lamina neuron interactions in the precise control of relative and likely also dynamic CadN levels.

The observation that the primary neurites of L3 neurons are located peripherally whereas their dendritic branches extend between R-cell axons and L1 and L2 neurites raises the question as to whether CadN-mediated differential adhesion also acts at the subcellular level to separately position primary neurites and dendritic arbors. Moreover, each of these neurites may act at the subcellular level to CadN-mediated differential adhesion and complex morphologies reflective of their functions, undoubtedly additional strategies await discovery.

References
12. Steinberg, M.S. (1963). Reconstruction of tissues by dissociated cells. Some morphogenetic tissue interactions and the sorting out of embryonic cells may have a common explanation. Science 147, 401–408.

Sleep: A Biological Stimulus from Our Nearest Celestial Neighbor?

Three studies have retrospectively analysed different data-sets to assess whether there is an effect of lunar phase upon human sleep. The results and conclusions differ. Until specifically designed experiments, controlling for key variables, are undertaken this issue will remain open.

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So does the moon really affect our sleep? There is a strong and pervasive belief across many societies that the moon has an impact upon different aspects of human biology, not least upon our patterns of sleep. This has prompted scientists to return again and again to this question and a considerable literature has accumulated. Reporting either some effect or absolutely no impact of the lunar cycle upon our physiology and behavior [1]. Three recent studies [2–4] published in Current Biology, including two in this issue, have correlated objective measures of several sleep parameters with changes in lunar phase. Perhaps unsurprisingly the results are inconsistent and controversy will undoubtedly follow, triggering further studies. However, before yet more research is undertaken perhaps it would be worthwhile to consider why these recent studies may have generated inconsistent results.

Whether the moon affects our sleep has intrigued our species since ancient times, but in the last decades only relatively few attempts have been made to address this issue with scientific rigor, and solid conclusions have been elusive [1]. A new cycle of research on the lunar effects on sleep...
was triggered by a retrospective study which carefully re-analyzed the sleep data collected under laboratory conditions in 33 subjects (age range 20–74 years) and showed clear cut effects of the lunar phase on several subjective and objective sleep parameters [2]. Specifically, EEG slow-wave activity (SWA), total sleep time and subjective sleep quality were reduced around the time of the full moon, while sleep latency and latency to REM sleep were prolonged. This study corroborated an earlier report [5], which found a significant decrease in the amount of subjective sleep around the full moon in 31 subjects (mean age of 50 years). This report triggered two further studies, published in the current issue, which either contradict or report novel effects of lunar phase [3,4].

One of these studies, a re-analysis of existing large data sets, could not confirm any of the findings made by Cajochen et al. [3]. By contrast, a second retrospective study [4], in which 47 young volunteers were analyzed, confirmed a decreased total sleep time around the full moon, but REM sleep latency was longer around the new moon. This contradicts the Cajochen et al. study as they found that the latency to REM was longest around the full moon [2].

Are we ready to reject the null hypothesis about the effect of lunar phase on sleep? While previously there was a paucity of well executed studies [1], we now have three that have passed the rigors and dissection of particularly robust peer review and yet they still yield inconsistent findings. Such a result triggers curiosity and suggests that further critical scrutiny is needed to try and find the basis for these discrepancies. Here we address two issues: (i) what would be the ideal experimental design to address the effects of lunar phase on sleep and (ii) what kind of effects may be expected in view of the potential influences of the moon on sleep.

First and foremost, to us it seems essential that the same subject must be recorded at each of the lunar phases, ideally more than once. Comparing data obtained from different subjects at different lunar phases is inherently prone to biases and imbalances in terms of age, gender, and many other factors. Indeed, one potential reason for the discrepancy between the studies by Cajochen et al. [2] and Smith et al. [4], with respect to REM sleep latency, is that in the former the data set collected around the full moon was dominated by older subjects and made up of approximately twice as many women as compared to the group recorded around the new moon [2]. By contrast, the study by Smith et al. studied only young subjects, with balanced proportions of males and females within both full moon and new moon phases, although it should be noted that there was a substantially lower number of subjects included in the full moon group. The study by Cordi et al. [3] was different again. In this case a large cohort was used but calculations were derived from different subjects, participating in different experiments and recorded under different conditions. As is well known, there is substantial inter-individual variability in many sleep parameters even within the same gender, age or race, including large differences in sleep duration, the response to sleep deprivation or EEG characteristics [6–9]. We suggest that studies based upon large heterogeneous populations could potentially miss relatively subtle effects, especially if present only in a subset of sensitive individuals. Furthermore, individuals may respond differently, both in terms of the specific sleep parameters affected and with respect to the influence of a specific lunar phase. Finally, of course, the response may differ on a day-to-day basis, introducing yet another confounding factor.

Our second point is that the experimental design for studying lunar effects on sleep would benefit from a hypothesis-driven approach, or at least some specific a priori expectations about the potential mechanisms. Proposed mechanisms behind the purported lunar effects on sleep include the endogenous oscillation with a periodicity corresponding to the lunar cycle, effects of nocturnal illumination on the circadian clock and waking behavior, as well as the cognitive aspect (a placebo or ‘nocebo’ reaction). Taking these possibilities into account is essential for developing a valid, well-controlled experimental design. We will now consider these mechanisms in more detail.

First, and perhaps the most intriguing possibility, is that we possess an endogenous circa-lunar clock, which has, or used to have an important adaptive role. Endogenous ~29.5 day molecular clocks exist within marine species and seem to have considerable adaptive value in both synchronizing reproductive events and predicting exposure and cover in the intertidal zone [10]. Such selection pressures would not have been a dominant feature in human evolution, yet the menstrual cycle in women is often thought to be linked to the phase of the moon and the tides, although the evidence supporting this is, at best, inconsistent [11]. Comparisons of the menstrual cycle length across the great apes has been informative. The human menstrual cycle is, on average, 28 days long but it can range from 21–35 days. In the great apes the menstrual cycle is ~29 days in orangutans, ~30–32 days in gorillas, ~32–35 days in bonobos and ~31–37 days in chimpanzees. The differences in cycle length across the apes, including humans, relates to food supply, social stress and nutritional status [12]. It is unclear how the phase of the moon might influence such factors, and what orangutans and humans might share in common to warrant lunar regulation of their menstrual cycles, the lengths of which are very similar (~29 and ~28 days, respectively).

Lunar phase has also been suggested to affect human sleep by providing a powerful source of light. It should be emphasized that most of the studies reported in the three papers [2–4] were performed in laboratory conditions where nocturnal illumination was controlled. Interestingly, in one of the studies, evening melatonin levels were decreased around full moon even though subjects were isolated from lunar light [2].

Lunar light at night raises the fundamental question of whether it is acting upon sleep rather than wake. As an essential defining characteristic of sleep is a withdrawal and sensory ‘disconnection’ from the environment, diurnal species such as ourselves would ideally prevent any influence of the moon on sleep. Therefore, it would be more accurate to suggest that if there is an effect of the moon, it is unlikely to concern sleep directly, but rather those neural networks that regulate excitability and wake. Indeed, there is tantalizing evidence for a link between lunar phases and epilepsy, with several studies having...
identified a correlation between the frequency of epileptic seizures and lunar phase or nocturnal illumination [13], and recently it has been argued that the word lunatic originally meant a person affected by epilepsy, rather than insanity [14]. We do not know whether light reflected from the lunar surface makes the brain more ‘excitable’, elicits certain types of behavior at its specific phases, or merely prolongs waking to cause secondary effects on sleep. A further complication is whether humans have a conscious awareness of specific phases of the moon.

The ~3,000 year-old Golden Hat in the Museum of Prehistory and Early History in Berlin illustrates that humans were aware of the moon and used it as a stable ‘calibration’ signal, presumably to time and synchronize their ongoing activities, as well as for making future plans. The presence and position of the moon seems to have been used by human societies as a navigation tool and for extending activity into the night, with the full moon in November, traditionally referred to as the ‘Poacher’s Moon’, allowing the capture of winter game for the pot. In addition to these practical applications many myths and legends draw upon the moon. One such myth, that humans can transmogrify into werewolves or vampires during a full moon, transmitted from generation to generation and likely served as a safeguard for preventing people from leaving their homes when they would be exposed to greater dangers. So although lunar phases do not play a significant role in the life of the industrialised nations, in the past the moon did feature as an important signal, and seems to have become a meme, which still propagates through generations, inadvertently affecting our daily life. Thus, if a subject is consciously aware of the lunar phase, it may be sufficient to influence their pattern of sleep through changes in behavior or mood and so the moon could trigger a potent placebo or a nocebo reaction [5].

How does waking behavior affect our sleep? It is well established that sleep is homeostatically regulated, which is reflected in longer and/or deeper sleep after extended waking [15,18]. Sleep thus appears to be a flexible behavior, as manifested in its exquisite sensitivity and fine tuning of its regulatory mechanisms to ‘sleep need’. The emergence of sleep could have been a major evolutionary leap in regards to increasing flexibility, as it allowed its functions to be fulfilled not only when the time is appropriate to do so, but also in proportion to the need [17]. Thus, if lunar phases affect, directly or indirectly, waking duration, quality or specific waking activities, the changes in subsequent sleep can be expected merely as effects of preceding waking, rather than direct lunar effects on sleep. Notably, the effects of preceding sleep–wake history are long-term, such that even if the moon were to reduce sleep quality on a given night, this would likely lead to compensatory effects on the following night, which could be mistaken for the effects of the moon itself.

In summary, it appears that two main challenges must be addressed in future research on the lunar effects on sleep. First, it is mandatory to design an original within-subject experiment, rather than perform further retrospective studies. This would prevent inherent biases and imbalances typical for across-subject protocols, and would control for numerous confounds which are difficult to exclude in retrospective studies, such as the effects of season or preceding waking history. Important new insights can be obtained using well established experimental approaches, including forced desynchrony protocols [18] and cohorts such as congenitally blind subjects [19]. Second, future experiments would likely benefit from a hypothesis-driven approach, which would not only address and disentangle specific mechanisms (such as endogenous circa-lunar rhythmicity, brain excitability or light), but also include appropriate controls to account for confounding factors inherent for specific hypotheses under scrutiny.

More generally, the conundrum of ‘lunar effects on sleep’ represents an exemplary case of a scientific question which should be approached with caution, as it may seem much easier than it will likely be. Having said that, it is essential to remain open-minded, and it is possible that solving the ‘lunar madness’ or ‘lunar sleeplessness’ question will be rewarding and lead to novel, fascinating and unexpected insights into the effects of the environment on sleep and in particular on our physiology in general. Would any funding agency offer to support this kind of research? Well, this may not seem a top priority, but it may bring significant societal and economic benefits, especially over time. In fact, one study found significant effects of the lunar cycle on the patterns of stock returns in all major U.S. stock indexes over the last 100 years; specifically, around the new moon returns were approximately doubled compared to around the full moon [20]. In view of such findings perhaps philanthropists in the city might fund a project to determine the basis for this loss of income?

References

Autophagy: Close Contact Keeps Out the Uninvited

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Autophagy is a self-degradation system equipped in eukaryotic cells [1,2]. It delivers a wide range of intracellular material, including whole organelles such as mitochondria, into lysosomes (in mammals) or vacuoles (in yeast and plants), which contain various hydrolases including proteases, nucleases, lipases, and glycosidases. Therefore, autophagy, in principle, serves as a degradation system for most biological macromolecules. In the process of autophagy, small, flattened membrane vesicles called isolation membranes (or phagophores) are formed, and these expand while engulfing degradation targets, and finally close to become double-membraned vesicles called autophagosomes. The outer membrane of the autophagosome then fuses with the lysosomal/vacuolar membrane, leading to degradation of the inner membrane and the contents.

Autophagy was discovered by electron microscopy of cells under nutrient-deprived conditions, in which autophagosomes sequestered random portions of the cytoplasm [3,4]. Since then, autophagic degradation had long been regarded as bulk and non-selective, and this property was reasonable considering its physiological role, providing degradation products as nutrients under starvation conditions. However, we now know that autophagy is more useful; it can also selectively degrade various targets, including protein aggregates, damaged mitochondria, and even intracellular pathogens. This type of autophagy is called selective autophagy and has been studied extensively in recent years given its involvement in human diseases [5–8]. In most cases, selectivity is determined by receptor proteins that play dual roles in wrapping targets by the autophagosomal membrane. First, receptor proteins recognize a specific target and recruit the machinery for membrane formation to the target. Secondly, when the isolation membrane is thereby formed, receptor proteins also bind to Atg8 family proteins on the membrane to link the target to the membrane. Consequently, in at least some cases, targets are exclusively wrapped (without incorporating other cytoplasmic material) by the autophagosomal membrane. A new study by Sawa-Makarska et al. [9] now provides critical mechanistic insights into these functions of receptor proteins: how they act only in the presence of their targets and how they achieve exclusive sequestration of the targets into the autophagosome.

These authors started with an analysis of the interaction between Atg8 and Atg19, a receptor for the most well-studied, selective autophagy-related pathway — the cytoplasm-to-vacuole targeting (Cvt) pathway in the budding yeast Saccharomyces cerevisiae [10]. Atg19 binds to a large assembly of the vacuolar peptidase Ape1 and triggers its exclusive sequestration into the autophagosome [11]. Previous studies established that autophagic receptors, including Atg19, interact with Atg8 family proteins via motifs named the Atg8-family interacting motif (AIM) or the LC3 (a mammalian Atg8 homolog)-interacting region (LIR) [5–7,12]. Atg19 recognizes Ape1 with a coiled-coil domain in the central region and the AIM is localized at the carboxy-terminal end (Figure 1) [13,14]. The authors analyzed interactions of truncated Atg19 variants with Atg8, and they found that truncation of the coiled-coil domain enhances the Atg19–Atg8 interaction [9]. This suggested that, in the wild-type protein, this domain inhibits the binding of the carboxy-terminal region to Atg8. Since the coiled-coil domain is responsible for Ape1 recognition [13], the authors reasoned that Ape1 might relieve this inhibition, and they showed that this was indeed the case; Atg19 bound to Atg8 with a much higher affinity in the presence of Ape1 (specifically, an Atg19-interacting region of Ape1) [9]. In the Cvt pathway, the assembly of the machinery for membrane formation requires both Atg19 and Ape1 [15]. The enhancement of the Atg19–Atg8 interaction by Ape1 may represent part of this mechanism. The receptor–Atg8 interaction may be regulated by targets in the initiation of other selective autophagy pathways as well. In addition, if the interaction of receptors with Atg8 on the isolation membrane also depends on targets, this would be beneficial to prevent a receptor from being degraded wastefully in the absence of the target or from unnecessarily competing with receptors that mediate sequestration of other targets for Atg8 on the membrane.

On the other hand, the authors unexpectedly found that Atg19 lacking the previously identified AIM at the carboxyl terminus still interacts with...