muscles were involved in erecting the hood with two groups playing major roles: the levator costae and the intercostal muscles. Young explains that the large levator costae and the supracostalis lateralis superior muscles raise the first few ribs nearest to the head, intercostal muscles hold the ribs together while the hood is erect, and muscles connecting the ribs to the skin hold the skin taut.

Collecting X-ray images of a relaxed and hooded cobra, Kardong and Young could also see how far the ribs rotate away from the vertebral column and wondered if the joint between the rib and vertebra was different from that of ribs further down the body that are not involved in hooding. Scrutinising the joint section of neck and torso ribs with scanning electron microscopy, the team could see a protrusion on the neck rib's articular surface, which was missing on the torso ribs, that could displace the rib along the vertebra as it rotated, extending the range of the rib's movement. And when they looked at the joint structure, the team could see that the joint was encased by an elastic ligament that holds the joint in place. The duo realised that this ligament could also recoil and pull the ribs back when the levator costae and intercostal muscles relax, to tuck the hood away with the aid of additional intercostal muscles.

Young explains that cobras are not the only snakes that hood. 'Several groups of unrelated snakes show almost identical defensive behaviour,' says Young, and he is keen to find out how these other snakes raise their hoods.

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Young, B. A. and Kardong, K. V. (2010). The functional morphology of hooding in cobras. *J. Exp. Biol.* **213**, 1521-1528.

CHITOSAN REPAIRS DAMAGED SPINAL CORD

Richard Borgens and his colleagues from the Center for Paralysis Research at the Purdue School of Veterinary Medicine have a strong record of inventing therapies for treating nerve damage. From Ampyra, which improves walking in multiple sclerosis patients, to a spinal cord stimulator for spinal injury victims, Borgens has had a hand in developing therapies that directly impact patients and their quality of life. Another therapy that is currently undergoing testing is the use of polyethylene glycol (PEG) to seal and repair damaged spinal cord nerve cells. By repairing the damaged membranes of nerve cells, Borgens and his team can restore the spinal cord's ability to transmit signals to the brain. However, there is one possible clinical drawback: PEG's breakdown products are potentially toxic. Is there a biodegradable non-toxic compound that is equally effective at targeting and repairing damaged nerve membranes? Borgens teamed up with physiologist Riyi Shi and chemist Youngnam Cho, who pointed out that some sugars are capable of targeting damaged membranes. Could they find a sugar that restored spinal cord activity as effectively as PEG (p. 1513)?

Having initially tested mannose and found that it did not repair spinal cord nerve membranes, Cho decided to test a modified form of chitin, one of the most common sugars that is found in crustacean shells. Converting chitin into chitosan, Cho isolated a segment of guinea pig spinal cord, compressed a section, applied the modified chitin and then added a fluorescent dye that could only enter the cells through damaged membranes. If the chitosan repaired the crushed membranes then the spinal cord tissue would be unstained, but if the chitosan had failed, the spinal cord would be flooded with the fluorescent dye. Viewing a section of the spinal cord under the microscope, Cho was amazed to see that the spinal cord was completely dark. None of the dye had entered the nerve cells. Chitosan had repaired the damaged cell membranes.

Next, Cho tested whether a dose of chitosan could prevent large molecules from leaking from damaged spinal cord cells. Testing for the presence of the colossal enzyme lactate dehydrogenase (LDH), Borgens admits he was amazed to see that levels of LDH leakage from chitosan-treated spinal cord were lower than from undamaged spinal cords. The sugar had repaired membranes not only at the compression site but also at other sites where the cell membranes were broken due to handling. And when the duo tested for the presence of harmful reactive oxygen species (ROS), released when the membranes of ATP-generating mitochondria are damaged, they found that ROS levels also fell after applying chitosan to the damaged tissue: chitosan repairs mitochondrial membranes as well as the nerve cell membranes.

But could chitosan restore the spinal cord's ability to transmit electrical signals to the brain through a damaged region? Measuring the brain's response to nerve signals generated in a guinea pig's hind leg, the duo saw that the signals were unable to reach the brain through a damaged spinal cord. However, 30 min after injecting chitosan into the rodents, the signals miraculously travelled again to the animals' brains. Chitosan was able to repair the damaged spinal cord so that it could carry signals from the animal's body to its brain.

Borgens is extremely excited by this discovery that chitosan is able to locate and repair damaged spinal cord tissue and is even more enthusiastic by the prospect that nanoparticles of chitosan could also target delivery of neuroprotective drugs directly to the site of injury, 'giving us a dual bang for our buck,' says Borgens.

10.1242/jeb.045039

Cho, Y., Shi, R. and Borgens, R. B. (2010). Chitosan produces potent neuroprotection and physiological recovery following traumatic spinal cord injury. *J. Exp. Biol.* **213**, 1513-1520.

MECHANOSENSATION MORE THAN A PROXIMAL SENSE

Most of us use our eyes and ears to negotiate our environment, but what about other senses such as touch? Do we use mechanical contact and touch to perceive the lay of the land over the long range? Steven Harrison and Michael Turvey from the University of Connecticut are fascinated by the information our bodies derive through touch. 'Traditionally touch – the sense concerned with stimulation of the body's mechanoreceptors that occurs throughout the day – was thought to be the basis for passively knowing what you are coming in contact with only,' says Harrison. However, he and Turvey were curious to find out if we might use the sense to localise our position in an environment. In other words, can we use the sense of touch to actively extract information about the environment that will allow us to orient ourselves? Harrison and Turvey decided to find out by walking blindfolded students through a relatively simple landscape guided only by a long cane (p. 1436).

Recruiting 46 undergraduate volunteers from the university's Psychology department, Harrison constructed a 0.8 m channel that was crossed by three low removable steps and bounded by 2 cm high PVC tubing for the human guinea pigs to explore. Covering the eyes of each subject with a pair of blacked out goggles and providing them with a long cane, Harrison guided each individual to the channel and gave them time to adjust to navigating the channel and steps, guided by the canes alone.

Next Harrison trained the volunteers to move around and familiarise themselves with the simple landscape (a channel crossed by three steps) by telling them when to begin and stop walking. After directing the volunteers along the channel, Harrison asked them to return to an unmarked location that he designated as 'home'.

After an hour of familiarisation with the environment and the location of 'home', Harrison began testing the students' abilities to navigate their way around the channel. Telling the volunteers to walk back and forth along the channel as they encountered and negotiated steps that he positioned at various locations, Harrison eventually directed each student to a location and then asked the student to return to their starting point, guided only by the sensory information that they had gathered using the cane.

The students were surprisingly good at finding their way back to their starting point even when there was only one step and when Harrison added a second and third step to the channel, their accuracy improved even more. Harrison also secretly shifted one of the steps in each environment by a few metres for one group of students and found that these individuals shifted their 'home' position by the same amount. And when Harrison tested the students' responses to an environment with two steps after secretly moving the second step, he found that the students shifted their perception of 'home' to a location that preserved the relative positions of the two steps. The students were able to find their way home guided only by sensory information gathered about the environment through their sense of touch.

'We've got evidence that mechanosensation is not a proximal sense [restricted to direct contact], and in the same way that the visual system allows us to encode our location in the environment with respect to its structure, this same capacity seems to be something that mechanosensing has as well,' says Harrison.

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Harrison, S. J. and Turvey, M. T. (2010). Place learning by mechanical contact. *J. Exp. Biol.* **213**, 1436-1442.

MOUSE OLFACTOMETER DISTINGUISHES BABOON FAMILY MEMBERS

Family is a central concept for almost all species, not only to avoid the mishap of inbreeding, but also to help each other along (nepotism). But how do promiscuous species that live together in bands avoid the risks, and gain from the benefits, when it isn't always clear who is related to whom? According to Aurélie Célérier, from the CNRS and the University of Montpellier II, France, scent could be the key with related individuals carrying similar odours. However, as Célérier and her colleagues from France and the UK explain, 'there is no easy way to "record" and quantify the olfactory properties of objects and chemical signals,' so the team turned to a rodent with an excellent sense of smell to see if they could distinguish 'family' from 'outsiders' (p. 1399). Testing the responses of male Swiss mice to the odours of 16 female chacma baboons, some of whom were related while others were not, the team found that the mice could distinguish between related and unrelated baboons.

So it is possible that baboons could distinguish next of kin from outsiders based on their odours, and Célérier and her colleagues are optimistic that their mouse-based 'olfactometer' could help us find out more about the messages that body odours send.

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Célérier, A., Huchard, E., Alvergne, A., Féjan, D., Plard, F., Cowlishaw, G., Raymond, M., Knapp, L. A. and Bonadonna, F. (2010). Detective mice assess relatedness in baboons using olfactory cues. *J. Exp. Biol.* **213**, 1399-1405.

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