MODELLING WALKING WITH TWO HUMPS

Since the seventeenth century, researchers have divided running and walking into two different mechanical models. Running requires a springy leg that compresses under the body’s mass. This simple ‘spring-mass’ model of running does surprisingly well in capturing the important dynamics of running. In the walking model, by contrast, the body is said to vault over a stiff leg, like an upside-down pendulum. The pivot is where the foot touches the ground. However, this ‘inverted pendulum’ model of walking, despite its nearly universal application, misses important features of how people walk. First, it predicts that we should oscillate up and down much more than we do. Second, and more importantly, it predicts that the foot’s force on the ground should rise smoothly as the body vaults over the leg, then drop off producing a force profile with one hump. Yet, experimental results show a two-humped profile – one force peak at the beginning of stance, when the foot is on the ground, and one at the end.

Hartmut Geyer and his colleagues at Friedrich-Schiller University in Jena, Germany, set out to develop a simple model of walking that would replicate the two-humped force trace seen in experiments. Their key insight, stunning as it may seem, is that walking requires two legs. Both the spring-mass and the inverted pendulum models describe only one leg during the time it’s touching the ground. During walking, though, there are time periods when both feet are on the ground.

So the team put together a simple model with two legs, not one. More complex models had indicated that springy legs might be important not only for running, but for walking, also. Because of this, the group simulated each leg by a massless spring with a fixed resting length that swings around a point mass representing the body. To specify when the legs contact the ground, they set one simple condition – that they must touch down at a defined angle. This condition defines the body’s location relative to the foot when the foot contacts the ground. Once a leg-spring touches down, it begins to compress under the body’s weight. As the body moves forward, the spring begins to lengthen again; when it returns to its resting length, the leg comes off the ground and stops influencing the body until it touches down again during the next step. Modelling both legs in this way results in periods when both feet are on the ground and the body is supported by two legs.

The researchers were then left with three unspecified parameters in their model: the touch-down angle, the leg’s stiffness, and an initial energy which kick-starts the system. When they tuned the parameter values, they found a plethora of different stable gaits. As they hoped, the model can walk with a two-humped force trace. But, in fact, it shows three different versions of two-humped walking – two high-speed walking gaits, and a slower version – plus gaits with three-, four- and five-humped force profiles. Conveniently, at high initial energies, the model also converges to a one-humped gait that turns out to be the standard spring-mass model of running.

In the end, Geyer’s two-legged spring-mass model provides a simple, unified framework for describing the mechanics of both walking and running, as well as some weird patterns that humans don’t seem to use. One model for both gaits not only eliminates the need for the over-simplified inverted pendulum model of walking, but also provides a template for exploring the relative efficiencies of walking and running and the dynamics of transitions between gaits.

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SOCIABLE FLIES DAYDREAM

Sleep remains one of the most mysterious things that animals do. However, a fascinating new investigation by Indrani Ganguly-Fitzgerald and colleagues shows that sleep helps organisms process social information, and they made their discovery in a rather unexpected little creature: the fruit-fly, Drosophila melanogaster.

Most fruit-fly sleep (as defined by prolonged periods of inactivity) takes place at night, but flies can also doze off for several minutes at a time during the day, making up around 40% of their total sleep. Furthermore, if fiendish experimenters keep flies awake, the insects need to recuperate their lost sleep, just like us. Interested in the molecular connection between sleep, social experience and memory, the authors investigated whether flies living in groups slept for longer than their solitary counterparts. Keeping flies in groups and in isolation, the team found that flies kept in groups slept longer during the day than flies housed alone, and slept in bouts approximately 60 min long, compared with 15 min bouts for isolated flies. When the team removed flies from the group and isolated them, they found that this experience affected the amount of daytime sleep: it reduced compared to when the fly was in the group. But isolating crowded flies did not reduce their amount of night-time sleep, suggesting that different processes are involved in the two sleep phases.

Because an individual’s requirements for day-time sleep also increased with the number of flies in its group, the authors investigated the sensory stimuli that enabled flies to detect the presence of other individuals. Blind flies and flies that could not smell showed the same low amount of day-time sleep, whether they were in groups or isolated, suggesting that visual and olfactory inputs are required for social-experience-driven sleep changes.

Next the team investigated the levels of neurotransmitters in the insects’ brains to determine what kind of neurons are involved in sleep. They found that longer-sleeping, socially stimulated flies had three times as much of the neurotransmitter dopamine in their brains compared to isolated flies. When the investigators killed neurons that use this neurotransmitter, crowded flies slept the same amount when isolated, not less, suggesting that these neurons are required for socially driven sleep.

Because dopamine is also known to be involved in memory, the authors then investigated whether socially driven sleep patterns were altered in flies with gene mutations known to affect learning and memory. After studying mutations in 49 different genes, they concluded that flies with long-term memory gene mutations slept the same amount when isolated from the crowd, suggesting that they could not store social information. Some of these genes are expressed in the mushroom bodies in the fly’s brain, which are involved in olfactory learning, implying that social signals are partly processed in this structure. This suggestion was reinforced when the team found that daytime sleep increased in males that had learnt not to court an unresponsive fly. This process is probably mediated by cuticular hydrocarbons, which are also processed in the mushroom bodies, implying that chemical communication may interact with visual and olfactory signals to produce crowded flies’ sleep patterns.

This fascinating study of how social stimuli affect sleep shows the enormous opportunities that exist in investigating complex behaviours in the fruit-fly. It provides information about the specific structures and neural networks that underlie the fly’s day-time sleep, and also gives those interested in vertebrate sleep new tools and approaches for investigating a subject that is vital to every one of us.

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MALARIA SURVIVAL

Exploiting Erythrocytes

Malaria is one of the most deadly infectious diseases that affects humans. Its most severe form, caused by the protozoan parasite Plasmodium falciparum, is transmitted to people by mosquitoes. The parasite has many life-cycle stages, and during the stage when the parasite is free in the human blood stream it invades red blood cells (erythrocytes), where it reproduces asexually. Parasites are dependent on erythrocytes for all their nutrients and one key question scientists want to answer is how a parasite obtains nutrients after it has settled inside its host blood cell. In a recent Nature article Stefan Bröer, Kiaran Kirk and co-workers from the Universities of Canberra and Melbourne, Australia, provide an interesting insight into this question. They show that parasites exploit the high sodium (Na+) levels that they induce in infected erythrocytes to drive the uptake of inorganic phosphate (Pi), an essential nutrient that is required to synthesise important molecules such as nucleic acids.

First the team confirmed that parasites need Pi. They monitored parasite growth in the presence and absence of Pi by measuring the rate at which radiolabeled DNA precursors were incorporated into newly synthesised DNA molecules. Parasites, still inside their host erythrocytes, stopped reproducing almost completely when the researchers removed Pi from their laboratory culture, showing that they need Pi to reproduce. This also suggested that they possess an uptake system to extract Pi from the erythrocyte’s cytoplasm. To investigate the mechanism by which Pi is taken up, the scientists isolated the parasites from their host cells, placing them in another laboratory culture and measured them taking up radioactive 32Pi. They observed that the parasites took up forty times more 32Pi when there was
Na⁺ in the extracellular medium. Replacing Na⁺ with either K⁺ or choline stopped Pᵢ uptake almost completely, so the scientists concluded that Pᵢ uptake involves a Na⁺-dependent transporter in the cell membrane.

Analysing the transport characteristics in closer detail indicated that the parasite takes up two Na⁺ ions with each Pᵢ molecule. Since single negatively charged hydrogen phosphate turned out to be the preferred transport substrate, Pᵢ uptake appears to be electrogenic, meaning that it is driven by an electric potential caused by an ion imbalance across the parasite’s plasma membrane.

Next the researchers tracked down the gene coding the transporter. As the Plasmodium genome contains only a single gene for a plasma membrane Pᵢ transporter, called PfPiT, the scientists suspected that this transporter could account for Pᵢ uptake by the parasite. They discovered that PfPiT is found on the parasite’s surface and is expressed throughout the entire blood stage of its life cycle, which supported their assumption that this transporter allows parasites to take up Pᵢ. To provide final proof for PfPiT’s supposed function in parasites, the team expressed the protein in Xenopus oocytes by injecting its RNA. They measured the uptake of radioactive ³²Pᵢ in injected and non-injected oocytes, observing uptake of ³²Pᵢ in the eggs containing the parasite transporter RNA only, which was clearly caused by PfPiT expression.

The researchers think that the rise in Na⁺ levels in infected erythrocytes is vital for the parasites’ survival as it means they can take up solutes such as Pᵢ via Na⁺-dependent transporters. These results will help researchers understand further the malaria parasite’s physiology and could pave the way for new strategies to combat malaria.

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biologists, this approach clearly does not work on extinct animals. So just how can you measure an extinct animal’s body temperature? Gillooly and his team Andrew Allen and Eric Charnov solved this problem by developing a model based on modern warm and cold blooded animals that predicts their maximum growth rate according to the creature’s body temperature and size. The team then ingeniously applied this model to dinosaur growth rates and body sizes to estimate the extinct creatures’ body temperatures.

Gillooly and colleagues predict that larger dinosaurs had higher body temperatures than smaller dinosaurs, supporting Spotila’s 1970s ideas. When they put dinosaur body size data into their model, the results supported this relationship: larger dinosaurs would have been warmer than smaller dinosaurs. At 13,000 kg Gillooly estimates that Apatosaurus excelsus had a body temperature of approximately 41°C, while at the opposite end of the size range the 12 kg Psittacosaurus mongoliensis had a body temperature of about 26°C.

Finally, Gillooly’s team needed to test the model in a living cold blooded animal to see if their predictions were on the right track. They used temperature and body mass data for crocodiles, the dinosaur’s closest living relatives, to test temperature variations with body size. The body temperature changes estimated by the model closely agreed with the temperature fluctuations measured in crocodiles over a range of sizes. Their results provide some of the first evidence that dinosaur body temperatures increased with body size, supporting the idea that larger dinosaurs could keep their heat when the weather got a bit chilly.

Gillooly, J. F., Allen, A. P. and Charnov, E. L. (2006). Dinosaur fossils predict body temperatures. PLoS Biology 4, e248. DOI: 10.1371/journal.pbio.0040248.

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HYPOXIC BRAIN CELLS LOSE THEIR INHIBITIONS

The brain is the most energy hungry organ in mammals, and more than 95% of the ATP it consumes is normally derived from aerobic respiration; up to 40–50% of this energy is used by neurons just to drive ion pumps. Thus a fall in oxygen levels (hypoxia) can rapidly deplete ATP levels, with catastrophic consequences. Fortunately, cells can sense and respond to low oxygen levels: originally scientists thought that most cells detect low O2 when cellular ATP levels drop, but it is now apparent that many cells respond to hypoxia before ATP levels reach a critical low, implying that they need a specific sensor. One candidate is the enzyme cytochrome oxidase (COX). COX gives up two electrons to oxygen to create oxygen ions, which combine with hydrogen ions to form water in the final step of aerobic respiration. In this way, COX activity and oxygen levels determine ATP production. A recent Journal of Neurochemistry paper by Susann Horvat and her colleagues at the Max Delbrück Center for Molecular Medicine, Berlin, characterises how COX activity changes with changing oxygen levels in two neural cell types: neuron-supporting astrocytes and granule cells, which are a type of neuron.

First the team exposed the cells to hypoxia, 5% oxygen, and monitored cell death. The cell types responded very differently: cell death did not increase in astrocytes and their ATP levels decreased by only 9%, suggesting that when aerobic respiration is no longer sufficient to meet their energy needs, astrocytes can switch to anaerobic respiration. By contrast, approximately 29% of the granule cells died, and their ATP levels decreased by 31%. These results show that astrocytes change to anaerobic glycolysis in the absence of oxygen, while granule cells die because they are more dependent on O2.

Knowing that COX comprises several different subunits and the enzyme’s activity is regulated by expressing different forms (isoforms) of some of these subunits, the team looked at COX subunit transcription patterns and found significant changes in COX transcription and activity. mRNA levels of one of the subunits, the COX IV-1 isoform, did not change with hypoxia in either cell type. In contrast, the COX IV-2 isoform increased in both cell types with hypoxia, showing that changing O2 levels regulate COX by affecting transcription. While astrocytes had no detectable COX IV-2 isoform in normoxia, approximately 10% of total COX transcription in granule cells was the COX IV-2 isoform, which affects overall enzyme kinetics.

COX activity is also regulated by the amount of ATP in a cell, as it binds to the COX IV subunit. When the team measured COX enzyme kinetics under normoxic and hypoxic conditions, they found that ATP inhibition of COX activity changed in the different cell types depending on which COX IV isoform is expressed. In high O2 conditions ATP usually inhibits COX, but in granule cells, which express high levels of COX IV-2, this inhibition didn’t occur. In normoxic astrocytes, ATP inhibited COX, but the inhibition disappeared as COX IV-2 mRNA levels increased. COX activity, however, still decreased in hypoxic astrocytes, providing a mechanism for astrocytes to change from aerobic to anaerobic respiration.

The authors propose that even low levels of COX IV-2 prevent inhibition of COX by ATP in granule cells, keeping the enzyme active even at high ATP levels. Since they found COX activity was also high in hypoxic granule cells, the neurons probably continue to meet their high energy demands by maintaining aerobic enzyme activity and ATP levels. However, this continued energy production comes at a price: it may create damaging reactive oxygen species and increase cell death. Apparently then, as in so much in life, losing your inhibitions can be very bad for your health!

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