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

LEARNING WITH LEECHES

Learning is a complex process, involving subtle changes in the properties of neurons and the connections between them. But trying to determine what changes occur in the nervous system during learning, and relating a specific neural network to a behaviour, is difficult in intricate vertebrate brains. So Brian Burrell and Christie Sahley, from Purdue University, turned their attention to learning processes in a much simpler animal – the medicinal leech. These little bloodsuckers' nervous systems are far less complex than those of vertebrates, so it is easier to investigate how transmission at the connection between two neurons – the synapse – changes and may contribute to learning.

Burrell and Sahley chose a circuit that receives inputs from touch and pressure neurons on the leech's skin. The circuit is involved in the body shortening reflex, which the leech uses when it is touched and feels threatened. The touch and pressure cells transmit most of their signal *via* a connecting cell to the S-cell, a neuron in the central nervous system that is important for different forms of learning. Some of the branches from the touch and pressure cell bypass the connecting cell and link directly to the S-cell.

The team wanted to investigate the conditions under which the synapses between pressure and S-cells, and touch and S-cells, showed long-term potentiation – a maintenance or strengthening of a signal across a synapse over time – or long-term depression, which is a weakening of a signal across a synapse over time. Long-term potentiation and long-term depression are thought to contribute to learning and memory by reinforcing or weakening synaptic connections, respectively.

The team chose two touch or two pressure cells and sent each a different signal. One of the signals was tetanising, so the synapse was working at its maximum rate, and the other was not. They then measured the output that was occurring in the S-cell, and again 1 h later, to see if long-term potentiation or long-term depression had occurred. For both types of synapse, either pressure to S-cell or touch to S-cell, they found that the changes depended on the signal from the touch or pressure cell.

At the synapse between pressure and S-cells, long-term potentiation occurred only when the signal was tetanising and the individual pulses in the signal were small in amplitude. They also found that the signal in the S-cell was greater after 1 h. And when they blocked a type of receptor called the NMDA receptor with AP-5 in the neuron's membrane, long-term potentiation was also prevented. This shows that the leech's synapses can change their properties according to the signal they receive; similar to vertebrate neurons.

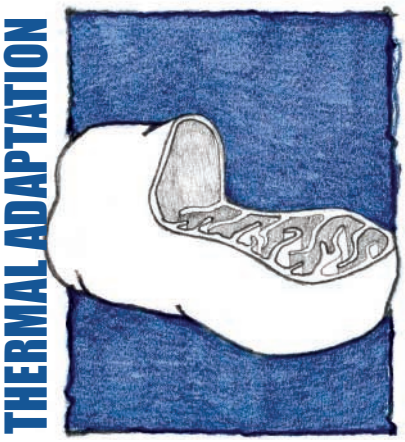
At the touch–S-cell synapse, a tetanising pulse caused long-term potentiation but only on one subset of the synapses tested. The signal size had no effect. Long-term depression occurred when the input was non-tetanising, but on a different subset of synapses. It was also blocked by AP-5. The leech's synapses were showing similar long-term potentiation and long-term depression to that seen in vertebrates. This means that researchers can study these processes far more easily in the simple leech to help understand how vertebrates might learn.

Burrell and Sahley don't yet know whether the synapse changes contribute to learning in the leech but hope that they will make more tantalising discoveries about the workings of neurons in our brains thanks to these little bloodsuckers.

10.1242/jeb.01160

Burrell, B. D. and Sahley, C. L. (2004). Multiple forms of long-term potentiation and long-term depression converge on a single interneuron in the leech CNS. *J. Neurosci.* **24**, 4011-4019.

Laura Blackburn
University of Cambridge
Lmlb2@cam.ac.uk



MITOCHONDRIA ON THE ROCKS

Temperature is one of the most important environmental factors for the regulation of animal physiology. This is especially true for ectotherms, a group of animals that cannot regulate their body temperature. Indeed, ectothermic animals display body temperatures similar to their surroundings. The fact that the rate of metabolic reactions doubles for a 10°C increase in temperature illustrates the profound impact of temperature on ectotherms' lives. Mitochondria are the powerhouses of the cell, and the effect of temperature on mitochondrial metabolism in ectotherms has been a subject of intense research for many years. Indeed, studies need to be carried out on many different species such that general principles can emerge. One fundamental question regards the comparison of changes in mitochondrial function that occur during seasonal temperature changes *versus* those occurring due to adaptation to life in different thermal habitats. Angela Sommer and Hans Otto Pörtner took up that challenge and examined mitochondrial functions during seasonal acclimatization in the lugworm, as well as mitochondrial characteristics during latitudinal adaptation, by comparing subpolar lugworms of the White Sea close to the Arctic ocean with boreal specimens from the North Sea.

Mitochondrial respiration is often increased in ectotherms exposed to cold temperature. This is thought to occur in order to maintain the level of metabolic activity present at warmer temperatures. Indeed, if there were no increase in respiration upon cold exposure, the effect of temperature on biochemical reactions would be such that oxygen consumption would be reduced compared with that at warmer temperatures. And the team's results supported this idea; mitochondria from both cold-acclimatized lugworms and subpolar specimens displayed increased oxygen consumption rate.

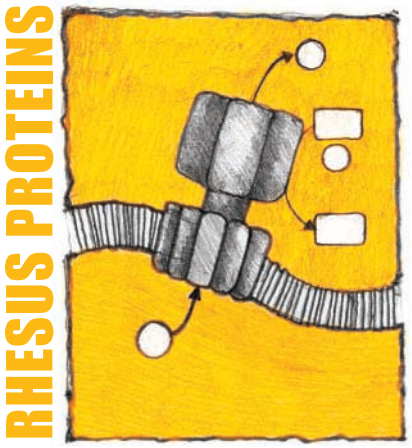
In order to determine which mitochondrial reactions caused the elevated oxygen consumption rate, the authors examined two important states of mitochondrial respiration: state 3 and state 4. State 3 represents the maximal oxygen consumption rate associated with ATP production, whereas state 4 represents basal respiration associated with maintenance costs. The ratio between state 3 and state 4 respiration indicates the coupling of respiration and mitochondrial metabolic efficiency. The team found that increased mitochondrial respiration in cold-acclimated lugworms as well as in subpolar animals was reflected in an elevation of both respiration states. However, different changes in mitochondrial properties occurred during latitudinal adaptation and seasonal acclimatization. During latitudinal adaptation, the increase in state 3 respiration was accompanied by a slightly larger increase in state 4 respiration, leading to a reduction in mitochondrial metabolic efficiency. In other words, the elevated capacity to produce ATP during latitudinal adaptation was paralleled by increased maintenance costs. However, during seasonal acclimatization, state 3 respiration increased more than state 4 respiration, resulting in elevated mitochondrial metabolic efficiency and lowered maintenance costs.

The different responses of mitochondrial metabolism to the two cold challenges examined in the present study illustrate nicely the plasticity of mitochondrial functions. The next fundamental question emerging in the field of temperature physiology is: how do mitochondria integrate all external stimuli into a finely balanced metabolic program?

10.1242/jeb.01163

Sommer, A. M. and Pörtner, H. O. (2004). Mitochondrial function in seasonal acclimatization versus latitudinal adaptation to cold in the lugworm *Arenicola marina* (L.). *Physiol. Biochem. Zool.* **77**, 174-86.

Julie St-Pierre
Dana-Farber Cancer Institute and
Harvard Medical School
julie_stpierre@dfci.harvard.edu



RHESUS PROTEINS CHANNELING GAS?

Discovered over 60 years ago, Rhesus (Rh) proteins are best known as antigens on red blood cells that cause immune reactions during blood transfusions and hemolytic disease in newborn babies. Studies over the past few years have shown that Rh proteins are not limited to red blood cells or to mammals and are involved in the active transport of the ammonium ion (NH_4^+) or the NH_4^+ analog, methylammonium, across cell membranes. Most recently, it has been suggested that these proteins are in fact bidirectional channels for biological gases, such as ammonia (NH_3) or carbon dioxide (CO_2). In support of this hypothesis, CO_2 and NH_3 exposure causes an increase in *Rh1* gene expression in the green alga *Chlamydomonas reinhardtii*. In order to follow up on this theory, E. Soupene, W. Inwood and S. Kustu decided to eliminate Rh1 function in *C. reinhardtii* using a technique called RNA interference (RNAi) and found that the protein functions as a CO_2 gas channel.

RNAi is a method that silences gene expression by the introduction of double-stranded RNA (dsRNA) specific for that gene; in the case of Soupene's study, the *Rh1* gene. Essentially, the team introduced dsRNA that bound to the naturally occurring *Rh1* mRNA, which ultimately caused rapid degradation of the *Rh1* mRNA and consequently reduced (or even silenced) *Rh1* expression. First, the team verified the expression of *Rh1* mRNA in *C. reinhardtii* through northern blot analysis and found that *Rh1* expression increased substantially when the green alga was exposed to high environmental CO_2. However, in strains of *C. reinhardtii* that underwent RNAi, the upregulation of *Rh1* expression seen in response to high

environmental CO_2 was eliminated. In addition, the alga failed to produce Rh1 protein. Through the use of RNAi, Soupene, Inwood and Kustu have successfully developed a strain of *C. reinhardtii* that lacks Rh1.

So, how did this new *C. reinhardtii* strain do without the Rh1 protein? The group determined that Rh1 is required for optimal growth at high environmental CO_2 and that a lack of Rh1 protein causes growth defects, in addition to affecting the expression of other genes that normally respond to environmental CO_2. The team deduced that algae without Rh1 were unable to rapidly equilibrate with the high CO_2 environment and, unlike algae with Rh1, did not benefit from the readily available CO_2, suggesting that Rh1 functions as a CO_2 channel. In addition, the group found that strains without Rh1 were able to transport the NH_4^+ analog methylammonium, suggesting that Rh1 is not involved in the transport of methylammonium, or potentially NH_4^+, in *C. reinhardtii*.

Soupene and colleagues have outlined evidence suggesting a possible physiological role for Rh1 as a biological gas channel, which would allow the effective uptake of CO_2 and permit optimal growth of *C. reinhardtii*. While Rh1's role in vertebrates is most likely in waste disposal rather than nutrient uptake, this study, as well as other studies from this group of workers, has given insight into possible alternative physiological roles for Rh proteins.

10.1242/jeb.01162

Soupene, E., Inwood, W. and Kustu, S. (2004). Lack of the Rhesus protein Rh1 impairs growth of the green alga *Chlamydomonas reinhardtii* at high CO_2. *Proc. Natl. Acad. Sci. USA* (doi:10.1073/pnas.0401809101).

M. Danielle McDonald
RSMAS, University of Miami
dmcdonald@rsmas.miami.edu

SYNAPTIC PLASTICITY

A NEW PLAYER IN SYNAPTIC PLASTICITY

The tidy circuitry of the cerebellum, a hindbrain region involved in balance, coordination and sensorimotor integration, provides an important model system for understanding cellular mechanisms of learning. Learning can involve either an increase or decrease in transmission at a particular synapse, and this change can either be a temporary or permanent increase. Collectively, these processes are referred to as 'synaptic plasticity'. In many regions of the central nervous system, NMDA receptors, neurotransmitter receptors located on postsynaptic sites, are involved in synaptic plasticity. In the cerebellum, however, the long-term depression of activity in the large output neurons, or Purkinje cells, is mediated by a different type of glutamate receptor, the AMPA receptor. NMDA receptors are present but in an unusual location: they are found presynaptically on interneurons that release the neurotransmitter GABA, which inhibits activity of the Purkinje cells. The unexpected location of NMDA receptors suggests that glutamate, previously believed to signal only in an anterograde fashion, i.e. from the presynaptic to the postsynaptic cell, could also be involved in retrograde signalling, from the postsynaptic to the presynaptic cell. A new paper by Ian Duguid and Trevor Smart demonstrates that the presynaptic NMDA receptors in the cerebellum are involved in a previously undescribed form of synaptic plasticity, which the authors call depolarization-induced potentiation of inhibition (DPI). Through a series of electrophysiological and immunocytochemical experiments, the authors revealed that the cellular mechanisms underlying DPI differ from those involved in a previously described form of plasticity, depolarization-induced suppression of inhibition (DSI), which occurs at the same synapse.

In DSI, repeated depolarization causes calcium to build up in the postsynaptic Purkinje cells, initiating release of endocannabinoids, a type of retrograde neurotransmitter, which activate cannabinoid receptors on the interneuron to temporarily suppress release of GABA. The resulting decrease in inhibitory input to the Purkinje cell lasts for tens of seconds.

Duguid and Smart found that both the underlying mechanism and the time course of DPI differ from those of DSI. In DPI, repetitive stimulation causes calcium to build up in Purkinje cells, leading to glutamate release, which in turn activates NMDA receptors on the presynaptic cell. This causes release of calcium from intracellular stores, resulting in enhanced release of GABA, increasing inhibitory input to Purkinje cells. The peak of DPI activity occurs after DSI has subsided and can last up to 10 min.

The ability of a neuron to strengthen activity at a synapse through DPI or weaken activity through DSI results in the capacity to weight incoming information; the impact of any given input will vary with the state of the neuron and the sum of all the synaptic inputs. Behavioural biologists have long known that behavioural responses can be context-specific, and neurobiologists understand that neuronal responses can be state-specific; for example, neurons in escape circuits will respond differently when an insect is walking than when it is flying. Given that the neural state of a circuit will vary with the behavioural state of an animal, it follows that synaptic events such as DPI and DSI contribute to context-specific behaviours. The new paper by Duguid and Smart demonstrates the existence of a novel form of state-dependent change in neural activity and adds to the growing list of mechanisms underlying synaptic plasticity.

10.1242/jeb.01161

Duguid, I. C. and Smart, T. G. (2004). Retrograde activation of presynaptic NMDA receptors enhances GABA release at cerebellar interneuron–Purkinje cell synapses. *Nat. Neurosci.* **7**, 525-533.

Heather L. Eisthen
Michigan State University
eisthen@msu.edu

Emma Coddington
Oregon State University
coddinge@science.oregonstate.edu