

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.

SELF AWARENESS



ARE ELEPHANTS SELF-AWARE?

Virtually everyone has wondered which animals are conscious. Post-graduate student Josh Plotnik and his colleagues have just extended the list of candidates to the elephant. Or, more precisely, to *an* elephant.

Part of the problem with studying consciousness is measuring it. One method is to test for 'mirror self-recognition' (MSR), where an animal is marked on a part of its body it cannot see and is then put in front of a mirror. If the animal shows interest in the mark on its own body, then scientists assume that the animal recognises itself in the mirror. MSR is seen only in the hominoids (humans and apes), and possibly in dolphins. MSR may be related to the existence of empathy – the ability to understand another's feelings – since human children develop these two abilities more or less simultaneously. Plotnik and his collaborators, Frans de Waal and Diana Reiss, therefore decided to test for MSR in the elephant, which is reputed to be highly empathetic.

Three female Asian elephants at New York's Bronx Zoo – Happy, Maxine and Patty, all in their 30s – had a jumbo-sized mirror placed in their enclosure. Video recordings revealed behaviours not seen in the mirror's absence: the elephants did not show aggressive or social behaviours to their reflection, but would instead bring food to eat in front of the mirror, or would inspect parts of their body with their trunk. These behaviours suggested that they realised the elephant in the mirror was themselves.

In the final phase of the experiment, to try and test more conclusively for MSR, each elephant was marked with a cross above each eye. One cross was painted with white pigment, the other with a compound that

was chemically identical, except that it was invisible. The mirror was then uncovered, and each animal's behaviour observed.

A striking movie published as an on-line supplement to the article shows one of the elephants – Happy – standing in front of the mirror and repeatedly touching the visible mark with her trunk. In fact, Happy touched her face significantly more often during the mark test than in other phases of the experiment. Furthermore, Happy tried to touch only the visible mark, not the invisible control cross above her other eye. The authors conclude that Happy showed MSR, during this experiment at least. However, neither Maxine nor Patty displayed a similar ability, although they showed what appeared to be self-directed behaviour in front of the mirror. When all three were tested again on two subsequent occasions, none of the elephants touched the marks, and were not considered to show MSR.

The authors argue that the fact that all the elephants were interested in the mirror strongly suggests they do have the capacity for self-awareness, but that this particular test may not be an appropriate measure of MSR. This is because elephants regularly cover themselves with dust, changing their appearance. A small cross on their brow might be irrelevant to them. Also, it's possible that not all individuals are self-aware.

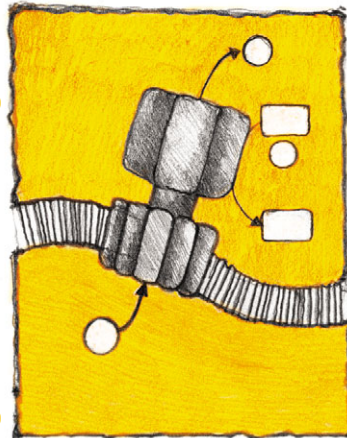
This study highlights that while evidence from single individuals is striking, it can also be ambiguous. Happy's behaviour could have been a statistical freak, but the data, and the accompanying movie, are very impressive. It is extremely difficult not to get the very strong impression that she is, indeed, studying her face, using her reflection as a guide. Happy challenges our preconceptions about animal behaviour and should encourage researchers to investigate MSR further in both elephants and other animals. Hominoids may not be as unique as we like to think.

10.1242/jeb.000745

Plotnik, J. M., de Waal, F. B. M. and Reiss, D. (2006). Self-recognition in an Asian elephant. *Proc. Natl. Acad. Sci. USA* **103**, 17053-17057.

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SPIDER VENOM



A HOT VENOM

Searing pain can drive sufferers mad, and many poisonous animals such as spiders exploit this fact for their defence. When they feel threatened, they bite and inject venoms that are extremely painful, warding off any potential predator. Recent research published in *Nature* by David Julius and his co-workers from the University of California, San Francisco, has now identified toxins in a spider's venom that resemble molecules from hot chilli peppers, in that they target the same pain receptors as these molecules.

Pain is caused by the activation of specialized nerve cells carrying receptors that are susceptible to capsaicin, the molecule that causes the burn of hot chilli peppers. One of the scientific highlights of 1997 was when Michael Caterina and his colleagues showed that the capsaicin receptor is a heat-activated ion channel involved in the pain pathway. Subsequent research revealed that this receptor belongs to a family of proteins called transient receptor potential (TRP) channels. When a TRP channel in a nerve cell membrane is activated by capsaicin or heat, it opens and forms a pore; calcium ions flow into the cell, generating electrical signals that are transmitted to the brain, signalling pain.

While the components of spider venoms that cause paralysis, inflammation and shock have been extensively studied in the past, little is currently known about pain-generating molecules. Julius and his co-workers addressed the question of which molecules in the spider's venom actually produce pain by designing ingenious experiments allowing them to test many different types of venoms for their ability to activate TRP channels.

For this purpose, the team cultured human kidney cells, which had been genetically altered to produce different varieties of

TRP channels on the cell surface. They monitored the activation of TRP channels using a fluorescent dye that lit up when calcium ions flooded into the cells. When the scientists tested the venom of *Psalmopoeus cambridgei*, a West Indian tarantula, they observed calcium influx in kidney cells carrying the capsaicin variety of TRP receptor. To isolate the venom molecules causing this response, they broke the venom down and identified three peptides, which they named vanillotoxins; each of them activated the capsaicin channel separately.

Next, the team wanted to know if the vanillotoxins also stimulated sensory nerve cells that have the capsaicin receptor on the cell surface. They added the isolated peptides to the laboratory culture of nerve cells from normal mice and from genetically manipulated mice lacking the capsaicin channel and measured their response. Again using the calcium-sensitive fluorescent dye, they observed significant calcium influx only in nerve cells from normal mice but not in those from deficient mice. Looking at the effect of the toxins in the live mice, the mutant mice lacking the capsaicin channel appeared to be insensitive to pain and inflammation that could be provoked in normal mice by capsaicin injection.

Julius and his team found out that organisms as distantly related as hot peppers and tarantulas produce molecules that activate the same receptor channel, producing strong pain. The discovery that vanillotoxins open these channels may provide new tools that could help in understanding TRP channel properties. Understanding the mechanisms that activate TRP channels may also help researchers exploring the pain receptors that are involved in certain types of chronic pain in humans.

10.1242/jeb.000521

Siemens, J., Zhou, S., Piskorowski, R., Nikai, T., Lumpkin, E. A., Basbaum, A. I., King, D. and Julius, D. (2006). Spider toxins activate the capsaicin receptor to produce inflammatory pain. *Nature* **444**, 208-212.

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FLY MUSCLE



LIMITS OF INSECT MUSCLE FUNCTION

Muscles contract when one protein molecule in a muscle fibre, myosin, pulls on another protein, actin, similar to a team of people pulling a chain (actin) hand over hand. But, the myosin arms can only bend at the elbow joint to achieve movement. To relax the muscle, the arms unbend and let go of the chain. Extraordinarily, some insects can contract and relax their flight muscles at a rate of 200 times per second, around 10 times faster than muscles in a similar sized non-flying insect. Because the muscles from most animals are incapable of such an Olympian performance, biologists are interested to know how insect flight muscles operate so quickly and what prevents them from operating at even higher speeds.

Douglas Swank, Vivek Vishnudas and David Maughan from the Rensselaer Polytechnic Institute, New York and the University of Vermont explore this question in a recent article using *Drosophila melanogaster* flight muscles. Muscles consume energy for contraction by breaking down adenosine triphosphate (ATP), which is bound to myosin in the presence of calcium, to adenosine diphosphate (ADP) and inorganic phosphate (P_i). This allows the myosin arms in a muscle fibre to bend and pull the actin chain, causing contraction. The concentration of ATP, ADP and P_i affect the speed of this chemical reaction, and hence contraction speed. Furthermore, P_i can bind to myosin, which prevents ATP binding and indirectly inhibits contraction.

The team already knew that flies with a mutation in their myosin, causing it to behave like a myosin found in slow twitch muscles, had flight muscles that couldn't contract or relax as fast. But, the length of pull for each myosin arm on the actin chain remained constant, suggesting that

biochemical, not mechanical, factors were limiting contraction speed.

To investigate what these biochemical factors were, they manipulated the ATP and P_i concentrations in muscle fibres containing the slow myosin and fast flight muscle myosin and made two striking observations. First, fast flight muscle fibres needed a very high concentration of ATP to work and a 7-fold higher ATP concentration to contract by the same amount as slow fibres.

Second, P_i caused the two fibre types to respond differently to ATP. For example, the contraction frequency at which slow muscles achieved maximum force output increased as P_i concentration increased. In fast muscles, the maximum force output decreased as P_i concentration increased, and the contraction frequency producing this maximum force remained constant. This suggested that P_i wasn't competing with ATP for myosin binding sites in slow muscles. Fast muscle bound less ATP as P_i concentration increased, due to competition between the molecules for binding sites on the myosin. These results suggested that ATP affinity in flight muscle fibres is much lower than in slow muscle fibres and also that different biochemical factors were limiting contraction rates in the two fibre types. Using a model for biochemical reaction kinetics, the team confirmed that P_i release was indeed the rate-limiting factor of the high-frequency contractions of insect flight muscles.

Swank and colleagues reasoned that *Drosophila* could compensate for low ATP affinity by increasing intracellular ATP concentration in the fast muscles to promote ATP binding over P_i binding. They suggested that controlling ATP concentration could be a unique mechanism whereby *Drosophila* achieve an optimum balance between muscle contraction frequency and power production, since contraction frequency is dependent on ATP concentration. This enables insects to achieve superior muscle performance and ultimately overcome the energetic demands of aerial flight.

10.1242/jeb.000752

Swank, D. M., Vishnudas, V. K. and Maughan, D. W. (2006). An exceptionally fast actomyosin reaction powers insect flight muscle. *Proc. Natl. Acad. Sci. USA* **103**, 17543-17547.

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INSIGHT FROM DELETIONS

Many times in experiments, there will be a few pieces of data that don't make sense. Confronted with an odd data point or two, most researchers will think, 'Huh. That's weird,' and move on. David McCrea from the University of Manitoba, on the other hand, has found a way to make his weird data points work for him.

He works on the neural circuitry for walking in cats, called the central pattern generator (CPG), which is contained in the spinal cord. Using the right sort of stimulation, the cat spinal cord (or in fact almost any vertebrate spinal cord) will 'walk' even with the muscles paralyzed and with no connection to the brain. Activity in nerves running to the limb muscles looks more or less like activity during walking: flexor muscle nerves generally alternate with extensor muscle nerves, and within that alternation, muscle activity is ordered more or less appropriately. But there is often a strange effect: sometimes certain nerves 'forget' to turn on or off. The rest of the pattern may stop as one nerve fires anomalously or the rhythm may keep on going, roughly as usual. And sometimes the CPG keeps time throughout the 'deletion', but sometimes it doesn't.

Most other researchers had shrugged off deletions as just another weird effect to be avoided, but McCrea realized that they might tell him something about the underlying structure of the CPG. So he teamed up with Ilya Rybak of Drexel University College of Medicine to produce a mathematical model of the CPG that could occasionally 'forget' to turn on one muscle, but not lose its rhythm.

They decided that they'd have to make a CPG model with two levels: a higher-level

'rhythm generator' to produce the basic timing and a lower-level 'pattern generator' to give the characteristic ordering of muscle activity. Using a standard Hodgkin-Huxley neural model, which models the characteristics of electrically excitable cells like neurons, they made a simulation of a pair of generic flexors and extensors, with one excitatory neuron at each level for the flexor and one per level for the extensor, a total of four main neurons. They then added in four inhibitory neurons, so that the flexors would inhibit the extensors and *vice versa*. This crossed inhibition, combined with a slow self-inactivation built into each neuron, results in rhythmic alternation; first the flexor neurons start firing, inhibiting the extensors, but they slowly deactivate themselves until the extensor neurons can come on, inhibiting the flexor, and so forth.

Deletions, they thought, might come from temporary fluctuations in the excitability of the CPG neurons. Increasing the excitability of an extensor neuron in either level, for example, keeps the extensors on, swamping the usual alternation with the flexors. But exciting the extensor neuron in the rhythm generating level resets the rhythm, while exciting the same neuron in the pattern generating level doesn't: the CPG keeps time, even though the flexors don't fire.

McCrea and Rybak's model can replicate some of the subtleties of deletions. For example, in the cat data, they would sometimes see the flexors turn on, firing continuously for a while, with little or no activity in the extensors. Other times, the flexors might go silent while the extensors kept up their rhythm as usual. In the model, exciting the pattern generating flexor neuron creates the first effect, while inhibiting it results in the second.

The two-level model is still a hypothesis – no one knows whether there really are 'rhythm generator' and 'pattern generator' neurons in the spinal cord – but it predicts differences in the behaviour of the two classes of neurons, particularly during deletions. Now the goal is to see whether they can find spinal neurons that match their predictions.

10.1242/jeb.000505

Rybak, I. A., Shevtsova, N. A., Lafreniere-Roula, M. and McCrea, D. A. (2006). Modelling spinal circuitry involved in locomotor pattern generation: insights from deletions during fictive locomotion. *J. Physiol. (Lond.)* **577**, 617-639.

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HIBERNATION



HIBERNATING HAMSTERS REALLY DISCONNECT

When the weather closes in and winter threatens to bite, many animals hibernate to see them through the harsh winter months. During hibernation, metabolism is down-regulated by decreasing energy expenditure for long periods and reducing an animal's heart rate and blood flow, causing hypothermia, or torpor. These periods of torpor are periodically interrupted by short intervals of rewarming to euthermia, or normal body temperature. These rapid increases in metabolism and oxygenation can cause physiological stress and cellular damage. Scientists are interested in how animals survive during both torpor and rewarming, but the triggers are not fully understood. One candidate is the brain's hippocampus, as it is one of the first brain areas to regain normal EEG activity as the arousal process begins. This led Ana Magarinos and her colleagues at the Rockefeller University in New York and the Université Louis Pasteur in France to

investigate changes in the hippocampus during the stress of torpor and arousal.

Structures within the hippocampus of birds and mammals mediate spatial behaviors such as the storage and retrieval of food within their territory, and the connections between neurons can be altered by repeated stress. During torpor, exploratory behavior such as searching for food halts temporarily, so the research team wondered if this might be caused by reversible changes in hippocampal structure during the stress of hibernation and arousal.

To investigate, they placed wild-caught European hamsters (*Cricetus cricetus*) in a 7°C cold room on a 24 h cycle of 8 h light and 16 h dark in the late autumn, monitoring hibernation bouts using implanted thermosensitive transmitters that measure body temperature. After normal torpor and arousal bouts were established, they removed the brains from active euthermic, hibernating or recently aroused animals and made slices, staining them to reveal neuronal structures.

To find out if hippocampal structure changed during hibernation, the team first analyzed the length and branching patterns of the neurons' dendrites, which link one neuron to many others and facilitate communication. They discovered that in torpid hamsters a type of hippocampal neuron called CA3 cells, which play a critical role in spatial memory, had shortened dendrites with less complex and less dense branching patterns than in active hamsters. This simplification of neuronal connections could limit excitatory input and be linked to behavioral suppression during torpor. In recently aroused hamsters, however, the dendritic simplification was

rapidly reversed and was similar to branching patterns seen in active hamsters.

The team also analyzed the number of visible 'spines' on the neurons, which receive synaptic inputs, and synaptic vesicle density, which would tell them how strongly neurons could communicate with each other. Not only were dendritic spines on the post-synaptic CA3 cells smaller, but the pre-synaptic cells sending the signals had fewer synaptic vesicles, further reducing excitatory input to the CA3 neurons. By contrast, they saw no changes in pyramidal neurons which are outside the hippocampus, and are not thought to play a role in hibernation.

The authors suggest that the simplification of dendritic branches helps limit excitatory input during torpor and may also interfere with the processing of incoming information. However, these changes are rapidly reversible, restoring normal connections, and function, during arousal. Research in other labs has shown that rodents perform better in water mazes as day length increases and that there is a direct correlation between hippocampal size and spatial ability. Which means that if you feel slow and stupid in the winter, blame it on your inner hamster!

10.1242/jeb.000513

Magarinos, A. M., McEwen, B. S., Saboureau, M. and Pevet, P. (2006). Rapid and reversible changes in intrahippocampal connectivity during the course of hibernation in European hamsters. *Proc. Natl. Acad. Sci. USA* **103**, 18775-18780.

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