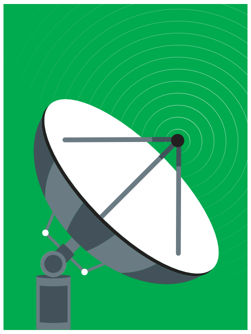
OUTSIDE JEB

Caution! A firefly approaches…



For humans, there is something soothing and cozy about the meandering flickers of light that come from fireflies on a warm summer evening. But what do those little green flashes mean? Researchers had thought that these signals developed as a part of firefly mating and courtship. However, new research by Brian Leavell and colleagues at Boise State University and the University of Florida indicates that these seemingly friendly pops of light are also a grave warning to nearby predators: these little insects are *not* a tasty meal. In fact, they are downright toxic. However, there is one catch. Bats, which are notorious insect predators, are certainly not famous for their visual abilities, although they can perceive light. Could they learn that firefly flashes should be approached with caution?

The research team began their work by confirming that firefly-naïve big brown bats (collected from an area with no fireflies) can learn to avoid these little glowing insects. The researchers put a bat and a firefly in a dark flight room and recorded their interactions using a high-speed camera. The bats learned quickly: over the course of a couple of days, they began spitting out and avoiding the fireflies altogether. Next, the researchers tested whether it was the firefly's flashes that bats were learning to avoid. They painstakingly painted the abdomens of fireflies with opaque paint to conceal their glow and repeated the experiment. Bats interacting with these 'blacked-out' fireflies also learned to avoid them, but they learned at a slower rate than with the fully illuminated fireflies.

The scientists reasoned that the bats must be detecting some other signal produced by the insects when their abdomens were painted over in order to learn that they are toxic. A scent cue might be a logical guess; however, the authors discounted this because the bats always tasted the fireflies before learning to avoid them, indicating that there is no scent that indicates toxicity. Could the bats be using a sound-based cue? Amazingly, bats can distinguish different types of insect prey based on their wing-beat rates and flight patterns. The authors returned to the flight chamber. This time, they tethered the fireflies by delicately strapping down their wings to prevent any flight-associated sounds, but still allowed their abdomens to glow. The researchers now 'swung' the flightless fireflies on their tethers in front of the bats to mimic flight. Again, the bats learned to avoid the bitter insects, but at a slower rate that was similar to the rate of learning when the fireflies were blacked out. They concluded that the bats learned best when they could both see and hear the fireflies.

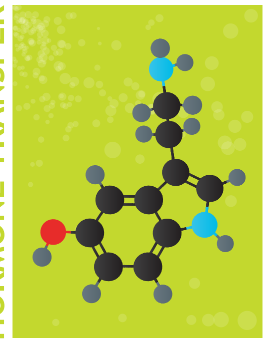
This study is the first to show that predators learn most effectively with multisensory cues emitted from their prey; in other words, two cues are better than one when it comes to avoiding a poisonous meal. Leavell's team also points out that only a few firefly species glow as adults, and they propose that the insects may have evolved to light up as a way to deter predators. Because other non-glowing fireflies can use scent cues to attract their partners, the team suspects that the pleasant green twinkles of glowing fireflies were later co-opted for attracting mates. Indeed, it seems that a firefly's neon flicker can simultaneously signal 'danger!' and beckon 'come hither', depending on who is watching.

10.1242/jeb.170266

Leavell, B., Rubin, J., McClure, C., Miner, K., Branham, M. and Barber, R. (2018). Fireflies thwart bat attack with multisensory warnings. *Sci. Adv.* **4**, eaat6601.8.

Erin McCallum (0000-0001-5426-9652)
Swedish University of Agricultural Sciences (SLU)
erin.mccallum@slu.se

Eating poop makes naked mole-rats motherly



Naked mole-rats are simultaneously the ugliest and cutest animals in the zoo. They've been described as hotdogs with teeth. And yet, so cuddly! What makes them so endearing to me and my kids is the fact that they live together as a big family unit and that they pile up when they get chilly. Also, uniquely for a mammal, naked mole-rat colonies are eusocial, like ants and termites. They have a queen that controls colony reproduction and several subordinate females that help to take care of the pups. But why do these females so willingly offer their care? A recent paper in *PNAS* led by Akiyuki Watarai from Azabu University, Japan, and his colleagues from several Japanese institutes determined that the cue to help lies in the queen's feces.

Watarai and his team observed that subordinate females were most willing to provide care to the queen's offspring, also called alloparental care, during a limited time window after pups were born. In other words, help was induced by something transient. But what was the trigger? They reasoned that the queen was manipulating subordinates using chemical cues transmitted in her feces. This isn't as odd as it sounds. Naked mole-rats regularly consume feces to supplement their diet and the subordinates were routinely nearby the queen,

Outside JEB reports on the most exciting developments in experimental biology. The articles are written by a team of active research scientists highlighting the papers that JEB readers can't afford to miss.

Journal of Experimental Biology (2018) 221, jeb170266. doi:10.1242/jeb.170258

providing ample opportunities to partake of the tasty morsels. To test this idea, the researchers fed subordinate females pregnant queen's feces and found that they increased their responsiveness to pups. This provided unambiguous evidence that poop was the culprit, but didn't yet clarify which part of the poop elicited the response.

Feces contains many things: digested and undigested food, bacteria, and lots of bits and pieces of everything else going on in your body, both good and bad. This is why doctors so often take fecal samples as a read-out of your health. It turns out that the feces from pregnant mole-rat queens also contains elevated levels of the hormone estradiol, the chemical that stimulates parental care behaviors. Strikingly, when the team fed subordinate females food pellets or feces from non-pregnant queens that were supplemented with estradiol, these both induced parental care behaviors in subordinates. By contrast, unsupplemented feces from non-pregnant queens had no effect at all – other than providing a nutritious snack. Thus, by transferring hormones to subordinate females via their poo, queens can make subordinates motherly.

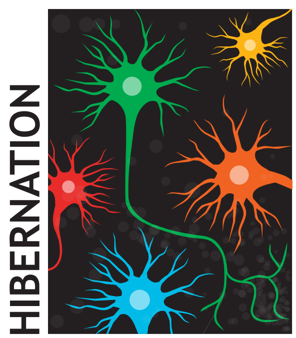
As cool as this is, it is unlikely that the mechanism of hormonal transfer in naked mole-rats is generalizable. Most animals are rightly averse to consuming feces. But the process of chemical coercion in social organisms is undoubtedly widespread, as long as there is a route of transfer. Social insects and some rodents carry out similar behaviors using pheromones or other odorants, but this may not work in the poorly ventilated tunnels where mole-rats live. Coprophagy – eating poo – by contrast, is both effective and reliable in this context because it takes advantage of an intrinsic eagerness to consume feces to supplement their diet and will occur preferentially in animals that are close to the queen and therefore already socially vetted. But did fecal hormonal transfer evolve as a means of behavioral manipulation, or simply as a fortuitous byproduct of the fact that queens naturally secrete pregnancy-associated hormones and mole-rats eat poop anyway? If the former, you might predict that queens produce excess estrogen beyond their own physiological requirements, or that the amount is conditional on the presence or number of subordinates. Regardless of the answers to the many remaining questions in this system, the result is fascinating. And bizarrely, it only serves to make these hideous beasts even more adorable.

10.1242/jeb.170183

Watarai, A., Arai, N., Miyawaki, S., Okano, H., Miura, K., Mogi, K. and Kikusui, T. (2018). Responses to pup vocalizations in subordinate naked mole-rats are induced by estradiol ingested through coprophagy of queen's feces. *Proc. Natl. Acad. Sci. USA* **115**, 9264-9269.

Daniel E. Rozen (0000-0002-7772-0239)
Leiden University
d.e.rozen@biology.leidenuniv.nl

Hibernating squirrels tweak sodium channels to rest their brain



When it comes to energy conservation, solar panels and bicycle commuting pale in comparison to hibernation. Hibernating animals limit energy-expensive processes, such as breathing and digestion, to help them squirrel away precious resources for the coming inactive season. Although some tropical animals hibernate to beat the heat, most temperate animals do it to endure the cold and chill out for months at a time in near-freezing temperatures. Preparation for the long energy hiatus includes bulking up, cooling down, and slowing down heart rate to fewer than six beats per minute. These peripheral changes are remarkable as they are extreme, but what's less clear is how central machinery, such as brain cells, are impacted by idleness.

A new report published in *Current Biology* by Lydia Hoffstaetter and colleagues in the collaborating labs of Slav Bagriantsev and Elena Gracheva at Yale University, USA, suggests that one central feature of hibernation is suppressed brain activity during dormant months in thirteen-lined ground squirrels. Squirrels hibernate for 8 months of the year and rapidly reanimate within hours during the active summer season, making them exemplar creatures to study.

To investigate how the nervous system changes during hibernation, Hoffstaetter measured how electrical properties differed between neurons of hibernating versus active squirrels. In particular, she focused on cells in the dorsal root ganglia near the spinal cord, which convey temperature, tactile and pain information to the brain, such as blistering cold conditions. Initially, Hoffstaetter found that neurons were similar between active and hibernating squirrels. For example, it took the same amount of prodding to elicit an electrical response from either hibernating or active neuronal cells, suggesting that brain activity and machinery are maintained during these periods of extreme cold and energy conservation.

However, clear differences emerged between active and hibernating cells once Hoffstaetter counted how often cells fired: neurons from hibernating squirrels fired at half the rate as their active counterparts. One reason may be due to voltage-gated sodium channels, which are required for neurons to fire. Careful follow-up recordings by Hoffstaetter determined that hibernating squirrels have greatly diminished sodium channel activity compared with active squirrels. Surprisingly, gene expression only partially explained this difference: hibernating cells expressed ∼20% less sodium channel genes than active cells, which is too modest a difference to explain the dramatic electrical differences. Therefore, a host of changes in addition to sodium channel expression must be altered to change how brain cells fire during torpor.

Taken together, these exciting findings suggest that despite hibernation seeming like a months-long nap marked by inactivity, squirrels, at least, have developed a way to keep their brains suppressed but ready for action when needed to save energy, thanks in part to sodium channels.

10.1242/jeb.170258

Hoffstaetter, L. J., Mastrotto, M., Merriman, D. K., Dib-Hajj, S. D., Waxman, S. G., Bagriantsev, S. N. and Gracheva, E. O. (2018). Somatosensory neurons enter a state of altered excitability during hibernation. *Curr. Biol.* **28**, 2998-3004.e3.

Journal of Experimental Biology (2018) 221, jeb170266. doi:10.1242/jeb.170274

Daniel M. Vahaba (0000-0003-2960-3756)
University of Massachusetts Amherst
dvahaba@gmail.com

Widespread ‘gassing off’ in killifishes

About 400 million years ago, when fish first crawled out of the primordial ooze and onto land, they faced a slew of new challenges. One pressing issue was getting rid of ammonia, a toxic waste product of protein metabolism, without water flowing across their gills to flush it out. Although terrestrial lineages have long since converted to a land-friendly form of waste management, some 40 families of fish continue to relive the glory days of tetrapod evolution by taking short sojourns onto land. Amphibious fishes rely on many strategies to dodge ammonia toxicity, including converting ammonia into less-toxic urea, temporarily suppressing protein breakdown and even releasing ammonia as a gas. Intrigued by the diversity of solutions to this recurring physiological problem, a group of Canadian researchers from the University of Guelph and Wilfrid Laurier University looked for rhyme and reason in the ammonia disposal strategies of six aplocheiloid killifishes selected from across a 1000-plus-member family tree.

First, the team had to verify that their killifish showed amphibious behaviour. They ‘encouraged’ the animals to be adventurous and emerge from the water by cranking up the thermostat or dropping the oxygen level in the water, mimicking the environmental challenges common in their natural habitat of swampy, stagnant pools. Five of the six species emerged when the water got too hot and all of them vacated the water when exposed to hypoxia, suggesting that the ability to pack up and leave inhospitable conditions may be a driver in the evolution of an amphibious lifestyle.

As expected, air exposure strongly handicapped ammonia excretion across the gills in all six species. The researchers then investigated each of the alternative ammonia handling strategies that the killifish could use while out of water: retention of ammonia in the tissues for ‘washout’ upon return to the water, conversion to urea, temporary suppression of protein metabolism, and the release of ammonia as a gas. Although one species showed evidence for ammonia retention and another for urea excretion, ‘gassing off’ was the primary strategy of all six killifishes, accounting for 57% to 89% of total ammonia excretion during emersion.

We know of very few fish that release ammonia gas, but the process seems to depend on ammonia-transporting Rhesus glycoproteins embedded in the skin or gut lining. Using dye that is specially targeted to Rhesus glycoproteins, the team found that the skin of all six killifish species stained positive for two of the proteins, Rhcg1 and Rhcg2, which likely act as release valves for the ammonia gas. However, the positioning of Rhcg1 and Rhcg2 varied between species: some fish glowed with an abundance of Rhesus proteins all over the skin, while others showed only pinpricks of stain, with their proteins being closely associated with ion-transporting cells. These different patterns may reflect some specialization in excreting ammonia in habitats with different salinity, or in how long a species typically spends out of the water.

Fish are remarkable colonists and have evolved many clever solutions for dealing with the new habitats. Despite the many possible options for handling ammonia on land, the aplocheiloid killifish adopted a common strategy for nitrogenous waste management when exposed to air. Whether this shared mechanism comes from evolutionary ‘family resemblance’, convergent evolution or both, there is no denying that the ability to survive on land is a powerful tool for an otherwise aquatic organism. Perhaps the secret to being a successful fish out of water is to just let the nasty stuff roll off your back.

10.1242/jeb.170340

Livingston, M. D., Bhargav, V. V., Turko, A. J., Wilson, J. M. and Wright, P. A. (2018). Widespread use of emersion and cutaneous ammonia excretion in aplocheiloid killifishes. *Proc. R. Soc. B* **285**, 20181496.

Brittney G. Borowiec
(0000-0003-0087-7577)
McMaster University
borowibg@mcmaster.ca

Enemy at the bat cave door

TV shows such as ‘Dracula’ and the ‘Batman’ series have made bats famous over the years. We all know that they are the only mammals adapted for flight, but did you know that some plants depend solely on bats for pollination and seed dispersal; or that they consume so many insect pests in a few hours that they reduce the need for insecticides? Bats are true engineers of their environment, but the populations in North America are currently threatened by an outside enemy: the European fungus, *Pseudogymnoascus destructans*, which causes white nose syndrome, leading to death. How the fungus spreads and adapts to new environmental challenges (e.g. colder or warmer climate) has not been studied extensively.

With this in mind, Adrian Forsythe, a PhD student at McMaster University in Hamilton, Canada, along with a team of colleagues from the same institution, have investigated how well the white-nose syndrome fungus adapts to varying temperatures, and how far and how fast it can spread in new regions that are warmer or cooler. Over the course of 5 years (2008–2013), the team collected samples of the fungus from bats in different locations across North America. Analysing the fungus colony samples, the authors were able to establish that they all varied in terms of colony size, colour and how the pigment was distributed. The group examined colony size because it is an indicator of the ability of the fungus to obtain nutrients from the environment and reproduce, but

Journal of Experimental Biology

Journal of Experimental Biology (2018) 221, jeb170266. doi:10.1242/jeb.170274

they also looked at pigmentation as an indicator of resistance to environmental challenges and the ability of the fungus to spread rapidly.

Comparing the new samples with the very first sample that was collected when the infection was initially identified in North America, 10 years ago in New York State, it was clear that the fungus had changed (or had adapted) to each specific location. The authors found that the colony size and pigmentation varied significantly the further the sample was from the original infection site, meaning that the fungus was adapting to its new environment; it was coping well with new environmental challenges and it was spreading at an alarming rate. To further test the resilience of the fungus, the research team picked four samples representing the differences in colony size and pigmentation within all groups and exposed them to 4°C, 13°C and 23°C for varying lengths of time, after which they looked at growth and genetic differences in the fungi. The team found that different strains of white-nose syndrome fungus from different locations preferred different temperatures and grew at different rates, but the most interesting finding was the presence of genetic mutations among the fungal groups, suggesting that it is adapting rapidly to its environment.

Bats are crucial for the health of ecosystems, from hunting harmful insects, such as mosquitos, to plant pollination and providing fertilizer for agriculture; a world without bats would be catastrophic. Through their work, Forsythe and his team have provided additional information on the spread of white-nose syndrome fungus and on the factors that influence and contribute to its rapid adaptation to the North American climate, but more research is needed to better understand and prevent its spread, which seems to have North American bats cornered.

10.1242/jeb.170274

Forsythe, A., Giglio, V., Asa, J. and Xu, J. (2018). Phenotypic divergence along geographic gradients reveals potential for rapid adaptation of the white-nose syndrome pathogen, *Pseudogymnoacus destructans*, in North America. *Appl. Environ. Microbiol.* **84**, e00863-18.

Oana Birceanu (0000-0002-3345-8769)
Wilfrid Laurier University
obirceanu@gmail.com