OUTSIDE JEB

Acetylcholine boosts turtle sense of direction

In the classic Aesop's fable, the tortoise triumphs over the smug hare by patiently making its way along the race track. While reptiles such as turtles and tortoises are famously ambling voyagers, how they learn their routes is less well known. One chemical that may be required is acetylcholine. Acetylcholine is a brain chemical that is broadly important for cognition and appears to be particularly relevant for spatial navigation. It's needed by rats for negotiating mazes, by birds when caching seeds, and homing pigeons flying home. Recently, Timothy Roth II (Franklin and Marshall College, USA) and Aaron Krochmal (Washington College, USA) found that acetylcholine is also important for painted turtles (*Chrysemys picta*) that are journeying to new water sources. However, acetylcholine acts on a variety of receptors in the brain; therefore, it was unclear which one might be responsible for regulating spatial learning.

In a follow-up study recently published in Proceedings of the Royal Society B, Roth and Krochmal set out to determine if M1, a specific type of acetylcholine receptor, facilitates turtle migration. To do so, the researchers first radio-tagged adult turtles residing at a man-made pond that is regularly drained. Once the scheduled drain happened, Roth and Krochmal tracked the turtles' routes to nearby ponds as they ventured in search of alternative watering holes. After mapping these routes, the team returned the following year to intercept the turtles mid-way to their new watering holes after their home had been drained. They then administered a drug that would block the M1 receptor and prevent acetylcholine from contributing to the turtles' ability to recall the route that they had learned the previous year. While the turtles had been trotting along the predicted route just fine beforehand, blocking the M1 receptor immediately sent the turtles off course. Therefore, M1 receptors are needed for turtles to recall directions; however, it was unclear whether M1 was also necessary to memorize the route.

To test whether turtles rely on M1 to memorize the directions, Roth and Krochmal next turned off the M1 receptor while juvenile turtles were in the midst of learning their route. Knowing that juvenile turtles must memorize watering hole routes within the first three years of life, the researchers caught radio-tagged three-year-old juvenile turtles that were still learning their way as they set sail for a new pond, and temporarily blocked the M1 receptors with a drug. The juvenile turtles were unfazed by the disruption and kept on trucking right along to the pond. However, when the researchers tracked the turtles during the following annual water drain, the presently sober turtles – which had been drugged a year earlier while memorizing their route – struggled to find their way to the pond. Thus, while juvenile turtles are unperturbed when the M1 receptor is blocked during real-time navigation, the receptor is essential for them to create spatial memories early in life.

Taken together, Roth and Krochmal's findings suggest that painted turtles require acetylcholine acting specifically on M1 receptors for both memorizing and recalling navigation routes.

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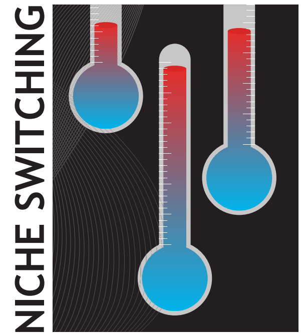
Roth T. C. and Krochmal, A. R. (2018). Of molecules, memories and migration: M1 acetylcholine receptors facilitate spatial memory formation and recall during migratory navigation. *Proc. R. Soc. B.* **285**, 20181904.

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

Nocturnal living beats the heat



A sweaty brow, dry lips and unslakeable thirst – we all know what it's like to swelter through a hot summer. While we can ease our discomfort by switching on an air conditioner or visiting an ice cream shop, animals don't have that luxury. They can, however, change their activity times to avoid high temperatures. This can be as simple as staying hidden during the middle of a hot day or, at the other extreme, changing tack and switching to being active at night. It was this latter strategy that interested Ofir Levy from Tel Aviv University, Israel, and his collaborators, so they set about examining the phenomenon of 'temporal niche switching', in the context of surviving increased temperatures associated with climate change.

In general, a warming climate will reduce how much food mammals and birds need to eat. However, daytime water loss will increase under hotter conditions, making the night an attractive option for day-active animals. In fact, saving water is probably the most important factor in stopping animals from overheating. Levy and his team identified three factors with the potential to drive animal activity patterns – heat from the environment, access to shade and the availability of water – and examined how changing the time of day when they're active may help animals cope with the impacts of climate change.

The researchers built a computer simulation looking at how climate change

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may cause a shift in the activity patterns of golden spiny mice. They expected that a shift to a nocturnal lifestyle would make life easier for the tiny rodents by reducing their energy expenditure and water loss. Dialling their calculations 80 years into the future as the temperature increases, they found that nightly energy expenditure of the mice decreased and their daily water costs increased. Additionally, the team realised that the amount of time when it will be cool enough for the animals to be active during the day will decline, and the amount of shade provided by vegetation, as well as free water to drink, are also predicted to decrease. This could spell trouble for day-active mice, as individuals that are unable to meet their daily energy demands will need to compete over scarcer resources, find new areas to live and may even be in danger of dying out in areas where it's simply too hot to trot. Changing to a nocturnal lifestyle seems like a no-brainer.

Unfortunately, switching from a day- to night-active lifestyle will not solve all of the mice's problems. Switchers must compete with animals that have better night vision, be aware of nocturnal predators and have the ability to stay warm when active by moonlight, instead of sunlight. To top things off, climate change is probably happening too quickly for switchers to completely adapt to the nightlife, or for those that stay day-active to get used to the daytime heat.

Levy and his colleagues leave us with the thought that time – whether minutes, days or millennia – is an ecological resource similar to space. They argue that the importance of time is under-appreciated and that time use is likely to be impacted by climate change: animals will need to shift when they go about their daily business as temperatures rise. Changing activity from day to night may help species endure a warmer world but more research is still needed to understand the breadth of this potential. Fortunately, thanks to the team's work, we now know that some species could go into the night to save the day.

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Levy, O., Dayan, T., Porter, W. P. and Kronfeld-Schor, N. (2018). Time and ecological resilience: can diurnal animals compensate for climate change by shifting to nocturnal activity? *Ecol. Monogr.* https://doi.org/10.1002/ecm.1334

James M. Turner (0000-0001-8699-7750)
Charles Sturt University
jaturner@csu.edu.au

Social networks buffer sickness

Crowded shopping malls, fully booked airplanes, daycare; these places are hot-beds for spreading illness. The frequent and close-knit nature of human interactions only helps disease to hop from person to person. How we behave when we are sick (staying home from work) or how we behave towards others who are sick (running away) affects the spread of disease by changing how often and with whom we interact. Interestingly, such changes in individual behaviour can also change the social structure of entire communities to reduce disease transmission, according to a new study by Nathalie Stroeymeyt from the University of Lausanne, Switzerland and colleagues.

Before you ask, no, Stroeymeyt and her co-workers did not go around experimentally infecting human societies to reach their conclusion. Instead, the researchers used the highly social garden ant as their species of choice. Humans are very similar to group-living animals like ants or bees when it comes to social organization. We come in frequent contact to communicate with each other and we should reduce this contact when sick. Also, like humans, ants live together in structured societies where everyone has a job. Foragers leave the colony to find food; nurses stay in the colony to care for the brood; and the queen – who produces all the offspring – is the most important ant in the colony. The queen cannot get sick.

Stroeymeyt's team assessed how ant colonies reacted to a spreading disease using a nifty research tool called social network analysis. They attached very small ID codes to each ant, returned them to the colony and followed them for 24 hours. Then, they used software to visualize and analyse the network of ant interactions. The team found that healthy, uninfected ant colonies are structured to prevent disease transmission in the first place. For instance, forager ants, who are more likely to pick up pathogens while roaming outside, had less contact with ants inside the colony. The colonies were also very 'modular', meaning the ants tended to congregate together and mainly interact with other ants in job-specific cliques.

Next, the researchers infected some ants to see how the colony responded to a disease outbreak. They dusted 10% of the foragers from each colony with fungal spores to make the ants sick. With the colony on the cusp of an epidemic, the structure shifted to reduce pathogen exposure: the ant cliques became even more tightly aggregated and the groups moved further apart. The ants never lost sight of their goal to save the queen and protect the brood. Infected foragers distanced themselves from the others and spent more time outside the colony. Nurses also moved the brood deeper into the nest, reducing contact with the infected foragers. This new anti-infection social organization reduced spore transfer to the nurses and the queen. And, when the researchers tracked the ants' survival for 9 days after the infection, they found that more foragers than nurses perished, but the queen never died.

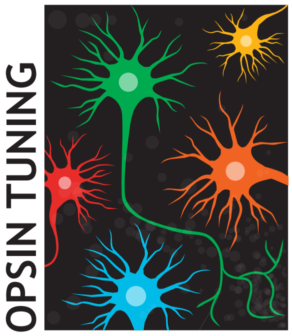
We tend not to think that ants have many similarities with humans, but Stroeymeyt's findings give us insight into the spread of disease in complex social groups. We can all identify with how the ants changed their behaviour in a time of sickness. The researchers show that such changes are reflected in the social network of an entire community, which reduced the spread of disease and protected valuable members of the population. Long live the queen!

doi:10.1242/jeb.192641

Stroeymeyt, N., Grasse, A. V., Crespi, A., Mersch, D., Cremer, S. M. and Keller, L. (2018). Social network plasticity decreases disease transmission in a eusocial insect. *Science*, **362** 941-945.

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

Some guppies only have eyes for orange



Roses, shopping trips, diamond earrings…lonely hearts go to great lengths to impress that special someone. Male animals make similar efforts to woo bachelorettes with dramatic markings and extravagant feathers. For her part, the bachelorette filters these signals through her eyes, and makes a decision based on what she perceives. A recent paper in PNAS from a trio of researchers from Tohoku University and the University of Tokyo, Japan, investigated how genetics and rearing environment shape the visual system of female guppies and whether that variation influences which future Mr Guppy she likes best.

Colour vision relies on opsin proteins in cone cells in the retina, and each opsin reacts to certain wavelengths (colours) of light. Always flamboyant, guppies sport nine opsin genes including red-sensitive opsins (LWS-1 and LWS-2) and green- or blue-sensitive opsins (SWS-1, SWS2-A, SWS2-B, and RH2-2). Variation in the amino acid sequences of each opsin influences its sensitivity to different coloured light: for example, if you replace the alanine amino acid at residue 180 in the guppy red LWS-1 opsin (‘Ala-type’) with a serine amino acid (‘Ser-type’), the opsin becomes sensitive to deeper shades of red. The team suspected that genetics and environmental changes could fine-tune guppy colour vision, and that this could shape the dating habits of female guppies.

First, Yusuke Sakai and colleagues compared how the genes that encode the different opsin proteins are converted into mRNA, a key step in the process where genes are eventually decoded to produce proteins; in this case, the opsins in the retina. The team compared mRNA production from the two forms of the LWS-1 opsin gene – Ala-type and Ser-type – in fish reared under white, green or orange light. They found that the Ser-type fish produced more red- and blue-sensitive opsin mRNAs. This suggests that Ser-type guppies pack more opsins into their eyes and may discriminate better between different colours. Regardless of which type of LWS-1 opsin the fish were producing, guppies that were raised in green or orange light produced less mRNA encoding UV- and blue-tuned opsins, probably because these opsins are not used as much in orange and green environments. So, guppies can alter how they express genes in response to the prevailing colour scheme in their environments.

However, gene expression data can be misleading because not every piece of mRNA gets involved in protein synthesis. The researchers then tested which opsin proteins were being produced from the mRNAs in the fish’s eyes by testing how well each fish tracked moving visual patterns of orange or green light. Guppies with Ser-type LWS-1 opsin benefited from their opsin-packed eyes and tracked the moving visual patterns better than fish that had the Ala-type opsin. However, when fish reared under orange or green light were tested, the Ser-type fish lost their clear advantage. More opsin mRNA does lead to better colour vision, but only in some environments.

Having linked changes in opsin proteins to differences in colour vision, the team wanted to know how this influences what ‘she’ looks for in a husband. They had female guppies choose between two digitally modified videos of a male guppy: one with a big, sexy, ‘high orange (HO)’ marking and the other with a small, dull, ‘low orange (LO)’ marking. The females with more red- and blue-sensitive opsin mRNAs spent much more time near Mr HO than Mr LO. In other words, the females with extra opsins and good colour vision, loved their eye candy and found brightly coloured males more attractive than dull-coloured males.

Miss Guppy’s genetics and rearing environment shape her colour vision and this relationship between visual physiology and mate preference may drive the evolution of extreme colouration in gentlemen guppies. For guppies, beauty is in the eye of the beholder and ladies with good eyes want bright orange eye candy.

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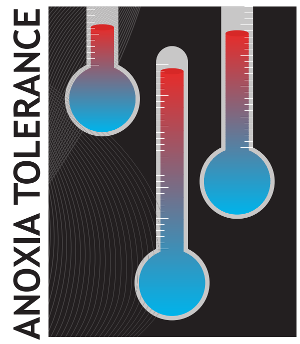
Sakai, Y., Kawamura, S. and Kawata, M. (2018). Genetic and plastic variation in opsin gene expression, light sensitivity, and female response to visual signals in the guppy. *Proc. Natl. Acad. Sci. USA.* **115**, 12247-12252.

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

Recovering from oxygen deprivation, froggy style!



In the animal kingdom, certain animals avoid the cold winter weather by either migrating to warmer regions, growing thick fur or hibernating, while others tolerate bitter temperatures by literally freezing solid. In addition to allowing 60–70% of their body to freeze, animals like the wood frog (*Lithobates sylvaticus*) have an amazing superpower, which we have yet to understand: they can survive for months without oxygen, because when they freeze, they stop breathing. How they do it and how they recover from such a challenge are just two of the questions that today’s researchers are asking.

Undeterred by working in cold weather, or perhaps just not appreciating the 4–6 week period of warm weather that Ottawa, Canada, enjoys each year, Rasha Al-Attar and Ken Storey from Carleton University, Canada decided to explore the mechanism behind the wood frog’s ability to tolerate no oxygen (anoxia) for a day at 5°C and then recover. To this end, the team captured male wood frogs from ponds around Ottawa and acclimated them to 5°C for two weeks prior to testing. To induce the anoxic condition, the team bubbled nitrogen gas in an experimental chamber for 15–20 min to displace the oxygen; they then added the frogs, placed the boxes at 5°C and started their timer. At the 24 h mark, they immediately collected liver and muscle samples from a group of frogs, while allowing another group to recover in air for 4 h, before collecting the same tissues.

Based on previous research on wood frogs performed by Storey's team, which had shown that the animals are able to decrease their metabolism when they have no access to oxygen, Al-Attar and Storey decided to determine what causes this reduction in metabolism by exploring the expression levels of a protein called nuclear factor of activated T-cell (NFAT), which plays a key role in regulating cell metabolism and cell death. The authors found that the four isoforms of the protein (known as NFATc1–NFATc4) are expressed differently in the liver compared with the muscle in response to anoxia and recovery. In addition, the team determined that NFATc4 may play an essential role in helping the liver recover from oxygen deprivation, but that the muscle may use other metabolic pathways to prevent tissue damage from the lack of oxygen. It appears that NFAT proteins may not be as essential in muscle as they are in the liver.

Al-Attar and Storey's study is unique, because it highlights some of the mechanisms that wood frogs use not only to survive anoxia and freezing, but to do so while incurring minimal tissue damage. Now imagine if we could harness this frog superpower and use it to reduce tissue damage in humans following a stroke – when the brain is deprived of oxygen – which may cause paralysis, or use it to preserve organs during transport from a donor to a recipient. The possibilities are endless! Who would have thought that we would have so much to learn from such a small creature as the wood frog?

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Al-Attar, R. and Storey, K. B. (2018). Effects of anoxic exposure on the nuclear factor of activated T cell (NFAT) transcription factors in the stress-tolerant wood frog. *Cell Biochem. Function* **36**, 420-430.

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