

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.

SENSITIZATION



I FEEL YOUR PAIN

Pain is not pleasant. We try to avoid it at all costs. Even witnessing another in pain can cause one to feel pain, particularly if it is someone we know. This ability to empathize has been thought to be uniquely human. However, Dale Langford and colleagues provide evidence in a recent *Science* publication that this ability may also exist in mice. Other studies had shown that pain-related distress of a conspecific can serve as an aversive stimulus in non-primates, but Langford and colleagues have demonstrated that the *degree* of pain can be modulated by the presence or absence of a conspecific in similar distress.

The authors first injected mice with a substance that induced pain behaviour, in this case writhing. One group of mice was left in isolation while individuals from a second group were placed in the company of another mouse that had either been injected with the same substance or was uninjected. The team noticed that the injected mice that were left alone and the mice that were placed with an uninjected mouse both exhibited the same levels of pain behaviour. However, mice placed in the company of another injected mouse displayed significantly more pain behaviour than the other groups; i.e. seeing another mouse in pain increased their own pain behaviour. There was just one catch though: pain behaviour only increased if the accompanying injected mouse was a cagemate and not if it was a stranger. Thus, injection and the mere observation of a cagemate in similar distress produced significant hyperalgesia, suggesting that the pain system can be sensitized by the observation of pain in someone familiar.

In a separate test, another pain behaviour, paw licking, was induced by injecting either a low or high dose of formalin. In those that received a low dose and observed a cagemate that received a high dose, there was an increase in pain

behaviour compared to mice that received a low dose and were left in isolation; an effect similar to that seen in the previous experiment. Again, the changes in pain behaviour were only seen if the other mouse was familiar. Surprisingly, there was a reduction in pain behaviour in those mice that received a high dose but observed a cagemate that received a low dose. Thus, observation not only enhances pain but can also reduce it.

What would be the advantage of not just identifying and feeling the pain of another, but to have your own pain compounded? Perhaps it evolved from a need to recognize pain in others in order to avoid noxious stimuli, an ability valuable to all organisms. There have been reports of 'mirror neurons' in the brain, which fire not only when one is performing an action but also when the same action is performed by another, and may provide a possible mechanism for the 'mirror pain' seen here. Interestingly, autistic children show no mirror neuron activity while imitating and observing emotional expressions and it has been suggested that a dysfunctional mirror neuron system may underlie the social deficits observed in autism.

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KEEPING TRACK OF THE STEPS

Many insects are known to navigate between their home and feeding sites with remarkable precision. This is also the case for the desert ant *Cataglyphis fortis*, which navigates in the vast and largely uniform Sahara desert by continuously keeping track of the direction and distance to their nest. This is a process known as path integration, which allows them to take the direct route home once they have found food, instead of following the sometimes tedious route used during their outbound search. While it has been shown that these ants use visual cues from the sky to keep track of the direction they have taken, less is known about how they judge the distance needed to return, but it seemed certain that they must use an odometer.

There are several hypotheses that could explain how their inbuilt odometer works but the most likely is that they assess distance by a step integrator (a pedometer) that in some way keeps track of how many steps they use. However, this hypothesis had not been tested until a group of scientists from University of Ulm and University of Zürich examined what happened to the ant's sense of distance when their legs were artificially shortened or lengthened.

The group trained ants to travel along a straight 10 m channel between the nest hole and a feeding site, so that the ants walked directly to the feeding site and collected a food item before turning around and heading home. When the ants were caught at the feeding site they remembered the direction and distance to home for a considerable time afterwards. So their idea of distance could be tested by placing them in an adjacent channel, without a nest hole, and measuring how far they walked before beginning to search for their 'fictive' nest. In this experimental setup the group showed that 'control' ants walked an

average of 10.2 m before searching for the nest hole. The group then examined what happened when ants had their legs shortened or lengthened artificially before starting the homebound journey, and found that the ants misjudged the distance to their nest considerably when their stride length was manipulated.

One group of ants had their legs elongated by gluing pig bristles to the legs and these overshot the distance by approximately 5 m. Another group had their legs shortened by severing the outer part of their legs, and this group undershot the distance by more than 4 m. This strongly indicates that the ants use a 'step integrator' to gauge distance. To validate this the group assessed the number of steps taken by control ants and multiplied this with the relative change in stride length of ants with shortened or elongated legs. Indeed, when stride length was corrected for body size and walking speed the expected/calculated distance travelled was very close to that observed for ants with elongated or shortened legs, confirming that they use some kind of 'step counter' to measure distance.

Although ants probably do not 'count' in a conventional sense they must be able to continuously keep track of how many steps they have used on their way out. Thus, when ants with manipulated legs took the outbound route from the nest to the feeding station with their 'new' legs, they returned at the right location, since they 'counted' the right number of steps on the way out.

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HOW TO TELL WHOSE BITE IS BIGGEST

Many male lizards use a variety of head and jaw movements in elaborate displays when courting potential mates and/or confronting conspecific rivals. In male collared lizards (*Crotaphytus*), for example, conspicuous gaping displays are an important component of the behavioral repertoire of territorial encounters. During these displays, males open their jaws widely, providing potential rivals with both a head-on and lateral view of their gaping mouths. Kris Lappin and his colleagues were curious about the functional role of such displays and suspect they may be involved in relaying information about the lizard's fighting prowess.

Lappin and his research group proposed that a head-on look into a gaping lizard's mouth provides an excellent viewpoint from which to size up the animal's primary jaw-closing musculature, its adductor mandibulae complex (AMC). Moreover, both head-on and lateral views of a gaping mouth reveal bright patches overlaying the AMC that highlight its extent. Biting is the key mode of attack among territorial adult male lizards, so their jaws and associated closing musculature are hence their major weapon. So Lappin and coworkers tested the notion that this gaping behavior does indeed encode information about the size of a collared lizard's jaw muscles and, in turn, its potential fighting/biting ability.

The team caught wild lizards in the Wichita Mountains of Oklahoma, USA, and took various morphometric body measurements, including the head and AMC size. Next they measured the animals' bite force by encouraging them to chomp down on a specially designed force transducer, recording each individual's maximum bite force. Among adult males, the AMC size, as seen from a frontal view,

was the best predictor of the animal's maximum bite force (i.e. better than any measurement of head or body size), and was not correlated with other morphological measures. Importantly, AMC breadth was not a good predictor of bite force among adult females and juvenile males, which generally are not territorial and do not fight.

In a parallel set of studies, Lappin and coauthors addressed the size and spectral properties of the bright patches found at the corners of the lizards' mouths, which cover the AMC when viewed from the side. As was the case with the frontal size measures, the size of the lateral patches was also important in explaining variation in bite performance in adult males, but not in juvenile males or adult females. Finally, reflectance spectra of the lateral patches revealed that they are the brightest parts of the skin, are in the iguanian lizard's visual range and contrast strongly with the adjacent integument and oral epithelium. It would appear that the patches are highly visible and can help to amplify or improve the visibility of the AMC, possibly giving rival males ample warning that they could be in for a good mauling.

In summary, gaping displays in adult male collared lizards may well be critical signals communicating jaw-adductor muscle size and, more practically, fighting ability, so animals don't have to find out the hard way. I look forward to manipulative experiments in which lateral patch size is artificially augmented to see how conspecific rivals react to the little lizard with the (apparently) big bite.

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MALE NEURONS RESPOND TO FEMALE PHEROMONE

Instead of sending a Valentines card on February 14th, female snakes produce a pheromone when meeting a handsome partner. Once vapourised, the snake love potion is detected by the recipient's vomeronasal organ, the vertebrate's most important chemoreceptive organ. While behavioural studies have already shown that this organ is involved in pheromone perception (males lacking the organ cannot respond to pheromones), the physiological basis of the vomeronasal organ's response remained unclear. To address this issue, Guang-Zhe Huang, from the Downstate Medical Center in New York, and his colleagues, tested the effects of the female pheromone on male vomeronasal neurons. They isolated vomeronasal organs from male red-sided garter snakes, exposed them to pheromones isolated from females of the same species and measured the electrophysiological response of the neurons.

Unfortunately, the pheromone is insoluble in water so Huang and colleagues tried solubilising the pheromone in a homogenate of the Harderian gland, a large reptilian gland whose secretions directly pass into the vomeronasal organ. Amazingly, this inspired step worked, allowing the team to test the vomeronasal organ's receptor cell responses.

Initially, Huang and colleagues measured vomeronasal organ levels of the cell secondary messenger, IP₃, in response to the pheromone and found that it increased by more than 200%, demonstrating that the pheromone activates neurons in the vomeronasal sensory epithelium. They also found that the pheromone induced measurable inward currents in male vomeronasal organ neurons, which increased as they raised the pheromone

dose. Even more interestingly, the team found that the amplitude of the pheromone-induced current was dose dependent, so they concluded that there are multiple pheromone receptors on male garter snake vomeronasal neurons and that pheromone binding to neurones facilitates further binding cooperatively. Stimulation with female pheromone also increased the neurone's membrane conductance, indicating that ion channels open in response to the pheromone, membranes depolarise and action potentials are initiated.

Finally, Huang and his colleagues examined whether the female's pheromone also affected female vomeronasal organ neurons, and found that female snake vomeronasal neurons did not respond at all to the pheromone. The researchers concluded, that the pheromone response originates at the vomeronasal organ and not in the central nervous system. Interestingly, behavioural observations underline this finding, as female snakes have never been observed to follow trails left by other females or respond to the female perfume.

Huang's study provides the first evidence of an electrophysiological response to a purified pheromone in a reptile and demonstrates that the response is sexually dimorphic, a finding which corresponds to behavioural observations in snakes. So, it seems that mate choice is a matter of smell rather than taste from the male red-garter snake's perspective.

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OSMOREGULATION



ACIDIC ABSORBATE INTEGRAL TO OSMOREGULATION

Think you know all there is to know about marine teleost fish osmoregulation? Well, you might want to think again. You may be familiar with the fact that marine fish continuously drink seawater to counteract their constant water loss to the environment – a consequence of the dramatic difference in salt content between the extracellular fluid and the more concentrated seawater environment. You may also know that the marine teleost intestine plays an important role in osmoregulation, contributing to water balance through the absorption of fluids *via* the active transport of Na⁺ and Cl⁻ in response to their high seawater consumption. But, did you know about the importance of intestinal Cl⁻/HCO₃⁻ exchange in osmoregulation? The secretion of HCO₃⁻ into the intestinal lumen contributes to the absorption of Cl⁻ and water and is so substantial that it results in basic intestinal fluids with HCO₃⁻ concentrations high enough to precipitate solid calcium carbonate in the gut lumen.

Incredibly, this ‘gut rock’ phenomenon appears to be common to all fish living in a seawater environment, but until now the mechanism of Cl⁻/HCO₃⁻ exchange in fish intestine was relatively uncharacterized. In a recent study published in the *American Journal of Physiology*, Martin Grosell and Janet Genz have elucidated the details of intestinal Cl⁻/HCO₃⁻ exchange and determined its overall contribution to marine osmoregulation in the toadfish.

To measure HCO₃⁻ across the toadfish intestinal epithelium, Grosell and Genz took a segment of intestinal epithelia and divided an Ussing chamber in two with the lumen facing a chamber filled with a luminal fluid-like saline and the blood side of the tissue facing a chamber filled with a blood-like saline. As the intestinal epithelium secreted HCO₃⁻ into the luminal fluid, acid was titrated into the fluid to maintain the luminal pH at 7.8. The amount of acid added was equal to the amount of base secreted giving a direct measure of the tissue’s HCO₃⁻ secretion. Using this approach, the temperature, pH and composition of the saline solutions in the chambers were manipulated and pharmacological agents, such as ATP or transport blockers, added to characterize the mechanism of HCO₃⁻ secretion.

The pair was able to determine that intestinal HCO₃⁻ secretion into the lumen occurs *via* a secondary active mechanism that is very sensitive to changes in temperature and requires energy in the form of ATP, which suggests that Cl⁻/HCO₃⁻ exchange in toadfish intestine is a transporter-mediated process. While half the total HCO₃⁻ secreted by the intestine appeared to come from either CO₂ or HCO₃⁻ extracted from blood, the team determined that metabolic CO₂

produced by the tissue accounted for the other half of HCO₃⁻ secretion. Furthermore, inhibiting the carbonic anhydrase inside the intestinal cell, which catalyzes CO₂ and H₂O to form HCO₃⁻ and H⁺, substantially reduced HCO₃⁻ secretion, suggesting that the hydration of CO₂ is important to HCO₃⁻ secretion. Grosell and Genz determined that the acid (H⁺) from the hydration reaction was absorbed into the extracellular fluid on the blood-side of the intestine, likely through a Na⁺/H⁺ exchanger located on the basolateral (blood-side) membrane. They also found that the elimination of H⁺ from the intracellular space was necessary for the continued secretion of HCO₃⁻. What is astounding about this finding is that the extrusion of H⁺ across the intestine’s basolateral membrane results in the absorption of a very large acid load; the theoretical pH of the absorbed fluid was calculated as being close to 1!

As more details of Cl⁻/HCO₃⁻ exchange are revealed by the Grosell group, the similarities between Cl⁻/HCO₃⁻ exchangers in fish and mammals become more apparent. That being the case, it is likely that the teleost intestinal epithelium may provide a valuable model for mammalian HCO₃⁻ secretion and/or fluid absorbing epithelia. And you thought you knew everything...

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