OUTSIDE JEB

Big consequences of small changes for stroke sufferers

For many stroke victims, the road to recovery is often a gruelling mental and physical challenge. During a stroke, the affected area of the brain is starved of blood, and therefore oxygen, causing the surrounding tissue to die within minutes, which can leave the patient with a variety of motor problems. One common post-stroke condition is weakness or paralysis down one side of the body, often leading to walking difficulties. Individuals with such problems are at higher risk of tripping during walking and the resulting injuries can lead to further setbacks in their rehabilitation and mental health.

Understanding the potential biomechanical causes of these stumbles would be highly valuable to those designing rehabilitation plans for stroke victims and could prevent fall-related injuries. Gait changes such as slower walking speeds and asymmetrical movements between the paretic (affected with paralysis) and non-paretic leg have been identified, but the biomechanical characteristics of a naturally occurring trip were previously unknown.

With this in mind, Jessica Burpee and Michael Lewek, from the University of North Carolina at Chapel Hill, USA, decided to investigate the biomechanics of the lower limb in stroke patients. They wanted to know whether there were differences between the affected and unaffected legs during unsuccessful steps, which might begin to explain exactly how these missteps occur. To do so, the authors analysed the leg joint movements of 26 patients recovering from stroke while they walked unaided on a treadmill, tethered with a harness to prevent them from falling when they tripped. The duo attached markers to the pelvis, legs and feet of each participant and filmed them with eight cameras while they walked at comfortable speeds. Using the markers, they then modelled each individual's three-dimensional motion and calculated the joint angles at the hip, knee and ankle during both normal walking and when they stumbled.

In steps that resulted in a trip, the scientists found a number of small but significant biomechanical changes in the lower part of the leg affected by the stroke; in particular, just as the foot leaves the ground. During this period in unsuccessful steps, the knee of the stroke-affected leg bends more slowly and the ankle is at a higher angle, so the toes are more pointed towards the floor. Though these differences are seemingly trivial by themselves, together they create a functionally longer limb, resulting in a stumble when the stroke-affected foot does not clear the ground successfully when stepping. Interestingly, there appeared to be no significant biomechanical changes in the upper leg that would also cause a more extended limb.

These results highlight characteristic biomechanical patterns during trip steps that span multiple joints of the lower limb. Currently, ankle–foot orthoses are often used during stroke recovery to aid stability and foot clearance during walking, but this targets only a single joint. This work suggests that, in fact, treatments addressing multiple joints would increase the success of rehabilitation, preventing trips and falls.

For chronic stroke victims, each successful step is a small victory. Studies like this help to increase our knowledge of the effects of stroke, and in turn the patients' physical and mental strength, one step at a time.

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Burpee, J. L. and Lewek, M. D. (2015). Biomechanical gait characteristics of naturally occurring unsuccessful foot clearance during swing in individuals with chronic stroke. *Clin. Biomech.* doi:10.1016/j.clinbiomech.2015.08.018

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A crow's lesson in death

Humans have always had an uneasy relationship with crows. While admired for their legendary intelligence, they are also despised as agricultural pests and feared as harbingers of death – human death, that is. But what about crow death? Here too, crow behaviors have long puzzled and fascinated. Draped in black, crows attend funerals for fallen conspecifics. Is it because they are simply morose? Or might it be that dead crows can still speak to the living, silently imparting wisdom from the grave about the manner and context of their demise?

To address these issues, Kaili Swift and John Marzluff from the University of Washington, USA, carried out a series of experiments that are as clever as they are creepy. First, to gain the trust of breeding pairs of crows, Swift plied the birds with a favorite snack of peanuts and cheese puffs. Then, a few days later and in sight of the resident pair, volunteers in expressionless facemasks showed up next to the food pile carrying dead taxidermy-stuffed crows in their outstretched hands! As if this weren't traumatic enough (to both crows and people alike), these macabre scenes were sometimes paired with a stuffed

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hawk, one of the crow's most feared predators.

The responses to these stimuli were markedly different. While a dead crow held aloft by a masked volunteer drew the unquestioned ire of the resident pair of crows, their 'scolding' only occasionally attracted the attention of neighboring crows. By contrast, when crows were confronted with a dead crow paired with a stuffed hawk, 'mobs' of crows invariably formed that contained crows recruited from outside the territory of the breeding pair. Moreover, these mobs tended to be larger and more persistent, thus maximizing the scolding.

Perhaps this isn't overly surprising. After all, hawks eat crows and crows are smart enough to put 2 and 2 together: dead crow+hawk=killer hawk! But what is especially neat about this experiment is that this contextual learning extended to the volunteer in the mask.

In the 6 weeks following the main experiment, a volunteer appeared once a week with food while wearing the same mask as in the original exposure. Remarkably, even after a 6 week interval, more than a third of crows continued to mob and scold the masked person. In short, the crows had learned that this person was a potential predator, both through her association with the dead crow and also through her association with the hawk. Thus, not only are crows capable of assigning guilt by association but also their memory of these associations can potentially persist for months or longer.

So what does this mean for crow funerals? Are crows morose or unduly saddened by death? Perhaps – but probably not. When the team repeated the experiment with a dead pigeon instead of a dead crow, the crows weren't much bothered. Instead, it seems that crows gather around their dead to gain insight into how to avoid a similar fate. And by this approach, even if the threat is a new one, say a creepy masked PhD student, crows can quickly learn to respond with suitable caution. After all, why risk death for a pile of peanuts?

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Bacteria eat first at the dinner table

There are some biological topics it can be unpleasant to think about immediately prior to lunch. For some people, it's the realization that bacteria are everywhere – living and growing not only on and in your body but also most likely on that lunch. Insects face the same reality, living in close association with bacteria in and around their bodies.

Researchers are just beginning to unravel how some of these associations work, finding that gut bacteria can: synthesize B vitamins, which are then used by insects; digest complex macromolecules that insects cannot; and otherwise modify the relationship between an insect and its food. As past research has shown that bacteria that cycle between the gut and the environment can reduce the amount of body fat that the fruit fly *Drosophila melanogaster* has tucked away, Jia-Hsin Huang and Angela Douglas from Cornell University, USA, wanted to try to unravel what the underlying mechanisms behind this waist-shrinking effect might be.

Using lines of flies that were bacteria-free, the researchers first set out to show that infection with particular strains of bacteria as adults could change the fat content of the flies. They found that flies infected with the bacteria *Acetobacter tropicalis* were much slimmer than their counterparts that were infected with *Lactobacillus brevis* or remained uninfected.

So how were the bacteria accomplishing this slimming effect on the flies? One possibility was that the flies infected with *A. tropicalis* were unable to digest as much sugar as the other flies. The researchers compared how well the flies with the different infections were able to digest sugar by measuring how much sugar they ate as well as how much was left undigested in their feces from a clean food source that was not able to be infected with the bacteria. They found that the *A. tropicalis*-infected flies were actually more able to digest sugar.

Next, the researchers looked at the sugar content of the food the flies were eating. While the researchers had provided all the flies with the same food, the food that was infected with *A. tropicalis* had a significantly lower sugar content and the researchers concluded that the bacteria were actually consuming the sugar in the food before the flies had a chance to get at it.

Fruit flies in the wild consume high sugar diets that can make it hard to consume enough micronutrients for a balanced diet, so having the right species of bacteria around is important for the insects. And this research shows that having bacteria around can not only change the amount of fat in a fly but also change the way that food is digested, which is probably the case for other animals as well.

While it might make us uncomfortable to think about it, bacteria are everywhere, growing not only on and inside us but also in our food. Understanding the complicated interactions between ourselves, our passengers and our food is only just beginning.

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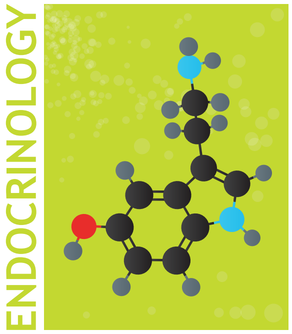
Goldfish pump up the volume with Nesfatin-1



It is often said that ‘The way to a man’s heart is through his stomach’, and, although the original author of this quote is unknown, I bet he/she was an endocrinologist – someone who understood that many of the hormones that communicate the sensations of hunger and thirst in our bodies can also modify our heartbeat. New on the list of such appetite-altering hormones is Nesfatin-1, a potent appetite-suppressing hormone found in mammals that was recently ascribed an additional role in cardiac regulation when researchers at the University of Calabria, Italy, demonstrated that it reduces the pressure generated by the left ventricle of rat hearts. As Nesfatin-1 is known to be an anorexic agent in other vertebrates, like the goldfish, the Italian team wanted to know whether the hormone could similarly play on a fish’s heartstrings.

To get to the heart of the matter, the team began by isolating goldfish hearts and then connecting them, separately, to a series of tubes to allow each heart to pump saline solution through its chambers just as if it was pumping blood inside a fish. Next, they added Nesfatin-1 to the fluid perfusing the hearts to see whether the hormone changed the volume and pressure of saline ejected from the heart during a heartbeat (stroke volume and stroke work, respectively). The team found that even small amounts of Nesfatin-1 increased both the stroke volume and stroke work of the hearts, meaning that Nesfatin-1 stimulates goldfish hearts to increase performance – which is the opposite of the effect described in mammals. To confirm the specificity of this response, the team then perfused the hearts with a combination of Nesfatin-1 and its specific inhibitor and found that, as expected, the function of the hearts decreased back to initial resting levels.

Confident that Nesfatin-1 is indeed a cardiac regulating hormone in fish, the team began probing the underlying mechanism by which this hormone sets the goldfish heart aflutter. They perfused their isolated goldfish hearts with various combinations of Nesfatin-1 and specific pharmacological inhibitors that target discrete steps in intracellular signaling pathways to see which drugs blocked the stimulating effects of Nesfatin-1. With this approach, the team identified several key players in Nesfatin-1 signaling in heart muscle cells, beginning with adenylate cyclase activation, which causes a rise in intracellular cyclic AMP and subsequent activation of protein kinase A – an enzyme that phosphorylates specific proteins to rapidly modify their function. Protein kinase A then targets several muscle proteins to ultimately facilitate the rapid cycling of calcium ions that is essential for cardiac contraction.

Together, the Italian team provides a thorough account of the action and process of Nesfatin-1 signaling in goldfish hearts, showing us that this appetite-altering hormone is also at the heart of the endocrine regulation of cardiac function in goldfish.

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