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OUTSIDE JEB

Doped to the bills – dopamine helps birds sing in tune

Singing karaoke makes most of us quickly realize how poorly we can carry a tune. Typically, when we're confident enough to sing on stage, we're deaf to the negative feedback. Recently, however, neuroscientist Jesse Goldberg at Cornell University, USA, discovered a brain region that listens and produces a chemical 'booing' when birds sing off key. Vikram Gadagkar and colleagues in the Goldberg lab report that a special group of dopamine neurones in bird brains critically evaluate and encourage when birds chirp well.

Like karaoke, birdsong sounds great when performed well. Learning to hit the right notes is hard work, though. To sing properly, songbirds must first memorize a model tweet early in life, continue to repeat and refine their imitation of that song during development, and eventually master the model song reproduction. Practice makes pitch perfect, but how the brain rewards good singing and discourages mangled squawks is unclear. One thought is that dopamine, a chemical messenger important for reward and reinforcement in the brain, may play a role.

To test whether dopamine neurones provide neural feedback on song production, Gadagkar made birds bungle their song. Placing male zebra finches (*Taeniopygia guttata*) in acoustic isolation chambers equipped with a microphone and speakers, Gadagkar set up a program that automatically detected and disrupted a single note in a bird's song. When the finches belted out the target note, a discordant tone was played over their own rendition, making the birds believe they had tweeted off key. All the while, Gadagkar monitored their activity through delicately implanted recording wires in the ventral tegmental area (VTA), a midbrain dopamine region.

Gadagkar soon discovered that a subset of VTA neurones were keenly aware of the birds' synthetic song errors. The VTA 'error neurones' quickly shut down brain activity whenever the birds perceived that they had sung a note incorrectly. Even more remarkably, when the same target note was uninterrupted, the VTA error cells 'rewarded' the correct production and rapidly increased firing during the note's production. Taken together, VTA error neurones appear to reward birds by bathing the brain in dopamine when they hit the right note. What remained to be seen was whether VTA error neurones tracked how often birds performed correctly – did VTA error neurones 'cheer louder' (more activity) when a note was accurately sung occasionally versus frequently?

In follow-up experiments, Gadagkar assessed whether VTA error neurones produced graded 'feedback' in proportion to how often birds sang a note correctly by interrupting two different notes in the bird's song with different probabilities. Birds perceived they sang one note incorrectly 50% of the time (note A), whereas they perceived singing a different note wrong only 20% of the time (note B). Singing either note incorrectly suppressed VTA firing to a similar degree, irrespective of how often it was mispronounced. However, when the notes were sung correctly, tweeting note A ramped up neural activity three times higher than when birds sang note B. Therefore, when a bird correctly utters a note it struggles with more often (note A), VTA error neurones provide more neural reinforcement to encourage the accurate imitation.

Taken together, Gadagkar and colleagues' findings demonstrate an error-responsive group of neurones that dishes out dopamine activity as a reward when birds sing well. While everyone is their own worst critic, it turns out the VTA is the brain's harshest audience but most supportive instructor.

10.1242/jeb.147355

Gadagkar, V., Puzerey, P. A., Chen, R., Baird-Daniel, E., Farhang, A. R. and Goldberg, J. H. (2016). Dopamine neurons encode performance error in singing birds. *Science* **354**, 1278-1282.

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Turtle shells have built-in shock absorbers

Many organisms evolved natural armour to protect themselves from threats, such as predators. A common strategy is to layer the armour with materials possessing different functions in structures known as 'hierarchical bio-composites' that serve a wider range of purposes than an individual material. While many invertebrate skeletons and seashells have a hard exterior to protect a soft interior, the chink in this armour design is that the harder outer layers can also be brittle, making them more prone to damage if an impact is too intense. Turtles, armadillos and alligators take an alternative approach; their body armour is composed of hard internal layers covered by a bi-layer of soft materials (keratin and collagen), but what benefits could such a soft-coated armour provide? Yaniv Shelef and Benny Bar-On from Ben-Gurion University, Israel, sought to answer this question by studying the mechanical properties and engineering of turtle shells.

Although cushioned armour may sound like a bad idea, the benefit of this strategy is

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that the softer, outer bi-layer serves as a shock absorber that minimises damage to the internal layers. Shelef and Bar-On wanted to understand how the soft bi-layer produces these shock-absorbing properties. They studied the upper portion of the shell (the carapace) in red-eared slider turtles (*Trachemys scripta elegans*), which is composed of a deep bony layer covered by soft skin containing collagen towards the middle (the dermis) and keratin on the surface (the epidermis). They used mechanical testing equipment that made small dents in the surface to quantify stiffness and hardness, and then built a digital model of the turtle shell to simulate how these tissue layers were affected by hydration, the sharpness of the object used to dent the surface and changes to the tissues' stiffness and hardness. These simulations were done using finite element analysis, which quantified the stresses and strains (deformations) that the shells would experience during an assault or impact.

The authors found that the skin protected the underlying bone by absorbing the energy from impacts and localising damage to the surface. In car terms, the keratin layer acts as a bumper that crushes as it absorbs most of the impact energy and then the collagen layer acts as an air bag that compresses to further buffer damage to the inner regions. This 'bumper-buffer' function decreases stress to the bones by 50% and persists regardless of how sharp the object impaling the tissues is and whether the tissues are wet or dry. The multifunctional skin of the shell also produces different responses depending upon the type of impact. For instance, the damage to the surface of the keratin layer was greatest when the impact object was sharper and the collagen layer sustained the greatest damage when the impact object was blunter. Overall, the individual layers of the skin exhibited unique functions that, together, could protect the turtle from a range of attacks.

This study of the resilience of turtle shells identifies the benefits of soft-coated armour and demonstrates how the coordinated functions of biomaterials can produce diverse defence mechanisms; so the secret of turtle shell strength may only be skin deep.

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Shelef, Y. and Bar-On, B (2017). Surface protection in bio-shields via a functional soft skin layer: lessons from the turtle shell. *J. Mech. Behav. Biomed. Mater.* http://dx.doi.org/10.1016/j.jmbbm.2017.01.019

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Blubber news: whale fat is active!

In the world of physiology, scientists use model organisms, such as fish, rats and mice, to study how the complex relationship between the body and its environment influences behaviour. Yet, few have the courage to tackle the challenges of working with the largest mammals on Earth. Undeterred, Hope Ball from University of Akron, USA, and her collaborators from several US institutions decided to investigate how bowhead whales (*Balaena mysticetus*) and belugas (*Delphinapterus leucas*) tackle the physical challenges of survival in the extreme environment of the Arctic Ocean and their lengthy migrations.

Whales have a unique layer of tissue under their skin called blubber, which provides buoyancy and thermal insulation in cold waters in addition to allowing them to store large amounts of fat to fuel migration while fasting. Other animals that undergo long periods of fasting also possess a specialized protein hormone, called leptin, that varies seasonally to suppress appetite and stimulate fat breakdown when the animals have stopped feeding. While whales are known to produce leptin, little was known about production of the hormone and how the levels vary in these animals.

Ball and her colleagues partnered with residents of several Inupiat settlements during their annual subsistence hunt, to collect blubber and tissue samples from bowhead and beluga juveniles and adults during the autumn migration and in the spring. In samples collected from the whales in the autumn – following an intense summer feeding period – the authors reported that leptin gene expression was approximately 10- to 50-fold higher than in the spring whales, suggesting that extreme seasonal variation in leptin plays a role in regulating fat breakdown during fasting periods. The authors also reported that the largest amount of leptin was found in the deepest layer of blubber, where fat is actively broken down for energy production when food is unavailable. This suggests that leptin levels, in addition to seasonal cues, may drive the whales' migration to and from summer feeding grounds.

To determine the influence of season on fat utilization, the authors analysed the activity of the enzymes that break down fat. They found that in the autumn, when the whales were well fed, the blubber was composed of large fat cells and the activity of these enzymes was low. However, in winter, following migration from cold waters back to summer feeding grounds, the blubber contained smaller fat cells and more connective tissue, and the activity of the enzymes that break down fat was high. This suggests that the whales break down fat to maintain the energy demands of the body when they migrate. In addition, Ball and colleagues reported low levels of leptin in juveniles, which were similar to those found in the spring fasting adults, suggesting that the metabolism of the young whales is low to minimize energy loss and it is directed at stimulating appetite to ensure maturation.

It appears that these extreme seasonal and life stage variations in leptin levels are unique adaptations that Arctic whales have evolved to drive fat utilization, feeding behaviour and initiation of migration in cold waters. In whales, leptin controls appetite and fat breakdown, thus making blubber an active player in a whale's physiology.

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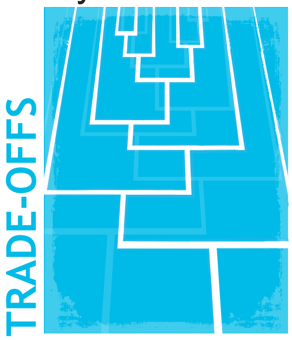
Salmon pay a price for costly carotenoids



If you were to catch a Chinook salmon, it might be red or it might be silver-white and this is because red Chinook salmon take up pigments called carotenoids. Carotenoids come with benefits in the animal kingdom: they can enhance how attractive colourful signals are to mates and even help boost the immune system. Time and time again, carotenoids have been shown to be beneficial for animal fitness. How is it, then, that the white Chinook salmon, which have little to no carotenoids, persist in the wild?

This question engaged Sarah Lehnert and her colleagues from the University of Windsor and the Department of Fisheries and Oceans, Canada, who wanted to know whether there was a hidden cost associated with carotenoids making fish bright and rosy red. The two colour variants in Chinook salmon are present throughout the entire life cycle; the red salmon produce vivid red eggs while the white salmon eggs are uncoloured. Salmon eggs are particularly susceptible to predation because salmon parents do not care for their offspring after spawning. Might the red eggs from the carotenoid-rich red salmon be more vulnerable to predation than the colourless eggs of the carotenoid-deficient white salmon?

Lehnert's research group decided to test whether white Chinook salmon eggs enjoyed a survival benefit over their red counterparts when faced with predators. As rainbow trout naturally prey on salmon fish eggs, the team simultaneously released white and red Chinook salmon eggs into tanks full of hungry trout. They then monitored how many of the eggs were snapped up by the famished fish. The researchers found that the red eggs were more likely to be eaten first by the trout. In fact, the red eggs were twice as vulnerable as the white eggs and they were consumed much faster.

The vulnerability of the red eggs to predatory fish may therefore allow the white Chinook salmon to persist in the population, despite the fact that red fish are likely to benefit from being carotenoid rich. Before the team's study, researchers knew very little about how the two colours of Chinook salmon were maintained in the population. Their findings show that white salmon have a knack for surviving early in life, as flashy colourful carotenoids are in fact quite costly for Chinook salmon eggs.

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