

## OUTSIDE JEB

## How crabs enjoy a hot meal



Crabs are renowned for their cranky demeanor, but when one considers the extreme conditions under which they must survive, it becomes easier to sympathize with these crotchety crustaceans. One hardship that crabs must endure is changing temperature: a daily swing of 20°C is not unusual for the intertidal zone. Because crabs do not actively maintain a constant internal body temperature, environmental fluctuations pose a serious problem for the crab's nervous system, whose component parts are all uniquely temperature dependent.

A recent study from Wolfgang Stein's lab at Illinois State University, USA, has uncovered some of the neural mechanisms that allow crabs to tolerate capricious weather. Stein, Carola Städele and Stefanie Heigle investigated the effects of temperature on the gastric mill, a neural circuit in the crab stomach whose rhythmic firing controls the chewing movement of three internal 'teeth'. A convenient feature of the gastric mill is that it continues to produce rhythmic activity even when it is removed from the animal. In this study, the authors used an *in vitro* preparation to ask how chewing rhythms change when the temperature rises.

Surprisingly, they found that a relatively minor temperature increase, from 10 to 13°C, caused the spontaneous chewing rhythm to completely collapse. When the authors recorded intracellularly from the lateral gastric motoneuron (LG), a key component of the gastric circuit, they

found evidence that the temperature change increased the amount of ions that leak across the neuron's membrane, most likely through potassium 'leak' channels.

To further test this idea, they manipulated the leak conductance with a technique called dynamic clamp, which allows an electrophysiologist to artificially add or subtract particular ionic conductances while recording from a neuron. When they subtracted the increased leak conductance from the LG neuron at 13°C, they found that the gastric rhythm was restored, consistent with the hypothesis that the temperature-dependent collapse was due to increased leak.

If a temperature increase of just 3°C is enough to disrupt a crab's ability to chew, how does a hot crab ever enjoy a decent meal? To answer this question, the authors recorded extracellularly from the gastric mill in live, chewing crabs. These experiments revealed that the circuit functions over a much wider temperature range in the intact animal, up to 16°C. Thus, under natural conditions, there is active compensation for the temperature-induced collapse of the gastric mill rhythm.

In the second half of the paper, the authors present evidence that robustness to temperature relies on the modulatory commissural neuron (MCN1), a descending neuron that initiates the gastric rhythm. They found that increases in MCN1 input allowed the LG neuron to function at higher temperatures, and bath application of the neuromodulator released by the LG neuron rescued gastric mill rhythms at 13°C.

So, an increase in temperature boosts the leak conductance of the LG neuron and, at the same time, triggers release of a neuromodulator from the MCN1 neuron. This neuromodulator opens a conductance in the LG neuron that compensates for the temperature-dependent increase in leak conductance. Without the intervention of the descending MCN1 neuron, the gastric chewing rhythm would fail at high temperatures, leaving the crab hot and hungry.

It is humbling to consider that the crab has millions of neurons whose intrinsic properties must be constantly fine-tuned to compensate for external temperature fluctuations. The neuromodulatory mechanism elucidated in this paper may exemplify a general solution to this ubiquitous problem.

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Städele, C., Heigle, S. and Stein, W. (2015). Neuromodulation to the rescue: compensation of temperature-induced breakdown of rhythmic motor patterns via extrinsic neuromodulatory input. *PLoS Biol.* 13, e1002265.

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## Pesticide resistance thanks to transcriptional noise



Our genetic code – our genome or DNA – is what defines our biology, how we interact with the environment and which traits give us an advantage in adapting, surviving and reproducing. That was the staple of genetics for years, but it was not the complete story. Later, we found out that it is not only the genome but also something more dynamic that is important, something 'above' the genome: the epigenome. Conrad Waddington coined the term epigenetics in the 1940s to refer to the interactions between the environment and the genetic code. It is how our DNA quickly 'learns' during development, under changing environmental conditions, without any modifications to the genetic code. DNA methylation, histone modification, alterations to chromatin structure and small non-coding RNAs are some of the

ways in which genes can be up-regulated or down-regulated, leading to different phenotypes without any changes to the genetic code. Recent studies have pointed to yet another epigenetic method, which may contribute to the development of resistance to various chemicals in insects: long non-coding RNAs (lncRNAs).

Recent advances in deep sequencing techniques have made it possible to identify and classify various types of lncRNAs. Although in the past they had been considered nothing more than transcriptional noise, it is now becoming clear that lncRNAs are involved in a multitude of biological pathways, such as growth and development, cell differentiation and gene expression. Through their study, Kayvan Etebari and colleagues have provided, for the first time, a glimpse of the lncRNA profile of the cabbage moth (*Plutella xylostella* L.). They also analysed the role that long intergenic non-coding RNAs (lincRNAs), a class of lncRNAs, play in insecticide resistance.

The authors found significant correlations between lincRNAs and the number of protein-coding genes in the genome scaffolds that they examined. As lincRNAs are generally expressed with their neighbouring proteins, the authors determined that cabbage moth lincRNAs play a role in protein-binding activities, such as DNA and RNA binding, and transcription regulation. When cabbage moth lincRNA sequences were compared with those of two closely related species, the silk moth (*Bombyx mori*) and the fall armyworm (*Spodoptera frugiperda*), only 14 similarities were detected among all three species. This lack of conservation among identified lincRNAs has been reported in vertebrates as well, making it difficult to identify the specific roles they play across various species.

When the lincRNA expression profiles of pesticide-resistant and *Bacillus thuringiensis* (Bt) endotoxin-resistant cabbage moths were compared with those of susceptible individuals, Etebari and colleagues noticed that approximately 70% of the lincRNAs examined were over-expressed in insecticide-resistant populations, and only 50% were up-regulated in Bt-resistant individuals. While the same lincRNAs were commonly altered in the pesticide- and

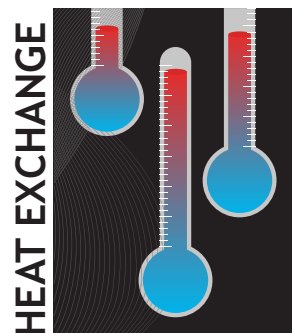
toxin-resistant individuals, the way in which they were modified differed from one population to another, suggesting that their roles in resistance are chemical specific. The authors also found that direct exposure of cabbage moth larvae to insecticides significantly impacted the transcript level of several lincRNAs in insecticide-resistant individuals when compared with non-exposed controls. Whether lincRNAs contribute to resistance development through epigenetic mechanisms, or are a part of the chemical detoxification pathways, remains to be resolved. However, it is now clear that what was previously known as transcriptional noise may indeed play a larger role in gene regulation than was initially thought.

10.1242/jeb.129874

Etebari, K., Furlong, M. J. and Asgari, S. (2015). Genome-wide discovery of long intergenic non-coding RNAs in diamondback moth (*Plutella xylostella*) and their expression in insecticide-resistant strains. *Sci. Rep.* 5, 14642.

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## Counter-current exchange gone wild



Studying counter-current heat exchange is akin to a rite of passage for budding young physiologists. After all, counter-current exchange systems are the quintessential example of an anatomical solution to a physiological challenge: elegant networks of interwoven vasculature whose arrangement alone maintains the internal milieu separate from a harsh and variable environment. The undergraduate physiology student may be bombarded with fascinating examples of heat exchangers: temperate ducks whose bodies stay toasty while their feet are on ice, tuna with red muscles keeping their core warm, etc. In these, as in most other examples, the counter-

current heat exchanger serves to keep heat in the core of the body while letting the limbs cool down to the temperature of the surroundings.

Given our intimate acquaintance with the *rete mirabile*, the ‘wonderful net’, one would hardly imagine a case of counter-current heat exchange that would be surprising. However, leave it to the enigmatic leatherback sea turtle to provide us with an exception. John Davenport at University College Cork, Ireland, and colleagues at NOAA Fisheries and the US Geological Survey have uncovered a fascinating and unusual counter-current exchange system in these turtles and, frankly, it seems just a bit backward. A look at the turtle’s biology suggests why that might be the case.

Leatherback sea turtles swim continuously and they often swim in cold temperate waters and dive down to water that is only just above freezing. The turtle’s core temperature is warmer than the surrounding ocean, but its metabolic rate is low. Because of this low metabolism, there is good reason to suspect that the turtle depends on exercise – specifically, the heat produced as a by-product of muscular work – to keep itself warm. As a result, the temperature within the limbs is likely higher than the core temperature, and heat travels from the limbs to warm the core.

Davenport and his colleagues noticed that there were networks of blood vessels – venous retes – at the base of each of the turtle’s limbs and examined the pelvic retes in detail. But the vessels seemed to run counter to what one would expect from previous examples, where heat would be kept out of the limbs to heat the core of the body. Instead, the turtle’s rete appear to act to retain heat within the limb, because of the heat gradient between the warm limb and the cooler body. Our hypothetical undergraduate physiologist may now be puzzled as to how this arrangement makes sense. But recall that the leatherback depends on swimming for warmth. The researchers suspect that the rete functions to maintain high limb muscle temperature for swimming in chilly water, while the core depends on insulation and thermal inertia to retain heat.

The authors also note that hyperthermia, overheating, is a true risk for leatherbacks,

in particular when nesting; turtles exercise their hindlimbs while moving over the sand and digging, which could potentially cause them to overheat. In this case, counter-current exchange would serve to protect the body from excessive heat generated by the limbs.

The leatherback's unusual employment of counter-current heat exchange is a welcome reminder that the solutions to physiological challenges are just as varied as the challenges themselves – even when they superficially look alike.

10.1242/jeb.129841

Davenport, J., Jones, T. T., Work, T. M. and Balasz, G. H. (2015). Topsy-turvy: turning the counter-current heat exchange of leatherback turtles upside down. *Biol. Lett.* **11**, 20150592.

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## Brain density and size help us catch our Zs



All vertebrates sleep at some point during the day or night, including humans. As you tuck yourself in at night for your 8 h of bliss, have you ever wondered why we sleep for that amount of time? Why not 12 h every day, or only 2? Mammals vary greatly in the number of hours they spend sleeping each day. For example, giraffes

sleep for as little as 3 h per day, while many primates sleep for 8 h. Scientists actually don't know what causes this variation in sleeping hours across species. To solve this problem, Dr Suzana Herculano-Houzel of the Federal University of Rio de Janeiro, Brazil, hypothesized that the time spent sleeping might be related to differences in neuron density in the brain and the brain's surface area across species.

To understand why neuron density and surface area are important, we must first understand one of the functions of sleep. Sleep allows our bodies to clear metabolites – by-products of our body's daily physiological processes – from our brains. The metabolites accumulate during the day, and while some are toxic, others – like adenosine – signal a need for sleep. These metabolites are washed away during sleep by cerebral spinal fluid, the liquid that coats our nervous systems. However, during waking hours the space between cells and neurons in our brains contracts, so the fluid can only clear metabolites at the surface of the brain and not those further below. Similarly, if the neurons in the brain are densely packed beneath the surface, this makes it even harder for the fluid to clear these metabolites.

Across evolutionary history, mammals have developed larger bodies and brains. Previous researchers have shown that larger brains (with more surface area for metabolite rinsing) and less densely packed neurons (allowing metabolites to be rinsed away more readily) are often linked to less time sleeping. However, they couldn't explain cases where large-brained animals like cattle only sleep for a few hours each day, while primates with

similar-sized brains sleep for up to 8 h. Herculano-Houzel made an important prediction, suggesting that it is the ratio between neuron density and the surface area of the brain that is rinsed with spinal fluid during waking hours that determines the amount of time spent sleeping each day. To explore this idea, Herculano-Houzel looked up average daily sleep hours for 24 mammalian species from the literature and related it to their average neuron density and brain surface area. She found exactly what she was expecting: as the ratio between neuron density and surface area increased, so too did the amount of time spent sleeping each day.

Her results explain the previous 'cattle-primate' sleep discrepancy, as primates have more densely packed neurons, requiring more sleep each day to clear away metabolites that accumulate faster in their brains compared with those of cattle. She found that this ratio – and not surface area or neuron density alone – most accurately predicts sleep time, making it important to consider the relationship between these two factors in future studies on sleep and brain evolution. Her findings suggest a 'self-reinforcing spiral' in the evolution of larger brains and bodies: increased neuron density leads to decreased sleep time, thereby allowing animals more time to look for food to support their larger bodily structures.

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Herculano-Houzel, S. (2015). Decreasing sleep requirement with increasing numbers of neurons as a driver for bigger brains and bodies in mammalian evolution. *Proc. R. Soc. B.* **282**, 20151853.

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