SA01

Neural circuits in sleep and anaesthesia

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Putting a patient to sleep has been used as a metaphor for drug-induced sedation and anaesthesia ever since general anaesthetics were first used clinically during the 1840s. Only relatively recently, however, has the possibility that general anaesthetics may act, at least in part, by affecting some of the natural pathways of sleep and arousal, been investigated in detail. I will discuss some of the evidence that sleep and anaesthesia may affect common neuronal pathways, and then go on the describe experiments showing that overlapping ensembles of neurons in the hypothalamus are involved in both deep sleep and drug-induced sedation, and that the same networks may also be responsible for the hypothermia seen in both states.

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"A Neuronal Hub Binding Sleep Initiation and Body Cooling in Response to a Warm External Stimulus" Harding EC *et al. Current Biology* 28:2263-2273 (2018).

Funded by the Wellcome Trust and the UK Dementia Research Institute.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA02

Sleep function and control in Drosophila melanogaster

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Sleep appears to be a universally conserved phenomenon among the animal kingdom but whether this striking evolutionary conservation underlies a basic vital function is still an open question. Using novel technologies, we conducted an unprecedentedly detailed high-throughput analysis of sleep in the fruit fly *Drosophila melanogaster,* coupled with a life-long chronic and specific sleep restriction. Our results show that some wild-type flies are virtually sleepless in baseline conditions and that complete, forced sleep restriction is not necessarily a lethal treatment in wild-type *Drosophila melanogaster*. We also show that circadian drive, and not homeostatic regulation, is the main contributor to sleep pressure in flies.

We propose a three-partite model framework of sleep function, according to which, total sleep accounts for three components: a vital component, a useful component, and an accessory component.

Most sleep does not serve a vital function. Evidence from Drosophila melanogaster Quentin Geissmann, Esteban J. Beckwith, Giorgio F. Gilestro doi: https://doi.org/10.1101/361667

Quentin Geissmann, Esteban Beckwith, Anne Petzold

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA03

Neural Networks and Sleep

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Sleep has been found in all animal species carefully studied to date; yet, the biological function of sleep remains unclear. Sleep can be defined on at least two distinct levels: the behaviour of the whole organism and the spatiotemporal patterns of neuronal activity in the brain. Upon falling asleep, cortical networks alternate between periods of generalized population firing and periods of relative silence. This pattern of neuronal activity gives rise to electroencephalogram (EEG) oscillations at a frequency of approximately 1-4 Hz, which are termed slow waves. Contrary to the widely-held notion, waking and sleep are not global, mutually exclusive states, and research over the last decades has revealed that spontaneous brain activity during sleep can be locally modulated. For example, slow wave activity (SWA) is more intense in frontal compared to more posterior areas, especially in early sleep or after sleep deprivation, and regional differences are apparent at the level of individual sleep slow waves. Although the alternation of periods of increased neuronal activity and silence is usually correlated across cortical regions and individual neurons, up-states can sometimes be seen in one region of the cortex while another region is in a down-state, with these states often spreading as travelling waves. Sleep deprivation is associated with increased low-frequency EEG activity during waking in both animals and humans, and recordings in rodents suggested that this EEG pattern reflects local neuronal OFF periods in the neocortex. Although the role of subcortical neuromodulatory areas in generating and maintaining sleep and wakefulness is well established, the possibility remains that the neocortex is also involved in sleep regulation. Consistent with this hypothesis, we observed a marked increase in the amount of wakefulness and a diminished increase in SWA after sleep deprivation in transgenic mice, in which a subset of pyramidal cells in layer 5 is functionally silenced by removal of the t-SNARE protein SNAP25 (Rbp4-Cre;Ai14;Snap25$^{fl/fl}$). These notions suggest that

sleep need accumulates at the level of local cortical networks, which are directly implicated in global sleep regulation.

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SA04

Sleep and synaptic down-selection

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The synaptic homeostasis hypothesis (SHY) proposes that sleep is an essential process needed by the brain to maintain the total amount of synaptic strength under control. SHY predicts that by the end of a waking day the synaptic connections of many neural circuits undergo a net increase in synaptic strength due to ongoing learning, which is mainly mediated by synaptic potentiation. Stronger synapses require more energy and supplies and are prone to saturation, creating the need for synaptic renormalization. Such renormalization should mainly occur during sleep when the brain is disconnected from the environment and neural circuits can be broadly reactivated off-line to undergo a systematic and yet specific synaptic down-selection. In short, according to SHY, sleep is the price to pay for waking plasticity to avoid runaway potentiation, decreased signal-to-noise ratio, and impaired learning due to saturation. I will discuss the rationale underlying this hypothesis and summarize electrophysiological, molecular and ultrastructural

studies in flies, rodents and humans that confirmed SHY's main predictions, including the recent observation, obtained using serial block face scanning electron microscopy, that most synapses in mouse primary motor and sensory cortices grow after wake and shrink after sleep. I will then present unpublished ultrastructural data obtained in the hippocampus and in the cortex of mouse pups. Finally, I will examine recent studies by other groups showing the causal role of cortical slow waves and hippocampal ripples in sleep-dependent synaptic down-selection, and discuss some of the molecular mechanisms that can mediate this process.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA10

Sleep in space

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With rapid scientific development and interest in interplanetary travel in the 20th century, there has been an increasing focus on the effects of spaceflight and microgravity on human physiology. Whilst the effects of spaceflight on cardiovascular and respiratory physiology are well recognized, the effect on the brain has not been widely studied. Astronauts have long been reported to experience impairments in sensorimotor function including posture control, spatial orientation, manual tracking, and cerebellar dysfunction.

Significantly shortened and disrupted sleep has also been consistently reported in space missions. Sleep serves a restorative function for the brain and cognition and involves dramatic changes to our perception, consciousness, cognition and health. The perpetual activity of the brain is largely supported by a variety of oscillations and rhythms it generates. Sleep and sleep stages are also characterized by specific brain oscillations, which, unlike those of wakefulness, are maintained free of external inputs. This means that a transient perturbation during sleep can have a lasting impact. Recent studies on astronauts have shown that weightlessness

induces neurophysiological changes including a cephalic fluid shift which alters cerebrospinal fluid volume, cerebrovascular flow and autoregulation, and intracranial pressure. These changes may also induce various neuroplastic changes and significant structural and functional remodeling in the central nervous system including the effect on the oscillations of sleep, positing that spaceflight might be associated with structural, functional and cognitive deficits of, as of yet unclear, longer-term impact.
In that background, the results of our recent study that investigated the effects of seven days of supine unloading on a hyper-saline filled water bed (hyper buoyancy floatation), a novel Earth-based novel analogue of microgravity will be discussed. In our study, twelve healthy male subjects (flotonauts), aged (27.2±4.2 years), with no previous neuro/psychiatric history, underwent a multimodal imaging and an overnight in-laboratory polysomnography (PSG/EEG) recordings during seven days of an unloading period. For the duration of the intervention period, the subjects lied supine, followed controlled sleep/wake schedules, they were fed a controlled diet and allowed a maximum of 15 minutes per day off the hyper buoyancy floatation bed (for personal hygiene etc). Several significant changes in sleep rhythms were found, with associated changes in neuroanatomy, cognition and neuroelectrical activity and connectivity. These changes will be presented with particular emphasis on changes in slow oscillations during sleep, which have recently been associated with amyloid load in the brain. The proposed underlying mechanisms will be discussed in some detail.

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SA11

Strategic Decision Making and Potential Drug Targets for Obstructive Sleep Apnea Pharmacotherapy

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The root cause of some of the most common and serious sleep problems is impaired breathing. Of the sleep-related breathing disorders, obstructive sleep apnea (OSA) is the most prevalent and is associated with significant clinical, social and economic consequences. OSA is ultimately caused by closure of the pharyngeal airspace during sleep due largely to relaxation of the tongue muscles whose activity normally keeps the airspace open. The hypoglossal motoneuron pool is the source of motor output to the tongue, and in theory strategies to modulate its activity may lead to identification, development and testing of new pharmacological treatments for OSA. The first part of this symposium presentation will identify the four principal factors underpinning the pathophysiology of OSA. Two of these factors are strongly influenced by upper airway muscle activity, and as such are amenable to targeted

manipulation. The presentation will then identify the two major mechanisms operating at the hypoglossal motoneuron pool in rats to modulate tongue muscle activity across natural sleep-wake states. Such studies have identified that there is a functional endogenous noradrenergic drive to the hypoglossal motoneuron pool that activates motor output to the tongue muscle in wakefulness via an α_1 receptor mechanism, with this drive being withdrawn in sleep. The presentation will also identify the mechanism of tongue muscle inhibition in REM sleep in rats, this being an acetylcholine mediated G protein-coupled inwardly-rectifying potassium (GIRK) channel mechanism. In addition, modulation of certain K^+ channels can reactivate tongue muscle activity throughout sleep in rats. Others have manipulated (with success) such mechanisms as potential OSA pharmacotherapies in humans (e.g., trial IDs: NCT02428478, NCT02656160, NCT02908529 at clinicaltrials.gov). The presentation will conclude with the identification and description of a resource of potential drug targets for OSA pharmacotherapy. Some of these targets and their pharmacological agents (e.g., thyrotropin releasing hormone analogs) have been studied in pre-clinical rodent models. Overall, these basic science findings inform current and future studies in humans to identify the potential beneficial effects of pharmacological agents for breathing during sleep and OSA.

The work presented in this symposium presentation was supported by funds from the Canadian Institutes of Health Research (CIHR, Grant MT-15563 awarded to RLH), and the National Sanitarium Association Innovative Research Program (fund number 00144051 awarded to RLH). RLH is supported by a Tier I Canada Research Chair in Sleep and Respiratory Neurobiology (fund number 950-229813).

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SA15

Cellular and molecular basis to circadian rhythms in mammals and its relevance to metabolic and neurological disease

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Circadian rhythms are cycles of metabolism, physiology or behaviour that persist with a period of approximately one day (hence circa- -dian) when organisms are held in temporal isolation. Their persistence is evidence of an internal timing mechanism, a circadian clock. The award of the 2017 Nobel Prize in Physiology or Medicine to Hall, Rosbash and Young provided the climax to a decades-long pursuit to identify the molecular-genetic basis of such clocks, in their case that of the fruit-fly Drosophila. In mammals, as in flies, the circadian mechanism is a transcriptional/ translational negative feedback loop (TTFL). The positive regulators CLOCK and BMAL1 drive expression of *Period* and *Cryptochrome* genes, the protein

products of which, PER and CRY, subsequently inhibit CLOCK/BMAL1-dependent transcription. Progressive degradation of PER and CRY then releases the negative regulation and a new cycle is initiated approximately 24 h after the previous one. Remarkably, the self-sustaining TTFL mechanism is present in just about every cell-type and major organ system. These local TTFLs drive cell-type-specific circadian programmes of gene expression that are the determinants of the circadian cycles of metabolism, physiology or behaviour that anticipate, and thereby adapt organisms to, the solar cycle of light and darkness.

Circadian regulation of cellular functions is therefore pervasive, and *ab initio*, critical to health. It is regulated in a hierarchical manner, with the principal circadian clock of mammals being the suprachiasmatic nucleus (SCN) of the hypothalamus. The 10,000 or so neurons and astrocytes of the SCN are capable of maintaining circadian cycles of TTFL function and electrical activity indefinitely when isolated in culture. It is a powerful circadian timing circuit that in vivo is entrained to solar time by direct innervation from retinal ganglion cells. In turn, via its innervation of the hypothalamus and brain stem, the SCN directs a complex series of endocrine, autonomic and behavioural cues that synchronise the innumerable local TTFLs across the body, forging them into a single adaptive temporal programme. This presentation will review recent developments in understanding the cellular and network-level properties of SCN time-keeping, and highlight how the new understanding of the TTFL and the hierarchical organisation of the mammalian circadian timing system will provide a platform for the next challenge to circadian biologists: how to apply circadian knowledge to understand and treat metabolic and neurological disease.

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SA17

REM sleep in blind people

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Abstract Body : Study objectives: There is an ongoing controversy regarding the role of rapid eye movements (EMs) during REM sleep. One prevailing hypothesis is that EMs during REM sleep are indicative of the presence of visual imagery in dreams. Congenital blindness appears as a good model to test empirically the validity of this claim since congenitally blind (CB) individuals never developed a visual repertoire. We therefore tested the validity of the scanning of visual dreams hypothesis by measuring EMs in CB individuals, individuals that became blind later in life (LB) and sighted controls (SC) and correlated these with visual dream content. Methods: Eleven blind, of whom 5 were blind from birth (CB; 40.8±16.1 years of age) and 6 that became blind later in life (LB; 47.5±14.7 years of age) and 11 matched sighted control (SC; 43.9±14.8 years of age) subjects participated in this study. The Blindness Duration Index (BDI), calculated as the duration of blindness over age, represents the relative amount of time a subject has been blind, where high scores indicate that they have been blind for the majority of their life, and low scores indicate a recent onset of blindness. All participants underwent full-night polysomnography (PSG) recordings staged manually following American Academy of Sleep Medicine (AASM) scoring criteria. Periods with any kind of EMs were detected automatically by using a validated EM detector, and the EM coverage was measured as the percentage of time containing EMs, during wakefulness, N1, N2, N3 and REM sleep. Frequency of sensory dream elements was measured in dream recall questionnaires over a 30-day period.
Results: Both blind groups showed a lower EM coverage during wakefulness, N1, N2 and REM sleep than did controls. CB and LB participants did not differ in EM coverage. Post-validation of the detector applied to blind subjects revealed an overall accuracy of 95.6±3.6%. There were no significant correlations between the incidence of nocturnal EMs and BDI. Analysis of dream reports revealed that CB

participants reported very few or no visual dream elements, which was significantly lower as compared to both LB and SC participants.
Conclusions: We found dissociation between EMs and visual dream content in the two groups of blind participants. The quasi absence of nocturnal EMs in LB individuals despite preserved visual dream content does not support the visual scanning of dreams hypothesis. It might be argued that extended blindness in LB has led to an uncoupling of EMs from visual dream content.

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SA18

Light, sleep and circadian interactions: Biology to new therapeutic targets

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By studying how circadian rhythms and sleep are regulated by the dawn/dusk cycle we demonstrated that there exists a "3rd class" of photoreceptor within the eye based upon a small number of photosensitive retinal ganglion cells (pRGCs) that utilise the blue light sensitive photopigment melanopsin (OPN4). Whilst there has been remarkable progress in understanding the complex intracellular mechanisms that generate circadian rhythms, the molecular pathways whereby the pRGCs entrain circadian biology and sleep has remained poorly understood. The suprachiasmatic nuclei (SCN) are the site of the primary circadian pacemakers within the mammalian brain. Until recently, the model for entrainment involved a simple linear pathway whereby glutamate release from the pRGCs resulted in Ca2+ influx and raised intracellular cAMP in SCN neurones, which in turn resulted in CREB phosphorylation leading to increased transcription of two key clock genes, Per1 and Per2. This signal then advanced or delayed the molecular clockwork. However, an important feature of entrainment is that circadian responses to light are limited – as typified by jet-lag. Full recovery from jet-lag requires a day for every time-zone crossed. We addressed this issue and have identified and characterized a key role for Salt Inducible Kinase 1 (SIK1) and the CREB-regulated transcription co-activator 1 (CRTC1) in clock re-setting. However, our more recent and unpublished

findings have shown that light entrainment also involves the parallel activation of a Ca2+-ERK1/2-AP-1 signalling pathway. Thus both CRE and AP-1 regulatory elements drive light-induced clock gene expression. In addition, whilst light activation of the Ca2+-ERK1/2-AP-1 signalling pathway increases Per1 and Per2 expression, sleep/wake behaviour alters the effects of light on the clock. Our proposed mechanism suggests that adenosine acts as a signalling molecule that encodes wake duration. Adenosine acts via inhibitory A1 receptors on the SCN to inhibit the Ca2+-ERK1/2-AP-1 signalling pathway, which in turn, reduces the expression of Per1 and Per2. Thus sleep/wake history, encoded by adenosine, reduces the phase shifting effects of light upon the circadian system, altering sleep/wake timing. These new pathway will be presented and placed into an ecological context. Furthermore, we will explore the possibility of how such signalling mechanisms provide a potentially new target for the regulation of circadian rhythms and the "pharmacological" replacement of light for sleep/wake re-setting in individuals lacking eyes or other individuals with severe circadian rhythm disruption.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C01

Interrelationship between sleep stability and glymphatic function

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It was recently discovered that waste products, such as amyloid-b, are cleared from the brain by cerebrospinal fluid via what was named the glymphatic system, and that this cleaning of the brain is only active during sleep [1], [2]. Neurodegenerative diseases, such as Alzheimer's disease, are often comorbid with sleep disturbances [3], and impaired glymphatic clearance has been proposed as a possible risk factor of amyloid build-up [1]. Therefore, a better understanding of the link between the glymphatic system and sleep could have important implications for treatment of diseases that share this common link. In this study, we explored the possibility that glymphatic flux might not only be controlled by sleep, but in itself participate in stabilization of the sleep state.

To test this, sleep was measured in mouse models of impaired glymphatic flux previously used in our laboratory, namely aquaporin 4 knockout mice (AQP4 KO), mice subjected to cisterna magna puncture (CM puncture, performed under anesthesia with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) intraperitoneally (i.p.)), and mice subjected to acetazolamide treatment (20 mg/kg i.p. every 6 hour for a total of 4 injections) [4]. To investigate whether glymphatic impairment leads to decreased sleep stability, sleep was monitored using EEG and EMG electrodes that had been implanted under isoflurane anesthesia and i.p. injections of carprofen (5 mg/kg) and buprenorphine (0,05 mg/kg) 2 weeks prior to recording. Furthermore, sleep characteristics of a second group of mice were monitored non-invasively using immobility-defined sleep analysis.

Non-invasive sleep analysis and EEG did not show any overall differences in sleep pattern between mice treated with saline, acetazolamide, CM puncture or sham surgery (figure 1A-C). However, AQP4 KO mice were found to have shorter nREM bouts than all other groups, and shorter REM bouts compared to saline-treated mice. Spectral analysis of nREM sleep during the first 4 hours of the light period after glymphatic manipulations did not show any differences between mice treated with acetazolamide, saline or sham surgery (figure 1D). However, AQP4 KO mice displayed a right shift in delta power with significantly higher power in the high end of the delta spectrum. Furthermore, mice subjected to CM puncture displayed increased power in the low delta frequencies.

Our study did not show a causative relationship between glymphatic flux and sleep stability. However, it does not rule out that water transport through AQP4 water channels could have implications for sleep, as AQP4 KO mice were found to have fewer bouts of nREM sleep and REM sleep, and displayed a right shift in nREM delta frequency. In conclusion, this study supports a model where sleep drives

glymphatic clearance, but glymphatic flux does not have a direct and acute effect and sleep stability or sleep quality (figure 1E).

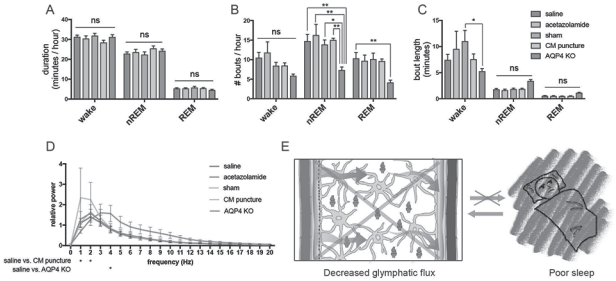

Figure 1. EEG analysis of sleep in control mice (saline injections or sham surgery) and mice with impaired glymphatic function (acetazolamide injections (20 mg/kg), CM puncture, AQP4 KO). (A) average duration/hour of wake, nREM, and REM sleep. (B) average number of bouts/hour for each state. (C) average bout length for each state. Two-way Anova and Tukey's test, n=5. (D) normalized power spectrum of nREM sleep during the first 4 hours of the light period following glymphatic manipulations. Two-way Anova and Dunnet's test, n=4-5. (E) these results indicate that there is a one-way relationship between sleep and glymphatic flux, as sleep drives glymphatic clearance but impaired glymphatic flux does not acutely decrease sleep stability. Error bars are mean +/- SEM. Significance is shown as * = $p<0.05$, * * = $p<0.01$ and * * * = $p<0.001$. CM = cisterna magna, AQP4 = aquaporin-4, KO = knockout, ns = not significant.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C02

Improving Student Sleep Quality and Quantity to Improve Higher Educational Experience

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Background: Poor sleep hygiene negatively impacts cognitive and physical abilities in students (Mah et al., 2018) and is common among students of higher education (Curcio et al., 2006). Moreover, a wide-range of literature explores the detrimental effect of poor sleep quality on learning.
Methods: An anonymous, voluntary and self-administered questionnaire was made available to a cohort of medical students at Imperial College London (n = 113; 60 female). Demographic information was collected to determine existing sleep quality. Questions regarding understanding of, and desire for sleep hygiene interventions to improve their experience of medical education were created on a 5-point Likert scale, ranging from 5 (strongly agree) to 1 (strongly disagree). Students were also asked to rank, the aspects in their lifestyle that warranted the most attention for improvement.
Results: Students from across all years strongly agreed that their sleeping habits could be improved (Mean [SD]: 4.13±0.86). Quantitative analysis revealed that there was strong consensus about the need to sleep better with sleep ranking top out of the six suggested categories for improvement. Equipping students with the time and energy management tools needed to maintain consistent sleep of adequate duration would be well received (3.73±0.97). Students understand the beneficial relationship between sleep with learning, and agree that a concerted intervention effort, such as having sleep promotion activities across campus would be beneficial for their education (3.65±0.90).
A low quality of sleep negatively impacts activities in the morning (linear model, $p=0.0006$, overall adjusted $r^2=0.17$) and in the afternoon ($p=0.02$), but not in the evening ($p=0.06$): more precisely, subjects reported to be more tired in the morning (1.03±0.97; 0=always tired, 3=never tired) than in the afternoon (1.52±0.83; Wilcoxon rank test, $W=4011$, $p=0.0002$). This study identified the main factors influencing sleep quality as the latency to fall asleep ($p=0.00001$), sleep duration ($p=0.002$) and frequency of dreams ($p=0.03$), together accounting for 29.0% (r^2) of the variation in sleep quality using a linear model ($p<0.0000001$). The presence of a bed partner, pain, temperature, breathing problems and waking up at night did not significantly influence sleep quality (generalised linear model, $p>0.05$ for all variables).
Summary: Students understand the importance of sleep and would be receptive of initiatives to improve sleep quality. Efforts in improving sleep quality should be directed in providing the resources to decrease the latency to sleep onset (Bartel et al., 2018) and to deep sleep to prevent dreams and increase sleep duration via

naps (Hayashi, Motoyoshi & Hori, 2005). This may further improve the tangible results of the ever-increasing drive and innovation occurring in the higher education landscape.

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C03

Sleep-wake regulation in mice: insights from a synaptobrevin-2 mutant line and computational modelling

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The alternation between waking and sleep is regulated by the internal circadian clock and sleep-wake history, and is also influenced by the external environment. Although our understanding of the circadian aspect of sleep regulation has increased, the mechanisms underlying sleep homeostasis are still largely unknown. Independent of the circadian clock, only a limited number of genes have been associated with specific sleep-wake properties. Forward genetics provides an unbiased approach, which seeks to identify genes involved in specific biological processes[1]. This project has focused on the *Sleepy6* mouse line, obtained via a forward genetics sleep screen, with a mutation in synaptobrevin 2, giving rise to decreased sleep duration. We aimed to further characterise the sleep phenotype of this line, at a molecular and behavioural level, to gain novel insights into the regulation of sleep. Using molecular techniques (high-performance liquid chromatography, quantitative PCR) to evaluate neurotransmitter levels and gene expression, we found no significant difference in the neurotransmitter pathways investigated (n(wild-type-WT)= 8, n(mutant)=10, measured compounds and levels of expression of genes involved in serotonergic and dopaminergic pathways, mixed-design ANOVA followed by t-tests, p-values all > 0.05). Behavioural assays highlighted hyperactivity, with a mild learning impairment (n(WT)=28, n(mutant)=21, mixed-design

ANOVA revealed a significant main effect of genotype: F(1,45)=8.93, p=0.005). Electrophysiology recordings revealed striking differences at global (electroencephalography - EEG) and neuronal levels, with *Sleepy6* homozygous mice showing a decreased ability to switch between vigilance states, and notable alterations in neuronal firing patterns during slow-wave sleep (surgeries were performed under isoflurane anaesthesia, inhalation, 1.5-2.5%; n(WT)=5, n(mutant)=5, mixed-design ANOVA, p-values ranging from 0.001 to 0.041 when analysing time spent in vigilance states and episode durations). Finally, the successful adaption of an elaborated version of the "two-process" model[2] of sleep regulation furthered our understanding of sleep/wake control in both wild-types and *Sleepy6* homozygotes (n(WT)=5, n(mutant)=5, non-parametric Mann-Whitney tests, $p<0.01$ for the rate of decrease (WT: $60\pm8\ ^{*}10^{-5}$ $(4s)^{-1}$; mutant: $23\pm3\ ^{*}10^{-5}$ $(4s)^{-1}$, mean±SEM) and upper asymptote (WT: 447±33 %; mutant: 172±15 %, mean±SEM) of the simulated homeostatic process in the frontal EEG derivation). This combination of *in vivo* and computational work provides new insights into the mechanisms that underlie the homeostatic regulation of sleep, and more particularly, the alternation between vigilance states. It furthers our comprehension of a putative "sleep-switch", which allows animals to transfer between sleep and wakefulness in a biologically relevant manner.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C04

Heart rate dynamics during NREM sleep in rats under cold environment

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Autonomic activation caused by contact to low temperature environment may increase the number of activation phenomena on sleep EEG, sleep fragmentation and sleep onset latency, as well as depress sleep at all.
It should be note that the episodes of autonomic activation are clearly seen in NREMS even at thermoneutral zone and characterized by the pseudo-rhythmic appearance of arousal phenomena in EEG. To what extend the cold influences the vegetative background of such phenomena is still poorly understood. The research aimed to study the long-term heart rate (HR) variations as a marker of autonomic activity changes in NREM after periodic cold exposures in rats.
The experiments were performed in 7-8-month male white breedless rats males (m=250-300 g, n=14). Heart rate (HR) and EEG were recorded and processed using a Poly-Spectrum-8 ECG system and Neuron-Spectrum-2 EEG system (Neurosoft).

Under general anaesthesia (i/p, thiopental sodium–oxybutyrate sodium mixture, 30 and 100 mg/kg) 4 electrodes for HR registration were secured under the skin in the paws' areas, tunnelled subcutaneously, fixed to the skull (together with 2 EEG electrodes) by dental acrylic and connected to the registration systems via electric swivel (Moog). Animals were exposed to cold over two days to +10°C or -12°C in the light period for 15 min hourly, for a total of nine exposures per day. Sleep stages were scored following the standard criteria on 4-second epochs. Continuous (45 min) HR registration after every cold exposure was performed. To minimize a transition effect, after each cold exposure the corresponding 46 s' interval during NREMS toward the end of the registration period were chosen for the analysis. Artefact-free, visually corrected R-R intervals data were imported into Kubios 2.2 software (Kubios Oy). Data were means±SD, compared by ANOVA.
Every cold exposure awaked the animals and different time was needed for them to enter sleep and achieve the stable HR level in NERMS again. However, RR temporary dynamics in NREMS consisted of rather stable episodes (40-200 s) interrupted by short (10-20 s) activation-deactivation periods. The mean distance between acceleration-deceleration episodes in control NREMS was 86.78±35.47, n=6, but decreased in NREMS both after +10°C (37.69±21.88, n=9, p=0.0001) and -12°C (77.37±38.16 s, n=6, p=0.0003). The mean HR increased significantly during NREMS after -12°C (from 336.3±9.7 at control to 352.3±29.2, n=6, p=0.05) and had a positive tendency after +10°C (347.2±27.5 ms, n=9, p=0.07). According to LF/HF ratio calculations the slight shift in sympatho-vagal balance was shown from 0.40± 0.19, n=6, at control, to 0.55± 0.36, n=6, p=0.06 and 0.53± 0.24, n=9, p=0.02, after -12°C and +10°C, correspondently. Thus, the studied periodic cold effects slightly change the sympatho-vagal balance and probably influence brain arousability as well as recuperative value of sleep.

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C05

How is valence encoded during sleep?

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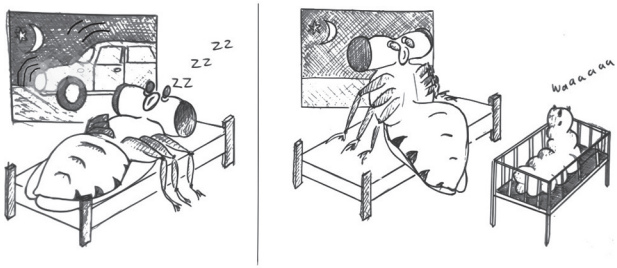
Sleep, with a few exceptions, is characterised by a state of immobility and reduced awareness of the surrounding environment. While sleep is a fundamental process, it has drawbacks in that a sleeping individual is less likely to detect cues signalling danger, food or mating opportunities which has a direct impact on fitness. While increased arousal thresholds are typical during sleep, some stimuli are more arousing than others. This begs the question: how do certain sensory pathways remain sensitised during sleep and differentially interact with sleep centres?

Here we present a new paradigm in order to study arousal in Drosophila (Geissmann et al., 2017). Acetic acid, which is produced by fermenting fruits, was delivered to sleeping flies. Our results show that different concentrations, which signal ripe to rotten fruit, and activate different subsets of olfactory neurons differ in their ability to wake sleeping flies (Semmelhack and Wang 2012). The most arousing concentrations were those with appetitive connotations and were not the most intense. Further we show that valence is an attribute that is highly plastic: both experience and internal state (changes in satiety, sleep pressure and intoxication) can shift arousal thresholds.
The mechanisms through which our sensory systems encode valence during sleep remain elusive and the subject represents an exciting yet understudied field.



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Quentin Geissmann, Esteban Beckwith and Giorgio Gilestro all contributed to this project.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C06

"Flying on Empty" – The Effects of Sleep Loss on Mood and Task-Specific Competencies in Commercial Airline Pilots

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Introduction: Current commercial airline flight operations work on a pressurised 24/7 timetable due to the unrelenting escalation in international long-haul, short-haul, regional and overnight flights. As a result, commercial airline pilots are highly suspectible to sleep loss and fatigue. Loss of sleep is proposed to be a key cause of pilot error and could pose a serious threat to flight safety (Caldwell et al., 2009). Therefore, this study examined the effects of 24 hours' sleep loss on mood, pilot-specific competencies and flying performance.
Methods: Seven short-haul commercial airline pilots completed the Samn-Perelli Crew Status Check, Profile of Mood States, Psychomotor Vigilance Task, Dual-N-Back, NASA Task Load Index and aviation-specific mathematical calculations. Participants also flew a 32-minute flight profile on a computerised flight simulator during which they were required to answer mid-flight fuel calculations and situational awareness questions. Testing occurred at 3 hour intervals during the final 12 hours of a 24 hour period of continuous wakefulness.
Results: One-way repeated measures ANOVA found that feelings of fatigue decreased ($F(6, 36)=6.585$, $p<.001$) whilst total mood disturbance increased ($\dagger F(1.756, 10.539)=8.734$, $p<.01$) with increasing time awake. Furthermore, sustained attention ($\dagger F(1.193, 7.157)=6.491$, $p<.05$), speed and accuracy of problem solving ($F(6, 36)=5.897$, $p<.001$; $\chi 2(6)=20.463$, $p<.01$), multi-tasking ability ($\dagger F(2.509, 15.055)=3.486$, $p<.05$), perceived workload ($F(4, 24)=2.740$, $p<.05$), speed of mid-flight fuel calculations ($F(4, 24)=8.561$, $p<.001$) and situational awareness ($F(4, 24)=2.923$, $p<.05$) were all significantly impaired following 24 hours continuous wakefulness. Significant reductions in performance were observed on nearly all tests following 20 hours continuous wakefulness. Flying performance was not significantly impacted.
Conclusion: Commercial airline pilots' mood and pilot specific competencies were significantly impaired following 24 hours' sleep loss with some impairments becoming evident following 12 hours continuous wakefulness. Whilst most participants were able to maintain flying precision during the period of wakefulness, it appears they are doing so by overcoming large increases in sleep loss and fatigue which considerably degrades cognitive performance. Alas, the number of serious accidents as a result of operator error in various industries due to sleep loss and

fatigue is large and appears to be increasing (Lopez et al., 2012) thus warranting further investigation into this area.

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The authors would like to sincerely thank those who were involved in and participated in this study. This research was funded by the Irish Research Council.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C07

Circadian control of paraventricular hypothalamic activity by suprachiasmatic VIP neurons

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The co-ordination of our internal physiological rhythms with external time relies on the suprachiasmatic nucleus (SCN) of the hypothalamus. This entrainment with the environment allows organisms to optimise their physiology according to the predictable changes that occur across the 24 hour day. While it is known that the SCN is necessary for this process, the precise nature of the timing signals supplied by the SCN remains unclear. One hypothesis is that the heterogeneity within this small nucleus may allow for the co-ordination of whole body physiology, with subsets of SCN neurons possessing unique circadian profiles that direct specific physiological processes.
We tested this hypothesis using optogenetic manipulations of vasoactive intestinal polypeptide (VIP) neurons in the mouse SCN using selective channel rhodopsin-driven manipulation of VIP cells in the mouse SCN while monitoring hypothalamic network activity. Acute ex vivo brain slices were obtained following cervical dislocation, and continuously perfused with aCSF. Recordings were made on both multielectrode arrays, and with penetrating Buszaki-style octodes. These approaches allowed us identify SCN VIP cells, characterise their daily electrophysiological output profiles and also determine how the spike output from these cells influenced neuronal activity in known SCN target regions such as the sub paraventricular zone (SPZ), the paraventricular nucleus of the hypothalamus (PVN), and the ventral thalamus. We identify a subset of cells (~10% of total; n=60) across these SCN target regions that respond to VIP cell activation with robust inhibitions (VIPin+ cells; 50-200ms). Pharmacological challenge reveals that this involves a GABAergic mechanism, since responses are eliminated by application of bicuculline. VIPin+ cells can be found throughout the SPZ, PVN and ventral thalamus. The majority of VIPin+ cells (~80%)

show evidence of circadian modulation of firing activity and, collectively, VIPin+ cell activity is lowest during the mid-late afternoon to early night (ZT 5-12). This relative absence of firing corresponds to peak firing phase for SCN VIP cells (~ZT6). Taken together, results from our electrophysiological studies therefore suggest that SCN VIP cells drive rhythmic activity in a subset of responsive downstream neurons in the SPZ, PVN and ventral thalamus. These VIPin+ cells are located in output areas crucial for the control of physiological rhythms, such as the daily rise in corticosterone. These data therefore establish a route by which electrophysiological output from a defined population of SCN neurons could influence a range of downstream physiological rhythms.

This work was funded by the BBSRC

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C08

Age, Alzheimers, circadian rhythms and sleep in *Drosophila*

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All organisms on earth are subject to predictable daily environmental changes caused by the earth's rotation, therefore they have evolved circadian clocks that regulate changes in behaviour, i.e. activity and sleep, as well as in physiology and metabolism to ensure they occur at certain times allowing adaption to the environment. All studied animals, from jellyfish to humans, show some form of sleep and while it is still not resolved why we sleep, it is essential for life since sleep deprivation is detrimental to health and shortens lifespan. We use *Drosophila* to study this fundamental behaviour because flies offer numerous advantages for investigating sleep and the clock, not least the strong history of circadian research in the model organism, genetic tractability, short lifespan, rapid generation time as well as clearly defined and manipulatable neural circuits.
Capitalising on fly genetics we are using both behavioural tests of circadian and sleep activity and electrophysiological recordings from the wake-promoting, pigment dispersing factor (PDF)-positive, large lateral ventral neurons (l-LNV). As in humans where it is well established that elderly individuals have increasing difficulties sleeping at night and have an increase in daytime sleep episodes, we show that sleep in aged flies is increased at day but not at night with significantly increasing sleep duration whilst reducing sleep latency. We demonstrate that the age-dependent decline in circadian output is combined with changes in the daily activity of *Drosophila*, namely a reduction in morning and evening anticipatory behaviour. Furthermore, the arousal specific l-LNVs change their electrical properties with age with a significant decrease in input resistance but no significant changes in spontaneous electrical activity or membrane potential. We also demonstrate a

reduction in the daily plasticity of the synaptic architecture of the s-LNv neurons, likely to underlie the reduction in circadian rhythmicity during ageing.
Alzheimer's Disease (AD) is the most common cause of dementia, and is associated with sleep and circadian rhythm defects. We show that driving expression of human 4R0N tau – that is associated with AD pathology – in the *Drosophila* clock gives rise to a phenotype which closely matches the behaviour seen in human AD patients. Tauopathic flies exhibited greater locomotor activity throughout the day and night and displayed a night-time-specific loss of sleep. Under constant darkness, the locomotor behaviour of tau-expressing flies was less rhythmic than controls indicating a defect in circadian rhythm. Current clamp recordings from l-LNVs revealed elevated spontaneous firing which likely underlies the observed phenotype.
These results provide further insights into the effect of ageing and AD on circadian biology, demonstrating changes in electrical activity in conjunction with the decline in behavioural outputs.

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C09

Do women experience more sleep deprivation when the clocks go forward, compared to men?

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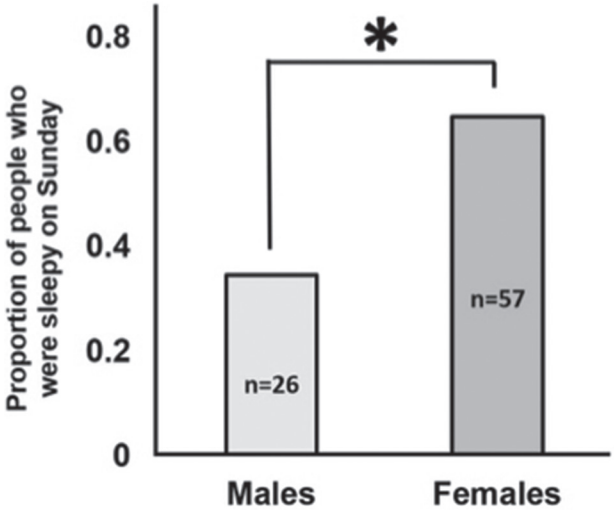
Background: The impact of, and necessity for the Daylight Saving Time (DST) transition is a debated topic due to the effect of a 'shifted sleep state' on the general population. Studies show a significant increase in fatal road traffic collisions[1] and heart attacks[2] the following day. Females tend to be more affected by shifted sleep states, exhibiting a greater impairment in key cognitive skills such as attention span and working memory[3]. Chronotype i.e. the behavioural manifestation of underlying circadian rhythms, varies between sexes and also influences the effects of a shifted sleep state.[4]
Aims*: 1. To explore the initial impact of the DST transition on the different sexes, 2. To explore the differences in adjustment to the DST transition from the Sunday-to-Monday between the different sexes* and *3. To establish if variation in chronotype confounded our findings*.

Methods: Questionnaire data was collected in 4 sessions over 2 days (Sunday and Monday) following DST transition, March 2018. We questioned 528 (265 females, 27 excluded) participants (157 on Sunday, 371 on Monday) out of a total estimated daily footfall of 10,500 people on Exhibition Road. Self-designed, piloted questionnaires determined: a) if they felt sleepy (yes or no) and their self-identified chronotype (whether people identified as 'morning larks' or 'evening owls'), b) in-depth analysis of sleeping habits such as bedtime, wake time and, daytime sleepiness scored on the Karolinska Sleepiness Scale.
Results: A significantly greater proportion of females were sleepy on Sunday as shown in Figure 1 (p=0.015). There was no significant difference in proportion of sleepy males and females on Monday. However, females slept significantly earlier (p=0.011) and significantly longer (p=0.023) than males on Sunday night. The proportion of sleepiness amongst 'evening owls' was significantly higher than other chronotypes, but there was no significant variation in chronotype proportions between the sexes.
Conclusions: Our results suggest that initial impact of the loss of sleep from the DST transition is greater on females. However, our data is insufficient to draw conclusions on how males and females adjusted over the Sunday-to-Monday period immediately following the transition. Variation in chronotype did not confound our study findings. To validate our findings, the study must be repeated, with a refined questionnaire to gather data about potential confounding factors (e.g. age, children in the family, sleep related disorders). These results could potentially be used to generate specific public health guidelines, inform further research, and raise awareness about potential adverse effects of DST transition.



A bar graph showing the proportion of males and females, out of a total of 83 people, who responded 'yes' to the question 'Are you feeling sleepy today?' on Sunday. Significance was assessed using a z-test for comparing two proportions. There was a significantly higher proportion of sleepy females on Sunday compared to sleepy males (p=0.015).

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C10

Effects of selective silencing of layer 5 pyramidal neurons on sleep-wake regulation and cortical network dynamics

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Sleep and wakefulness are controlled by neuronal clusters in brainstem, hypothalamus, and basal forebrain in the mammalian brain (1). Although local cortical regulation of sleep depth and sleep slow waves has been shown (2,3), it remains unclear whether cortex contributes to global sleep-wake regulation. Cortical layer 5 pyramidal neurons are a key population in the generation and propagation of cortical slow oscillations (4,5). Slow waves are an electroencephalographic (EEG) hallmark of non-rapid eye movement (NREM) sleep and their spectral power (slow wave activity, SWA, 0.5 – 4 Hz) is a precise marker of sleep pressure. In this study, we probe the role of layer 5 pyramidal neurons in sleep-wake regulation and cortical network dynamics in mice.

We performed EEG and 16-channel laminar cortical recordings in a transgenic mouse model, in which a subpopulation (~15-30 %) of pyramidal cells in layer 5 is functionally silenced by removal of the t-SNARE protein SNAP25 (Rbp4-Cre;Ai14;Snap25$^{fl/fl}$). Male adult mice (10-17 weeks, 5 homozygous, 4 Cre-negative controls) were single-housed on a 12h/12 h light/dark cycle (light onset at 9 am). Sleep deprivation (SD) was performed on one day by exposure to novel objects for 6 hours starting at light onset.

In undisturbed 24-hour recordings, layer 5 silenced animals presented anincreased total amount of wakefulness (13.28 hrs, SEM 0.55 hrs) compared to controls (10.52 hrs, SEM 0.36 hrs). In addition, the maximum duration of individual wake

episodes was longer in layer 5 silenced animals (282.1 min, SEM 84.0 min) compared to controls (89.6 min, SEM 17.5 min). Following six hours of sleep deprivation, the increase of slow wave activity during NREM sleep relative to baseline was diminished in layer 5 silenced animals (134.9%, SEM: 5.7%) compared to controls (190.1%, SEM: 6.9%). The laminar profile of cortical activity revealed recurrent spike-wave patterns in transgenic animals, which did not occur in controls.
Our preliminary results indicate altered sleep-wake regulation in transgenic mice with a silenced subpopulation of layer 5 pyramidal neurons. Layer 5 silenced mice exhibit extended wakefulness, a greater capacity to stay awake, and a diminished homeostatic response to sleep deprivation. Furthermore, the fine orchestration of cortical activity appears disturbed. We tentatively interpret this data as first evidence that layer 5 pyramidal neurons contribute to the global regulation of sleep and wakefulness. This specific cortical cell population might represent a core element in a homeostatic circuit, which tracks the cortical need for sleep and translates it into a sleep signal.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C11

The impact of colour on circadian photoentrainment in mice.

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The large daily changes in ambient illumination associated with the earth's rotation are a major source of timing information for the mammalian circadian clock. These 'irradiance' signals are encoded via the integration of extrinsic (rod) and intrinsic (melanopsin) photoreceptive signals in the retinal ganglion cells (ipRGCs) that innervate the suprachiasmatic nucleus (SCN). By contrast, cone photoreception makes minimal contributions to this process, despite the appearance of cone photoreceptive signals at the level of ipRGCs and the SCN. Instead cones may play

at least one alternative function: to provide information about daily changes in the colour of ambient illumination. We previously showed that a subset of SCN neurons exhibit chromatic responses and that naturalistic changes in colour influence phase of entrainment. Here we more thoroughly investigate the contribution of chromatic information to the entrainment mechanism.
Human-cone knockin mice (Opn1mwR) were used in conjunction with polychromatic lighting environments, to allow us to experimentally isolate cone signals. Animals were subjected to a variety of entrainment paradigms (constant light, shifted LD cycles, and brief light pulses) whilst locomotor activity was assessed using running wheels, or a passive infrared system. We observed that stimuli of identical irradiance (melanopsin, rod and net cone flux) but different colour (ratio of L cone to S cone activation) differentially influenced circadian responses in a paradigm dependent manner. For example, while responses to discrete light pulses were independent of colour, the period lengthening effect of constant light was significantly greater when L cone signals 'yellow' were dominant (as in during natural daylight). These data therefore support the view that chromatic information supplied by cones influences entrainment and challenge the popular assumption that the circadian system is especially sensitive to 'blue' light.

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C12

Can a Visual Analogue Scale (VAS) be used to measure sleepiness in patients diagnosed with Obstructive Sleep Apnoea (OSA)?

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Motivation: The Epworth Sleepiness Scale (ESS) is the most commonly used questionnaire for the assessment of sleepiness. However, it is variable between individuals, not applicable in all countries, and requires high level of literacy. Visual analogue scales have been validated in the assessment of chronic symptoms such as anxiety and pain. The aim of this study was to investigate the efficacy of a newly developed Visual Analogue Scale (VAS) for the measurement of sleepiness, compared to ESS, in patients with Obstructive Sleep Apnoea (OSA) and healthy participants.
Methods: A prospective, observational study was carried out in patients with OSA and healthy participants. All of the participants completed 2 visits during which they filled in both ESS and VAS. Between visits 1 and 2 the patients diagnosed with OSA were treated with Continuous Positive Airway Pressure (CPAP). The agreement between the VAS and ESS results was assessed using Bland-Altman plots.

Secondary outcomes were the ease of use (0-10 Likert scale) and the time taken in each measurements.
Results: 32 patients diagnosed with OSA (age [Mean±SD] 55.80 ± 13.49 years) and 32 healthy participants (age: 36.74 ± 11.65 years) were recruited. Both ESS and VAS detected a reduction in sleepiness after CPAP treatment in patients with OSA (ESS: 11.16 ± 5.53 to 4.74 ± 5.01 a.u., $p<0.001$, VAS: 50.22 ± 30.08 to 21.90 ± 26.52 mm, $p<0.001$). There were no significant differences between visit 1 and visit 2 in healthy participants using both ESS and VAS (ESS: 3.91 ± 3.41 to 3.07 ± 3.27 a.u., $p=0.31$, VAS: 15.58 ± 21.21 to 9.12 ± 10.93 mm, $P=0.138$). The Bland-Altman agreement is shown in Figure 1. In patient with OSA, the time taken to complete the VAS (visit 1: 14.92 ± 13.76 seconds, Visit 2: 5.67 ± 3.39 seconds) was significantly less compared to ESS (visit 1: 38.75 ± 20.01 seconds, Visit 2: 31.33 ± 23.68, $p=0.001$).
Conclusion: The findings of this study suggest that the new VAS is an effective measure of sleepiness in patients with OSA and healthy participants. The VAS is also able to detect changes in sleepiness in patients with OSA treated with CPAP.

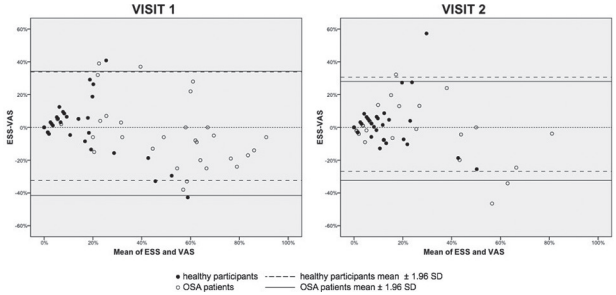

Figure 1. Bland-Altman plots of agreement between ESS and VAS results of healthy participants (n=32) and OSA patients (n=31) collected at visits 1 and 2.

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C13

Exploring the molecular clock in sympathetic preganglionic neurons

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Cardiovascular physiology exhibits a diurnal rhythm e.g. blood pressure dips at night and increases in the morning. Loss of diurnal rhythm of blood pressure is correlated to an increased risk of developing cardiovascular diseases. Blood pressure is to a large part controlled by sympathetic nervous system activity, which exhibits diurnal activity. Since sympathetic preganglionic neurons (SPNs) are the final common

pathway the central nervous system influences blood pressure, this project aims to determine if SPN function could be regulated by diurnal expression of genes.
The diurnal expression of genes encoding proteins involved in determining neuronal activity were investigated in RNA extracted from the whole spinal cord and from micro-punches that included the location of the majority of SPNs, the intermediolateral cell column (IML) at 7:30 AM and 7:30 PM of C57/Bl6 mice (N= 10) that were terminally anaesthetised with 80mg/Kg intraperitoneal sodium pentobarbitone and had their spinal cords removed and RNA extracted using an RNA extraction kit. qPCR revealed mRNA levels of Bmal1 and Per2 varied with time of day in the punch and spinal cord samples (N=10); Bmal1 mRNA was higher in the morning while Per2 mRNA was higher during the evening. Diurnal rhythm of Bmal1 and Per2 protein levels in SPNs were then examined using immunofluorescence. C57/Bl6 mice (N= 15) were terminally anaesthetised as described above at morning and evening time points, perfused with 4% paraformaldehyde and the spinal cords removed and sectioned at 50 μm on a vibrating microtome. Bmal1 and Per2 protein levels within the SPN nucleus vary with time of day with Bmal1 levels being higher in the morning (N=5 animals, n=15 sections). To investigate a broader sample of genes, RNAseq was performed on micropunches obtained as above. Diurnal rhythm of expression was observed in potassium channel subunits (e.g. Voltage-gated potassium channel subunit beta-2, Kcnab2), sodium channel subunits (e.g. Sodium channel subunit beta-1, Scn1B) and glutamate subunits (e.g. Glutamate [NMDA] receptor subunit 3B, Grin3B) consistent with increased neuronal activity. Single cell patch-Seq is currently underway to examine if genes identified in micropunches are present in single SPNs.

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C14

High intensity interval running increases cardiac autonomic activity but does not disrupt subsequent night's sleep in trained runners.

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Background: Observational studies have shown sleep quality in athletes is reduced after training sessions. As such, this period is considered a big obstacle to athletes' recovery, which could hinder future performances. High intensity exercise training in the evening is one of several factors that may explain this phenomenon through its effect on cardiac autonomic activity. Yet, no research has studied the impact of high intensity exercise on sleep within a trained cohort, making it impossible to discern whether it has a positive or negative effect on sleep. The aim of this study was to investigate the effect of exercise intensity on cardiac autonomic activity and

subsequent night's sleep in trained runners. Methods: Eight trained male runners (age: 27.8±6.9yrs; height: 1.8±0.1m; weight: 73.5±5.3kg and $VO_{2\ max}$: 57±4ml.kg^{-1}.min^{-1}) completed three experimental trials in a randomised, counterbalanced study design. Following a standardised afternoon meal (2g CHO.kg^{-1}BM) participants either performed: i) a 1h high intensity interval running session (6x5 min @60% $VO_{2\ max}$ interspersed with 6x5 min @90% $VO_{2\ max}$); ii) a 1h low intensity running session (45% $VO_{2\ max}$); iii) no exercise. Exercise sessions were performed at 18.00h prior to a fixed bedtime of 22:30h. Sleep was assessed in a temperature controlled laboratory using overnight polysomnography and cardiac autonomic activity was recorded via electrocardiography. A one-way repeated measures ANOVA was performed to compare sleep variables and measures of cardiac autonomic activity between exercise intensities. Results: There were no changes in average nocturnal heart rate variability after exercise but average nocturnal heart rate was higher after high intensity interval running than low intensity running (50±5 bpm v 47±5 bpm, $p = 0.02$) and no exercise (50±5 bpm v 47±5 bpm, $p = 0.028$). In the polysomnography analysis, total sleep time, sleep efficiency and wake after sleep onset were improved after high intensity interval running and low intensity running compared with no exercise ($p < 0.05$). Conclusions: High intensity interval running increases cardiac autonomic activity but does not disrupt subsequent night's sleep compared to no exercise in trained runners. It should be considered that poor sleep on the night following a single training session in the evening is not caused by exercise intensity. Future research is warranted to determine if other stressors that are encountered by athletes after intense exercise training cause sleep disruption such as muscle damage and glycogen depletion.

The authors would like to acknowledge S-MED for providing the polysomnography.

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C15

Effects of sleep extension and sleep restriction on the performance and cardiac autonomic function of endurance cyclists

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Sleep is considered the most important recovery behaviour for athletic success(1). While there is evidence that sleep may affect endurance performance(2), no study has investigated the effects of sleep on the sport-specific performance of trained endurance athletes. In addition, while indices of cardiac autonomic function are increasingly being used to monitor athlete wellbeing(3), little is known of the effects of sleep on such indices. In a balanced crossover experiment, endurance cyclists (n=9) completed three trials; normal sleep (NORS), sleep restriction (SRES),

and sleep extension (SEXT). Each trial required cyclists to complete a time trial (TT) - based on predicted work achievable in one hour when cycling at anaerobic threshold - on four consecutive mornings (TT_1 _ TT_4). Cyclists slept habitually prior to TT_1 of each trial. However, on the three subsequent nights time in bed either; remained as normal (NORS), was restricted by 30% (SRES), or was extended by 30% (SEXT). A 7-day washout period separated each trial. Actigraphy (sleep-wake threshold 40 counts/min) was used to monitor sleep. Performance time (to the nearest second) and rating of perceived exertion (6-20 scale) were recorded for each TT. Prior to each TT, resting heart rate (HR), HR variability (Ln rMSSD), maximal rate of HR increase during submaximal exercise, HR recovery following submaximal exercise, mean response time during a psychomotor vigilance task (PVT), and mood disturbance were recorded. Data were analysed using a Generalised Estimating Equations approach. On each of the three sleep intervention nights, total sleep time was higher (P=0.000) in the SEXT trial compared with both NORS and SRES, and lower (P=0.000) in the SRES trial compared with NORS. TT_4 was faster in the SEXT trial (mean±SD, 3409±187sec) compared with both the NORS (3521±204, P=0.013), and SRES (3718±312, P=0.010) trials. Rating of perceived exertion, resting HR, resting Ln rMSSD, maximal rate of HR increase at the onset of submaximal exercise, and HRR following submaximal exercise were unchanged between trials. Prior to TT_4, total mood disturbance was higher in the NORS (13±18au, P=0.002) and SRES (28±12, P=0.000) trials compared with SEXT (4±10). Prior to TT_3, mean response time was faster in the SEXT trial (346±27ms) compared with SRES (374±31, P=0.008) and NORS (360±28, P=0.021). Prior to TT_4, mean response time was faster in the SEXT trial (332±29) compared with both NORS (363±28, P=0.000) and SRES (392±40, P=0.000). Sleep extension for three nights enabled cyclists to better maintain performance compared with normal sleep and sleep restriction. Cardiac autonomic indices were not sensitive to changes in sleep duration. Better mood and vigilant attention following sleep extension suggests psychological factors may explain the effects of sleep on endurance performance.

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C16

The use of melatonin in the treatment of paediatric sleep disorders in the UK

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Sleep disorders such as obstructive sleep apnoea and sleep onset insomnia occur in 3.7% of children. [1] Melatonin is a sleep-promoting pineal hormone, regulated by the suprachiasmatic nucleus [2] that is sometimes prescribed off-label for sleep disorders in children. [3] Oral melatonin is moderately expensive, costing £15-75/month dependent on dosage. [4] Side effects are uncommon, but include hyperactivity, nightmares and constipation. [5] Alternative management strategies for paediatric sleep disorders include behavioural therapies.

Aim: To carry out an exploratory study to investigate clinical perspectives on the use of melatonin in the treatment of paediatric sleep disorders.

Methods: A Qualitative exploratory study was carried out using semi-structured interviews of 15-30 minutes, either face-to-face or via video or telephone calls; dialogue was transcribed during interviews. Chain sampling was used to select interviewees. Inclusion criteria: professionals with experience of paediatric sleep disorders: 14 contacted, 10 respondents interviewed (Figure 1). Data thematically analysed via open coding (Figure 2).

Results: Misconceptions about melatonin and its use in treating paediatric sleep disorders were reported in healthcare professionals and parents, possibly producing suboptimal prescription practices and unrealistic expectations. This could impair the quality of care in paediatric patients with sleep disorders, and may incur costs upon the NHS, as shown in Figure 2. Behavioural interventions could also be useful and implemented prior to or in conjunction with melatonin treatment, but access to behavioural treatments appears to be limited in many parts of England and Scotland. Melatonin has become the “sleeping aid of choice” for paediatricians, and high prescription rates may not be detrimental provided prescribers are well-informed, as sleep deprivation has profound effects in children.

Conclusions: Current melatonin prescribing practices could be improved in physicians who treat paediatric sleep disorders but who are not sleep experts. Behavioural interventions may be more effective than melatonin in paediatric sleep disorders. There is an apparent lack of awareness of paediatric sleep disorders amongst medical students, and therefore awareness of treatment options and the role of melatonin vs behavioural treatments.

Future work: Investigate current prescribing practices of melatonin in a larger sample of paediatric professionals. Raise awareness of paediatric sleep disorders and the necessary treatments for them. Investigate sleep education across UK

medical schools and raise awareness of paediatric sleep disorders and treatment options amongst medical students who may become paediatricians.

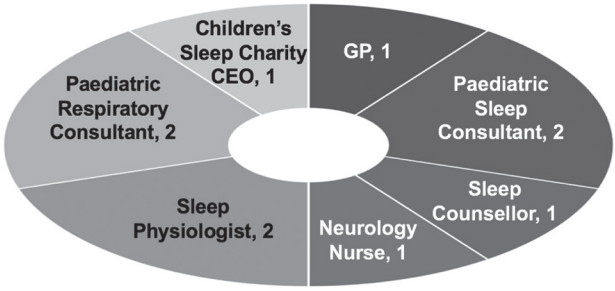

Figure 1: Interviewees and their roles

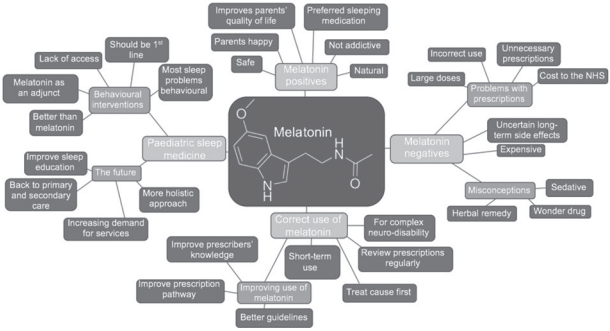

Figure 2: Codes (blue), concepts (green) and categories (yellow) from analysis of interviews.

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C17

Amyloid Beta Oligomeric Structure Governs Sleep/Wake States in Zebrafish

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Introduction - Despite decades of research, the endogenous function of amyloid beta (Aβ), the hallmark protein of Alzheimer's Disease (AD), remains unknown. Knowing what Aβ does physiologically would enable us to understand what is going wrong in the disease state. Recent studies have highlighted links between Alzheimer's disease and sleep[1]. Sleep is disrupted in AD patients, often years before cognitive deficits[2]. Since Aβ levels cycle across the sleep/wake cycle[1], we hypothesized that one in vivo function of Aβ may be to directly modulate sleep/wake states. Several features of zebrafish biology make it an excellent model to investigate the role of Aβ in sleep: Zebrafish have a complete repertoire of amyloid precursor protein (APP) processing machinery and most of the Aβ receptors are highly conserved in zebrafish. In addition, the zebrafish brain is anatomically and molecularly similar to the mammalian brain, and many behaviours like sleep, are controlled by similar neuronal mechanisms.

Materials and Methods - Using CRISPRs we mutated the two zebrafish APP genes to downregulate Aβ levels. To upregulate Aβ levels acutely, we injected different oligomeric forms of Aβ with final brain concentrations in the picomolar (i.e. physiological) range to anesthetized zebrafish larvae (in 1 mM MS222). Different oligomers/fibrils were obtained by incubating Aβ preparations at 4°C or 25°C and the length of oligomers were assessed by TEM (Transmission Electron Microscopy). We determined the effects of Aβ oligomer/fibrils on sleep/wake behaviour via video monitoring and used whole brain activity mapping to identify neurons that differentially respond to Aβ.

Results and Conclusions - While APP loss of function mutants had an 11±1.0% decrease in waking activity, Aβ in its shorter oligomeric forms (median oligomer length 45±4 nm); caused strongly increased activity (14±4.9%) and decreased sleep (-12±9.2%). In contrast, longer Aβ oligomers/fibrils (median oligomer length 75±7 nm) acutely increase sleep (30±12.0%) without neurotoxicity. Consistent with their effects on wakefulness, short Aβ oligomers induced neuronal activity in a subset of neurons in the posterior hypothalamus, which is a major wake-promoting center in the vertebrate brain. In contrast, longer oligomeric forms do not activate these wake-promoting neurons but instead globally dampen neuronal activity.

Our experiments suggest that physiological and temporary upregulation of Aβ levels can directly promote both zebrafish sleep and wakefulness depending on the oligomeric state of Aβ via activation of discrete subpopulations of neurons. We are now performing a CRISPR-mediated genetic screen in zebrafish to identify the receptors that interact with Aβ to mediate these effects on sleep and wake.

Understanding neural and molecular mechanisms of Aβ's effect on sleep/wake behaviour may provide a mechanistic understanding of what goes wrong in AD.

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C18

The impairment in insulin sensitivity after sleep restriction does not increase with more nights of sleep restriction.

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Voluntary sleep curtailment is common in modern society and has been linked to poor glucose control. Many experimental studies have shown impaired glucose regulation after sleep restriction, ranging from one to five nights. However, it remains unclear whether the impairment in glucose regulation is related to the number of nights of sleep restriction, as methodological differences make comparisons between existing studies difficult. The current study aimed to explore glucose regulation following each night of sleep restriction for four consecutive nights to identify if there is a linear effect of number of nights of restriction on the impairment in glucose regulation. We hypothesised that the level of glucose control would decline with each subsequent night of sleep restriction.

10 healthy, non-diabetic humans aged between 18 and 50 years were recruited for this randomised crossover study with 4 nights of control sleep (8 h/night) and 4 nights of sleep restriction (4 h/night), separated by a 3 wk washout period. Participants stayed in the laboratory overnight but were permitted to leave during the day, during which time wrist actigraphy was used to ensure no sleep or physical activity was undertaken. Each morning upon wakening an oral glucose tolerance test was conducted and venous blood samples were collected at regular intervals for 120 min. Glucose and insulin concentrations were determined from the blood samples and area under the curve (AUC) was calculated for each day using the trapezoidal rule. ANOVAs were conducted to compare glucose and insulin AUC for the four days in each condition.

Glucose AUC displayed trends for an effect of trial ($P = 0.063$) and interaction effect ($P = 0.098$) but no effect of day ($P = 0.773$). Insulin AUC showed a significant effect of trial ($P = 0.020$), however there was no effect of day ($P = 0.861$) or interaction effect ($P = 0.129$).

Our findings agree with previous studies which have shown that sleep restriction impairs glucose regulation. However, contrary to our hypothesis, there does not appear to be a significant linear effect of impairment with an increasing number of nights of sleep restriction.

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C19

Modelling Local Sleep Homeostasis

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Sleep homeostasis refers to the process by which a need for sleep accumulates during wakefulness and dissipates during subsequent sleep. Homeostatic sleep need is typically measured using slow wave activity (SWA); oscillatory power at 0.5 – 4 Hz present in extracellular field potentials during NREM sleep, which is generated by synchronous alternating bouts of neuronal spiking activity ("on periods") and silence ("off periods"). Existing quantitative descriptions of sleep homeostasis describe the dynamics of slow wave activity only as a function of recent sleep-wake history. However, homeostatic sleep pressure is non-uniform across the brain, originates locally, and changes in association with neuronal activities. This project aims to develop quantitative models that predict homeostatic sleep need markers from multi-unit firing rate history within the same channel. Models were developed and applied to an existing dataset of electrophysiological recordings from 16-channel microwire arrays implanted in mouse frontal cortex (McKillop et al., 2018). Data were obtained continuously for several days while mice were freely behaving, including periods of spontaneous sleep and wake, and periods of 6hr sleep deprivation (by gentle handling and novel object presentation). To assess model fit quality, an error metric was defined as the sum of absolute differences between median simulated and empirical values over continuous NREM episodes at least 1 minute duration, weighted by episode length. Model parameters were algorithmically optimised to minimise this error metric. A simple model, in which homeostatic sleep need increases in proportion to multi-unit firing rate and decreases exponentially over time, can often describe the time course of SWA with high accuracy (n=28; 7 mice x 4 best quality channels per mouse). An alternative model, which employs a firing rate threshold, with a saturating exponential rise in homeostatic sleep need above threshold, and exponential decrease below, provided an even better fit to SWA, yielding a lower minimal error metric ($p < 0.001$, n=28, Wilcoxon signed rank test). The time course of off period occupancy shows qualitatively similar temporal dynamics to slow wave activity and is a

viable alternative sleep homeostasis metric. Preliminary results using this suggest that restricting simulated homeostatic sleep need decay to detected off periods improves model fit ($p < 0.05$, n=6, Wilcoxon signed rank test). In conclusion, the dynamics of local homeostatic sleep need can be well described by models dependent solely on local neuronal activities, independent of any information about the animal's global wake-sleep state. The advancement of models of sleep homeostasis will likely provide means to test competing theories of its mechanistic origin within neurones and local networks.

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C20

Investigating the neural circuit basis of sleep disturbance-induced cognitive deficits using the larval zebrafish (*Danio rerio*)

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Sleep is vital for brain function, with insufficient or poor sleep inducing severe deficits in cognitive performance. Although these impairments have serious negative effects on health, economic productivity and quality of life, the mechanisms underlying them are not well understood. My work aims to investigate how sleep deprivation leads to performance deficits in simple sensorimotor behavioural tasks. I am employing *in vivo* functional imaging to characterise the activity of identified task-related circuits as zebrafish larvae perform visually guided behaviours, to attempt to detect the alterations in this activity that are caused by sleep disruption and are associated with impaired task performance. Using an array of different environmental and pharmacological sleep-deprivation paradigms, I then plan to explore the precise pathways leading to this cognitive impairment. Subsequently, by using optogenetic, chemogenetic and pharmacological tools, I aim to mimic, prevent, and reverse these processes to test whether these impairments can be dissociated from sleep loss *per se*, and determine the sleep-related processes that support efficient cognitive and neurological function. This research will have important implications for understanding of sleep neurobiology, and the development of therapeutics to effectively manage sleep deprivation induced cognitive problems in healthy individuals and in the numerous neuropsychiatric disorders in which sleep dysfunction is implicated.

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C21

Torpor Preferentially Induces c-Fos Expression in Dorsomedial and Posterior Hypothalamus in Mice

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Torpor is the naturally occurring hypothermic, hypometabolic and hypoactive component of hibernation(1). It is an adaptive, controlled reduction in temperature and metabolic demand in response to reduced availability of substrate. If such a centrally-driven hypothermic and hypometabolic state could be mimicked in a clinical setting it may represent an improved strategy for therapeutic hypothermia(2). The purpose of this study was to identify regions of the brain active during torpor in the mouse. Female mice (C57BL/6J, Charles River) were maintained on a 12-hour reversed light/dark cycle, and acclimatised to an ambient temperature of 30 °C for 5-7 days. Torpor was induced by reducing the ambient temperature to 18°C at lights off, then after 24 hours cold acclimatisation, food was removed for 12 hours(3). Torpor was detected by monitoring surface temperature changes using a thermal imaging camera (Flir C2, ResearchIR 4 software), and defined as a surface temperature greater than 2 standard deviations below the mean during the 24 hours prior to fasting. Controls were fasted at 30°C for 12 hours, or exposed to 18°C ambient temperature with access to food. No control mice entered torpor, and all mice exposed to cooling and fasting entered torpor. The mean nadir surface temperature for torpid mice was 24.2 +/- 0.9°C, 27.8 +/-0.06°C in the cooled controls ($p < 0.001$) and 34.2+/-0.2°C in the fasted controls ($p<0.0001$) (one-way ANOVA). Two hours after torpor entry (n=3), at the end of a 12 hour fast (n=4), or after 36 hours cold exposure (n=4), mice were terminally anaesthetised with pentobarbitone (175mg/kg i.p), then transcardially-perfused with formalin. Brains were post-fixed for 24 hours at 4°C before being cryoprotected in 30% sucrose for 48 hours. Sagittal sections of subcortical structures and brainstem (40μm) were cut, blocked with 5% normal donkey serum, and incubated overnight at room temperature in 1:2000 anti-c-fos primary (Cell Signalling Technologies, 2250s) followed by a fluorescent secondary antibody (donkey anti-mouse, AlexaFluor488, 1:1000). Sections were tile-scan imaged using a Leica DMI6000 Widefield fluorescence microscope. Images were analysed using ImageJ software with a semi-automated image processing protocol to count c-fos positive nuclei. Figure 1 shows an example plot of surface temperature and activity of a mouse entering several short torpor bouts of increasing depth. Figure 2 shows that cooling increased c-fos expression in the medial preoptic, parabrachial nucleus, and bed nucleus of the stria terminalis. Fasting induced c-Fos expression in the arcuate, the dorsomedial, posterior, and paraventricular hypothalamus. The dorsomedial and posterior hypothalamus increased c-fos expression during torpor compared

to either cold or fasting alone, and as such they represent potential nodes within the torpor induction circuitry.

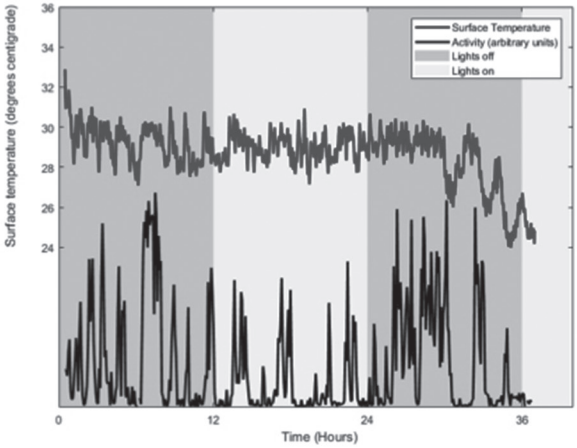

Figure 1. Skin surface temperature and activity of a mouse entering torpor

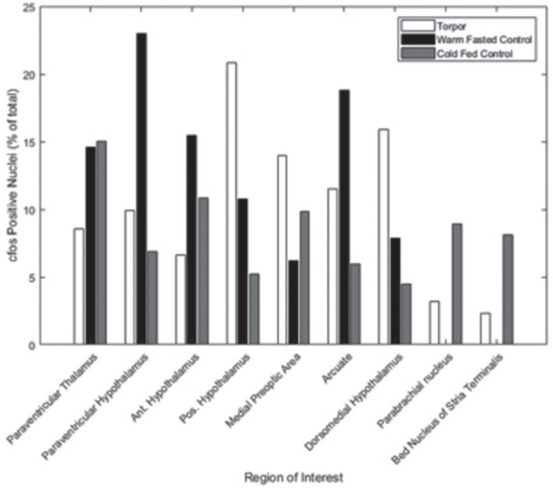

Figure 2. C-fos expression by brain region of interest.

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C22

Can blue light exposure influence mood in night shift workers?

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Background: Night-shift work is becoming more common as society moves towards services being available 24 hours a day. This is problematic as poor sleep quality in shift workers can lead to mood disorders such as depression and seasonal affective disorder. Phototherapy can be used to treat mood disorders that originate from circadian disruption.
One form of phototherapy is the Re-timer light therapy glasses, which emit 500nm of blue-green light. Although the glasses have been shown to be effective in phase-shifting circadian rhythms, it is not known if they are effective in improving sleep or other downstream processes such as mood changes. We aimed to investigate whether the glasses had an effect on the mood of a cohort of night shift workers.
Methods: A hypothesis generating, prospective cohort study was conducted in permanent night-shift workers at a supermarket distribution warehouse. Of the 200 warehouse workers working nights, 150 visited the 'nightclub' project*. Volunteers were given the glasses and data was collected in 6 night shift workers. Workers were excluded if they had any photosensitive conditions such as epilepsy or were unable to wear the glasses for safety reasons. The positive and negative affect schedule (PANAS) generates a positive and negative arbitrary mood score, and was used to monitor mood, on a twice-daily basis for 18 days. After 9 days of baseline monitoring, phototherapy was administered via the Re-timer glasses for 30 minutes before each subsequent night-shift for another 9 days. The baseline and post-interventional mood scores were compared via a paired two sample Student's t-test.
Results: There was a significant decrease in the negative mood scores with light therapy (baseline: 15.39±5.67 vs intervention: 12.88±2.98 (mean±1 SD), $p<0.001$).

Due to the study design, monitoring phototherapy compliance was unfeasible and the participants were not blinded to the intervention. Sample size and study duration were also limited, with little information on demographic stratification. These points need to be considered in future studies.
Summary: The results suggest that light therapy was was able to alleviate negative moods in night shift workers. Whether this was due to a physiological response to light or an ensuing effect from circadian stabilisation is unclear. However, these preliminary data do suggest that potential improvements in mental health of shift-workers can be achieved with light therapy. Therefore, making similar adjustments in night shift environments and increasing awareness of available therapies may have positive implications, in the interests of both employers and employees.

Acknowledgements: *These data were collected as part of a wider study funded by the Wellcome Trust, in collaboration with Liminal Space and the Sleep & Circadian Neuroscience Institute, University of Oxford. Ethical approval was obtained from the University of Oxford CUREC.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C23

Does exposure to blue light reduce sleepiness in night shift workers?

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Background: Approximately 12% of all employees in the UK undertake night shifts to serve an increasingly 24/7 modern society, according to the Labour Force Survey (2016). Night shift work is thought to cause circadian phase-shift, associated with decreased alertness, workplace accidents, diabetes and cancer.[1] Light plays a crucial role in circadian rhythm entrainment and regulating melatonin release.[2] Previous experiments[3] have shown that blue/green light of wavelength 470-525nm shows the greatest melatonin suppression, reducing and postponing sleepiness within individuals, but this has not been explored outside controlled conditions in a real-world setting.
Methods: A prospective cohort study was conducted in long-term night shift (22:00 – 06:00) workers, at a Co-op warehouse in Thurrock. Approximately 150 workers were invited to take part and those who had planned annual leave, a diagnosed sleep disorder or were non-English speaking were excluded. Participants (n=10) who gave their consent filled in two questionnaires at the start and end of each shift every day for two weeks: the Karolinska Sleepiness Scale (KSS)

scored from 1-9 with 9 indicating extreme sleepiness, and the Epworth Sleepiness Scale (ESS) which is scored from 0-24 (24 being very sleepy).[4,5] The first week was assigned as the baseline week in which there was no intervention. During the second week, goggles (Re-Timer™) emitting blue-green light (500nm) were worn for 30 minutes before the start of each shift. Ethical approval was obtained from the University of Oxford CUREC.

Results: KSS and ESS scores from 6 participants were analysed as median and interquartile range (IQR). Data from 4 participants were neither returned nor completed properly. The average scores of weeks 1 and 2 were compared. The median KSS score decreased from 4.5 (IQR:3) a.u in week 1 to 4 (IQR:3) a.u in week 2. The median ESS score decreased from 9.5 (IQR:5) a.u to 8 (IQR:8) a.u. Both questionnaires revealed that the workers were alert, and their sleepiness was found to be low, albeit higher than normal. It was observed that exposure to blue light may reduce self-perceived sleepiness, however further studies are needed to increase the sample size and add an effective control group.

Conclusions: It is important to note that to our knowledge this is the study first to examine the direct real-world effects of blue light on subjective sleepiness in shift warehouse shift workers. The project hopes to raise awareness on improving occupational lighting, encouraging the installation of blue-enriched white lights to boost mental performance and productivity in the workplace setting.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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The relationship between fasting-induced torpor and sleep in mice

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Torpor is a regulated and reversible state of metabolic suppression employed by many animals mainly to conserve energy. Torpor can be induced by fasting or by changes in photoperiod (i.e. seasonal torpor, which includes hibernation and daily torpor). Previous studies on seasonal torpor revealed notable similarities and differences between torpor and sleep. Behaviourally, both states are associated with immobility and reduced responsiveness. However, both hibernation and daily torpor induced by shortening of photoperiod appear to be sleep-depriving states. Specifically, animals emerging from torpor usually enter sleep characterised by high encephalographic (EEG) slow-wave activity, an established marker of homeostatic sleep pressure. Much less is known about the relationship between sleep regulation and *fasting*-induced torpor, which can be readily induced in laboratory mice.

In this study, we established a model of fasting-induced torpor in C57BL/6J mice (n=8, male; 12-weeks old; mean weight 26.9 g), and performed continuous electrophysiological and surface body temperature ($T_{surface}$) recording (via infra-red cameras) across successive days of food restriction. The animals were implanted with epidural EEG electrodes above the frontal and occipital cortices and electromyogram (EMG) electrodes in the nuchal muscle, and allowed to recover for at least 7 days after surgery prior to starting the experiment. Mice were kept at a 12:12 light-dark cycle throughout the experiment and provided with approximately 1 g of food daily between ZT6 and ZT9. Ambient temperature was kept at 22 to 24 °C, and body weight was carefully monitored to ensure that it remained above 85% of *ad lib* feeding weight.

Our preliminary analyses revealed that all animals entered torpor bouts (defined as $T_{surface}$<28 °C for at least 1 hour) within 5 days of food restriction. Torpor bouts were invariably initiated via a state that, based on EEG and EMG signals, resembles NREM sleep, but EEG amplitude subsequently showed a prominent and progressive reduction during entrance into torpor, in some cases reaching below 10% of the values during euthermic NREM sleep. We did not observe REM sleep or extended spontaneous wakefulness periods during torpor bouts. However, in all animals the torpor bouts were punctuated by prominent EMG bursts, associated with a transient EEG activation, at a regular periodicity of <5-10 min. Upon spontaneous return to euthermia, the torpor bouts were typically followed by periods of wakefulness and often further torpor bouts until the animals were fed. The animals entered deep sleep with high EEG SWA shortly after feeding.

Our study tentatively suggests that fasting-induced torpor and sleep are closely related yet distinct neurophysiological states. It remains to be determined whether fasting-induced torpor is a sleep-depriving state functionally similar to seasonal torpor.

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Vladyslav Vyazovskiy Group

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Is self-reported chronotype associated with caffeine intake in male night shift workers?

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Caffeine is a widely consumed psychoactive drug that increases alertness[1]. While moderate caffeine intake (<400 mg[2]) can prevent certain chronic diseases, chronic high caffeine intake increases cardiovascular risk factors such as blood pressure and cholesterol[1]. Working night shifts is associated with increased caffeine consumption[3]. Individuals' chronotypes (that which determines preference for day or night) can be categorised into 'early lark', 'intermediate', and 'night owl'[4]. The aim of this study was to investigate if self-reported chronotype is associated with caffeine intake in male night shift workers.

The study was carried out as part of a wider project to engage night shift workers, in collaboration with Sleep and Circadian Neuroscience Institute (SCNi), University of Oxford and Liminal Space. Night shift workers at a supermarket distribution warehouse were recruited. Self-reported chronotype was determined via a questionnaire provided by SCNi. Participants were then categorised as 'lark', 'intermediate', or 'owl'. Consumption of caffeinated food and beverages (classified by the National Health and Nutrition Examination Survey[5]) over a 24-hour period from the end of 1 shift to the end of the next was determined by pictorial questionnaire

developed by the research team for ease of use. Caffeine intake was then estimated using values made available by the Government of Canada[2].
Data was collected from 38 male night shift workers over 2 night shifts; approximately 200 staff were on duty per shift. 'Intermediates' (n=20) consumed the most caffeine, with 25% consuming more than the 400 mg recommended limit, but the difference between the 3 categories was not statistically significant. Duration of time working night shifts seemed proportionally associated with caffeine intake, with those having worked night shifts for longer consuming more caffeine than those who had recently begun working night shifts.
The findings of this study suggest that self-reported chronotype is not associated with caffeine intake in male night shift workers. Duration of time working night shifts could be a better predictor of caffeine intake than self-reported chronotype, and follow-up studies should be performed to investigate this. In addition, association between genotypic chronotype and caffeine intake could be looked into. These studies could also be performed with the inclusion of female night shift workers. As night shift workers already tend to consume more caffeine than day shift workers, it is hoped that through these studies, public health messages could be tailored to sub-groups in the night shift worker population that may be especially at risk of consuming unhealthy levels of caffeine

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Circadian disruption and sleep regulation in mice

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Light is the primary entraining cue for the circadian system, adjusting biological time to the external environment, however it also has direct effects on arousal and

sleep. The demands of our modern 24/7 society, with increasing exposure to artificial light at inappropriate times of day, is widely considered to be detrimental to our physiology. Evidence comes from studies using aberrant light/dark (LD) cycles to produce circadian disruption in mouse models. A range of different protocols have been used, including constant light (LL), jet-lag (JL), dim light at night (DLAN), and non-24 hour LD cycles (T-cycles). To date, no detailed comparison of the effects of these different protocols has been conducted, and it remains to be determined whether and how circadian disruption affects sleep regulation.
Methods: We used passive infra-red (PIR) sensors to simultaneously measure activity and immobility-defined sleep in wild-type C57BL/6J mice under different protocols, including LL, JL, DLAN and T20 (10h light: 10h dark). We compared the effects of these different protocols on commonly used measures of circadian disruption including periodogram power (Qp), intradaily variability (IV) and interdaily stability (IS), as well on the architecture of immobility-defined sleep. In a separate cohort of C57BL/6J mice (n=7) we examined the effects of 14 days constant light on sleep architecture and EEG.
Results: Different LD conditions produced different effects on circadian activity. Whilst IV is increased and IS were decreased under all conditions (n=24, IV ANOVA $F(1,20)=28.8$ $p<0.0001$, IS ANOVA $F(1,20) = 295.6$, $p<0.0001$) decreases in Qp were only observed under LL and T20 (LL $p<0.0001$, T20 $p=0.0053$). All conditions change the distribution of immobility-defined sleep. Furthermore, our preliminary results suggest that constant light acutely increases the amount of sleep in the first day, which results in an altered distribution of EEG slow-wave activity – the established marker of sleep homeostasis.
Conclusions: Our data suggests that commonly used protocols exert different effects on sleep and circadian rhythms. These data provide a framework to understand the effects of these protocols on other biological processes such as cognitive functions and physiology.

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GRIA1 knockout mice show reduced global EEG sleep spindles, preserved local LFP spindles an retain long-term memory

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Sleep spindles have been implicated in cognitive functions and memory consolidation[1,2]. Deficits in spindles have been reported in brain disorders (e.g. schizophrenia) associated with polymorphisms of the GRIA1 gene[3], which codes for the GluA1 AMPA receptor subunit. Here we investigated the dynamics of sleep

spindles and their relationship with memory performance in GRIA1-/- and wild type (WT) mice.
Chronic electroencephalogram (EEG) and the electromyogram (EMG) were recorded during spontaneous sleep in n=14 mice. Multichannel recordings of local field potentials (LFP) were also collected in a subset of mice from layer-V somatosensory cortex (SCx). Surgical procedures were performed under isoflurane anaesthesia. EEG and LFP power spectra were calculated with a Fast Fourier Transform using 4-second epochs. For individual spindle event detection, an automated algorithm based on autoregressive modelling was applied to the LFP and EEG signals. Spatial reference memory was assessed in an additional group of mice using a plus maze task.
Frontal EEG spectral power during NREM sleep was significantly reduced in the spindle-frequency range (10-15 Hz) in GRIA1-/- relative to WT mice. Furthermore, individual EEG spindle events were readily detected in WT mice with the automated algorithm, while they were absent in GRIA1-/- mice. Interestingly, despite the absence of EEG spindles in GRIA1-/- mice, preliminary analyses of LFP signals revealed an occurrence of local spindle events in the SCx in both genotypes. A repeated measures analysis revealed no significant differences between GRIA1-/- and WT in memory performance (main effect of genotype and interaction by day; $F< 1;p>0.20$). This is consistent with previous evidence indicating that long-term memory formation is preserved in GRIA1-/- mice.
The deletion of GluA1 in mice is associated with a profound reduction of EEG sleep spindling activity; yet local cortical sleep spindles may be preserved. Global EEG spindles do not seem necessary for memory consolidation, although a role for local LFP spindles cannot be excluded. These results suggest an important role of the GRIA1 gene in mediating the link between sleep and cognitive function.

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GABA and glutamate networks in the VTA regulate govern vigilance state

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We screened for novel circuits in the mouse brain that determine vigilance states. Using chemogenetic activation and EEG recordings, we converged on glutamatergic/nitrergic (NOS1) and GABAergic neurons in the VTA. Activating glutamatergic/NOS1 neurons, which were wake- and REM-sleep active, produced wakefulness via the nucleus accumbens and the lateral hypothalamus. Lesioning the glutamate cells impaired the consolidation of wakefulness, with many extra transitions to NREM sleep. In contrast, activation of GABAergic VTA neurons elicited a long-lasting NREM-like sleep akin to sedation. Lesioning them produced a large increase in wakefulness, which persisted for at least 4 months after lesioning. The VTA GABAergic neurons, however, are selectively wake- and REM sleep-active. Our findings suggest that VTAVgat neurons limit wakefulness by inhibiting the arousal-promoting VTA glutamatergic and/or dopamine neurons, as well as by projections to the lateral hypothalamus. Thus, the VTA, widely investigated for its contribution to goal- and reward-directed behaviors, contains circuitry with an unexpected role in regulating wakefulness.

Xiao Yu and Wen Li contributed to the study equally

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