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

C-STARTS WITH MAUTHNER CELLS – AND WITHOUT

If you scare a fish, it will typically bend strongly to one side, making a C-shape, then kick away from you. A pair of neurons called Mauthner cells – one left, one right – can trigger the behavior. But a recent paper in the *Journal of Neuroscience* shows that Mauthner cells aren't the whole story.

Tsunehiko Kohashi from Nagoya University and Yoichi Oda from Osaka University used advanced microscopy techniques to describe the behavior of other cells, similar to the Mauthners, that also trigger escape behavior. The non-Mauthner escapes are a little slower to get started than normal Mauthner cell-mediated escapes, but once the behavior starts, the researchers couldn't tell the two apart.

Kohashi and Oda's results show that touching a fish's head tends to activate these other cells – called Mauthner homologs – more often than the Mauthner cells. The researchers also showed that the Mauthner homologs can modulate the strength of a Mauthner-mediated escape.

They used well-established optical techniques to measure the cell's electrical activity by injecting a fluorescent dye that changes fluorescence depending on the calcium levels in a cell. Calcium tends to increase when a cell fires an action potential, so monitoring the change in fluorescence let them see the activity in the neurons of interest. To get the dye into those neurons, it was linked to a sugar molecule that tends to be transported along axons to the cell body, and so the long axons of the Mauthner cells and their homologs – which extend all the way down the spinal cord – conveniently took up the dye and labeled the cell bodies in the hind brain.

The main innovation was to use a special high-speed confocal microscope that could rapidly scan between the different locations of the cells. Typically, Mauthner cells are located somewhat below the homologs, requiring rapid, precise control over the microscope's focus in order to get reliable measurements of changes in fluorescence in the two sets of cells simultaneously. To keep the fish stationary in the microscope to obtain a clear image, Kohashi and Oda embedded the fishes' heads in agar, leaving the tails free to flex during the C-start.

They were able to divide the behavior into two types, based on latency. Poking the ear or the tail produced a C-start with activity in both the Mauthner cells and the homologs about 4 ms later. Poking the nose, in contrast, produced an escape indistinguishable from the others, except that it took about 8–10 ms to get going and the Mauthner cells didn't spike.

Instead, these longer latency escapes were driven by the Mauthner homologs. Unlike the Mauthner cells, which fire a single action potential to set off the escape, the data showed that the homologs fire multiple times. And because their axons are much thinner than the Mauthner axons, the signal takes longer to travel down the spinal cord, which may be the reason why the movement starts a little later in non-Mauthner escapes.

Not only can the homologs trigger escapes on their own without the Mauthner cells but they can also work together with the Mauthner cells to modulate the strength of an escape. When a Mauthner cell and its homolog fire together, the researchers found that the resulting escape is stronger than when the Mauthner cell fires alone.

Other researchers had suspected that the homologs were involved in escape responses. Kohashi and Oda provided the first proof, and – more importantly – the first description of the neural activity underlying an escape response that doesn't require the Mauthner cells.

10.1242/jeb.021535

Kohashi, T. and Oda, Y. (2008). Initiation of Mauthner- or non-Mauthner-mediated fast escape evoked by different modes of sensory input. *J. Neurosci.* **28**, 10641-10653.

Eric Tytell
University of Maryland
tytell@umd.edu

DEVELOPMENT

A LITTLE STRESS FOR A FETUS GOES A LONG WAY

Many animals experience hypoxia – low oxygen levels – in their environment on a regular basis. Fish living in shallow coastal rockpools experience hypoxia on a daily basis, and humans can also feel the effects of hypoxia when hiking at altitude in thin air. Developing embryos also experience bouts of hypoxia, some due to the mother's uterus contracting, which can reduce blood oxygen by 25%. During hypoxia, norepinephrine (noradrenaline) is released into the bloodstream to protect us from the negative effects of hypoxia by increasing the heart rate and either relaxing or constricting blood vessels to ensure blood supply to crucial organs. Because hypoxia provokes norepinephrine release, and developing embryos experience hypoxia, Margie Ream and her colleagues from Duke and Tufts Universities wondered whether norepinephrine protects developing fetal mice from the damaging effects of hypoxia.

Ream and her colleagues exposed pregnant mice to either normal oxygen levels or hypoxia and identified the embryos within the litter that had genetic mutations preventing norepinephrine synthesis. Then the team monitored the fetus's physiological responses to the oxygen conditions.

Compared with their normal siblings, the norepinephrine-deficient fetuses did not tolerate hypoxia well, and if they survived suffered from low heart rate, blood-flow problems, excessive bleeding, and even heart failure. Ream's team also concluded that, even under normal oxygen conditions, the cardiovascular systems of the norepinephrine-deficient fetal mice performed as if they were normal mice experiencing hypoxia. The team could only ensure the norepinephrine-deficient embryo's survival beyond 15 days if they supplied the mother with extraordinarily high oxygen levels, over twice the amount of oxygen found in normal air.

So, why not prevent hypoxia all together if the damaging effects are so profound? Evidently, exposure to hypoxia is crucial for some developmental processes; hypoxia is the only way to activate a special protein called HIF. When HIF is activated, it attaches to DNA and allows 155 genes to be decoded. If HIF is not activated in a developing fetus, certain genes are not turned on, and the animal may suffer eye damage, abnormal heart and liver development, and even shortened arms and legs. Therefore, it may be important for an animal to experience brief episodes of hypoxia to ensure HIF is activated so it can activate important genes and ensure proper development. Of course, excessive hypoxia is definitely problematic, and if not immediately fatal, can result in growth restrictions, pre-term delivery and even sudden infant death syndrome, as well as heart problems that can persist into adulthood. Ream and her colleagues concluded that an animal protects itself from the damaging effects of hypoxia by releasing norepinephrine, which helps maintain heart rate, blood flow and oxygen levels throughout the body. Meanwhile, developing fetuses that experience hypoxia are able to activate essential developmental pathways and develop properly because HIF is activated. However, if a fetus cannot synthesize norepinephrine, then the effects of hypoxia are magnified and death is almost certain.

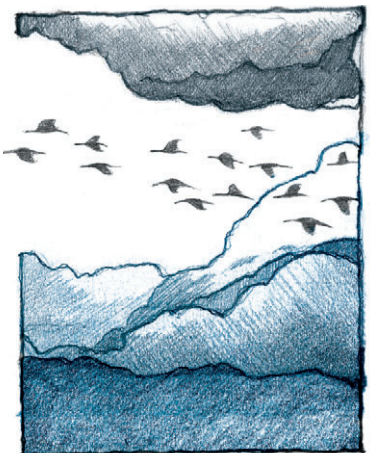
Hypoxia clearly plays a key role in many cardiac developmental diseases. This study is the first to document the genes that are turned on in response to norepinephrine availability and hypoxia, and the findings provide a springboard for further understanding the essential role of the stress response to hypoxia and fetal development. Stress… some of us can't seem to live with it, but our lives apparently can't begin without it.

10.1242/jeb.023812

Ream, M. A., Chandra, R., Peavey, M., Ray, A. M., Roffler-Tarlov, S., Kim, H., Wetsel, W. C., Rockman, H. A. and Chikaraishi, D. M. (2008). High oxygen prevents fetal lethality due to lack of catecholamines. *Am. J. Physiol.* **295**, R942–R953.

Jodie L. Rummer
University of British Columbia
rummer@zoology.ubc.ca

FLIGHT PERFORMANCE



MATERNAL STRESS IMPROVES FLIGHT PERFORMANCE OF FLEDGLINGS

The link between a mother and its offspring goes much deeper than simply sharing genetic material. The condition of the mother and the environment she lives in also influence the development of her offspring, through mechanisms such as exposure to maternal stress hormone levels during development. In birds, the levels of stress hormones in the mother's blood are reflected in her eggs and exposure to stress hormones during development can have detrimental effects on her young. However, it has also been proposed that stress hormone exposure might prepare the offspring to face its new environment. The evolutionary and ecological consequences of these 'maternal effects' have recently been considered by Eunice Chin, Oliver Love and their colleagues from Trent and Simon Fraser Universities in a paper published in the *Proceedings of the Royal Society B*. To study the impact that maternal effects can have on fitness, the researchers studied flight performance of European starlings. They hypothesized that exposure to higher levels of the stress hormone corticosterone during development would improve fledgling flight performance.

Using nest boxes located in the field, the team injected newly laid European starling eggs with corticosterone hormone. They then marked individual hatchlings, tracked them until they fledged at 21 days and took various physical measurements. Taking a flight performance chamber to the field the team filmed the fledglings during a short flight so that they could calculate the mechanical energy gain during take-off. They also measured the birds' body mass and wing morphology and were able to calculate the animals' wing loading, which is the body mass supported by a given surface area of wing. After the flight trials, the team measured the mass and

composition of the fledglings' pectoral flight muscles, as well as their metabolic enzyme activity, which is an indicator of the metabolic energy production potential of the muscle. Having quantified the fledglings' physical condition, the research group looked to see whether exposure to elevated corticosterone levels had affected the chicks' development.

Initially they found no differences in hatching success, brood size, sex ratio, fledging success, body mass or size between birds that had been exposed to corticosterone and those that had not. However, exposure to elevated levels of stress hormone did improve the fledgling's flight performance. Their energy gain after take-off (an indicator of the bird's take-off velocity and height gained after take-off) was greater in both male and female fledglings treated with hormone. Their improved flight performance was associated with physical differences between the treated and untreated birds. The corticosterone-treated birds had larger pectoral flight muscles and a larger wing surface area, which reduced their wing loading during flight. In addition to the physical differences, the properties of the flight muscle were also different. Two enzymes involved in metabolic energy production, citrate synthase and creatine phosphokinase, had higher activity per unit muscle mass in the corticosterone-treated group.

Chin, Love and their colleagues have shown that fledglings exposed to maternal stress hormone during embryonic development have improved flight performance due to differences in wing size and muscle tissue, adding maternal effects to the role call of phenotype fine-tuning for future consideration.

10.1242/jeb.021618

Chin, E. H., Love, O. P., Verspoor, J. J., Williams, T. D., Rowley, K. and Burness, G. (2009). Juveniles exposed to embryonic corticosterone have enhanced flight performance. *Proc. R. Soc. B* **276**, 499-505.

Charles Darveau
University of Ottawa
cdarveau@uottawa.ca

HYPOXIA TOLERANCE

DOR-MANT BRAINS MAINTAIN ION BALANCE

Delta-opioid receptors (DORs) in the brain normally bind painkilling neurochemicals called enkephalins as well as opiates like morphine. Recent research has shown that these receptors are also protective when oxygen or blood flow to neurons is reduced. The protection occurs in part by reducing inflammation, but other effects are likely. One possibility is reducing ion flow across the cell membrane. Ion leak is a critical step in hypoxic damage to neurons, leading to depolarization, the toxic release of neurotransmitters, and cell death. Potassium leaks out of the cell while sodium and calcium flow in, thus all three ions are potential therapeutic targets for neurological disorders. Ying Xia's group at the Yale University School of Medicine recently demonstrated that protection by DORs occurs by inhibiting the outflow of potassium ions, and this inhibition was linked to the suppression of calcium movement. But potassium efflux is associated with sodium as well as calcium influx, so the investigators set out to test whether DORs also protect against potassium imbalance in mammalian neurons by inhibiting sodium influx.

They utilized brain slices from the mouse cortex and exposed them to decreasing sodium levels, replacing it either with a compound that cannot cross into the cells or with lithium ions that cross into the cell like sodium. They then exposed the slices to anoxia (no oxygen), which normally triggers a massive potassium efflux within minutes.

The investigators first showed that potassium efflux from neurons is linked to sodium influx. When sodium levels were decreased around the brain slices by replacement with a membrane impermeable substitute, it took longer for potassium ions to leak out of the cell during anoxia, and fewer ions in total crossed the membrane. By contrast, lowering external sodium levels using sodium-like lithium ions caused the oxygen-deprived neurons to lose greater levels of potassium even more rapidly than usual. They concluded that the loss of potassium ions from anoxic neurons is indeed linked to the inward flow of sodium ions.

The group then investigated whether the protection of anoxic neurons by DORs could occur by a similar mechanism, by decreasing the intracellular effects of sodium ions. If DOR activation decreases potassium efflux by inhibiting sodium influx, then low sodium levels should abrogate the protective effect of DORs. And indeed, receptor activation under low sodium conditions had no effect on potassium and DOR protection was lost. DOR activation, then, may protect mammalian neurons by decreasing sodium as well as calcium influx, thereby slowing the catastrophic loss of potassium.

Interestingly, one of the most anoxia-tolerant of vertebrates, the freshwater turtle, has very high levels of these receptors compared with mammals. In anoxia, turtles are also known to reduce ion flux through calcium, potassium and sodium channels. A recent article in JEB by Matthew Pamenter and Leslie Buck (*J. Exp. Biol*. **211**, 3512-3517) showed that the inhibition of calcium channels during anoxia was linked to DOR activation; it would be of interest to see if changes in sodium and potassium ion flux are also linked to these receptors as part of the turtles' ability to withstand long periods without oxygen.

10.1242/jeb.021576

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Sarah Milton
Florida Atlantic University
smilton@fau.edu