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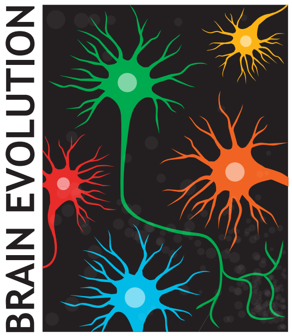
OUTSIDE JEB

Listening to Bach lights up crocodile brains



In a ground-breaking first, researchers have measured brain activity in live crocodiles to understand how brains evolved to process sights and sounds. As crocodiles are a recent ancestor of birds and, more distantly, mammals, seeing how far back in evolutionary time brain circuits originate in animals provides a clue for when brains first started operating as they do in vertebrates. Although many animals share brain molecules and cell types, the real test of brain conservation is how similarly they function across species. For example, despite mammal and bird brains being hugely different in appearance, both are similarly wired, resulting in them perceiving their environment in analogous ways, namely in a hierarchical fashion.

Hierarchical processing starts with simple sensory information (e.g. tones, noises) being initially perceived in 'earlier' brain regions, whereas complex sounds (e.g. music, language) solely activate downstream, 'higher-order' brain regions. Hierarchical processing is present in birds and mammals, but until recently it was unclear at which point the brain pathways were gained. Enter the crocodile, stage left. Crocodiles are the closest relatives to birds and haven't shared a common ancestor with mammals for nearly 300 million years. If crocodiles, birds and mammals share similar brain regions important for processing sights and sounds, it would suggest that these brain circuits were established early on in evolutionary time and were passed down to all subsequent species. In an extraordinary new paper in the journal *Proceedings of the Royal Society B*, Mehdi Behroozi from Ruhr University Bochum, Germany, and Brendon Billings from the University of the Witwatersrand, South Africa, recorded brain activity from mildly sedated crocodiles while the animals listened to tunes and watched a laser-light show, and the results hint that these brain regions may have emerged before birds and mammals went their separate evolutionary ways.

After ironing out a number of technical kinks, the international team of researchers led by Felix Ströckens carefully placed juvenile Nile crocodiles into a brain scanner in order to measure brain activity. Once the crocs were relaxed, the researchers presented a brief flash of red or green lights twinkling at different speeds while Behroozi and Billings watched how their brain responded. Overall, the lights excited two suspected visual areas, including one 'higher-order' region that responded more to red lights, suggesting subtle selectivity. These results are the first to show visually triggered brain areas in crocodiles; however, there was a lack of clear evidence for hierarchal processing as observed in other animals or senses, such as hearing.

In order to expand the diversity of senses tested, the team then broadcasted several sounds to the crocodiles in a separate part of the study. The crocs listened to two different simple sounds (random chord noises at around 1000 or 3000 Hz), as well as one complex sound: a snippet from Johann Sebastian Bach's Brandenburg Concerto No. 4. Although 'lower' auditory brain areas responded to all three of the sounds, a 'higher-order' brain area responded solely to the symphony in a similar region found in birds and mammals that is similarly selective for complex sounds such as music and birdsong.

Taken together, these findings elevate crocodiles' status from fear-inducing, cold-blooded, modern-day dinosaurs to like-minded, informative and capable animals that could teach us about brain function and evolution through non-invasive methods. Crocodiles may have split from the mammalian evolutionary tree nearly 300 million years ago, but their brains appear to be more in sync with those of birds, and possibly humans, than previously thought.

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Fly breath sets mites up for success

For parasites to thrive, they must first locate and then derive resources from a host. Some parasites can use chemical cues – such as CO_2 released through respiration – to find hosts, and when multiple hosts live in the same area, parasites often preferentially infest certain individuals (or certain hosts are more resistant to parasite infestation). There are several characteristics that can make hosts more preferable for (or, alternatively, more vulnerable to) infestation by parasites, including specific behaviours, poor overall health and being injured. However, whether parasites might be able to select hosts based on their metabolic rates – and, if so, whether they might select hosts with high metabolic rates (which would produce resources for the parasites at high rates) – remained

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unknown. Because many parasites can detect CO_2 produced by hosts and because CO_2 production increases with metabolic rate, Collin Horn and two colleagues from the University of Alberta in Canada wondered whether parasitic mites might be able choose specific fruit fly hosts based on their relative CO_2 production. If so, they also wondered whether higher metabolic rates in injured flies might underlie the mites' documented preference for hurt hosts.

To answer their first question, the researchers first determined the resting metabolic rate of individual flies by measuring each one's CO_2 production in a tiny respirometer. Then, they placed a parasitic mite at the base of a Y-connector and immobilized two fruit flies with different metabolic rates at the other end of the connector – one fly in each arm – creating a two-choice tunnel for the mite. After an hour, the group inspected the flies under a microscope to see which fly the mite had chosen. Horn and his colleagues found that the mites did indeed tend to infest the fly with the higher metabolic rate, suggesting that these parasites can detect not only the presence of CO_2, but also small differences in CO_2 concentration.

To determine whether mites might be attracted to injured flies because of increases in the flies' metabolic rates during healing, Horn and his colleagues measured the metabolic rates of flies that had been punctured with a pin. Unexpectedly, they found that metabolic rate did not change in injured flies, indicating that mites must use another cue to detect injured hosts.

To further determine what might attract mites to wounded flies, the scientists placed either haemolymph (equivalent to fruit fly blood) or water on the bodies of uninjured flies. They then allowed mites to choose between haemolymph- and water-treated flies in the same Y-connector setup used in the first experiment. The researchers found that the mites preferentially infested flies that had received a drop of haemolymph, suggesting that these parasites rely on chemical cues from the injury itself to find vulnerable hosts.

Overall, Horn and the other researchers discovered that this common parasite of fruit flies is able to use both exhaled and 'leaked' chemicals to pick the perfect host. Their results also suggested that the mites can discriminate tiny differences in odours produced by hosts and select the victims that will produce the resources that they require most rapidly via higher metabolic rates. If fruit flies think they're going to escape these bugs, they shouldn't hold their breath – or maybe they should!

10.1242/jeb.170100

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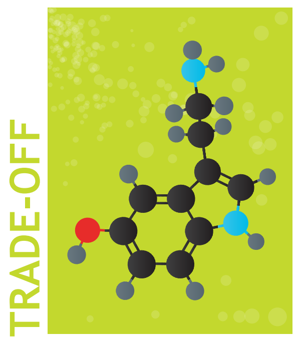
Cavefish compromise colour for catecholamine-kick



We're all the same colour with the lights off. This sentiment is literal for many animals that live in constant darkness because the loss of the skin pigment, melanin, is commonplace. For example, certain populations of the Mexican tetra fish, *Astyanax mexicanus*, live in isolated underground caves that never see the light of day and have evolved to have little to no pigment in a very short time. But other populations of *A. mexicanus* living in sun-lit surface pools still produce normal amounts of melanin. In fact, albinism is an extremely rare condition because it leads to a host of health complications beyond a sunburn. There must be some advantage, then, to shutting off melanin synthesis in a lightless world. One intriguing explanation is the melanin–catecholamine trade-off hypothesis. Catecholamines are neurotransmitters that play a key role in brain function. Because both of these molecules are synthesized from the same amino acid, L-tyrosine, less melanin production leaves more L-tyrosine available for catecholamine synthesis. But why are cavefish willing to sacrifice melanin for a boost in catecholamines? That's what a group of researchers headed by William Jeffery from the University of Maryland, USA, wanted to find out.

Jeffery and his colleagues had a hunch that if cavefish brains had more of the catecholamine noradrenaline (norepinephrine), it might help the fish stay alert longer to seek out food and mates, both of which are in scarce supply deep underground. Noradrenaline is a stimulating neurotransmitter best known for heightening an animal's senses during stress. Jeffery already knew that it takes a lot longer for an albino laboratory mouse to succumb to anaesthesia than a regular mouse, and he faced similar challenges with his albino cavefish compared with the fully pigmented surface fish. So, the researchers used the length of time it takes a fish to reach unconsciousness under anaesthetic as a proxy for alertness and set about testing the trade-off hypothesis.

The team began by measuring noradrenaline in *A. mexicanus* brains and confirmed that albino cavefish brains do have more of this catecholamine than the brains of surface fish, as predicted by the trade-off hypothesis. When the team gave albino cavefish a drug to block noradrenaline receptors, they found that the cavefish's tolerance for anaesthesia decreased, meaning the cavefish struggled to stay alert without noradrenaline signalling in the brain. In contrast, when the team supplemented the pigmented surface fish with an extra dose of noradrenaline, these fish resisted the anaesthetic longer, becoming more like their albino relatives.

Jeffery's team now needed to link decreased melanin production with their observations of heightened alertness in noradrenaline-loaded brains. Albinism is caused by a mutation in a gene called *oca2*, which encodes a protein that regulates L-tyrosine delivery to melanin-producing cells. So, the scientists used a molecular tool to knockout the *oca2* gene in surface fish, making them albino. When the researchers presented these mutated surface fish with anaesthetic, they found that it took these fish a long time to reach unconsciousness, just like in the naturally albino cavefish.

So Jeffery's group has confirmed that decreasing melanin synthesis in cavefish enables the fish's brain to make more noradrenaline from L-tyrosine, and this

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additional noradrenaline helps them fight off drowsiness, a great advantage for cave life. Unfortunately, we surface dwellers will have to settle for a double espresso to eke out a few more hours from the day!

10.1242/jeb.170092

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Whisper in the dark

In 1938, a young student from Harvard University solved a mystery that was first described in the 18th century, when zoologists discovered that bats with experimentally blocked ears crash into objects, whereas blinded bats can navigate just fine. The student, Donald Griffin, found out what is general knowledge today: bats echolocate to navigate, i.e. they send out sound waves and use the echo that is produced when the sound waves hit an object to find obstacles in their flight path. But it appears that we still do not know everything about bat navigation. A recent study suggests that bats 'whisper' or even completely switch off echolocation under certain circumstances and fly 'blind', which could explain the high percentage of bats killed by wind turbines.

Aaron Corcoran from the Wake Forest University, USA, and Theodore Weller from the USDA Forest Service investigated the use of echolocation in hoary bats, the species that is killed most frequently by wind turbines in the USA. The researchers performed a series of three experiments recording bat calls and their flight paths: (1) under natural conditions, (2) in response to an introduced obstacle and (3) in response to echolocation playbacks. Previous field recordings had suggested that bats might not always use echolocation, but these observations had been challenged because it was not clear whether the bats were always in the range of the microphones. Therefore, the researchers of the current study used a combination of time-synchronised ultrasound microphones and infrared cameras. The cameras captured the exact flight path of the bats and, together with the known position and the recording direction of the microphones, they were able to determine the exact location that each recorded call was made from.

Surprisingly, Corcoran and Weller only recorded examples of previously known hoary bat echolocation calls in 8% of the recorded flights and the bats flew about half of the time without any recordable echolocation. Furthermore, the scientists detected a previously unknown call type that the bats used in the remaining 43% of recorded flights. The call, which they termed a 'micro' call, was shorter and had a much lower sound pressure level (volume) than known echolocation calls. Importantly, the low pressure level drastically reduces the detection range, making the call ineffective for flight navigation at high speed or insect detection. And, in the cases in which the bats flew into the mist-net that the researchers deployed as an obstacle, they were either using the micro calls or no calls at all when approaching the net and echolocation was only used shortly before the collision occurred – too late to avoid being caught.

As the study took place during the hoary bat mating season and the researchers recorded the micro calls when the bats were approaching members of their own species or when flying towards a speaker that was playing bat calls, the researchers concluded that bats might produce micro calls to avoid other bats eavesdropping on their conversations during the mating season. Although micro calls would still allow bats to detect trees from a distance of 7.5 m, which is early enough to avoid a collision, the situation might be different, unfortunately, when it comes to rotor blades moving at high speed, and might partly explain the high number of hoary bats killed by wind turbines in the autumn breeding season.

10.1242/jeb.170076

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