Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

COCAINE STOPS VICTIMS FROM MASKING MEMORIES

Cocaine is a powerful and highly addictive drug that has wrecked countless lives since its isolation in the 19th Century. Addicts are left with powerful cravings for years after kicking the habit, but no one knows how the drug retains its hold. Barbara Sorg and her colleagues from Washington State University and the University of Calgary explain that cocaine profoundly affects regions of the brain associated with memory formation; could the addicts' drug-altered memories hold the key to their addiction? Sorg suspected that cocaine either produced an extremely powerful initial memory that was hard to eradicate, or the drug impaired the brain's ability to 'extinguish' the addiction memory by learning new habits that mask the old. But memory formation in human brains is highly complex, so Sorg and her colleagues decided to investigate the effects of cocaine on learning and memory extinction by turning to a creature with a much simpler memory network: the snail *Lymnaea stagnalis*, which regulates its breathing patterns with a scaled down 3-neurone network (p. 4273).

Knowing that dopamine is a key neurotransmitter involved in memory formation, the team began by testing the effects of cocaine on dopamine uptake in the snail's brain, and found that even a dose as low as 0.1 mol l^{-1} of cocaine reduced the snail's dopamine uptake significantly. Having established the level of cocaine that affected dopamine uptake and possibly memory formation, the team needed to test the drug's effects on the snail's breathing behaviour.

Sorg explains that in well oxygenated water, *Lymnaea* breathes through its skin, but as the oxygen levels drop the mollusc extends its pneumostome (a breathing tube) above the surface to breathe air and supplement the water's dwindling oxygen supply. After exposing the snails to 0.1 mol l^{-1} of the drug for an hour a day over a 5 day period, the team monitored the snail's breathing patterns when transferred to de-oxygenated water. The molluscs used their pneumostomes more often than they had before cocaine exposure, possibly because the drug subtly changed their metabolism.

Knowing that the snail's behaviour changed a little after drug exposure, the team tested the drug's effects on the snail's ability to form memories. They taught the molluscs to keep their pneumostomes closed at low oxygen levels by gently tapping them on the breathing tube when they came up for air, and then checked the snails' memories a day later. Both the cocaine-treated and 'clean' snails remembered to keep their pneumostomes closed. Cocaine hadn't altered the treated snails' ability to form a memory, so Sorg turned to her second hypothesis; had the addicted snails lost the ability to 'extinguish' the trained memory (to keep their pneumostomes closed) with a new memory to keep it open?

The team retrained the molluscs, this time allowing them to breathe uninterrupted when they extended their breathing tubes. When the team once again tapped the 'clean' snail's pneumostomes, the snails continued breathing through them. The extinction training had worked and masked the original training. But when they tested the cocaine-treated molluscs with a gentle tap, the molluscs instantly closed their pneumostomes. Instead of recalling their new training, they returned to their old habits. They'd failed to extinguish the memory.

Having found that cocaine strongly interferes with memory extinction, the team are keen to identify the 'fundamental processes underlying extinction learning' says Sorg, and adds that this may 'have important applications for treatment in cocaine addiction'.

10.1242/jeb.02569

Carter. K., Lukowiak, K., Schenk, J. O. and Sorg, B. A. (2006). Repeated cocaine effects on learning, memory and extinction in the pond snail *Lymnaea stagnalis*. *J. Exp. Biol.* **209**, 4273-4282.

HOW SPIDERS SPIN

There are almost as many anecdotes about man trying to harvest spider silk as web styles; even Napoléon tried farming the territorial arachnids in a bid to harness the robust material. However, despite decades of intense effort and millions of research dollars, no one has successfully spun synthetic spider silk to the specifications that spiders achieve effortlessly. Gareth

McKinley realised that although a great deal was known about the spun thread's composition and mechanical properties, few had studied the silk solution's behaviour as it is extruded from the animal's major ampullate gland. Knowing that polymer solutions often behave in strange and unexpected ways when squeezed thorough microscopic apertures, McKinley decided to study the material properties of natural spider silk solutions on a microscopic scale. When biological engineer Nikola Kojiç joined the MIT lab, McKinley knew he'd found the right person to begin unlocking the spinner's secrets (p. 4355).

But before Kojiç could begin testing the fluid's flow properties, he had to learn how to extract the microscopic volume of silk protein solution stored in the golden orb spider's major ampullate gland. Kojiç visited Marian Goldsmith's lab at the University of Rhode Island where he learned to extract silk protein solutions from silk worms before he tried his hand on the rarer golden orb spider. Having successfully extracted a few microlitres of the scarce solution Kojiç explains that he had to work fast, keeping the gel-like solution under water to prevent evaporation, to measure the fluid's flow properties.

First Kojiç measured the thick solution's viscosity using Christian Clasen's flexure-based micro-rheometer. Shearing 0.7 μl of the sample between two glass plates, the pair found that the silk solution's viscosity decreased dramatically as the plates moved faster. The silk solution was becoming increasingly slippery until it was even slipperier than silicone oil. McKinley admits that he was surprised at how slippery the material became, but adds that it explains how spiders extrude the thick gel to produce the delicate thread. According to McKinley, the shearing process aligns the long protein polymers in the silk solution until the molecules begin slipping easily past each other and the viscosity drops dramatically.

Next the team tested the protein solution's stickiness by placing 1 μl of the sample between two small plates on an extensional rheometer, built by José Bico. Pulling the rheometer's plates 5 mm apart to produce a thin filament of the gooey solution, Kojiç measured the filament's diameter as it thinned and found that the silk solution was as sticky as egg white, becoming stiffer as the thread dried.

McKinley explains that many of the solution's unique properties boil down to the protein's long molecules, which are relatively entangled at high concentrations making the solution highly viscous. However, as the solution is squeezed through the spider's ampullate gland the molecules become aligned, sliding past each other easily as the viscosity drops before the sticky silk is extruded into a fine filament, drying to form one of the toughest materials known to man.

10.1242/jeb.02570

Kojić, N., Bico, J., Clasen, C. and McKinley, G. H. (2006). *Ex vivo* rheology of spider silk. *J. Exp. Biol.* **209**, 4355-4362.

DEEP SEA DIVERS

Picture by Natacha Aguilar Soto

Dive a few hundred metres beneath the ocean's surface and the environment changes rapidly, becoming darker and colder as the pressure rises. It is amazing to think of warm-blooded mammals in such an inhospitable environment, yet this is the domain of whales and dolphins. Beaked whales are particularly at home in deep water, as Peter Tyack and his colleagues discovered when they tagged two species and monitored their dives. They found that beaked whales repeatedly make long dives as deep as 2 km in search of squid, making their average foraging dives deeper and longer than any other air-breathing animal (p. 4238).

It is no surprise then that little is known about beaked whales. 'They are not deep divers, rather they are occasional surfacers,' says Tyack. He explains that the animals only surface between dives for a few seconds, making it difficult to track them. To study two beaked whale species' normal diving behaviour, Tyack and an international team of collaborators used a tagging device recently designed by Mark Johnson to measure various aspects of the animal's dive, including their depth and pitch as they glide through the depths.

The team found that both species of beaked whales have similar dive behaviour. First, a deep dive typically reaches depths of between 800 and 1000 m and lasts 45 min to 1 hr. After surfacing briefly, both species start a lengthy cycle of increasingly shallow dives before the whole process starts again. 'Once they are deeper than 100 m, the rest of the dive is thought to have little impact in terms of nitrogen saturation of the blood,' explains Tyack as he describes how the lungs collapse due to water pressure, preventing gases passing between them and the blood. He suspects that these relatively small whales cannot store sufficient oxygen to fuel a dive aerobically, so they switch to anaerobic metabolism as their oxygen reserves dwindle. Tyack believes that the shallow diving between deep dives helps these whales clear the unfavourable by-products of anaerobic metabolism, such as lactic acid. He likens it to athletes who clear lactic acid from their overworked muscles by gentle exercise, to speed their recovery. Tyack also believes that these dives help beaked whales avoid predators such as killer whales and white sharks, which rarely venture deeper than 20 m.

So why do beaked whales expend so much energy plumbing the depths? Like other deep divers they dive to catch their favourite food: squid. Although the tags showed that they clearly hunt prey, they also revealed something unexpected. 'After diving so far, beaked whales pass hundreds of prey before selecting one to hunt down', he says, but why and how they choose their target remains unclear.

Having detailed the enigmatic mammal's diving behaviour, Tyack is keen to point out that there is a serious reason for studying these animals beyond simple curiosity. He explains that recent mass strandings of beaked whales have been associated with military use of sonar. Autopsies showed that the carcasses had symptoms consistent with decompression sickness, challenging the view that the whales' physiology had evolved to protect against decompression. Could the whales prevent decompression through their normal diving behaviour? Tyack's whale tracking data suggests this is the case, and the team predicts that if sonar does cause decompression sickness, it is because it alters the normal dive behaviour of beaked whales in a way that leaves them vulnerable to stranding.

10.1242/jeb.02572

Tyack, P. L., Johnson, M., Aguilar Soto, N., Sturlese, A. and Madsen, P. T. (2006). Extreme diving of beaked whales. *J. Exp. Biol.* **209**, 4238-4253.

Sarah Clare

TOADFISH CLOAKED IN UREA

Pete Jeffs is an illustrator living in Paris

When it comes to getting rid of your own waste, it's often best to go for the low cost option. However, when it comes to disposing of metabolic nitrogenous waste, the cheapest alternative, ammonia, is also the most toxic. This isn't a problem for most fish as the toxin is quickly diluted in their environment, but toadfish still seem to go with the high cost alternative: urea. Why toadfish excrete urea had puzzled scientists for decades, until John Barimo and Pat Walsh began wondering what effect urea has on species that prey on these coastal fish. Focusing on the gulf toadfish and one of its predators, the gray snapper, Barimo and Walsh tested how the gray snapper reacted to ammonia and urea in a natural setting. They found that ammonia attracted the predator to the toadfish's hiding place at levels as low as 55 nmol N l^{-1}. However, when the team released mixtures of ammonia and urea from simulated toadfish lairs, gray snappers were much less attracted to the ammonia; urea seemed to 'cloak' ammonia and the toadfish's presence (p. 4254). Barimo and Walsh suspect that the fish have opted for this costly waste disposal alternative to conceal themselves from predators, as the benefits far outweigh the expense.

10.1242/jeb.02571

Barimo, J. F. and Walsh, P. J. (2006). Use of urea as a chemosensory cloaking molecule by a bony fish. *J. Exp. Biol.* **209**, 4254-4261.

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