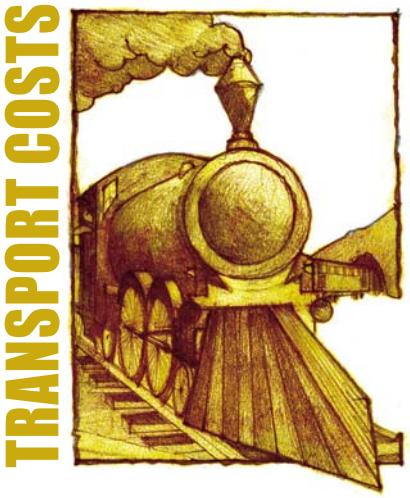
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB



WALK THIS WAY

Why is the preferred walking speed (PWS) of healthy adult humans so predictable and consistent? A commonly given explanation is that we unconsciously select a speed that minimizes the energy expended per unit distance (the energy cost of transport). However, a recent study by Arizona State University researchers suggests that the amount of energy required may not be as important as the source of the energy.

The traditional 'minimal energy hypothesis' correctly predicts that there is a U-shaped relationship between the energy cost of transport and walking speed, with the lowest energy cost occurring at the PWS (about 4.5 km h^{-1}, or 2.8 miles h^{-1}) and higher costs occurring at slower and faster speeds. A possible weakness of this hypothesis is the fact that the slope of the U curve is quite shallow; in other words, large deviations from PWS cause only slight increases in energy cost. Could the body really set PWS so precisely if the energetic penalty for straying from PWS is so small?

To explore the issue further, Willis and colleagues studied the perambulations of 12 young, healthy adults. After each subject's PWS was established, he or she walked on a treadmill for 10 min at each of six speeds ranging from 3.2 to 7.2 km h^{-1}. At each treadmill speed, Willis and colleagues measured the subject's expired O_2 and CO_2 levels and asked the subject to rate his or her perceived exertion. The team then used the expired gas measurements to calculate carbohydrate and fat oxidation rates at each speed.

The researchers were able to reproduce the usual U-shaped relationship between energy cost of transport and walking speed, again noting its shallow slope. However, they found that the rates of carbohydrate oxidation varied dramatically as a function of walking speed, with carbohydrate use increasing substantially at speeds above the PWS. Furthermore, subjects' rates of carbohydrate use correlated with ratings of perceived exertion more strongly than did energy costs of transport.

Based on this analysis, Willis and colleagues propose that PWS is selected not to minimize energy expenditure but to minimize carbohydrate use. Carbohydrates are valuable to the body because they are the primary fuel for intense exercise, yet are stored only in small amounts, whereas fat reserves are ample even in slender individuals. From an evolutionary perspective, then, it may be advantageous to walk at speeds that primarily burn fat, thus saving the body's limited carbohydrate stores for the occasional fight-or-flight moments when they are really needed.

If Willis and colleagues are correct, the relationship between PWS and carbohydrate oxidation should hold true in different subpopulations with different PWSs. Indeed, they found this to be the case when they retrospectively analyzed data from a previous study of young and old active and sedentary people.

If the need to conserve carbohydrates really does dictate PWS, the next logical question to ask is, how does the central nervous system 'sense' the rate of carbohydrate oxidation? The tight correlation between this rate and perceived exertion ratings suggests that subjects' subjective sense of exercise difficulty is indeed somehow linked to carbohydrate use. The researchers' speculations on the mechanisms underlying this connection are not entirely convincing, in my opinion. Nevertheless, their carefully designed and well-executed study does offer reasonable evidence that PWS is selected to minimize depletion of the body's carbohydrate stores.

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Willis, W. T., Ganley, K. J. and Herman, R. M. (2005). Fuel oxidation during human walking. *Metabolism* **54**, 793-799.

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MENISCUS CLIMBING

SLIDING UPHILL

We often have great difficulties understanding how the world works on a miniature scale. For evidence, you have to look no further than the attempts of novels such as 'Gulliver's Travels' and Hollywood films like 'Honey, I Shrunk the Kids'. Despite being about the size of an insect, the humans in these novels and films move in almost exactly the same way as normal-sized humans. Yet when you're as small as an insect, your movements become quite different from those of larger creatures, and the substrates that you walk on may have quite different properties too. One obvious example of this is the meniscus of water. Insects have overcome many problems to enable them to move about on the water surface, and several recent studies have provided insights into some of the mechanisms they use, such as the water repellent hairs on the legs of water striders. At the water's edge, the meniscus bends just like it does in a test tube. Although this bending might seem small to us, to an insect the bending of the meniscus is a significant obstacle. The meniscus is smooth, so to an insect it appears to be like a glass mountain that provides no purchase to allow it to climb using its normal walking gait. Yet insects do manage to 'climb' this obstacle and get back onto land. How do they do it? The trick, it seems, is to keep perfectly still.

David Hu and John Bush from the Massachusetts Institute of Technology set out to discover the technique insects use to climb the meniscus. The pair filmed water-treading insects from several genera including *Mesovelia* (water treaders) moving on the meniscus and at the edges where the meniscus bends. Their high-speed videos revealed that the insects often made rapid movements to attempt to climb up the meniscus, but this strategy didn't get them anywhere. Instead, when the water-treaders held themselves completely rigid, Hu and Bush saw that the insects would glide effortlessly up the meniscus. In this posture, insects such as *Mesovelia* use small claws to pull up the meniscus at their front and back legs whilst pushing down with their middle legs – but why does this cause the insect to move forward? The answer lies in the lateral capillary forces that exist between small floating objects. These are the forces that attract bubbles towards one another on the surface of drinks, forming 'bubble rafts'. The insects that move up the meniscus are making use of these forces by adopting the rigid posture that Hu and Bush observed. The theory that the pair developed to predict the speed of the insects as they approach the edge of the meniscus is remarkably accurate, suggesting that the lateral capillary forces really do account for the insects' movements up the meniscus. Insects specialised for life on the water surface are not the only ones to exploit the lateral capillary forces; Hu and Bush also filmed terrestrial insects using this mechanism to scale the heights of the meniscus at the water's edge.

Meniscus climbing is an unusual form of locomotion quite different from the mechanisms normally used by insects or other animals. Although this is one of the more surprising – and least dynamic – mechanisms insects use to move through the world, no doubt there are many more out there to be discovered.

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Hu, D. L. and Bush, J. W. M. (2005). Meniscus-climbing insects. *Nature* **437**, 733-736.

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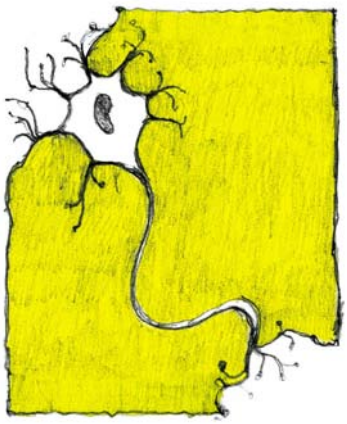
DECISION MAKING



A TONIC FOR UNPALATABLE FOOD?

Obviously, the search for safe, edible food is a lifelong pursuit of all animals. Although animals are born with innate preferences for certain foods, the ability to modulate these preferences dynamically is clearly adaptive. Put simply, any starving animal (including humans) will tolerate normally unpalatable food. Surprisingly, this complex-seeming behavioural trait is tractable to simple genetics, and studies in *Drosophila* have now implicated two signalling pathways that are conserved from fly to human: neuropeptide Y and insulin-like proteins.

Drosophila larvae must feed voraciously in order to gain sufficient weight for pupation. It is possible to score larval feeding behaviour quantitatively by feeding larvae a tasty liquid diet of yeast and sugar with a green dye and measuring how their bodies change colour. When quinine (the bitter antimalarial drug found in Indian tonic water) is added to their diet, larvae find it disgusting – perhaps because they have yet to discover gin! However, larvae starved for 40 or 120 min overcome their innate aversion to quinine and start feeding again. This assay provides a robust baseline from which to look for modulation of risk-taking behaviour in feeding.

Mammalian neuropeptide Y (or its *Drosophila* homologue NPF) is thought to modulate feeding behaviour in both fly and human, but is it involved specifically in the decision to overrule avoidance of unpalatable food? To find out, the experimenters produced lines of transgenic flies in which the promoters of either NPF or its receptor (NPFR) drove expression of the yeast transcription factor GAL4. In such fly lines, any transgene downstream of the UAS promoter (which binds GAL4) will be switched on in any cells where GAL4 is switched on. This potent and

widely used *Drosophila* technology thus provides a toolbox for expressing transgenes of choice in very specific populations of cells – in this case, just those cells expressing NPF or NPFR.

In this study, the authors used this approach to drive expression of a temperature-sensitive allele of the synaptic protein *shibire*, known (ironically for a study of feeding) as shi^{ts}, in cells expressing NPF or NPFR. This is an excellent way of disrupting synaptic transmission: the mutant dynamin protein encoded by shi^{ts} performs normally at 23°C but becomes dysfunctional in just a few seconds at 30°C. These manipulations didn't affect the behaviour of non-deprived larvae, but food-deprived larvae responded very differently. At the permissive temperature (23°C), the transgenic flies behaved like wild-type flies, feeding on aversive food, but at the restrictive temperature (30°C) they avoided it. These results suggest that the NPF/NPFR neuronal circuit is involved in suppressing avoidance of unpalatable food in hungry animals. To confirm this result, the authors used RNAi interference; by driving UAS-controlled constructs encoding double-stranded RNA to either NPF or NPFR, they were able to take down expression of each gene specifically. Again, they were also able to make hungry flies avoid aversive food. By contrast, flies over-expressing NPFR ate even more of the quinine diet after food deprivation. These results further show that the control of avoidance depends not just on the NPF neuronal circuit but also on signalling through NPF. Similar studies implicated the insulin receptor and DILP2, one of the seven insulin-like proteins encoded by the *Drosophila* genome.

So it seems as if the decision to overrule an innate aversion to noxious food when starving is attributable in *Drosophila* to the NFP and insulin-like signalling pathways, and to a relatively small neuronal circuit. Interestingly, neuropeptide Y in humans is associated with suppressing anxiety and fear, leading to a plausible anthropomorphic explanation for the behaviour. It will be interesting to see if this model is applicable beyond insects.

10.1242/jeb.01943

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ANOXIA TOLERANCE

EXCITOTOXIC CELL DEATH DENIED

Most vertebrates' brains die within minutes when deprived of molecular oxygen (anoxia), mainly due to excitotoxic cell death (ECD). ECD begins with a massive and uncontrolled influx of Ca^{2+} into neurons by over-stimulation of *N*-methyl-D-aspartate receptors (NMDARs), resulting in the activation of Ca^{2+}-dependent phospholipases and proteases that cause membrane depolarisation, uncontrolled cellular swelling and, ultimately, cell death. Some ectothermic vertebrates, such as the western painted turtle (*Chrysemys picta bellii*), are remarkably anoxia tolerant and can survive days to months without oxygen and recover without any apparent brain damage. One mechanism that is believed to enable these animals to accomplish such a feat is their ability to prevent ECD by reducing NMDAR activity during anoxia. However, the mechanisms underlying the attenuation of turtle brain NMDAR activity have not been fully elucidated.

Thomas Buck's team at the University of Toronto was determined to tease out how NMDAR activity is reduced in the turtle brain during anoxia. In order to do so, Buck's team obtained cortical slices from painted turtles' brains and used whole-cell patch clamp techniques to measure NMDAR currents from individual cortical neurons during normoxia and after 0, 20 and 40 min of anoxic exposure. As they expected, the team confirmed that turtle neuronal NMDAR activity is reduced during anoxia; during the 40-min anoxic period they observed a 56% decrease in whole-cell NMDAR currents from normoxic levels.

To discover which intracellular modulators are responsible for this reduction of neuronal NMDAR activity, the team pharmacologically blocked possible modulators of NMDAR activity in the turtles' brains and again measured NMDAR currents from neurons in normoxic and anoxic experiments. Suspecting that protein phosphatases play a role, the team incubated the turtles' cortical slices in inhibitors of serine/threonine protein phosphatases PP1 and 2A and found they could abolish the anoxia-induced reduction in NMDAR currents. To test the role of intracellular Ca^{2+}, they added the calcium chelator BAPTA to the recording electrode solution (which is continuous with the cytoplasm in whole-cell patch clamp experiments) and found that this also abolished the reduction in NMDAR currents. This indication that intracellular Ca^{2+} modulates NMDAR activity led the team to wonder about the role of calmodulin, an intermediary protein that senses calcium levels and relays signals to various calcium-sensitive enzymes, ion channels and other proteins. Sure enough, when the team pharmacologically blocked calmodulin during anoxia, they did not see a reduction in NMDAR current, indicating that calmodulin also controls NMDAR activity. The team concluded that protein phosphatases PP1 and 2A, intracellular Ca^{2+} and calmodulin all work in concert to decrease NMDAR activity during anoxia.

Synthesizing their findings with previously published data, the team proposes a novel mechanism of how NMDAR activity is attenuated during anoxia. The team suggests that during anoxia, protein phosphatases PP1 and 2A dephosphorylate the NR1 subunit of the NMDAR receptor. Dephosphorylation of the NMDAR receptor subsequently enables calmodulin, which must first be activated by Ca^{2+}, to bind to the receptor and disrupt NMDAR binding to α-actinin-2, a molecule that normally connects the NMDAR receptor to the cytoskeleton. Disruption of this connection results in the dissociation of the NMDAR from the cytoskeleton, ultimately leading to a decrease in receptor activity and prevention of ECD.

10.1242/jeb.01945

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CHILLY WATERS, HOT SHARKS

Elevating body temperature above ambient using metabolic heat is the norm among birds and mammals, but among fishes it is the exception. Two fish groups that buck the trend are tunas and lamnid sharks, and they do so using remarkably convergent adaptations for minimizing heat loss. One such adaptation for reducing heat loss across the skin is internalized oxidative swimming muscle. Most fishes swim using slow-twitch (or 'red') muscle located just under the skin, but tunas and lamnids have red muscle that is much closer to their body core. One advantage of burying red muscle deep within the body is that this high metabolic rate tissue is then insulated by the large mass of fast-twitch ('white') muscle surrounding it. The consequence of this arrangement is a steep thermal gradient, from warm red muscle at the core to relatively cold white muscle just under the skin. Bernal and colleagues were interested in the consequences of this thermal gradient on muscle function in salmon sharks, a lamnid that inhabits icy Arctic waters and is believed to maintain some of the highest thermal gradients among endothermic fishes.

To measure the magnitude of the thermal gradient in salmon sharks' muscles, the investigators caught three salmon sharks in the Gulf of Alaska. They found that, at its warmest, salmon sharks' deep red muscle can be 26°C, which is about 20°C higher than ambient. The white muscle under the skin was typically only a few degrees higher than ambient, whereas the deepest white muscle was almost as warm as the red muscle. These measurements confirmed that salmon shark white muscle exhibits a dramatic thermal gradient.

The investigators also measured the effects of temperature on muscle contractile properties in live salmon sharks using a portable stimulator/transducer. They found similar twitch durations in superficial white muscle all along the body, but significantly faster twitches in deeper (warmer) white muscle. When they stimulated isolated muscle fibre bundles *in vitro* over a range of temperatures, they found that salmon sharks' red muscle was remarkably sensitive to temperature, with twitch speed decreasing by a factor of 3.7 for a 10°C drop in temperature. By contrast, nearby white muscle was not nearly as sensitive to temperature, decreasing only 2-fold for a 10°C drop. The investigators also measured muscle power as a function of temperature and showed that white muscle can operate well over a wide range of temperatures, but red muscle simply can't.

The authors point out that the high thermal sensitivity of red muscle underscores the fact that these animals are obligate ram ventilators; they have to swim continuously in order to keep breathing. If a salmon shark were to stop swimming, its red muscle would stop generating heat and eventually would cool. Even a small drop in red muscle temperature could compromise its function enough to send it into a downward spiral from which the animal couldn't recover. When framed this way, it seems unfortunate for salmon sharks that they are saddled with such sensitive red muscle in such a challenging thermal environment. But the other side of the coin is that salmon shark red muscle likely achieves higher power outputs when warm than it could if it were less temperature sensitive. Furthermore, salmon sharks probably exert tight control over the temperature of their red muscle, which allows them to reap the benefits and avoid the pitfalls of having red muscle more typical of a mammal than a fish.

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