Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

NEWTS USE RIBS AS LETHAL WEAPONS

Amphibians have evolved a number of defence mechanisms against predators. Some secrete poisonous substances from their skin, some detach their tails and use them as decoys, and some have cryptic coloration; but of all amphibian antipredator mechanisms, the Spanish ribbed newt (*Pleurodeles waltl*) wins the trophy. In addition to some very noxious skin secretions, this little amphibian possesses a bizarre defence tactic. When faced with a threat the newt projects its ribs outside its body wall, turning them into sharp poisonous spines, giving a whole new meaning to the phrase 'concealed weapons'. F. Leydig described this phenomenon in 1879 and now, 130 years later, Egon Heiss and his colleagues from Austria set out to describe the mechanisms by which these animals perform this peculiar feat.

To understand how the ribs move during their defence displays and to determine the morphology of the ribs and the corresponding vertebrae, Heiss' team analysed x-rays and computed tomography (CT) scans of the newts before and after their defensive behaviour. Repeatedly touching the newts with a cotton bud evoked the curious self defence behaviour.

The authors observed that when threatened and unable to escape, the newts become immobile, enlarge their body and rotate their long, spear shaped ribs forward (from 27 to 92 deg. relative to the vertebrae axis). This rotating motion lacerates the skin of the flanks at orange warts situated on either side of the body. The orange warts also appear to play a role in the defence strategy, as they make the spiny ribs more conspicuous to potential predators.

Surprisingly, instead of the ribs emerging from permanent pores located on the skin, as the authors had thought, these self-inflicted injuries occur *de novo* each time the animal adopts the 'antipredator posture'. Meanwhile, the animal secretes a poisonous milky substance from the surface of its skin, which coats the tips of the ribs as they protrude from the body wall. The combination of sharp, spear-like ribs and the very poisonous secretions turns this newt's ribs into very effective and potentially deadly weapons.

Most amphibians have an extraordinary capacity to heal skin injuries and the Spanish ribbed newt is no exception. But even more remarkable is the fact that despite repeated self-laceration, the authors never observed signs of self-poisoning or infection in the animals. In 1969 Edmund Brodie, Jr and Linda Gibson observed that some species of urodels are immune to their own toxins and *P. waltl* can be added to that list. It is also likely that antimicrobial substances, similar to those seen by Ermin Schadich in 2009 in the African Clawed frog, are produced in the skin of this newt to protect it from infection. Furthermore, the tip of each rib is also covered with a thick periosteum layer which, the authors believe, may serve as a shield against microbial infections when the ribs protrude from the skin.

Self destructive but effective, this bizarre defence mechanism will put most predators in a very prickly situation.

10.1242/jeb.036400

Heiss, E., Natchev, N., Salaberger, D., Gumpenberger, M., Rabanser, A. and Weisgram, J. (2009). Hurt yourself to hurt your enemy: new insights on the function of the bizarre antipredator mechanism in the salamandrid *Pleurodeles waltl*. *J. Zool.* doi10.1111/j.1469-7998.2009.00631.x

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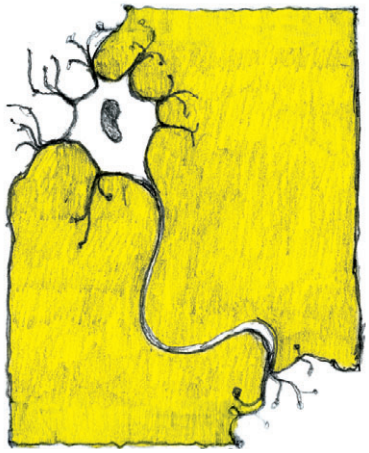
BRAIN ORGANISATION



IS THERE AN OCTOPUNCULUS?

In the 1940s, Wilder Penfield was working with epileptic patients, treating their epilepsy by excising portions of brain tissue where the seizures started. To target his surgery better, he stimulated the patients' brains while they were conscious, and observed what body parts moved, or where the patients felt a sensation. Based on his experience, he drew an image that would become famous: a little man, grossly distorted, with huge hands and lips – called a homunculus – that mapped which brain regions affected which body regions. Areas close to each other in the brain tended to relate to regions close to each other on the body, roughly preserving the same spatial organization.

This effect is called somatotopy, the correspondence between areas on the body and areas in the brain. While the homunculus that Penfield drew has proved to be an oversimplification, somatotopy seems to be a common organizing principle in the brains of both vertebrates and some invertebrates.

In a recent paper in *Current Biology*, Letizia Zullo and her colleagues looked for the same sort of organization in the octopus's brain, a ganglion called the supraesophageal mass. Octopi pose a particularly interesting problem for motor control. In contrast to human limbs, which can only bend at joints, octopi can move their arms in an almost unlimited number of ways. How is their nervous system organized to handle this bewildering complexity?

Zullo used microelecrodes to stimulate small areas in the higher motor centers of the octopus brain – not motor neurons that would stimulate muscles directly, but higher areas that control complex motions. They found that stimulation produced a variety of complex but discrete behaviors, including arm extension, crawling, and jetting behaviors. At low voltage, the stimulation produced relatively simple components of these behaviors; at higher voltages, these simple components were combined to produce more complex movements.

But even the simplest motions involved multiple arms or body parts. None of the stimulation sites produced movement in a single arm. Not only that, the researchers also found relatively little spatial organization to the behaviors themselves; the stimulation sites that produced the behaviors, while consistent, were often distributed throughout the brain.

In this sense, the supraesophageal mass of octopus seems to be similar to the integrative areas in the parietal cortex in vertebrates, in which microstimulation produces multijoint movements. But in vertebrates, the parietal cortex connects to the motor cortex, which does have a somatotopic representation – Penfield's homunculus. Octopi, in contrast, seem to lack somatotopy even in regions that control motor output fairly directly.

Zullo and her colleagues hypothesize that the missing 'octopunculus' might be related to the octopus's unique body plan, with eight long flexible arms that must be highly coordinated. Even more than other animals, the octopus must integrate its behavior across multiple limbs and senses. Perhaps its unusually integrated nervous system evolved to help it coordinate the practically unlimited number of ways it can move its body.

10.1242/jeb.036376

Zullo, L., Sumbre, G., Agnisola, C., Flash, T. and Hochner, B. (2009). Nonsomatotopic organization of the higher motor centers in octopus. *Curr. Biol.* **19**, 1632-1636.

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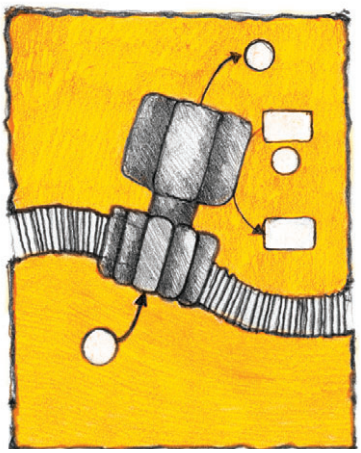
CALCIUM TRANSPORT



A MITOCHONDRIAL DOOR FOR CALCIUM

Calcium (Ca^{2+}) signalling is one of the main mechanisms that cells use to integrate and process intra- and extracellular signals. Signal transduction occurs when the cell is stimulated to release Ca^{2+} from intracellular stores. The resulting increase in the cytosolic Ca^{2+} concentration is sensed by various proteins and converted into an appropriate cellular response. For this system to function, cells must maintain a low cytosolic Ca^{2+} concentration by continuously extruding Ca^{2+} ions from the cytosol into intracellular stores. The mitochondrion is an important Ca^{2+} store that takes up, but also releases, Ca^{2+} ions on demand. Although the proteins that move Ca^{2+} in and out of mitochondria have been studied for decades, the molecular identity of these proteins remained unknown until David Clapham and colleagues from the Harvard Medical School identified one of the elusive calcium ion transport proteins in a recent *Science* paper.

Knowing that calcium must be transported by proteins such as calcium channels, or Ca^{2+}/Na^{+} and Ca^{2+}/H^{+} exchangers, across the inner mitochondrial membrane, the team decided to try to identify the genes that encode proteins involved in mitochondrial Ca^{2+} transport. They designed a clever high-throughput assay to detect calcium transporters based on a Ca^{2+} and H^{+} (pH) sensing fluorescent protein. Generating *Drosophila* cells that were equipped with this fluorescent protein, the team determined the concentrations of Ca^{2+} and H^{+} ions in the cells' mitochondria by measuring the amount of florescence at different excitation wavelengths. Then they treated these cells with double stranded RNA molecules (dsRNA) to each of the ~20,000 *Drosophila* genes in the hope of inactivating (knocking down) the expression of a gene involved in the mitochondrial Ca^{2+} transport.

The team identified one gene whose knockdown abolished mitochondrial Ca^{2+} and pH increases: a homologue of human *Letm1*, an evolutionarily conserved protein of unknown function. In a series of subsequent experiments performed in *Drosophila* and human cells, the team discovered that Letm1 functions in the slow uptake of Ca^{2+} into mitochondria in exchange for H^+ ions observed at submicromolar Ca^{2+} concentrations.

To test more directly whether Letm1 is a Ca^{2+}/H^+ exchanger, the team expressed Letm1 in bacteria, purified the protein and integrated it into the membranes of liposomes, tiny membrane-enclosed bubbles filled with and surrounded by defined buffers. Then they measured Ca^{2+} uptake and pH changes in the liposomes using fluorescent dyes. They showed that the Letm1 protein is responsible for the specific uptake of Ca^{2+} by liposomes, and that the Ca^{2+} transport was dependent on H^+ ions. Moreover, the exchanger turned out to be electrogenic, because a twofold positively charged Ca^{2+} ion is exchanged for a single positively charged H^+ ion resulting in the generation of a potential difference across the membrane.

By identifying the mitochondrial Ca^{2+}/H^+ exchanger, Letm1, the Harvard team has supplied an important piece to the puzzle of mitochondrial Ca^{2+} transport. The reported transport properties of Lemt1 indicate that Ca^{2+} entry is mediated by Lemt1 but is limited by the mitochondrial pH gradient. As *Letm1* is genetically linked to Wolf–Hirschhorn syndrome, a genetic disorder characterized by mental retardation, microcephaly, seizures and hypotonia, its discovery may also help to understand the underlying pathophysiology of this devastating disease.

10.1242/jeb.036392

Jiang, D., Zhao, L. and Clapham, D. E. (2009). Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca^{2+}/H^+ antiporter. *Science* **326,** 144-147.

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THERMOGENESIS

BRRROWN ADIPOSE TISSUE: SPECIAL FAT FOR COLD CRITTERS

There are two major strategies available to mammals to survive winter: hibernation, where large animals drop their metabolic rate, and non-hibernation, where small mammals utilise fat stores to maintain their body temperature. According to Stuart Egginton from the University of Birmingham, textbooks state that the liver is the main organ of heat generation, 'but this is based purely on the relative mass,' he explains. Egginton realised that the liver could only be a thermogenic organ if it generated more heat then expected for its size. Egginton, David Hauton and Andrew Coney decided to test the liver's lipid oxidation profile to find out whether the liver really does keep non-hibernators warm during winter.

Focusing on rats' body temperatures and fat metabolism as the animals were cooled, the team treated some of the rats with fenofibrate (which increases fat oxidation by the liver and could maintain the rat's body temperature during cooling if liver is the source of winter warmth) and others with dichloroacetate (which inhibits the liver's ability to oxidise fats and would force the animals to rely on other forms of heat generation to maintain their temperature) before cooling the rodents. Then they compared how the fenofibrate and dichloroacetate treated animals performed with a third group of rats that had been prepared for the cold conditions by cold acclimation.

If fat metabolism by the liver was key to keeping warm, the fenofibrate treated rats should survive cooling as well as the animals that were previously acclimated to cold conditions. However, the cooled rats that had been treated with dichloroacetate would not survive cooling in good condition, as their livers cannot metabolise fats and they must rely on other, limited, energy supplies (glucose) to maintain their temperature. As the fenofibrate treated rats did not maintain their temperature as well as the well-prepared cold acclimated rats, the researchers suggested that the liver is not the critical step to heat generation.

Some other fat related tissue must be responsible for the rodents' ability to defend their body temperature. Brown adipose tissue (BAT) is a fat storage tissue especially abundant in small mammals and newborn humans. BAT is highly vascularised, full of mitochondria and burns fat to produce heat in a special way. Maybe it could provide the warmth the rodents require to survive winter in addition to its supposed role in arousal?

The team found that the BAT of cold acclimated rats took up fatty acids that were oxidised to generate heat. Amazingly, these rats were up to 12 times better at the conversion than the other rats. Additionally, while the other rats slowed their ventilation, the cold acclimated rats increased their breathing rate to better supply BAT with oxygenated blood and hence maintain their temperature while being cooled.

The authors decided that BAT is the true 'thermogenic machinery' for non-hibernators, and that the liver may contribute very little to thermogenesis. Scientists think BAT fat metabolism that non-hibernators use to stay warm and remain alert during cold conditions may have been one key to the evolutionary success of early mammals.

10.1242/jeb.036384

Hauton, D., Coney, A. M. and Egginton, S. (2009). Both substrate availability and utilisation contribute to the defence of core temperature in response to acute cold. *Comp. Biochem. Physiol.* **154A**, 514-522.

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