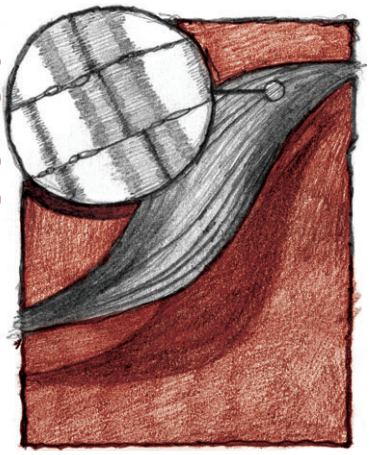
Outside JEB

PHARMACOLOGY



DRUG COCKTAIL COULD SUBSTITUTE FOR EXERCISE

Most of us have seen the infomercials in the wee hours selling us fitness in various forms. You need only take a certain pill for a couple of weeks and you will become as strong as Superman. It now appears that may soon be more than just a late-night claim. In a multi-centre study led by Ronald Evans from the Salk Institute, the pharmacological action of two drugs and how they are able to mimic the effects of exercise on mouse skeletal muscle have been revealed.

Within mammalian muscle tissue there are essentially two types of muscle cells. Type I are slow contracting, generate energy through oxidative metabolism and are responsible for the endurance typical of long distance running. Type II muscle cells on the other hand are fast contracting and rely on anaerobic glycolysis to produce energy. As such they are responsible for rapid, powerful movements, for example during jumping or weight lifting. Different training regimes and types of exercise will cause shifts in the relative proportions of these two types of muscle cells. Evans and his colleagues wanted to explore the cellular signalling pathways responsible for such shifts.

Starting several years ago, the team developed transgenic mice that over-express a cell signalling molecule (PPARδ) that is critical for the regulation of skeletal muscle metabolism. These mice exhibited increased oxidative metabolic enzymes and a shift in the fibre type, with muscles having proportionally more type I oxidative muscle cells than type II. The end result was an increase in the endurance of the transgenic mice of 60–75%, but this enhanced endurance was only seen in combination with exercise training, indicating that a second stimulus was necessary to enhance the development of type I muscle.

To better understand the mechanism of the training stimulus, Evans and his team compared gene expression profiles of trained non-transgenic mice, mice treated with a PPARδ stimulant and mice that received doses of the PPARδ stimulant along with a training regime. Although many of the gene expression patterns overlapped, each condition had a distinct expression signature. Consequently, Evans suggested that the pharmacological activation of a second critical signalling pathway (AMPK), which is known to be stimulated by exercise, might act as a substitute for exercise.

The team then gave non-transgenic mice two pharmacological agents that stimulated each of the two (i.e. PPARδ and AMPK) pathways. They found that the combined action of the two drugs led to an increase in expression of genes involved in oxidative metabolism and blood vessel growth and an associated reduction in the levels of enzymes involved in glycolytic metabolism. This was combined with a shift in the muscle fibre types from glycolytic type II fibres to oxidative type I fibres. And when the team tested the animals they found that pharmacological activation of these two cellular pathways led to a significant increase in their endurance.

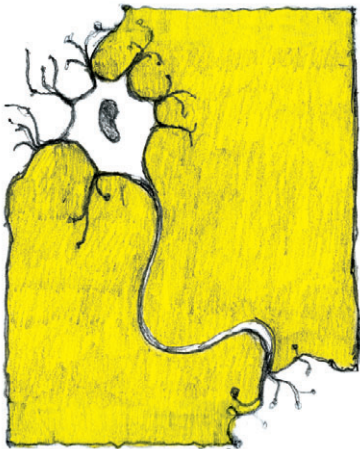
The potential applications of such pharmacological agents have not escaped the attention of clinicians and these drugs offer huge potential in the treatment of a number of diseases. On the other hand, this may well prove to be yet another headache for sport's World Anti-Doping Agency with secretive supermen competing alongside orthodox athletes.

10.1242/jeb.024265

Narkar, V. A., Downes, M., Yu, R. T., Embler, E., Wang, Y.-X., Banayo, E., Mihaylova, M. M., Nelson, M. C., Zou, Y., Juguilon, H., Kang, H., Shaw, R. J. and Evans, R. M. (2008). AMPK and PPARδ agonists are exercise mimetics. *Cell* **134**, 405-415.

Glenn Lurman
University of Bern
glenn.lurman@ana.unibe.ch

OLFACTION



BIRDS MAY HAVE BIGGER NOSES THAN WE THOUGHT

Although many birds are known to have acute vision and hearing, it has been widely thought that they have a very poor sense of smell. Part of the reason for this view is that it is relatively difficult to devise experiments to test the sense of smell in these animals.

Molecular genetic data appeared to have provided support for this assumption when the genome of the domestic chicken and of its wild progenitor, *Gallus gallus*, was published in 2004. Although around 550 genes were detected that could code for olfactory receptor (OR) proteins, only 15% of these genes (~80, compared to ~600 in humans) were thought to be functional. Furthermore, most of these genes seemed to be very closely related, suggesting the olfactory system would have a restricted functional range.

To investigate this assumption that animals in a whole phylogenetic class do not use their sense of smell much, a group from Germany and New Zealand led by Silke Steiger tried to amplify OR genes in DNA extracted from eight species of birds ranging from the prosaic (the blue tit and the mallard) to the exotic (the kakapo, the black coucal or the snow petrel). As a control, they also included the latest version of the *G. gallus* genome.

To their surprise, all the species – including *G. gallus* – seemed to have far higher levels of functional OR genes than was expected. The total number of estimated functional OR genes varied from around 70 for the galah (an Australian cockatoo) to around 600 for *G. gallus*, with most of the species appearing to have around 300 functional OR genes.

Because the degenerate PCR technique the group used will not distinguish functional genes from mutated genes, they checked the accuracy of their procedure by looking in the *G. gallus* genome to see whether the OR genes they had identified as functional were in fact complete. They found they were over-estimating the number of functional OR genes by about 11%. When they removed these false positives, their data fell into line with unpublished estimates of the proportion of functional ORs from the latest version of the *G. gallus* genome, which suggest that the chicken's nose may contain up to 500 different kinds of receptor.

These findings challenge the notion that birds do not use their noses much by revealing they possess a substantially larger OR repertoire than was previously imagined.

As a test of their findings, the authors compared the OR repertoires with the relative size of the birds' olfactory bulb and with the ecology of the species. In general, the larger the relative size of the olfactory bulb, the larger the OR gene repertoire, while nocturnal birds like the kakapo and the kiwi, which are more likely to use olfaction, had larger OR repertoires.

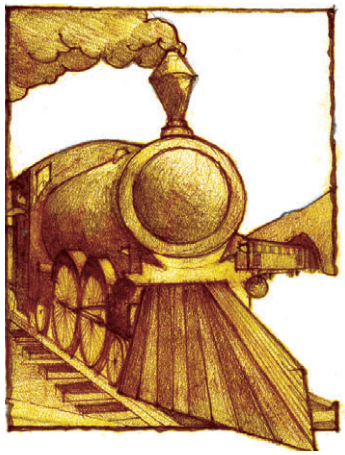
This study represents a challenge on a number of levels: it provides a reminder that the results of genomic studies need to be verified by studies of gene expression and it shows the importance of comparative inter-specific studies to test whether findings can be generalised across species. But above all it throws down the gauntlet to experimental biologists: attempt to measure the olfactory capacities of this important class of animals and to test the hypotheses that have emerged from this molecular study. In particular, some of the few experimental studies on avian olfaction suggest that birds with the smallest OR repertoires may not have the poorest olfactory responsive range or acuity. Explaining this apparent enigma will require extensive experimentation.

10.1242/jeb.011502

Steiger, S. S., Fidler, A. E., Valcu, M. and Kempenaers, B. (2008). Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? *Proc. Biol. Sci.* **275**, 2309-2317.

Matthew Cobb
University of Manchester
cobb@manchester.ac.uk

PERISTALSIS



PUMPING MUCUS

Animals are filled with tubes, pushing fluids from one place to another – blood, lymph, mucus. In many cases, the tubes do the pushing by a process called peristalsis, where waves of contraction slide along the tubes, driving the fluid along with them.

Understanding how peristalsis works has become particularly important in recent years with the growing use of *in vitro* fertilization, because both eggs and sperm are pushed around in the oviducts by peristaltic waves. Sometimes the waves push the egg and sperm together, aiding fertilization, but sometimes they drive the two apart.

Fluid dynamics engineers have analyzed peristalsis for decades, and it would seem that their expertise should be helpful. Unfortunately, most of the analysis doesn't apply very well to biological fluids like the fluid in the oviducts.

The trouble is, mucus (or other polymers) gum up the fluid, making its properties somewhat intermediate between a fluid and a solid. A normal solid, like a rubber band, resists being stretched and produces a force that's linearly proportional to how far it's stretched. A normal fluid, like water, doesn't care how far it's deformed, but responds linearly to the rate of deformation. Fluids that aren't 'normal' are called non-Newtonian, and can have some properties of solids. For example, many biological fluids, called viscoelastic fluids, have polymers in solution that stretch like rubber bands, while the fluid around them flows like water. Together they make for complicated, poorly understood non-Newtonian fluids.

To start to understand peristalsis of such fluids, Joseph Teran and Michael Shelley of the Courant Institute at New York University teamed up with Lisa Fauci at Tulane University, and developed a

computational simulation of a tube with rhythmically contracting walls. Starting with code they had developed to simulate Newtonian flows, they overlaid a mesh of elastic structures to simulate the rubber band-like polymers. Since a tube is symmetrical around its long axis, they only simulated a two dimensional slice through the middle. In this view, the walls on either side contract in a traveling wave.

To compare their results with previous ones on Newtonian fluids, the researchers had to assess how weird their fluid was. They used a dimensionless number called the Weissenberg number *Wi* – the ratio between the rate that the polymers spring back and the rate of fluid motion. For a Newtonian fluid such as water, *Wi* is always zero; for a solid, it's infinity. Teran and his colleagues tested a range from zero to five.

They found some interesting differences with Newtonian fluids. For Newtonian fluids, the higher the amplitude of the peristaltic wave, the more fluid gets pushed along, even if the two sides of the wave meet in the center of the tube. For viscoelastic fluids, this wasn't true at all. With more polymers in solution, high amplitude waves produced little or no net motion, since most of the energy went into squishing or stretching the polymers perpendicular to the tube, not squirting the fluid along the tube. But with low amplitude waves, adding more polymers (to increase *Wi*) initially decreased the flow, but then, as *Wi* increased even further, started to increase the flow.

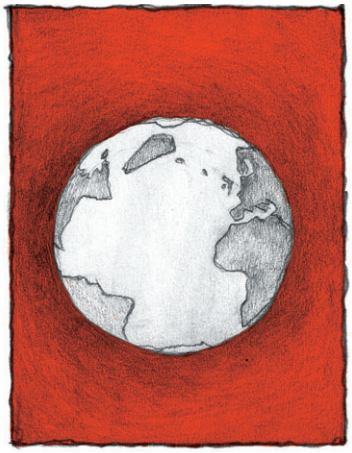
The group's research is still in its early stages, but they've set up a framework for studying non-Newtonian peristalsis. Now they can start to investigate practical questions, such as how are particles, like sperm, transported in such tubes? And for *in vitro* fertilization, when, relative to the peristaltic wave, would it be best to inject the sperm to obtain the optimal fertilization rate?

10.1242/jeb.011486

Teran, J., Fauci, L. and Shelley, M. (2008). Peristaltic pumping and irreversibility of a Stokesian viscoelastic fluid. *Phys. Fluids* **20**, 073101.

Eric Tytell
University of Maryland, College Park
tytell@umd.edu

LIFE EXPECTANCY



COLD VOLES LIVE FAST BUT DON'T DIE YOUNG

It is commonly assumed that there is a trade off between the pace of life and longevity, an assumption that is the basis for many theories of ageing. In a nutshell, it has long been observed in comparative studies that there is an inverse relationship between the rate of energy expenditure and lifespan of species. A candidate mechanism explaining this link is the oxidative stress theory of ageing, where damaging molecules, reactive oxygen species, are produced in proportion to metabolic rate, thereby damaging life's building blocks (nucleic acids, proteins, lipids) and shortening an individual's life. Luckily, protective and repair mechanisms exist and the balance between damage, protection and repair explains the extent of oxidative damage.

Despite extensive efforts in studying physiological mechanisms related to oxidative stress, the basic assumption linking metabolic rate and lifespan through oxidative damage remains unclear. To test the hypothesis that increased energy expenditure will induce greater oxidative damage and therefore reduce lifespan, Colin Selman, Jane McLaren and John Speakman from the University of Aberdeen and their colleagues from the Rowett Research Institute experimentally increased energy expenditure in short-tailed field voles by exposing them to the cold. By maintaining one group of voles at 22°C and another group of paired siblings at 7°C, the team managed to increase energy expenditure throughout the life of the cold-exposed group. It was then possible for the group not only to test the impact of increased energy expenditure on lifespan but also to monitor a collection of variables, such as oxidative stress indicators, levels of antioxidant molecules and the activity of antioxidant enzymes in a variety of tissues to see how the different life styles had affected the animals' physiology.

Exposing voles to the cold throughout most of their life increased energy use; all three measurements taken as an index of energy use (resting metabolic rate, daily energy expenditure and food intake) increased by approximately 50% or more. Even though a significant increase in energy expenditure was induced, the cold-exposed voles did not die younger than the warm-maintained group. Moreover, until late in their life the warm-exposed group had higher mortality risks than the cold-maintained voles. The fine details of the 'live fast, die young' assumption may thus need further scrutiny as it does not appear to simply apply to this species.

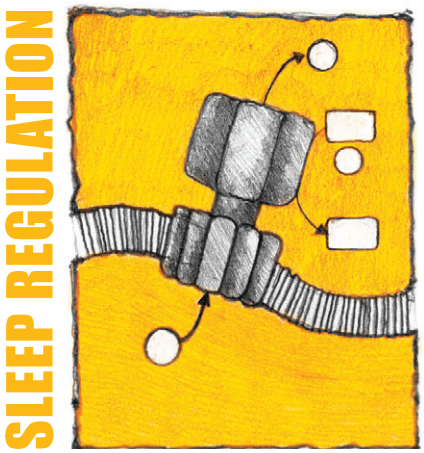
To get a good grasp of the impact of metabolic rate variation on oxidative damage, the group measured various metabolic parameters. Indicators of oxidative damage on lymphocyte and hepatocyte DNA and hepatocyte lipids showed no effect of cold exposure in almost all cases, except a possible increase in damage to hepatocyte DNA. The team also monitored antioxidant molecule levels in the liver of these animals, and found no differences between the cold- and warm-exposed voles for the three antioxidants that they analysed. Moreover, the group measured the activity of three antioxidant enzymes in the heart, liver, kidney, muscle, duodenum and brown adipose tissue and only found a significant increase in superoxide dismutase in the cold vole's brown adipose tissue. So despite running at a higher metabolic rate, the team found no evidence of increased oxidative damage in the cold voles.

Together, this study shows that integrative approaches are necessary to test current hypotheses connecting metabolism and lifespan. An important increase in energy expenditure did not shorten lifespan or induce an obvious increase in oxidative damage in short-tailed field voles and it had little effect on antioxidant molecules and enzyme levels in most tissues. We have yet to establish when the 'live fast, die young' rule applies.

10.1242/jeb.011528

Selman, C., McLaren, J. S., Collins, A. R., Duthie, G. G. and Speakman, J. R. (2008). The impact of experimentally elevated energy expenditure on oxidative stress and lifespan in the short-tailed field vole *Microtus agrestis*. *Proc. R. Soc. Lond. B Biol. Sci.* **275**, 1907-1916.

Charles Darveau
University of Ottawa
cdarveau@uottawa.ca



sleepless GENE HELPS FLIES TAKE 40 WINKS

Sleep is still an enigmatic phenomenon, mainly because we do not know its precise function and regulation. At least we know that it is essential to our physical and mental health. If we don't get enough, our performance really suffers. The need for sleep varies from person to person and it seems that this has a genetic basis. Identifying genes that affect sleep duration could therefore help us to understand the underlying principles. In a recent article published in *Science*, Amita Sehgal and her team from the University of Pennsylvania report on a gene whose loss of function renders fruit flies sleepless.

In contrast to the common view, sleep is not unique to vertebrates. At the turn of the millennium it became pretty clear that it also occurs in invertebrates, suggesting that sleep fulfils some really fundamental functions. Also, *Drosophila* fruit flies, which are easy to manipulate genetically, experience periods of sleep-like states. Therefore, Amita Sehgal's team dug in their bag of genetic tricks to dissect the sleep phenomenon in these animals. To identify genes involved in sleep regulation, they screened thousands of mutant fly lines for animals exhibiting a reduction in daily sleep. Eventually, they obtained a mutant with an extreme reduction in sleep caused by the functional loss of a gene that they named *sleepless*. The gene encodes a membrane-anchored, glycosylated protein that is significantly enriched in the brain of the flies.

The scientists were careful not to jump to conclusions. They knew that they first had to ensure that *sleepless* is really responsible for the observed phenotype. As the mutation was the result of the insertion of a transposon into the *sleepless* gene, they excised the transposon from the gene to see if its removal restored the daily sleep pattern. Indeed, after doing so the flies slept normally again. Additionally, they re-introduced the wild-type gene into the *sleepless* mutant insects, and this also rescued the sleep deficiency. These and further experiments indicated that *sleepless* is truly required for sleep in *Drosophila*.

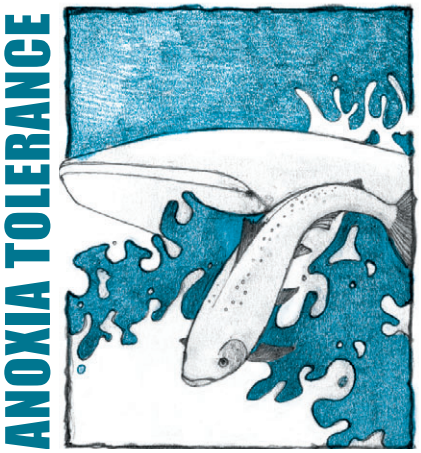
Sleep is controlled by circadian and homeostatic mechanisms. While the circadian clock regulates the timing of sleep, the homeostatic process controls the need for sleep, which – as we all know – increases with length of sleep deprivation. Therefore the team tested whether *sleepless* affects circadian or homeostatic sleep control. Because it is difficult to deprive the already sleep-deprived *sleepless* flies further, the team used other mutant flies, which show less sleep reduction due to incomplete disruption of the *sleepless* gene, to discover whether *sleepless* regulates homeostatic or circadian sleep mechanisms. The finding that these flies showed a significant reduction in rebound sleep, which allows animals to recover from sleep deprivation, but that sleep under normal conditions was minimally affected, suggests that *sleepless* impairs homeostatic sleep control.

A previous study provided exciting evidence that a Shaker-type potassium channel may regulate the need for sleep by affecting neuronal excitability. But how does the *sleepless* gene exert its effects on sleep? A potential mechanism by which *sleepless* controls sleep is suggested by the team's finding that the previously described *quiver* mutant, which contains a mutation known to affect Shaker-dependent potassium currents, is one form of the *sleepless* gene found in fly populations. Thus, is seems that the sleepless protein may convey a signal that connects sleep drive to lowered neuronal excitability. Validating this idea will require more experimental efforts and will certainly cause many additional sleepless nights for both scientists and fruit flies.

10.1242/jeb.011494

Koh, K., Joiner, W. J., Wu, M. N., Yue, Z., Smith, C. J. and Sehgal, A. (2008). Identification of SLEEPLESS, a Sleep-Promoting Factor. *Science* **321**, 372-376.

Hans Merzendorfer
University of Osnabrueck
merzendorfer@biologie.uni-osnabrueck.de



ANOXIC GOLDFISH ARE DEPRESSED, NOT DRUNK

The vertebrate brain is generally highly vulnerable to damage when deprived of oxygen (anoxia); without oxygen, mitochondrial energy production ceases, and ATP-dependent processes fail. Some vertebrates, however, including certain freshwater turtles, the goldfish and the Crucian carp, can survive for weeks or months without oxygen by decreasing ATP demand low enough for anaerobic metabolism alone (lactate production) to provide sufficient energy for survival. One key energy-consuming process known to be downregulated for energy conservation in anoxia-tolerant turtles is ion flux; decreases in ion leakage across cell membranes (channel arrest) reduce the need for ATP-dependent ion pumping, while decreases in calcium influx reduce the likelihood of glutamate release, which would in turn allow calcium into the neuron to trigger cell death. But while turtles essentially enter a 'reversible coma', with little electrical activity or movement, the goldfish and carp remain at least slightly active even in anoxic waters (presumably enabling them to seek out water with more oxygen), and avoid potential lactate poisoning during anaerobic metabolism by converting lactate into ethanol and excreting it across the gills. These differences led Michael Wilkie and his colleagues at Wilfrid Laurier University and the University of Toronto to investigate whether goldfish exhibit channel arrest to conserve energy like anoxia-tolerant turtles.

The team measured the activity of the key glutamate (NMDA) receptor in normoxia and found that NMDA receptor currents in the brain slice decreased by 40–50% within 20 min of anoxic exposure, the first direct evidence of channel arrest in oxygen-starved goldfish.

The team also made other goldfish anoxic in nitrogen-bubbled water, to measure the

activity of the main neuronal ATP-dependent ion pump (Na^+/K^+ ATPase) in the brain, as a decrease in ion flux should be mirrored by a decrease in ion pumping. While the team did find that the fish's Na^+ and K^+ ion pumping activity dropped, it did so more slowly than the NMDA calcium current changes. Immediate changes in NMDA activity and long-term changes in ion pumping together may save considerable ATP and thus allow the fish to survive anoxia, whereas reduced cellular activity in live anoxic fish is reflected by decreased activity and ventilation rate, and loss of balance.

Knowing that the anoxic fish produce ethanol and ethanol reduces neuronal activity in mammals, the team decided to test the activity of the NMDA receptor in the presence of ethanol. They found that it did not change receptor current amplitude, suggesting that ethanol does not change *in vivo* ion pump activity. Thus the reduction in NMDA currents is due to factors other than the general neurodepressant effects of ethanol.

Given that anoxic Crucian carp (a close relative of the goldfish) have shown a general metabolic decrease of about 40%, Wilkie and his colleagues suggest that the behavioural changes associated with anoxia may be explained, at least in part, by alterations in NMDA receptor currents, as decreasing NMDA currents would result in decreased neurotransmitter release and altered neuronal activity. However, the mechanism by which these receptor currents is altered is as yet unknown. Brief bouts of anoxia may raise tissue ethanol levels as high as 5–7 $mmol\,l^{-1}$, enough to depress mammalian neurons, yet Wilkie and his colleagues found that 10 $mmol\,l^{-1}$ did not alter NMDA activity, and others report that ethanol does not alter goldfish activity levels. The combined data suggest that goldfish neurons are resistant not just to anoxia but also to the effects of ethanol – so the blind, tipsy fish you meet on Saturday night gets to claim, 'It's just my NMDA receptors! What's your excuse?'

10.1242/jeb.011510

Wilkie, M. P., Pamenter, M. E., Alkabie, S., Carapic, D., Shin, D. S. H. and Buck, L. T. (2008). Evidence of anoxia-induced channel arrest in the brain of the goldfish (*Carassius auratus*). *Comp. Biochem. Physiol.* doi 10.1016/j.cbpc.2008.06.004

Sarah L. Milton
Florida Atlantic University
smilton@fau.edu