CellPress

Current Biology
Dispatches

Sleep Origins: Restful Jellyfish Are Sleeping Jellyfish

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http://dx.doi.org/10.1016/j.cub.2017.08.024

What is the 'simplest' animal that sleeps? When did sleep first evolve? Do all animals sleep? Tantalizing hints to answers come from new research showing that jellyfish, one of the earliest evolving groups of animals, have a sleep-like restful state.

Walk to the end of a pier and you may well see the unhurried undulations of jellyfish in the sea. Their pulsing movements are slow and rhythmic. The animal that creates them is ostensibly simple [1]. Jellyfish are members of one of the earliest branching lineages in the animal kingdom, the cnidarians, a phylum that appeared nearly one billion years ago [2]. Cnidarians are not widely known for complex behaviours: beyond their austere pulsing activity, they seemingly do little else. But Nath *et al.* [3] report in this issue of *Current Biology* compelling evidence that jellyfish exhibit a behavioural and physiological state that is commonly associated with more complex animals: sleep.

Sleep Behaviour

Sleep is typically a profound behavioural shutdown [4,5]. In and of itself, such restfulness does not uniquely identify sleep. Quiescent animals might be in other dormant states, such as diapause or hibernation, or simply be awake, but immobile. In contrast to wakefulness, sleep is a state of decreased responsiveness to stimulation; unlike dormancy, sleep is rapidly reversible to an awake condition. Sleep is also homeostatically regulated, such that an animal kept awake will, when sleep is allowed to proceed, sleep longer and more deeply, thus making up for lost sleep [6].

Sleep appears to be a phylogenetically widespread behaviour. A free-living platyhelminth flatworm (*Girardia tigrina*), for example, engages in two main behavioural states: they can glide through the water in exploratory or feeding pursuits, or contract and remain immobile for minutes or hours [7]. Their quiescence is largely relegated to the day, although they typically have a midnight nap. Patterns of predominantly nocturnal activity persist in the absence of photoperiodic cues, indicating that the rhythm is generated endogenously. Active flatworms respond more readily to stimulation, suggesting that immobile animals either lack the motivation to respond or fail to register the stimulus. Rest in flatworms is homeostatically regulated, as indicated by inactivity infiltrating the night following a period of sustained movement during the day. This response does not arise from muscle fatigue or stress caused by the experimental protocol, because the amount of movement induced, and the number of times an animal was stimulated to move, did not predict their response when they were allowed to behave freely. Lastly, melatonin, a hormone that promotes sleep in diurnal animals [8], similarly modulates inactivity in flatworms in a dose-dependent manner. Collectively, these observations suggest that at least some of the quiescence observed in flatworms is sleep [7].

Sleep Evolution

Every species that has been adequately studied by sleep scientists has been found to sleep. Sleep is widespread across vertebrates [9]. Studies into arthropods have likewise found that quiescent insects, arachnids and crustaceans are sleeping [6]. A handful of molluscs [10], one species of nematode roundworm [11] and a platyhelminth flatworm [7] also sleep. The indications are that sleep evolved no later than the appearance of the centralized nervous system, a brain or cephalic ganglion (Figure 1).

What then of animals with de-centralized nervous systems? Cnidarians (corals, sea anemones, hydra and jellyfish), along with ctenophores (comb jellies), arose early in the evolutionary history of animals, and both have the most rudimentary nervous systems. Nerve cells are formed into diffuse nets throughout their radially symmetric bodies [12]. Ostensibly sleep-like states have been reported previously in several cnidarians, including alternating rhythmic tentacular pulsation and rest in soft coral [13]. Additionally, the box jellyfish, *Chironex fleckeri*, lies prostrate for hours in the afternoon, during which no undulations of the bell occur, and its long tentacles nestle on the seabed [14]. Anecdotally, the animals can be stimulated to re-enter the water column, but appear reluctant to do so and quickly resettle on the seafloor to resume their quiescence. Another study [15] on two other species of box jellyfish reported similar observations: in wild and laboratory environments, *Copula sivickisi* were seen to rest by attaching themselves to the underside of stones and coral skeletons for much of the day; *Tripedalia cystophora* show an inverted rhythm and are found at the bottom of muddy mangrove lagoons only during the night. In both instances, immobility can be elicited following acute changes to the photoperiod, indicating that ambient light plays a role in regulating this enigmatic state.

In a series of elegant experiments, Nath *et al.* [3] report on the significance of reduced activity in jellyfish. They focussed on upside-down jellyfish (*Cassiopea* spp.), so named because they rarely leave the substrate, preferring instead to sit supine on the seabed in shallow seas. In this environment, stationary pulsing serves to ventilate their photosynthetic zooxanthellae endosymbiote [13]. With animals individually housed and a behavioural tracking system, Nath *et al.* [3] quantified jellyfish behaviour over successive 24-hour days. First, they

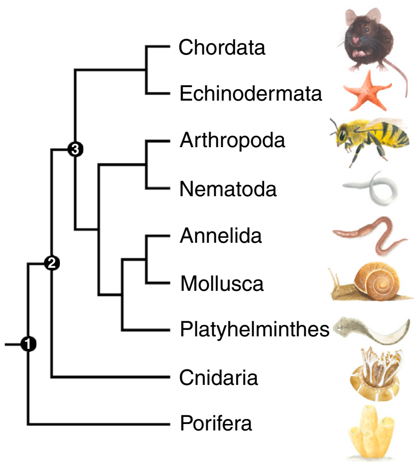

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Figure 1. A pruned phylogenetic tree of the animal kingdom.
Shown are evolutionary relationships among the nine most species-rich animal phyla, which collectively represent upwards of 95% of all animal life. Descendants of the numbered nodes: are animals (1); have a nervous system of varying complexity, which is diffuse and radially symmetric in cnidarians (2); have a centralized nervous system and bilaterally symmetric body plan (3). Sleep has been observed in vertebrates (phylum Chordata), multiple arthropods and molluscs, and one species of nematode roundworm, platyhelminth flatworm and jellyfish. Echinoderms, annelids, poriferans (sponges), and the other 27 minor animal phyla (not shown) have yet to be studied by sleep scientists. Modified from [7] by permission of Oxford University Press; paintings by Linh M. T. Ly.

found that *Cassiopea* showed reduced activity at night, manifesting as fewer pulses with longer, variable intervals between pulses, rarely longer than 20 seconds. Such diel variation in activity did not arise from daytime feeding as the pattern persisted in fasted animals. Nevertheless, night-time feeding could elicit an acute increase in the number of pulses, attesting to the rapid reversibility of the quiescent state.

Second, nocturnal restfulness was associated with reduced responsiveness. Specifically, animals were slower to begin pulsing after being released into the water column at night. Importantly, Nath *et al.* [3] then re-tested the animals, seconds after the first trial. The reasoning was that, if the jellyfish had been asleep, then they would have awakened upon steadying their descent, such that the latency to respond on the second test should be much shorter. Indeed, this was the case. Moreover, when tested during the day, jellyfish responded quickly and similarly irrespective of whether they had been perturbed once or twice.

Third, nocturnal quiescence was homeostatically regulated. Animals were kept pulsing during either the last half of the night or the entire 12-hour night. When the jellyfish were allowed to behave freely, the animals showed reduced activity during the first hours of the day. The magnitude of this reduction was more pronounced after 12-hours of sustained performance, consistent with dose-dependent responses to extended periods of wakefulness observed in other animals [6]. As with flatworms, this reduced activity in jellyfish is unlikely to reflect fatigue or stress, as the same protocol executed during the day did not influence subsequent activity levels.

And fourth, two compounds that promote sleep in other animals had similar soporific effects on jellyfish. In a reversible and dose-dependent manner, melatonin and pyrilamine were found to reduce activity, intimating the existence of conserved molecular machinery regulating sleep in cnidarians as well as other animals.

Sleep Homology?

Nath *et al.* [3] provide convincing evidence that restful jellyfish are sleeping jellyfish, the simplest animals that have been shown to sleep. Indeed, sleep might be widespread across cnidarians [13–15]. Whether sleep is found in yet simpler organisms, such as sponges (and zooxanthellae endosymbiotes), is uncertain; however, in the absence of a nervous system, it is doubtful that these organisms even have the capacity to be awake!

Do all animals with a nervous system sleep? There are an overwhelming number of animal species to study, and an impressive (but more manageable) number of animal phyla. To this end, it pays to be choosy. Of the 36 animal phyla, sleep has been demonstrated in six (Figure 1). Sleep appears to have evolved early in the lineage of animals and has seemingly persisted over evolutionary time. It remains unknown, however, whether these large-scale similarities in sleep reflect homology or convergent evolution. More comparative research is needed on the phyla (and species) for which no systematic sleep data exist. It is noteworthy, however, that sleep researchers have found sleep in all animals investigated. Even species that routinely sleep very little [16,17] or approach sleeplessness for days or weeks [18,19] retain some sleep. This grand evolutionary longevity suggests that sleep fulfils a fundamental — and inescapable — need.

Sleep Function

Sleep serves many functions. Sleep can mature developing nervous systems, strengthen or weaken synapses, consolidate memories for long-term storage, and clear waste generated by neural tissues [20], among other non-neurological benefits [4]. Some of these effects might reflect evolutionarily new sleep functions, present only in derived species. Others may reflect an ancestral (or core) function that was responsible for the evolutionary appearance of sleep. In light of evidence for sleep in jellyfish, that core function is unlikely to involve the brain *per se*. But given that cnidarians and animals with more complex nervous systems share neuropeptides, neurotransmitters, synapses and action potentials [1], these common features may well be the key to unlocking the reason for the origin of sleep.

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Plant Biology: Unravelling the Transient Physiological Role for PHO1 in the Seed

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http://dx.doi.org/10.1016/j.cub.2017.08.050

During seed development, an important transfer of nutrients occurs between the seed coat and the embryo. A new study reveals that, for inorganic phosphate (Pi), this function is transiently performed by PHO1, a protein associated previously with Pi loading into the xylem.

Phosphorus is an essential macronutrient for plant development and signalling. It is acquired in the plants exclusively in the form of Pi from the environment by the PHT1 family of Pi/H+ cotransporters [1], which mediates its transport across the plasma membrane. Interestingly, this family of transporters is involved in both the direct uptake of Pi from soils and the exchanges with arbuscular myccorhizal fungi (a trait shared by 80% of vascular flowering plants [2]). All of these transporters are mainly associated with the influx activities of Pi into the cell, and many additional Pi transporters have been identified for the allocation of this crucial resource in the different plant cell compartments [2].

For the efflux activity, other key components for Pi homeostasis are the members of the PHO1 family. Their physiological activity was difficult to demonstrate, as they exhibit no homology to known transporters [3], but show similarities with the yeast Syg1 protein (a component of the mating pheromone signal transduction pathway) or with the Rcm1 mammalian receptor for xenotropic murine leukemia retrovirus. Another problem with studying these proteins is the difficulty to detect the PHO1 protein at the plasma membrane. A GFP-labeled PHO1 can complement the *pho1* mutant but is observed only in the Golgi and trans-Golgi network [4]. This may be due to fine control of the level of this protein at the plasma membrane level through important recycling at the trans-Golgi network level.

Until now, PHO1 proteins were only known to support the Pi transfer from root to shoot [5]. Indeed the *pho1* mutant exhibits a strong reduction of Pi content in the leaves due to a failure in the loading of Pi into the xylem [6,7]. Experiments overexpressing this protein in plant tissue where it was normally not detected triggered Pi export, suggesting a direct role in Pi export for the PHO1 protein [3,4].