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

NEURONAL IMAGING

WHAT THE FLY'S NOSE TELLS ITS BRAIN

Every now and then, a seminal paper really leaves its mark. One of the papers that most impressed me during the 1990s was an early use of degenerate PCR to identify a massive set of olfactory receptors in rat, and thence to map their distribution within the nose. In this single paper (much imitated since), Axel and Buck cut through generations of speculation on the molecular basis of olfactory perception, providing a simple answer. And now Axel has resurfaced in a *Cell* paper published this January that actually images synaptic activity in live fly brain, through its proxy, calcium, when the fly is exposed to different odours. This is hardly an ambulatory, non-invasive procedure: the fly was decapitated, its head mounted in agar, and the cuticle dissected back to expose the brain. Nor is it technically simple: multi-photon confocal microscopy is still more in the realm of physics research than a routine biological tool. Nonetheless, it proved possible to expose antennal lobes to different compounds, and to image the response in deep structures of the brain, for up to five hours. In the longer term, development of these procedures might produce new, less invasive and more physiological access to brain function.

Real-time calcium measurement in genetically defined cells in *Drosophila* is not new. Previous studies have used the transgenic jellyfish calcium reporter, aequorin, and, more recently, calcium-sensitive green fluorescent protein (GFP) derivatives, such as G-CaMP and pericam. In common with aequorin, these calcium reporters are proteins. This means that their expression can be directed to specific cells within a particular tissue using the beautiful genetic tools unique to *Drosophila*.

In this paper, the gene encoding G-CaMP was placed under the control of a transcriptional promoter that is activated by the GAL-4 transcription factor, and transgenic flies made. These flies have the potential to make G-CaMP protein in any cell in which GAL4 is expressed. Then they constructed a second set of flies, where GAL4 transcription was controlled from olfactory-lobe-specific promoters. When the two strains were crossed, high levels of G-CaMP were thus confined to the lobes. However, the lobes are buried quite deep in a relatively large (by *Drosophila* standards) structure, so conventional epifluorescence or even confocal microscopy would not suffice to give a good signal. Instead, the authors used two-photon confocal scanning, which allows longer illuminating wavelengths, and thus deeper tissue penetration to visualise the G-CaMP-mediated calcium signal.

The output of these techniques was remarkable: the authors were able to show that particular odorants elicited responses in particular lobes (or combinations of lobes) and that these mappings persisted between individual flies. By directly stimulating the antennal nerve, the authors were also able to show that the calcium responses they were measuring were a direct function of spike frequency in the antennal nerve.

Thus, this study nicely complements the original Axel and Buck paper, which mapped out the other end of the sensory pathway and the distributions of the odorant receptors in the nose. Axel's current work also shows that calcium in the olfactory lobes could be taken as a faithful correlate of electrical activity in neural tissue.

10.1242/jeb.00410

Wang, J. W., Wong, A. M., Flores, J., Vosshall, L. B. and Axel, R. (2003). Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain. *Cell* **112**, 271-282.

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EUCALYPTUS PLEASE – BUT HOLD THE TANNINS

If you have ever visited Australia, you will no doubt be familiar with the phrase "Bloody possums!" Arguably the most successful of all marsupials, these agile vegetarians are renowned for both their resourcefulness and their big appetites. *Eucalyptus* remains an all-time favourite of both ringtail and brushtail possums; in fact, a particularly tasty *Eucalyptus* tree can be stripped bare of its leaves within days of discovery. Although tree-dwelling ringtails specialise in eating *Eucalyptus* leaves and brushtail possums eat a variety of other vegetation in addition to *Eucalyptus*, both species are picky about the *Eucaluptus* leaves they will eat. So why are certain *Eucalyptus* leaves more irresistible than others? Recent research by Karen Marsh and her colleagues at the Australian National University provides some insight into possum preferences. Marsh and her team examined the degree to which chemicals called plant secondary metabolites influence feeding preference in ringtail and brushtail possums. Their results reveal that, whilst both species adore *Eucalyptus* leaves, they prefer different leaves for different reasons.

Plant secondary metabolites can directly influence feeding behaviour in herbivores. Some of these compounds encourage feeding whilst others, such as tannins and formylated phloroglucinol compounds (FPCs), deter it. *Eucalyptus* leaves are especially famed for the variety of secondary metabolites they contain. The Australian researchers hoped to discover how tannins and FPCs affected feeding behaviour in ringtail and brushtail possums.

Marsh and her colleagues collected *Eucalyptus* leaves containing naturally different levels of FPCs. By collecting leaves from several trees, the researchers ensured that the FPC content of their leaves spanned the full range known for the species. Next, the team manipulated the tannin content of the same leaves by coating some with a substance able to neutralise tannin's deterrent effects. The scientists then monitored how popular the altered leaves were with the possums. Finally, they collected possum faeces in order to measure the digestibility of the different leaves.

The brushtail possums avoided eating leaves that contained tannins, while the specialist ringtail possums avoided eating leaves that contained FPCs. Although neither plant secondary metabolite affected how well ringtails digested the leaves, the specialist brushtails were able to gain more nutrients when the tannins were neutralised. Therefore, brushtails foraged to find *Eucalyptus* without tannins, while ringtails foraged to find *Eucalyptus* without FPCs.

So why do the two species avoid different plant secondary metabolites? One explanation is that the ringtail, which avoids FPCs, lacks the mechanisms to tolerate FPCs but has evolved anatomical and physiological adaptations that allow it to cope with ingested tannins. And why can't ringtail possums tolerate FPCs? Logic suggests that, as specialist leaf-eaters, ringtails should be better placed to cope with all leaf compounds, so perhaps ringtail possums aren't the 'specialised' leaf-eaters we thought they were. And finally, could the possum's differing digestive abilities reduce competition over limited food resources between the species?

As with most good studies, Marsh's work has generated almost as many questions as answers. But, from the *Eucalyptus* perspective, respite from plundering possums isn't likely.

10.1242/jeb.00409

Marsh, K. J., Foley, W. J., Cowling, A. and Wallis, I. R. (2003). Differential susceptibility to *Eucalyptus* secondary compounds explains feeding by the common ringtail (*Pseudocheirus peregrinus*) and common brushtail possum (*Trichosurus vulpecula*). *J. Comp. Physiol. B* **173**, 69-78.

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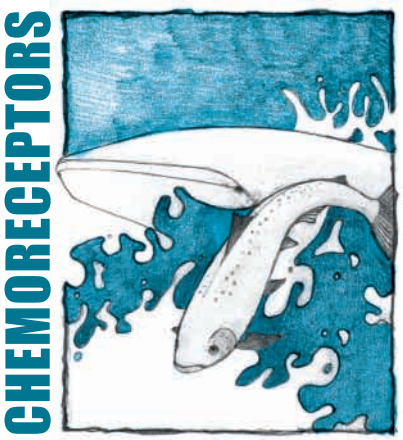

LOCATING OXYGEN CHEMORECEPTORS IN FISH

The necessity of oxygen for survival leads to a wide variety of physiological adaptations that are induced in vertebrates during periods of low oxygen availability. Mediation of a response to hypoxia critically depends on the ability to detect decreases in oxygen supply. In mammals, peripheral oxygen chemoreceptors that can recognise decreases in arterial blood oxygen and initiate physiological responses are located within the carotid body. Fish are known to possess analogous chemoreceptors on the gill arches that mediate an increase in breathing and a decrease in heart rate during hypoxia. Fish also respond to hypoxia by releasing catecholamine hormones into the bloodstream. However, the link between detection of environmental hypoxia and this hormonal reflex remains unknown. As gill chemoreceptors elicit cardiorespiratory reflexes in fish, Stephen Reid and Steve Perry posed the hypothesis that similar branchial chemoreceptors may initiate the reflex arc, leading to catecholamine release during hypoxia in fish.

They exposed rainbow trout to sodium cyanide, either in the external water or directly into the gill circulation. Sodium cyanide is known to pharmacologically stimulate oxygen chemoreceptors. Using two methods of administration, Reid and Perry targeted externally orientated (water sensing) and internally orientated (blood sensing) chemoreceptors, to test whether either, or both, types of receptor played a role in catecholamine release. They found that there are both externally orientated and internally orientated oxygen chemoreceptors on the gills that trigger catecholamine release.

To locate these chemoreceptors, the authors repeated the administration of sodium cyanide but ligated the first gill arch. In these fish, catecholamines were still released when sodium cyanide was internally applied but not when sodium cyanide was present in the water. They concluded that externally orientated oxygen chemoreceptors are confined to the first gill arch. As catecholamine release still occurred when sodium cyanide was internally applied, internally orientated oxygen chemoreceptors appear to be located within other gill arches.

In a second part of the experiment, fish were exposed to environmental hypoxia created by bubbling nitrogen through a water–gas column. Catecholamine release occurred during environmental hypoxia even when the primary gill arch was ligated. Reid and Perry therefore conclude that internally orientated oxygen chemoreceptors are the major vector for catecholamine release during environmental hypoxia. During hypoxia, some cardiorespiratory responses occur well before catecholamines are released into the circulation and must be triggered by oxygen chemoreceptors. By measuring cardiorespiratory responses during these experiments, the authors were able to show that the oxygen chemoreceptors responsible for an increase in ventilation amplitude are distinct from those that elicit catecholamine release or a decrease in heart rate.

A lot is known about the consequences of elevated catecholamine levels during respiratory stress in fish. However, Reid and Perry are the first to identify the site of chemoreception that initiates catecholamine release during hypoxia. They have shown that peripheral oxygen receptors on the gills, orientated both externally (on the first gill arch) and internally (on all gill arches), can initiate the reflex that leads to the release of catecholamines.

10.1242/jeb.00411

Reid, S. G. and Perry, S. F. (2003). Peripheral O_2 chemoreceptors mediate humoral catecholamine secretion from fish chromaffin cells. *Am. J. Physiol.* **284**, R990-R999.

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CARBS OR FAT? NERVE CELLS THAT CONTROL GLYCOLYSIS IN LOCUST FLIGHT MUSCLES

Catabolic activity in muscles is regulated with the amount of exercise a muscle performs, and muscle contractions are controlled by motor neurons. However, there could be a more direct link between the nervous system and muscle metabolism, as Tim Mentel and colleagues report in their recent *J. Neurosci.* paper. They have found that one of the cell signaling pathways that activates glycolysis metabolism during locust flight is under control of a set of identified modulatory neurons.

Migratory locusts are extremely determined flyers. Their flight muscles are fueled by carbohydrates during take-off and for short distances, but they switch to lipid metabolism for longer flights. Two key players regulate glycolysis in these muscles: octopamine, which is a neuromodulator and neurohormone in insects, and fructose 2,6-bisphosphate ($F2,6P_2$), which is a potent activator of glycolysis control enzymes. $F2,6P_2$ levels decrease during prolonged flight sequences and thus glycolysis is downregulated when the muscles switch to fat metabolism. Octopamine levels in the muscle also decrease during flight, and increasing octopamine levels experimentally keeps $F2,6P_2$ levels and glycolysis up.

But what regulates octopamine levels in these muscles? It could be supplied by the blood system, but hemolymph levels of octopamine rise during longer flights, so the source is unlikely to be hormonal. Instead, the authors suspected that muscular octopamine could be supplied by nerve cells as they knew that the thoracic central nervous system of insects contains neurons that release octopamine directly onto skeletal muscles. Some of the so-called dorsal unpaired median (DUM) neurons innervate flight muscles and, consistent with a putative role in regulating octopamine levels in flight muscles, this subset is active at rest and inhibited during flight.

First they tested whether the activity of DUM neurons could increase $F2,6P_2$ levels. They stimulated the DUM neurons at frequencies in the range observed in the resting animal, and consistently elevated the level of $F2,6P_2$ in the muscle. Therefore, DUM neuron activity would be sufficient to keep $F2,6P_2$ levels high in the resting animal, which would keep the flight muscles poised for the high carbohydrate oxidation rates used for take-off.

But how does octopamine released from the nerve trigger an increase in the levels of $F2,6P_2$ in the flight muscle? Octopamine receptors act through second messenger cascades, and Mentel and colleagues wanted to find the signaling molecules that link the neuromodulator to the metabolic enzymes. In another set of experiments they showed that DUM neuron activity affected $F2,6P_2$ levels partly through the cAMP/protein kinase A (PKA) pathway. Stimulating DUM neurons was sufficient to increase PKA levels in the muscles, and a PKA inhibitor blocked the increase of $F2,6P_2$ in response to DUM neuron stimulation. However, just increasing PKA levels in the muscles pharmacologically had no effect, indicating that a parallel pathway must also be involved.

This is the first case showing a neuromodulator's direct action on muscle metabolism. Modulatory neurons release substances into many different tissues, including the blood and the nervous system itself, so one might speculate that in many systems neuromodulators may provide a link between changing behavioral states and adjustment to changing catabolic demands.

10.1242/jeb.00408

Mentel, T., Duch, C., Stypa, H., Wegener, G., Müller, U. and Pflüger, H. J. (2003). Central modulatory neurons control fuel selection in flight muscle of migratory locust. *J. Neurosci.* **23**, 1109-1113.

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