More Fun Than Fun: Not Just Us – Dancing Bees Need a Good Night's Sleep, Too

09/06/2021

Barrett Klein with a swarm of honey bees being readied for shooting the BBC documentary Invisible Worlds. Photo: Eileen Inkson

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This article is part of the 'More Fun Than Fun' column by Prof Raghavendra Gadagkar. He will explore interesting research papers or books and, while placing them in context, make them accessible to a wide readership.

On the invitation of my former student Ruchira Sen, then a postdoctoral fellow working on fungus-growing ants under the mentorship of Ulrich G. Mueller, I visited the University of Texas, Austin, in April 2010, to give a seminar on the Indian paper wasp (*Ropalidia marginata*). The most memorable experience I had on that trip was to befriend Barrett Anthony Klein, then a PhD student.

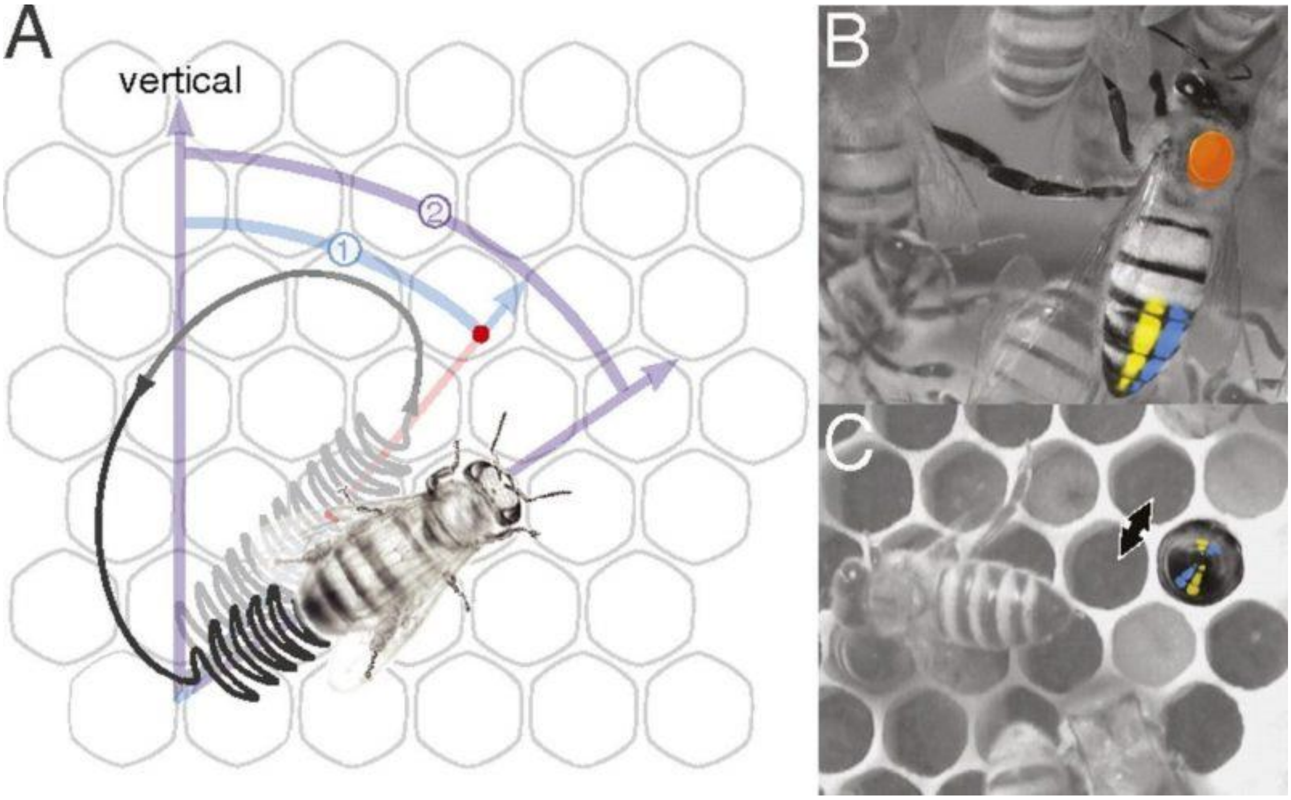
No one can forget Barrett, even if you had met him only once. I have since met him several times and invited him to visit my laboratory in Bangalore in October 2010. I discovered during our very first meeting that Barrett has two rather unusual passions, both concerning insects: insect-inspired art and insect sleep. I will reserve stories of his interest in insects and art for another occasion and focus here on his adventures with insects in slumber.

Sleepy dancers

In the December 28, 2010, issue of the Proceedings of the National Academy of Sciences, Barrett and a number of his collaborators, including his twin brother Arno Klein, published a paper entitled 'Sleep deprivation impairs precision of waggle dance signalling in honey bees'. It is now well known that honey bees have a unique dance language that comes closer to human language than that of any other animal, in using arbitrary symbols to encode information. Returning honey-bee foragers recruit naïve home bees to participate in the task of transporting newly discovered food (pollen or nectar) back to the nest. They do so by communicating the distance and direction to the food source.

The dance form that communicates distance and direction is called the 'waggle dance' because the dancing bees vigorously vibrate their abdomens during the waggle phase of the dance. (They have many dances.) The distance to the food is encoded in the duration of the waggle phase of the dance. The direction to the food source is encoded in the angle of the dancing bee relative to the hive's vertical. This in turn corresponds to the angle relative to the Sun's azimuth outside the hive. Potential recruits follow these dances, leave the hive and decode the information received, and locate the food source, without further guidance from the dancer or anybody else.

We can measure the precision with which the dancer transmits information by the consistency in the waggle duration and angles between repeated dances by the same bee for the same food source. Barrett and his colleagues measured the standard deviation of the waggle duration and angles of the dances of normal and sleep-deprived bees. They found that the standard deviation of the dance angles was higher for sleep-deprived bees than for normal bees. Sleep-deprived dancers were thus imprecise in their communication of the direction of the food.



A: Wagle dance of honey bees showing the variation in the angle of the waggle phase from dance to dance (compare 1 and 2). B: Image of a tagged sleeping bee. C: Bee sleeping inside a cell, identified by their dorsoventral discontinuous ventilatory motions, represented by arrows. Images: Barrett Klein

This is a most interesting result, especially because similar experiments have been performed in humans, yielding very similar results. When human volunteers who have enjoyed a normal night's sleep are compared with those deprived of sleep for 30 hours, sleep-deprived volunteers have been found to be less precise on cognitive tasks involving the use of memory and language.

But Barrett's study also raises many intriguing questions. When and where do honey bees sleep? Do honey bees also experience light and deep sleep states? How can we deprive honey bees of sleep? And when they dance imprecisely due to lack of sleep, what do the dance followers do?

More fundamentally, do insects really sleep? How do we define, detect and measure sleep in insects?

We now have answers to all these questions, many on account of the work of Barrett and others, who have all enriched this field before and after his paper.

Detecting sleeping bees

Barrett was lucky. Other scientists, especially Walter Kaiser and his colleagues in Germany, have previously defined behavioural criteria for detecting honey bee sleep and correlated them with neurological correlates of sleep. Sleeping bees are relatively immobile, with their body drooping in the direction of gravity, but they exhibit occasional, spontaneous antennal or leg twitches or proboscis (their sucking mouth part) extensions and perform discontinuous respiratory pumping motions of their abdomens.

Even deep sleep and light sleep can be distinguished by the absence or presence of antennal twitching and swaying. Sleeping bees can thus be identified in video recordings, except when the bees are inside the hexagonal cells of the comb. But Barrett and his colleagues had an ingenious solution for the problem of obscured bees.

The tips of bees' abdomens are usually visible even when they are otherwise hidden from view because the rest of their body is inside the cells. Barrett and his colleagues developed a method to detect sleeping bees just by looking at the tips of their abdomens. First, they constructed observation hives using plates of transparent glass so that they were able to observe the entire body of the bees even when they were inside the cells. This allowed them to detect sleeping bees among those inside cells. Now, merely by observing the tips of the bees' abdomens, they learned to distinguish a sleeping bee from one that was not sleeping.

The Insominator

Perhaps the cleverest thing that Barrett has done so far is to devise a way to deprive selected bees of sleep while letting the rest of the colony snooze. The trick is to keep shaking the chosen bees while leaving the rest alone. Barrett and a gifted in-house engineer at the University of Texas, Terry Watts, constructed a manually operated sleep deprivation device that they charmingly called the 'insominator'.

The device is as simple as it is clever. It consists of two plates of plexiglass, with several magnets attached to them, placed on either side of an observation hive. They glued small pieces of magnetic steel to the chosen bees (called treatment bees) and pieces of non-magnetic copper to a similar set of control bees. They then moved the magnet containing plexiglass plates from side to side three times a minute, all night long. Thus, the treatment bees were jostled all night and couldn't sleep – while the control bees remained undisturbed.

An observation bee hive fit with the magnetic insominator. Notice the plexiglass plates fitted with three columns of magnets on the left. These plates are moved from side to side to jostle test bees fitted with magnetic steel pieces but leave control bees fitted with non-magnetic copper pieces undisturbed. Photo: Barrett Klein

Can you take a sleepy dancer seriously?

If sleep-deprived dancing bees convey imprecise information, what should the dance followers do? Can they detect these glitches and ignore them, or do they inevitably get fooled and go off on a wild goose chase?

We might expect that they would get fooled during the experiment because it is reasonable to assume natural colonies wouldn't usually contain many sleep-deprived bees. Indeed, we may be justified in such a conjecture because Barrett and his colleagues found, in the previous experiment, that sleep-deprived bees compensated for their experimentally induced sleep-deprivation by sleeping more after the experiment. Very sensible indeed.

Barrett and a different set of colleagues reexamined videos of the dancing floors from the experiment described earlier, and turned their attention to the dance followers. They analysed 615 instances of dance, following 39 dances performed by 13 dancing bees. And they found that bees generally ignored imprecise dances. After watching such dances, namely those with similar angles between multiple dances, they left the hive in search of the promised food. But if the angle varied from dance to dance, they simply switched their attention to other more precise dancers.

Their disregard for imprecise dancers can be precisely computed. For every 1º increase in the standard deviation of the dance angle, the followers had a 6% higher propensity to switch their attention to another dancer.

Honey bees thus appear to have a built-in safety mechanism, a sort of lie detector, or a reliability detector, if you will. We don't know what happens if the followers are also sleep-deprived, but perhaps the chances that both dancers and followers are simultaneously sleep-deprived are small. But it is not just that followers simply ignore sleepy bees. The number of followers showing interest in the dances of sleepy bees is no different from those that show interest in normal bees.

There is also an intriguing suggestion in the results of this study that the followers were able to detect the sleep-deprived state of dancing bees independent of the imprecision of their dances. Such an ability may help them treat the information conveyed by sleepy bees with a pinch of salt, as it were.

We are only beginning to gauge the intricacies of the 'language' and intelligence of different species of animals – and it is telling that we are constantly surprised. If we make the mistake of assuming that animals lower down in the phylogenetic tree of life will necessarily be simpler, as many scientists and laypersons alike do, we will miss out on a great deal of knowledge and excitement.

Do bees take naps?

We have seen that experimentally sleep-deprived bees compensate by sleeping more and more deeply when the opportunity arises. We have also seen the nuanced and variable responses of dance followers to the dances of sleep-deprived bees. Therefore, we can conclude that experimental deprivation of sleep is not too artificial to be biologically relevant. Indeed, the experiments suggest that both sleep and sleep deprivation are integral to the lives of bees and their toolkit for survival. Nevertheless, it would be nice to study naturally sleeping and sleepless bees.

In one experiment, Barrett Klein and Thomas Seeley put their bees to work either early in the morning or late in the evening to see if they compensated by taking naps at other times.

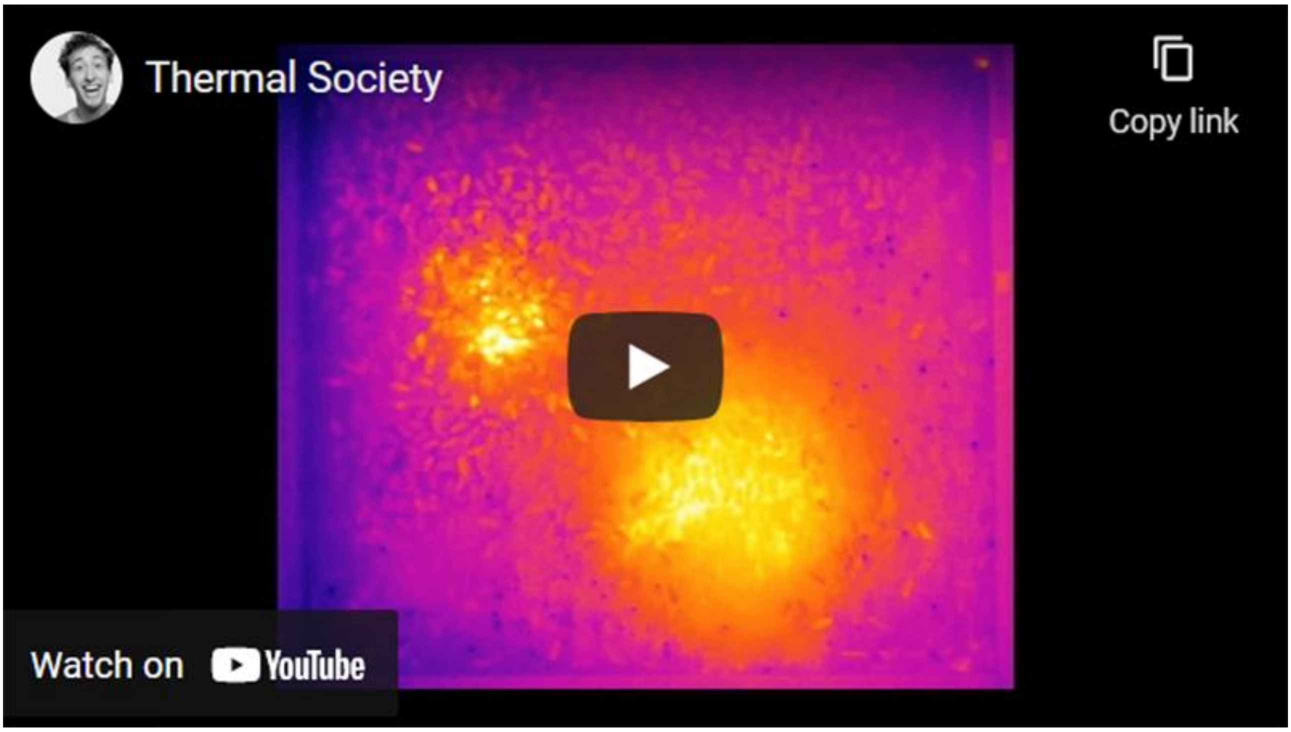
The Cranberry Lake Biological Station in the Adirondack Biological State Park, New York where Barrett conducted many of his experiments. This happens to be a favourite spot for Barret's mentor and collaborator, Thomas Seeley, because it contains very few flowers that bees can use; experimental bees are therefore easy to train to come to artificial feeding stations set up by the researchers. Inset: A typical inverted glass bottle from which bees are trained to take sugar solution, return to the hive, dance to advertise its location and recruit more bees to visit. Photos: Barrett Klein

They accomplished this by training some bees to forage only in the morning (around 6 am to 9 am) and others only in the evening (around 4 pm to 7 pm). While the total time devoted to sleep did not differ between bees that worked in the morning and those that worked in the afternoon, the timing of their sleep was very different.

Bees who were put to work in the morning slept more in the afternoon, while those put to work in the afternoon slept more in the mornings. The fact that bees can take naps whenever possible to compensate for missing out on sleep at other times reinforces the idea that sleep is essential for their well-being, just as it is for us.

Barrett has relentlessly chased sleeping honey bees for two decades. Shifting locations across continents and forging collaborations with colleagues, friends and family, he has shown that different classes of bees – cell-cleaners, nurses, food-storers, foragers – all sleep, that young bees sleep more and do so inside the cells, and that older bees sleep less and on the surface of the nest (barely avoiding falling off).

Barrett has provided increasing evidence of the importance of sleep in honey bees, even getting his PhD along the way. I can't fail to mention that Barrett studied sleep in a paper wasp for his MS thesis. In collaboration with Jürgen Tautz of the University of Würzburg in Germany, he has developed infrared photography to study honey bees while [causing little or no disturbance] (https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0102316).



Watch on YouTube : https://youtu.be/iYr158rwLBI

Now, let us turn to the more fundamental questions.

Do animals and insects really sleep?

Without being too strict in their definition of sleep and not being too judgemental about the rigour of the study, Scott Campbell and Irene Tobler of the University of Zurich surveyed the literature in 1984. They identified at least 150 species of (some) invertebrates and all classes of vertebrates (fishes, frogs, reptiles, birds and mammals) that may be displaying some form of sleep.

Counterintuitive as it may seem, such a liberal attitude towards the literature is very useful in surveying nascent fields of research. It protects us from too many prematurely judged false negatives. The 150 species thus preselected as possible candidates for sleep research provide a gold mine for further exploration. And others have since added more putative and some well-confirmed sleeping species.

For example, only two species of insects, the cockroach and the honey bee, were included in the Campbell-Tobler survey. Other insects like wasps and fruit flies have since been added as well.

Humans have become so accustomed to thinking of sleep in terms of what happens in our brains and in using neurological correlates of sleep to define light sleep, deep sleep, REM sleep, etc. that we tend to forget that sleep is a behaviour. It is, therefore, the subject of ethology. Long before we knew anything about its neurological correlates, people experienced sleep and detected it in others, and measured its duration in both. Even the sophisticated neurological correlates were initially calibrated with reference to the behavioural markers of sleep before they were considered reliable enough to stand on their own.

Therefore, it makes sense to begin to define and detect sleep in animals using behavioural criteria. Sleep can be reliably detected by certain postures: relative immobility and a high threshold for responding to external stimuli. If being in this state has a biological function, we should expect that animals should return to this state and get enough of it if we disturb them out of it. Adding the additional criterion of such 'compensation, which is sometimes called 'sleep rebound', we get a reasonably robust definition of sleep and a means of detecting and measuring its duration.

The modern study of sleep in insects can be said to have begun with the classic paper 'Sleep of Insects: An Ecological Study' by Phil and Nellie Rau, the inimitable amateur naturalist couple who lived all their lives near the city of St. Louis, Missouri, running a dry-goods store and a real estate business. They financed their own research and gave us such other gems as *Wasps Studies Afield* (1918) and *The Jungle Bees and Wasps of Barro Colorado Island* (1933).

Phil and Nellie Rau, 1929 photo by St. Louis Globe-Democrat, scanned from an original from Nellie Rau. Reproduced with permission, courtesy Mary Jane West-Eberhard

The Raus begin their paper with this charming passage:

> "An object in motion always attracts the attention of children, young and old; a butterfly flitting from blossom to blossom, a locust jumping before one in the dusty road, a bee rummaging in a flower, all arouse one's interest. But naturalists, like children, cease to pay attention to insects when the latter cease their activity. Thus, the interesting problem of when, where and how insects sleep has been all but neglected."

The rest of their paper is an inspiring naturalists' diary featuring sleeping wasps, bees, moths and other insects, described in the first person, in a now-forgotten style that does not separate the process of science from its product.

Fast forward to 2021, and we see that sleep research in one insect species (fruit fly, *Drosophila melanogaster*) has scaled the dizzy heights of identifying the neurophysiological, genetic and molecular control of sleep. And yet, barely a handful of insect species have received even a fraction of the attention that Phil and Nellie Rau bestowed on their favourite insects, back in 1916.

It is a paradox of modern biology that while expensive, sophisticated and technology-intensive research is conducted with great fervour, simple, low-cost, natural history observations are few and far between. And yet, we complain that lack of funding and access to sophisticated laboratory facilities prevent many people, institutions and countries, from participating in knowledge production.

It is not far-fetched to imagine that even school children, let alone zoology professors in India's numerous colleges and universities, could apply the Phil and Nellie Rau magic to thousands of insects in their backyards and gardens and expand the world's list of sleeping insects by orders of magnitude.

Why then does this not happen? I think it is because we have downgraded natural history in particular and all kinds of simple, low-cost, fun and adventure-filled research as being below the dignity of 'real' scientists.

But let me end on a cheerful note, and that is easy to do: we just have to shift our attention back to Barrett Klein, better still to the Klein brothers. Barrett is a great interlocutor. Once I asked him for his spontaneous thoughts about his life and work. Here are two from several he came up with; the others are safely stored away in my archives.

> "I clearly remember my five-year-old self, struck with the exciting but nebulous epiphany that insects would dominate my thoughts and my life."
>
> "Who isn't struck by the bizarre nature of sleep and dreaming? When I found a paper by Walter Kaiser suggesting that a honey bee might sleep, in a stack of readings by my Sleep and Dreaming professor, I knew that I wanted to bring sleep studies and my entomophilia together."

I know several famous scientific collaborations involving husbands and wives or fathers and sons, but I can't recall any notable collaborations involving twins. Barrett frequently collaborates with his twin brother Arno Klein. Arno is the director of innovative technologies at the Child Mind Institute, New York. He is designing a sensors and wearables programme to study mental illness and offer potential interventions. I haven't met him yet but I'm eagerly look forward to doing so.

Arno and Barrett Klein. Photo: Barrett Klein

I asked Barrett about his experience of working with his twin brother, and here's what he said:

> "Arno and I work together because we love spending time together, and our passions overlap in a number of ways. We are both fascinated by nature, especially obscure biological phenomena. We are both biologists and share a love of art and visualising science. Arno possesses talents and expertise I do not, so it has been an honour (and a relief!) to have him assist with my sleep research, as well as with the programming associated with my cultural entomology database and website. Arno is indispensable, hilarious, and inimitable – and I say that as his identical twin. I recommend that everyone have a twin. Arno has been one of my closest collaborators, and I hope to forge future projects in which we can further activate our wonder twin powers."

More power to Arno and Barrett and all collaborating scientist twins. May there be many more!

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Gadagkar, R., 2021. More Fun Than Fun : Not Just Us – Dancing Bees Need a Good Night's Sleep, Too. URL https://science.thewire.in/the-sciences/honey-bees-dance-language-sleep-deprivation-barrett-klein-simple-research/