

## OUTSIDE JEB

## This is Cerebrospinal Tap



**MECHANOSENSATION**

Of all the fluids produced by the human body, the cerebrospinal fluid (CSF) may be the least offensive. A clear, unassuming liquid, CSF circulates throughout the ventricular system of the brain and the central canal of the spinal cord, cushioning the nervous system from injury and rinsing away metabolic waste. The composition of CSF is very similar to blood, but lacks proteins. If you were to taste CSF, it would be salty and a little bit sweet, like a mix of Gatorade and seawater. And unlike blood (or feces), a cup of CSF would probably not make you feel afraid or disgusted.

But despite its prosaic reputation, CSF has some interesting quirks. One of them involves its relationship to the nervous system. Nearly a century ago, two neuroanatomists, W. Kolmer and E. Agdhur, discovered a population of neurons that innervate the central canal of the spinal cord, and make direct contact with the CSF. These CSF-contacting neurons, or CSF-cNs, appear to be ubiquitous among vertebrates, as Kolmer and Agdhur found them in over 200 species.

CSF-cNs are peculiar-looking neurons. Each cell body possesses a bushy protuberance, which extends into the central canal. This protuberance includes a primary cilium, which is characteristic of other sensory neurons, such as olfactory receptors and auditory hair cells. Thus, it has been proposed that CSF-cNs may be sensory neurons that monitor the chemical or mechanical properties of the CSF. There is also evidence that CSF-cNs can affect locomotion by inhibiting premotor interneurons in the spinal cord.

However, the specific sensory properties of CSF-cNs have remained elusive.

Now, a recent study from Claire Wyart's lab at the Institut du Cerveau et de la Moelle épinière, Paris, has explored the mechanosensory function of CSF-cNs in larval zebrafish. Using genetic tools for labeling CSF-cNs, Urs Lucas Böhm, Andrew Prendergast, and colleagues began by showing that the cilium that extends into the central canal is free to bend, which could allow it to detect CSF flow. They then used 2-photon calcium imaging to show that these neurons respond to active contraction of tail muscles, as well as passive mechanical bending of the tail. These mechanosensory responses were abolished in mutant fish that lacked an ion channel specific to CSF-cNs (called PKD2L1), indicating that the CSF-cNs may be directly mechanosensitive.

Böhm, Prendergast and the team then used fish with the same PKD2L1 mutation, as well as an independent genetic method, to examine how CSF-cNs contribute to zebrafish swimming behavior. Although fish lacking feedback from the mutated CSF-cNs were able to swim, they exhibited a marked reduction in their tailbeat frequency. Thus, CSF-cNs appear to provide mechanosensory feedback to motor circuits in the spinal cord, leading to an increase in tailbeat frequency during swimming.

Although these data provide the first evidence that zebrafish CSF-cNs respond to mechanical stimulation generated by CSF motion, many questions remain. For example, what are the dynamics and distribution of mechanical force generated by CSF movement during locomotion? Which aspects of CSF flow do the CSF-cNs detect and encode? The molecular mechanisms by which CSF-cNs sense mechanical signals are also not clear. PKD2L1, the ion channel required for mechanosensory responses in CSF-cNs, is highly sensitive to extracellular pH. In fact, a separate study by Elham Jalalvand and colleagues has recently suggested that a primary function of CSF-cNs is to monitor the pH of CSF and decrease

locomotion when it drifts outside the appropriate physiological range. Given all of these complex and provocative links to the central nervous system, CSF may soon shed its reputation as the most boring of the bodily fluids.

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## A bird must flap before it flies



Flight, the most power-demanding mode of locomotion, is seen as one of the greatest transformations in the history of vertebrate evolution. The way bone and muscle formations come together to help a bird take flight, glide through the air, dive onto prey, land on water and power over our tallest mountains has always fascinated researchers. Yet, the origin of flight is still not entirely understood.

The current perspective on how flight has evolved uses the adult bird skeletal and muscular structures as the hallmarks for defining whether ancestral birds had the ability to fly. Adult flying birds are equipped with large wings, strong pectoral muscles and tight, specialized joints and bones that allow them to fly. If a fossil

presents these adaptations, then it must have been capable of flight. However, if a fossil has small wings and less constrained joints and bones, then it is concluded that its skeletal structure would have supported underdeveloped muscles that would have been too weak for flight. While this approach appears to be well established in the avian literature, young developing flight birds challenge this notion. They do not have the flight structures of an adult bird, much like the early winged dinosaurs, yet they are still capable of aerodynamically active flapping behaviours. This means they have the moves (the flap), but not the capacity (skeletal and muscular ability) to take flight.

Ashley Heers, a postdoctoral fellow at the American Museum of Natural History, and her colleagues set out to examine how a developing bird goes from flapping its wings to flying. Using X-Ray Reconstruction of Moving Morphology (XROMM), they explored how flight movements correlate with the development of the skeletal, joint and muscular structures necessary for flight. The experiment examined wing and leg movements in a pre-flight behaviour test in the precocial ground bird *Alectoris chukar*, where adult and immature birds were required to flap-run over obstacles to mimic pre-flight scenarios. By analysing flight structures in adults and young birds, the authors tested three hypotheses: (1) immature birds have different flapping movements from adults; (2) differences in pre-flight movements between adults and youngsters are due to different wing size; and (3) immature birds move differently because they struggle more to go over obstacles.

The team was able to establish that immature birds flap-running on a 60–65 deg incline displayed significant differences in pre-flight behaviours when compared with adults, but that those differences were not due to the ratio of wing length to substrate width, nor to the different levels of effort. The authors determined that, initially, juvenile birds use their legs and wings to perform the same movements as the adults during locomotion, in spite of lacking the flight adaptations of the older birds. However, as the youngsters' skeletal structures develop and they begin to rely more on their skeleton for powered flight, their movements become more aerodynamic. Therefore, although immature and adult birds have different skeletal structures, the

youngsters are nevertheless capable of using adult-like flapping movements to mimic pre-flight behaviour.

These findings offer insight into how birds develop the ability to fly, but also provide additional information on how flight was first acquired in ancient vertebrates. The authors propose that the smaller ancestral flapping forelimbs, similar to those of immature flight birds, first evolved to improve leg movements (i.e. running from predators), which led to increased pectoral muscle strength, then to the aerodynamically active, larger forelimbs that we now call wings. Therefore, it seems that a bird always flapped before it flew.

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## Thrifty metabolism helps butterflies go the distance



Human activities like planting crops or managing land changes landscapes for wild animals. For butterflies, these changes might reduce access to food like wildflowers. When faced with a changing food supply, certain butterflies may be able to survive in environments with reduced resources if, for example, they have lower metabolic rates that would reduce the energy required to maintain their bodily functions. This is exactly what interested Julie Lebeau, Renate Wesselingh and Hans Van Dyck of Université Catholique de Louvain, Belgium. They used wild meadow brown butterflies to test whether human-modified landscapes affect the physiological characteristics of wild butterfly populations.

Butterflies typically live in meadows with plentiful nectar-rich flowers. However, some butterflies live on land fragmented for human use with fewer available flowers. Butterflies then have to travel to find a single flower resource. Lebeau and her colleagues were specifically interested in the effects of different landscape habitats on butterfly energy budget and flight ability. The researchers went to the field and collected butterflies: half from unmanaged wild meadows with infrequent mowing and abundant flowers, and half from managed meadows that were mowed frequently, had fewer flowers and were surrounded by agricultural land.

The researchers then placed the butterflies in flight cages to test their responses to environments of differing nectar quality. Each cage was provided with flowers to create conditions that varied from a high-quality environment simulating a wild unmanaged meadow – the butterflies were provided with 100 flowers of their preferred species (brown knapweed, *Centaurea jacea*) – to the poorest quality setting simulating a managed meadow – where the butterflies were provided with only 10 flowers of a species that they would not normally visit (red clover, *Trifolium pratense*). After 2 days in the cages, the researchers measured the butterflies' resting metabolism – the amount of energy consumed for baseline bodily functions – as well as their flight capacity using a flight mill that measured the distance flown by each butterfly in 10 min.

Looking at the effects of the different settings on the physiology of the insects collected from the managed and unmanaged settings, the team found that butterflies that had been collected from the managed meadows were physiologically better suited to a harsher lifestyle. To start, they had lower metabolic rates than the butterflies from wilder areas, required less energy to maintain baseline bodily functions and potentially used more energy when moving to find mates or flower resources in the wild. The butterflies that originated from the managed landscapes were also able to fly greater distances in the flight mill; on average, they flew 2.5 times farther than butterflies from the wilder areas. Being able to fly longer distances would be helpful when looking for infrequent nectar-bearing flowers in their managed habitat.

Interestingly, the team found that the quality of the nectar in the different cages

(the number and type of flowers available) had little impact on the butterflies' metabolic rates and flight distances, except when the nectar was of the poorest quality (10 flowers of a non-preferred flower). On this occasion, the butterflies from the wild areas had lower metabolic rates and flew very little. The authors suggest that butterflies from the wilder meadows are not prepared for poor-quality nectar and must reduce their metabolism and the amount of energy that they expend on flight, while butterflies from managed environments can maintain their physiology in the face of varying nectar quality.

This work shows that intensive land management can select a specific subset of butterflies that have a well-adapted physiology to persist in such environments and we are therefore shaping the characteristics of wild animal populations with our activities.

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## Oxygen diffusion capacity scales as oxygen consumption



Metabolic scaling is one of the central enigmas of comparative biology.

Oxygen consumption, as a proxy for aerobic metabolism, does not scale proportionally with body mass.

Expressed as an exponential function, oxygen consumption scales as mass to the  $2/3$ – $8/10$  power. This fundamental principle explains why the appropriate drug dose per kilogram of pet cat is higher than that of an average adult human, and why the cat's resting heart rate is over 140 beats  $\text{min}^{-1}$  when my own is closer to 70 beats  $\text{min}^{-1}$ , and a large whale's is closer to 15 beats  $\text{min}^{-1}$ . The scaling pattern holds in endotherms and ectotherms alike. Yet, in spite of the tremendous utility of metabolic scaling, the cause of this phenomenon remains elusive.

James Gillooly and colleagues at the University of Florida, Gainesville, used another fundamental principle of comparative physiology to look into one possible driver of metabolic scaling: Fick's law of diffusion. Passive diffusion of oxygen into the body may be a rate-limiting step in aerobic metabolism and, as the first step in the process, seemed a likely candidate. According to Fick's law, the rate of passive diffusion across a membrane is proportional to the surface area of that membrane, and inversely proportional to the thickness of the membrane. Given this relationship, if you know how membrane surface area and thickness relate to mass, you can relate passive diffusion capacity to mass directly.

Gillooly and his team compiled data on respiratory membrane surface area and thickness for a range of vertebrates, including animals from all major vertebrate classes, with representatives from all ecosystems and all forms of locomotion. This is particularly remarkable for the amphibians included in the study, as they obtain oxygen through their mouths and skin in addition to their lungs. Once the team had this information, they found that respiratory surface area scales with mass strongly, whereas membrane thickness changes little with body size.

Using the scaling exponents calculated from the published data, and estimates

of the physiological properties of respiratory membranes, the researchers applied Fick's diffusion equation to model how passive oxygen diffusion capacity varies with mass. Doing so revealed that the relationship between oxygen diffusion capacity and body size was statistically indistinguishable from the relationship between resting oxygen consumption and metabolic rate for both endotherms and ectotherms. In other words, the body's capacity for passive oxygen diffusion precisely matches its resting metabolic oxygen requirements. This supports the idea of symmorphosis, a hypothesis suggesting that no one bodily system is over-engineered relative to the others.

The researchers noted that the match between resting oxygen consumption rate and diffusion capacity is inconsistent with the idea that respiratory systems evolved to optimize performance at maximum activity levels: it would appear instead that the respiratory surface is acting at capacity during resting activity and that the jump between resting and active metabolic rate could be made by modifying the oxygen partial pressure gradient over the respiratory surface, consistent with their model.

It may be a jump to suggest that oxygen diffusion capacity is the rate-limiting step in oxygen consumption, but Gillooly and colleagues present intriguing evidence of a structure–function match: a step in the right direction on the path to explaining the existence of metabolic scaling. Of course, only time will tell whether the symmorphosis hypothesis will hold for other components of the metabolic pathway.

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