

## OUTSIDE JEB

### Slime molds transfer knowledge through fusion



Animals acquire an understanding of the world through experience and instruction. If we touch a hot stove, we burn ourselves and learn to keep our fingers away from glowing red coils. Alternatively, we can avoid the drama of direct experience by heeding the warnings of our parents. Both options are effective and help to guide our future behaviors. And until now, both were believed to require a complex and coordinated nervous system. However, in a recent issue of *Proceedings of the Royal Society, Series B*, David Vogel and Audrey Dussutour, from Toulouse University in France, show that even brainless slime molds can learn and teach.

Few people would look towards slime molds as the poster-child of intelligence. Yet, over several decades of work, these multi-nucleate bags of goo have been found to be capable of surprising feats of cleverness. They can navigate complex mazes, optimize nutritional challenges and even find their way through a miniature version of the Tokyo subway system; they show swarm, or collective, intelligence. But can they learn?

To test this, Vogel and Dussutour tempted slime molds with a tasty treat that could only be reached by traversing a bridge containing high levels of salt. At first, slime molds hesitated before crossing the salt bridge; they moved across it slowly and haltingly, like dipping a single toe into a frigid sea. However, after repeated exposure, they

learned to ignore the noxious, although harmless, stimulus. In other words, they had become habituated to the salt and learned to ignore it so that they could more rapidly reach the food. Further, after the salt was removed, the slime molds forgot what they had learned and reverted to salt aversion; they had recovered their justified caution.

Habituation and recovery may not seem especially advanced. However, they require a form of basic learning coupled to a type of memory. Given this, the team reasoned that this physical memory could potentially be transmissible. When slime molds meet each other they can undergo cellular fusion, whereby the two formerly independent individuals join forces to form a larger single entity. If fused cells can share nuclei and cytoplasm, why not their memories too?

When the team fused a habituated slime mold with an unhabituated one, they found that the unhabituated individual gained the salt habituation of the other. Something, as yet unidentified, thus carried durable memories from one individual to another. But the transfer took some time. If the pair was separated after only an hour of fusion, no transfer occurred, while by 3 h, transfer was complete. Furthermore, habituation persisted in the formerly naive slime mold even after it was separated from its habituated partner, confirming that transferred memories are at least as durable in the recipient slime mold as they are in the donor.

Our world is filled with sensory inputs that we experience and share. But imagine yourself stripped of sight, sound, taste or smell. How would you learn from others? How would you teach what you know? You couldn't simply push your brain, with all its knowledge, into another's head. Yet, this is essentially what slime molds do. By fusing together, they share information. And, as the authors have shown, as more individuals fuse, the faster they learn. But this is also an area filled with questions. Among many others, how are memories formed and

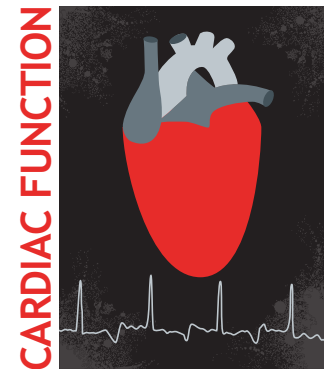
carried? Do some individuals cheat by acquiring information while withholding their own hard-won knowledge? Are all memories transmissible? And finally, does any of this matter in nature? I hope the authors are as eager to learn the answers to these questions as I am.

10.1242/jeb.147306

Vogel, D. and Dussutour, A. (2016). Direct transfer of learned behavior via cell fusion in non-neural organisms. *Proc. R. Soc. B.* **283**, 20162382.

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### [Cardiac] Pressure's on for thirsty trout



Because it is much saltier than their body fluids, the ocean is a very dehydrating environment for fishes. Species that can survive in both freshwater and saltwater, such as trout and salmon, must therefore undergo significant physiological changes when transitioning to seawater. To counteract water loss, they drink more and they use the salts in the water to help drive water absorption across the gut; the salts are then sent to the kidney or gills for excretion. This process requires extra blood flow to the gastrointestinal tract. Trout achieve this extra flow solely by increasing the stroke volume (the amount of blood the heart ventricle pumps with each beat), which in turn increases the cardiac output (the flow rate of blood from the heart). However, the exact mechanisms utilized to increase the stroke volume have long been uncertain. Jeroen Brijs and his colleagues at the

University of Gothenburg in Sweden, as well as collaborators at the University of Applied Sciences in The Netherlands and the University of South Bohemia in the Czech Republic, sought to clarify these mechanisms. The group also wanted to determine what might happen if the fish were challenged with warm temperatures, which typically also elicit increased cardiac output so fish can meet greater metabolic demands.

To address their questions, the researchers compared cardiovascular variables in rainbow trout acclimated to freshwater or seawater. Briefly, they measured the pressure of blood entering the heart as well as the cardiac output for 3 h at 10°C, then raised the temperature to 16.5°C and monitored these same variables for another 3 h. Finally, the scientists weighed the heart ventricles and determined the proportions of stronger and weaker muscle types in the hearts of the fish in each acclimation group.

Brijs and his colleagues discovered that seawater-acclimated trout increased their stroke volume, and thus cardiac output, by increasing the pressure of blood filling the heart. They also developed a higher proportion of strong muscle tissue in their ventricles than the freshwater-acclimated trout, likely to give their hearts the power to pump the extra blood. When challenged with warmer temperatures, however, the two groups converged on similar cardiac outputs and stroke volumes – even though the seawater-acclimated fish maintained higher pressures filling the heart – suggesting that osmoregulatory strategies in seawater environments may reduce the scope of heat-induced increases in cardiac output.

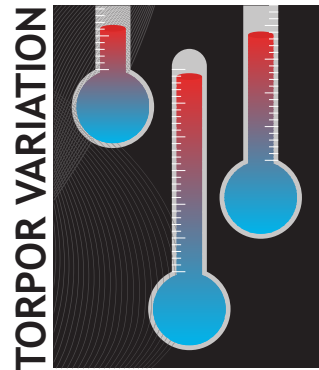
This study is the first to suggest that temperature and salinity may interact to influence changes in blood flow in fish that are tolerant of variable salinities. It is also the first study to attribute salinity-induced increases in stroke volume to higher heart filling pressures in rainbow trout, inviting further investigation into exactly which mechanisms are responsible for increasing pressure. Ultimately, it looks like these salinity champions feel the pressure when they're faced with a new environment – but they forge on with full hearts.

10.1242/jeb.147439

Brijs, J., Sandblom, E., Dekens, E., Näslund, J., Ekström, A. and Axelsson, M. (2017). Cardiac remodeling and increased central venous pressure underlie elevated stroke volume and cardiac output of seawater-acclimated rainbow trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **312**, R31-R39.

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## Too much hibernation isn't always good for you



Many animals undergo phases of torpor by reducing body temperature and metabolic rate, which ultimately lowers their energy demands during harsh periods. Torpor use and especially hibernation, during which animals remain torpid in protected burrows or nests for months, are generally viewed as very advantageous survival mechanisms. Hibernating animals are less often subject to predation than non-hibernators and can withstand long periods when food is scarce. But surprisingly, individuals often vary in their use of torpor, leading to the question why do some individuals use less torpor than others if torpor is such a beneficial strategy?

Melanie Dammhahn, a researcher from the University of Potsdam in Germany, and colleagues from the Université du Québec à Montréal and McGill University in Canada analysed torpor patterns of free-ranging eastern chipmunks (*Tamias striatus*) to find out why some individuals forgo torpor when it has clear survival benefits. Eastern chipmunks are relatively small seasonal hibernators that regularly interrupt torpor bouts during winter to feed on food reserves stored in their burrows. The researchers collected skin temperatures of 55 chipmunks over five winters, analysed the amount of time that each chipmunk

spent torpid and linked this to the animals' survival and birth rates.

Dammhahn and colleagues found that, as expected, the use of torpor varied between individuals. Even more interesting, chipmunks maintained a stable torpor pattern over the whole hibernation season; animals that used less torpor at the beginning of the hibernation season also used less torpor later in winter. Linking the chipmunks' skin temperature measurements with their survival rates, the scientists found that chipmunks using less torpor in autumn had higher death rates in years when food was plentiful, but not when food was scarce. Additionally, they found that the chipmunks that used more torpor early on and survived until the breeding season produced fewer offspring in spring.

While a lower birth rate in animals that use more torpor suggests that increased torpor use could be related to a slower pace of life – as animals increase their survival chances via torpor and spread their reproductive output over more years – the other results are not as easy to explain. Variation in torpor use might reflect individual personalities or could just be caused by differences in food availability in the animals' habitats.

Extended torpor use may also be beneficial as individuals entering torpor early in autumn can spend more time underground and have a lower risk of predation, whereas animals that remain active have a higher risk of falling prey to a predator. In a year when food is abundant, an animal retreating underground early will probably have enough food stored to survive the entire winter season. However, in years when food is scarce, an animal that reduces its use of torpor – remaining active for longer in autumn – would probably have the advantage of establishing a larger food cache, thereby increasing its survival chances.

While it remains a matter of speculation whether the variation in torpor use is part of a pace-of-life syndrome or is simply caused by differences in food availability around burrows, one thing is clear: variation in torpor patterns within a population guarantees that some individuals will survive the winter regardless of the environmental conditions.

10.1242/jeb.147322

Dammhahn, M., Landry-Cuerrier, M., Réale, D., Garant, D. and Humphries, M. M. (2016). Individual variation in energy-saving heterothermy affects survival and reproductive success. *Funct. Ecol.* doi:10.1111/1365-2435.12797.

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## Feisty fish from anoxic waters



What could you accomplish in just 4 h? Run a marathon, perhaps, or bake a pie? How about permanently alter the behavioral phenotype of a zebrafish? It turns out that depriving a zebrafish embryo of oxygen for just a few hours has a big impact on that fish's future. For example, a previous study from Nicholas Bernier's lab at the University of Guelph, Canada, showed that 4 h at zero oxygen (anoxia) during a critical developmental window resulted in larval zebrafish that were better able to tolerate low oxygen environments and adult zebrafish that were disproportionately male. In this follow-up study, students Catherine Ivy and Cayleigh Robertson from Bernier's lab tested whether this early exposure to anoxia also affected the behavior of these fish when they were reared to adults.

Ivy and Robertson began by cutting the O<sub>2</sub> to nil for one group of 36 h old zebrafish embryos, then returning them to fully oxygenated water (normoxia) 4 h later. They also kept a separate group of embryos in normoxic water throughout development. A few months later, when all of the fish were mature adults, the researchers ran a series of experiments to compare the behaviors of the anoxia- and normoxia-exposed fish. First, they isolated pairs of sex-matched fish – one from each of the developmental treatments – to see which treatment produced the feistier fish. Compared with the normoxia-reared fish,

fish that were exposed to anoxia during development more frequently chased and bit their tankmate to establish themselves as the dominant fish in the pair. Even when the researchers used a mirror in the tank instead of a second fish, to simulate a social interaction, the anoxia-exposed fish bit at their reflection almost twice as much as their normoxia-exposed cohort. This means that those 4 h of anoxia – way back when the fish weren't even hatched – produced a more aggressive adult fish, and this might help the fish out-compete others for limited resources like food and mates.

Next, Ivy and Robertson wanted to understand the physiology that might underlie this punchy personality, so they looked at the sex hormone testosterone, which is well known for its ability to turn a docile string bean into a raging muscle head when taken in excess. Testosterone is produced in the gonads of both male and female fish, but the amount released into the circulation depends on the activity of aromatase, an enzyme that helps convert testosterone into estrogen. When the researchers measured gonad aromatase expression, they found that fish exposed to anoxia as embryos had lower levels than those in the normoxia group, meaning that the anoxia group could secrete more testosterone from their gonads. In fact, the team found higher testosterone levels in the bodies of the fish from the embryonic anoxia exposure, and this could be responsible for the higher aggression in these fish. It also helps to explain the male-biased population that results from embryonic anoxia exposure, which the group discovered in their previous study, because high levels of testosterone can masculinize a female fish's ovaries.

This study from the Bernier group breathes new life into the topic of how early life environments influence future phenotypes. As the incidence of environmental stressors, including aquatic hypoxia, continues to increase, understanding these latent effects is critical to predicting how species will weather climate change.

10.1242/jeb.147330

Ivy, C. M., Robertson, C. E. and Bernier, N. J. (2017). Acute embryonic anoxia exposure favours the development of a dominant and aggressive phenotype in adult zebrafish. *Proc. R. Soc. B* **284**, 20161868.

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## Bumblebees adapt for turbulence



Everyone has heard the myth that bumblebees defy the laws of physics when they fly. Of course, we know that this is not the case, but we still know surprisingly little about how bumblebees fly in outdoor environments, where the weather can change abruptly. For a lightweight bumblebee, wind conditions are arguably the most important aspect of weather to consider and can alter very quickly – sometimes in seconds. So, how does the tiny bumblebee cope with these rapid and sometimes extreme changes, and what wind conditions do they actually experience while foraging outside the hive?

These are questions that a team of researchers from the USA wanted to address. Firstly, James Crall, Stacey Combes and colleagues fitted 87 *Bombus impatiens* bumblebees with radio identification tags and tracked them as they exited and entered the hive. Simultaneously, they measured wind speeds in an area outside the hive where the bumblebees were likely to forage. Studying these two datasets together allowed the team to determine the exact wind conditions during the bees' foraging trips. The researchers found that these bumblebees experience a variety of wind environments, in terms of both wind speed itself and turbulence – characterised as the amount of variability in the wind speed over a 10 s period. However, the bees did not appear to adjust their foraging times to avoid windier or more turbulent conditions.

Next, the authors attached triangular markers to the backs of bumblebees from a different group and filmed them at 5000 frames s<sup>-1</sup> flying in a wind tunnel that recreated some of the wind conditions that the bees had encountered previously:

completely calm conditions and at two different wind speeds (1.5 and 3 ms<sup>-1</sup>), with and without turbulence. By tracking the markers on the bees' bodies and the position of their wingtips in the videos, the team calculated the insects' body orientation and wing movements throughout each trial, thus determining how the different conditions affected their flight.

The results revealed that bumblebees react and adapt to wind conditions in a number of ways, most obviously during turbulent conditions at the highest wind speed tested. Here, the bees appeared to roll to a greater degree, while this behaviour was not seen at lower wind speeds. The bees

also beat their wings more often and with greater amplitude in these conditions. The authors suggest that this could be a way of increasing their control in changeable weather conditions; by reducing the time between wing strokes, the bees can respond more quickly to wind fluctuations when they occur and beating with a larger amplitude provides more power to their flight.

Though this study tested only a small subset of the wind conditions likely to be experienced by bumblebees, it provides a good insight into the strategies a bee can use to maintain stability in challenging weather. Work like this can also help to

develop more effective control systems for bioinspired flying robots, allowing them to cope with more severe wind conditions. These robots could be useful in a variety of situations, carrying cameras to track wildlife poachers or aiding in police surveillance – helpful bee-inspired robots that will certainly cause a buzz!

10.1242/jeb.147314

**Crall, J. D., Chang, J. J., Oppenheimer, R. L. and Combes, S. A.** (2016). Foraging in an unsteady world: bumblebee flight performance in field-realistic turbulence. *Interface Focus* 7, doi:10.1098/rsfs.2016.0086.

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