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Inside JEB

LATERAL LINE HELPS FISH DETERMINE SOUND DIRECTION

It's almost impossible to creep up on some animals, such as goldfish, that streak for safety when alarmed. Donald Faber from the Albert Einstein College of Medicine, USA, explains that the fish curl into a tight C shape and zip off in the opposite direction from a threatening sound. The big question was how do fish tell which direction the sound is approaching from to orchestrate the response. According to Faber, fish cannot use the time difference between a sound arriving at both ears to identify the direction, because they are transparent to sound waves and the sound arrives at both ears at the same time. However, he had a hunch that the fish's lateral line – a line of vibration sensors running along the fish's side – may help them to determine the origin of a threatening sound.

Mana Mirjany, Thomas Preuss and Faber designed their experiments to test this idea by taking advantage of the fish's natural behaviour (p. 3358). After inactivating the lateral line of a goldfish with cobalt chloride, Mirjany released the fish to swim naturally in a large circular tank equipped with two loudspeakers. Playing alarming sounds from each of the loudspeakers at random intervals, the team filmed the startled fish's reactions. Repeating the experiment using other techniques to inactivate the lateral line and also blindfolding the fish with custom-made eye caps, Mirjany then painstakingly analysed the fish's escape behaviour when it happened to be in open water and away from the tank sides.

Not surprisingly, the fish turned and fled, regardless of which sense they were deprived of: 'The auditory system is sufficient to trigger an escape,' says Mirjany.

Yet, the lateral line was essential for the fish to figure out which direction the threat was coming from, as the fish that had lost their lateral lines fled in random directions whereas the blindfolded fish (with an intact lateral line) successfully headed in the opposite direction from the threat. Yet, when Mirjany detached the body portion of the fish's lateral line from the nervous system, the fish were still able to escape in the opposite direction. So it was the anterior (head) section of the lateral line that was essential for the fish to locate the source of the scary sound and escape correctly.

However, Faber explains that the fish's startle response becomes more complex when the animals are close to an object that could block their escape. Instead, they override the startle response and turn towards the threat in order to avoid colliding with the obstacle. The team decided to find out how the lateral line affected the fish's escape response when near an obstruction.

Analysing the fish's reactions when they happened to be near the tank wall, the team realised that the lateral line and visual systems were both playing crucial roles in determining the direction that the escaping fish chose. The animals successfully overrode their standard escape response when their lateral line was inactivated and, when Mirjany repeated the experiment with blindfolded fish, they too successfully overrode the escape response. It was only when Mirjany blindfolded fish with inactivated lateral lines that the fish lost the ability to override the reflex. 'There is either compensation between the visual and lateral line systems or there could be integration of the two, but it is difficult to say which at the moment,' says Mirjany.

Having confirmed that goldfish use the lateral line to pin down the direction of a sound, Mirjany and Faber decided to determine how signals from the anterior portion of the lateral line contribute to the fish's escape response. Explaining that the tightly choreographed escape sequence is coordinated by a single neuron – the Mauthner cell (M-cell), which integrates all of the fish's sensory inputs and determines which direction the fish should flee – Faber and Mirjany decided to analyse the nerve input from the lateral line to the M-cell (p. 3368).

Stimulating the lateral line nerves electrically and recording the M-cell responses at various sites along the neuron, Mirjany found that the M-cell responded within 1 ms. 'There is only about 3–4 ms from the time the stimulus occurs to the time that one of the Mauthner cells fires an action potential to trigger the response,' explains Faber, so the M-cell response to the lateral line input was fast enough to control the fish's swift reaction. Also, the lateral line input was close to the M-cell cell body, allowing the coordinating cell to integrate the lateral line inputs with inputs from other sensory systems further out along the M-cell dendrites, to determine which escape strategy is best for each individual situation.

10.1242/jeb.065698

Mirjany, M. and Faber, D. S. (2011). Characteristics of the anterior lateral line nerve input to the Mauthner cell. *J. Exp. Biol.* **214**, 3368-3377.
Mirjany, M., Preuss, T. and Faber, D. S. (2011). Role of the lateral line mechanosensory system in directionality of goldfish auditory evoked escape response. *J. Exp. Biol.* **214**, 3358-3367.

Kathryn Knight

KOALAS' BELLOWS BOAST ABOUT SIZE

Koalas have a well-earned reputation for being dopey. Sleeping 19 h out of every 24 h, and feeding for 3 h of the remaining 5 h, there doesn't seem to be much time for anything else in their lethargic lifestyle: that is until the mating season. Then the males begin bellowing. Benjamin Charlton from the University of Vienna, Austria, explains that they probably bellow to attract females and to intimidate other males. But what messages could these rumbling bellows communicate about their senders (p. 3414)?

According to Charlton, they could be telling nearby listeners about their size. He explains that there was a possibility that koalas may be one of the few animals that have a descended larynx, which makes the vocal tract longer. Also, because all pipes – including vocal tracts – have frequencies where the air inside them vibrates naturally and amplifies sound, larger animals with longer vocal tracts produce lower resonances, giving their voices a more baritone quality. So, the longer vocal tracts of the largest koalas should produce deeper resonances to tell the listening koala audience just how big they are. Intrigued, Charlton, Tecumseh Fitch and their colleagues decided to find out whether male koalas have descended larynxes.

Teaming up with Allan McKinnon at Moggill Koala Hospital and Gary Cowin and William Ellis at the University of Queensland, Australia, Charlton investigated the anatomy of the marsupial's vocal tract. Using MRI and post-mortem studies, the team found that the koala's larynx had descended to the level of the 3rd and 4th cervical vertebrae, instead of being high in the throat. They were also surprised to find that the muscle that attaches the larynx to the sternum was anchored very deep in the thorax and they suggest that it could be involved in pulling the larynx even further down into the chest cavity.

But what effect does the koala's deeply descended larynx have on the acoustics of their bellows? Travelling to the Lone Pine Koala Sanctuary, home to 140 koalas, Charlton patiently recorded their rumbling bellows. He also measured the animals' head sizes, with the help of Jacqui Brumm and Karen Nilsson, as skull size is a good proxy for body size.

Back in the lab, Charlton analysed the bellows' spectra and found that the largest males always had lower resonances than the smaller animals. More surprisingly, when Charlton calculated the koala's vocal tract length based on their acoustics, he was astonished to find that the koalas were able to make themselves sound as if they had 50-cm-long vocal tracts, nearly the entire length of the animal. In fact, the diminutive animals sound even larger than bison. Charlton suspects that koalas use the resonances of the oral and nasal tracts simultaneously to sound much larger than they are.

So, koala males are able to communicate their size, with the largest animals producing the richest baritone bellows. Charlton also suspects that the males' boastful bellows could have driven the evolution of their descended larynxes. 'Individuals that could elongate their vocal tracts by lowering the larynx may have gained advantages during sexual competition by sounding larger, and this would drive the evolution of laryngeal descent,' he says.

10.1242/jeb.065706

Charlton, B. D., Ellis, W. A. H., McKinnon, A. J., Cowin, G. J., Brumm, J., Nilsson, K. and Fitch, W. T. (2011). Cues to body size in the formant spacing of male koala (*Phascolarctos cinereus*) bellows: honesty in an exaggerated trait. *J. Exp. Biol.* **214**, 3414-3422.

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MOSQUITOES SELECTIVE ABOUT PLUMES THEY FOLLOW

Listening to a mosquito's whining buzz can be an unnerving experience. At best you'll end up with an itchy bite, but in some parts of the world it could prove fatal. Mosquito traps offer some protection, luring the insects with plumes of attractive odours. However, when Teun Dekker and his colleagues tested how yellow fever mosquitoes responded to odour plumes with different widths and structures in 2001, they were astonished to see that although the insects were extremely attracted to continuous plumes of human odour, they completely ignored continuous plumes of CO_2. This was surprising because a sniff of CO_2 is usually one of the most attractive odours a mosquito can encounter. 'There was something wrong with the manner in which we presented these odours,' recalls Dekker. Wondering how the structure of the odour plume might affect the insect's behaviour, Dekker and Ring Cardé from the University of California, Riverside, USA, decided to find out which aspects of odour plumes yellow fever mosquitoes ignore and which features are irresistible (p. 3480).

'We built a wind tunnel with good laminar flow so we could predict where the plume was and where the mosquito was with respect to the plume and then analyse the behavioural patterns,' explains Dekker.

Releasing four ravenous females into the wind tunnel with a ribbon-thin plume of CO_2, Dekker filmed the insects' flight paths and was pleased to see that as soon as an insect encountered the odour, it switched direction and began heading upwind. Next, Dekker reduced the concentration of CO_2 in the ribbon plume and was surprised to see that the mosquitoes reacted even when the CO_2 concentration was barely above atmospheric levels.

However, when Dekker tested the mosquitoes' responses to a thin filament of the odour from his own forearm, he was surprised to see that the insects were oblivious to it. They just flew straight through without flinching.

Next he designed broader uninterrupted plumes and found that the mosquitoes were attracted to both odours, flying upwind in the human odour plume while flying in and out along the edge of the CO_2 plume. Dekker also disturbed the two plumes to produce intermittent turbulent puffs of each odour and found that the mosquitoes were most responsive to these intermittent plumes, turning into the flow and heading upwind.

Having discovered that yellow fever mosquitoes are oblivious to thin plumes of skin odour, Dekker suspects that the 50 ms encounter with the smell was insufficient to allow them to process the information and steer upwind. However, he explains that the insect's CO_2 receptors directly control their behaviour, allowing to respond almost instantly to even the faintest of whiffs.

He also adds that it makes sense for mosquitoes to respond strongly to fluctuating CO_2 because it indicates the presence of a live host, unlike skin odour that can linger after the victim has departed. However, mosquitoes do not follow CO_2 trails slavishly to the source – otherwise they'd fly into your mouth. He suggests that they lock on initially to a fluctuating CO_2 trail before switching to follow a broad skin odour plume to home in on lunch.

10.1242/jeb.065680

Dekker, T. and Cardé, R. T. (2011). Moment-to-moment flight manoeuvres of the female yellow fever mosquito (*Aedes aegypti* L.) in response to plumes of carbon dioxide and human skin odour. *J. Exp. Biol.* **214**, 3480-3494.

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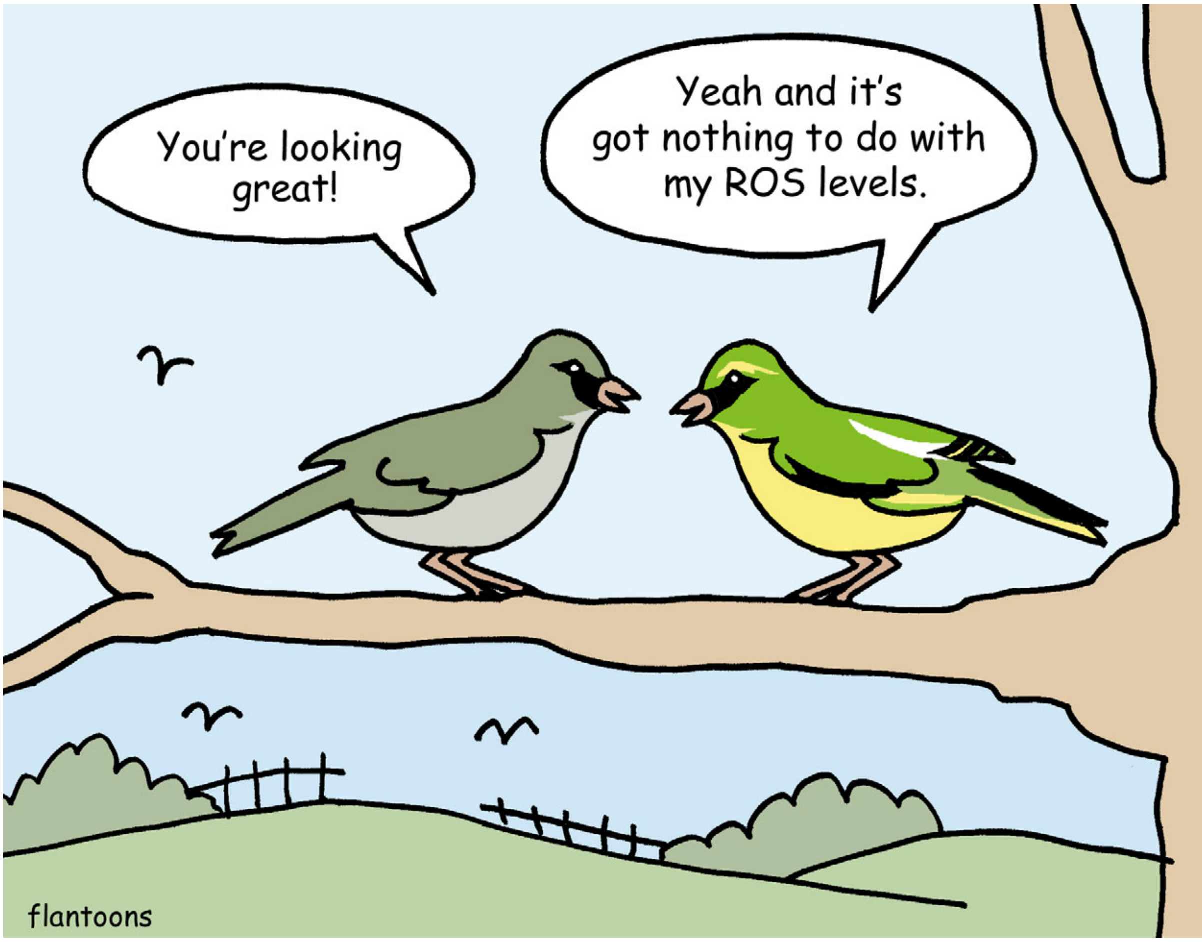
NO CAROTENOID LINK FOR GREENFINCHES



With their distinctive green-yellow plumage, greenfinch males are not bashful when boasting about their fitness. Elin Sild and colleagues from Tartu University, Estonia, explain that the bird's vivid colour is produced by antioxidant carotenoid pigments, which can only be incorporated into their plumage if not required for other purposes – such as reducing the levels of damaging reactive oxygen species (ROS) circulating in their blood. However, the team explains that this simple relationship has recently come under debate, as the birds' phagocytic cells (part of the immune system) also produce ROS to combat bacterial infections. In this case, the high carotenoid levels indicate by bright plumage could counteract the immune system. Evidence supporting both sides of the argument has been accumulating so, the team decided to test how carotenoids in the birds' diet affected ROS levels in the birds' blood (p. 3467).

Collecting adult male birds from a garden in Tartu, the team supplemented the birds' diets with two carotenoids, monitored their blood carotenoid levels and found that the carotenoid levels increased by 40–53% compared with birds that had not received the supplement. Next, knowing that the bird's immune systems produce ROS in response to bacteria infections, the team tested the effects of a simulated infection on the amount of carotenoids in the birds' blood and on their ROS levels. Injecting the birds with lipopolysaccharide (a component of bacterial cell walls), the team found that the bird's carotenoid levels fell. However, when they injected the birds with dead *Brucella abortus* bacteria and measured the amount of ROS produced by the birds' phagocytic cells in response to the fake infection, the team found no change in ROS production by the phagocytic cells. So the carotenoids did not counteract the production of ROS by the birds' immune systems, suggesting that carotenoids do not provide the link between bright plumage and ROS production during the immune response in greenfinches.

10.1242/jeb.065714

Sild, E., Sepp, T., Männiste, M. and Hõrak, P. (2011). Carotenoid intake does not affect immune-stimulated oxidative burst in greenfinches. *J. Exp. Biol.* **214**, 3467-3473.

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