The effect of disruptive light conditions on sleep and circadian rhythms



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A thesis submitted for the degree of

Doctor of Philosophy

Trinity 2019

Acknowledgements

First I wish to thank my supervisors, Professor Stuart Peirson and Professor Vladyslav Vyazovskiy. Their support over the last 4 years has helped me develop into the person I am today. In addition, Gareth Banks has provided me with invaluable advice and support during my degree.

I want to thank my family. My family have shaped me. They have always been there for me, they have supported me, and they helped me get further than I ever thought I would. The Langleys, who I have known as long as I can remember, definitely fit in the definition of family.

The friends I have made during the last 4 and a half years are incredible and I wouldn't have got through this without them. My friends from both labs have helped calm my panic when I struggled with experiments and writing and the whole process. I want to thank everyone in both the Vyazovskiy and Peirson groups for helping me over the last few years. Special thanks go to Ximena Omlin, Laura McKillop, Mathilde Guillaumin, Michael Gilhooley, Moritz Linder, Lewis Taylor, Teele Palumaa, Christopher Thomas, Yige Huang, Kirk Reardon and Chiara McDermott. Ximena, you managed to get me out when that was the last thing I wanted to do. Laura, you convinced me that I could actually get through this. Mathilde you were a companionion throughout the whole process and showed me that it is doable.

I have been incredibly lucky to be part of Brasenose College during this degree. The people and the place have been a space for me to escape to, to get away to when everything else got too much. Special thanks go to Thom Diment, Laura Diment, Ariane Laurent-Smith, Matthew Speight, Eric Haney, Sunniva Bostrand, Callum Brodie, Matt Slauson, Alex Dunn, and Tom Yems. Thom, you have been a brilliant friend, and you have introduced me to so many things I wouldn't know about without you, and I am extremely grateful for you. Eric, I am lucky to count you as a friend, you have taught me more obscure knowledge than I know existed, and you have entertained us for countless nights.

I want to thank Professor Elspeth Garman especially. You have been a source of support and knowledge and encouragement throughout the degree, reminding me when I didn't believe in myself.

Finally, in addition to all the friends I have made in the last few years, I have good friends that I have known for years, and even though most of them are in

Australia, they have supported me from the other side of the world. Specifically Laura Setyo, Angus Ades, Tim Herington, Chris Simpson, and Sophie Fenwick. You have all been friends for me for years, and have managed to be some of the best humans I know in the world, despite seeing you so rarely.

This is the hardest thing I have ever done. I spent years never thinking I would get to this point. I would not be here without the support of all the people above. I am incredibly grateful for everyone who has helped me through this.

Abstract

The rotation of our planet on its axis provides a rhythmic environment for almost all life on Earth, and most forms of life display daily cycles in physiology and behaviour. In mammals these circadian rhythms are entrained to the external environment by light through visual and non-visual pathways from the eyes. Circadian rhythms are important for many aspects of physiology and behaviour, and their disruption appears to cause many negative consequences, including heart disease, obesity, and cancer in humans. This is important as over the last two hundred years, the invention of artificial light has majorly changed our exposure to light, with increases in many of the negative side effects of circadian disruption.

Abnormal light cycles are commonly used in nocturnal rodents to understand the effects of disrupted circadian rhythms. Here, we used a variety of different abnormal light cycles to investigate their effects on locomotor activity and sleep. First, we show that each light cycle has a unique effect on locomotor activity and sleep, which appears related to the amount of light they get at night. This suggests that their effects cannot be directly compared. Next, we show that constant light induces sleep in nocturnal rodents, and that this is likely mainly due to the direct effects of light. This effect highlights where our quantitative models of sleep-wake regulation are lacking. Finally, we show that after prolonged circadian disruption, locomotor activity is not altered. This suggests that the circadian system is more robust to disruption than we previously assumed, and highlights the problems of using light to induce disruption. Overall, the data presented here in this thesis help us understand the effects of abnormal light cycles on locomotor activity and sleep, and demonstrate where our understanding of sleep and circadian rhythms are lacking.

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List of Abbreviations

cAMP . . . . . Cyclic adenosine monophosphate

CCG . . . . . Clock controlled genes

Ck1 . . . . . Casein Kinase 1

CORT . . . . . Corticosterone

CRY . . . . . Cryptochrome protein

Cry . . . . . Cryptochrome gene

CT . . . . . Circadian time

DD . . . . . Constant darkness

DLAN . . . . . Dim light at night

DMH . . . . . Dorsomedial hypothalamus

DPS . . . . . Disruptive phase shift

DR . . . . . Dorsal raphe nucleus

EEG . . . . . Electroencephalogram

EMG . . . . . Electromyogram

FOS . . . . . Fos protein, marker of neural activity

HPA . . . . . Hypothalamic-pituitary axis

IS . . . . . Interdaily stability

IV . . . . . Intradaily variability

Jetlag . . . . . Advance or delay of the LD cycle which requires animals to gradually re-entrain to the new LD cycle.

LC . . . . . Locus coeruleus

LD . . . . . Light dark. Used to refer to either any light dark cycle, or an experimental group where animals are housed under a standard 12:12hr light:dark cycle

LDT . . . . . Laterodorsal tegmental nucleus

LH . . . . . Lateral hypothalamus

LL . . . . . Constant Light

MCH . . . . . Melanin-concentrating hormone

MR . . . . . Median raphe nucleus

NIF . . . . . Non-image forming

NREM . . . . Non-rapid eye movement sleep

OPN4 . . . . . Melanopsin protein

Opn4 . . . . . Melanopsin gene

pRGC . . . . . Photosensitive retinal ganglion cells

PER . . . . . Period protein

Per . . . . . Period gene

PPT . . . . . Pedunculopontine nucleus

REM . . . . . Rapid eye movement sleep

RHT . . . . . Retinohypothalamic tract

ROR . . . . . Retinoid-related orphan receptors

SCN . . . . . Suprachiasmatic nucleus

Slow wave sleep NREM sleep during which slow waves predominate on the EEG

SPZ . . . . . Subparaventricular zone

SJ . . . . . Social Jetlag. Light cycle with 8:16h light:dark cycle for 5 days a week and 16:8h light:dark cycle for 2 days a week

SWA . . . . . Slow wave activity. Power in the 0.5-4Hz frequency band of EEG recordings.

SWE . . . . . Slow wave energy. Cumulative slow wave activity

T cycle . . . . Light dark cycle which deviates from 24 hours in total

T20 . . . . . T cycle made up of 10:10h light:dark

TMN . . . . . Tuberomamillary nucleus

TTFL . . . . . Transcriptional-translational feedback loop

VIP . . . . . Vasoactive intestinal peptide

Vipr . . . . . Vasoactive intestinal peptide receptor gene

VLPO . . . . . Ventrolateral preoptic nucleus

Zeitgeber . . . Rhythmic cue which can entrain circadian rhythms

ZT . . . . . Zeitgeber time

1 Introduction

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1.1 Introduction

Light exerts profound effects on physiology and behavior. These include entraining circadian rhythms, and direct effects on body temperature, melatonin, cortisol, and the cortical electroencephalogram (EEG) [1–4]. These effects of light are of particular concern in the modern 24/7 society as inappropriate light exposure affects an increasing proportion of the populace. This includes not only shift work and jet-lag, but exposure to light at night and the effects of light emission from mobile devices, such as laptops, tablets, and smartphones[5]. Extended periods of abnormal light exposure can result in circadian disruption, which has been implicated in changes in metabolism, sleep, and cognition, as well as increasing the risk of metabolic and cardiovascular disease [6]. For example, shift work involves abnormal light exposure, sleep, and circadian disruption, and is associated with increased risk of breast [7], colorectal[8], and prostate[9] cancer. Shift work is also associated with increases in cardiovascular disease [10], obesity[11], and impaired cognition [12]. To understand these effects, many studies have looked at the effect of abnormal light/dark (LD) cycles in animal models [13–16]. While these studies have been critical for understanding how circadian disruption affects different systems, the relationship between the direct effects of light and the long-term consequences of abnormal light exposure are not straightforward. Specifically, abnormal LD cycles may affect physiology via the direct effects of light as well as via its effects on the circadian system. Changes in circadian function may in turn influence sleep, which will subsequently affect additional processes.

1.2 Circadian Rhythms

Circadian rhythms are approximately 24 h cycles in physiology and behavior that enable an organism to predict and adapt to periodic changes in its environment. These rhythms provide a selective advantage, enabling anticipation and exploitation of predictable changes in the environment[17, 18]. Circadian rhythms have been described in virtually all organisms, from cyanobacteria to mammals [17]. Moreover,

they have been shown to coordinate numerous aspects of physiology and behavior, including locomotor and sleep/wake cycles [19, 20], hormonal rhythms[21, 22], metabolism[23] and cognitive performance[24]. Conversely, disrupted circadian rhythms impair fitness. Studies on ground squirrels with suprachiasmatic (SCN) lesions found that they were predated 20% more than sham-operated control animals[25], and cyanobacteria with differing circadian periods showed increased fitness when their period matches that of the prevailing LD cycle[26]. Due to the role of the circadian system in optimizing physiology and behavior in anticipation of predictable environmental changes, a mismatch between internal circadian time and the external LD cycle appears to be a key mechanism by which circadian disruption gives rise to negative health consequences[27].

1.2.1 The Suprachiasmatic Nuclei

In mammals, the master circadian pacemaker is the paired SCN located within the anterior hypothalamus. When the SCN are lesioned, animals lose rhythms in corticosterone secretion, locomotor activity, and drinking behaviour [28, 29]. Furthermore, if fetal SCN are transplanted into an SCN lesioned animal, circadian rhythms are restored[30], with a period determined by the donor animal[31]. The SCN show circadian variations in electrical activity and firing rate over 24 h, with high activity during the subjective day and low activity during the subjective night[32]. Individual SCN neurons oscillate with a period of roughly 24 h when dissociated from the rest of the SCN tissue, indicating that these rhythms are generated at an intracellular level rather than occurring as an emergent network property[33]. While the role of the SCN in driving circadian rhythms in physiology and behavior in mammals was established in the 1970s, it was not until the late 1990s that the molecular basis of these rhythms was established[34].

1.2.2 The Molecular Circadian Clock

In eukaryotic organisms, the underlying mechanism generating intracellular circadian rhythms is a transcriptional-translational feedback loop (TTFL) comprising positive,

negative, and accessory limbs[35], while in the prokaryotic cyanobacteria, it is a post-translational oscillator [36]. In eukaryotes, the specific genes involved differ between species, e.g. drosophila and mammals[37], however the basic structure appears similar. In mammals, the positive limb consists of the core clock proteins, CLOCK and BMAL1, which both contain a basic helix-loop-helix domain and bind together to form heterodimers. These bind to E-box enhancer regions of *Per1-2* and *Cry1-2* genes to promote their transcription, as shown in figure 1.1. The negative limb comprises the translated PER and CRY proteins which translocate back into the nucleus and directly interact with the CLOCK/BMAL1 complex to inhibit transcription of *Per1-2* and *Cry1-2*. This then reduces the amount of PER and CRY proteins, leading to re-activation of transcription by CLOCK/BMAL1. In addition to these core genes, others also regulate the TTFL. Casein Kinase 1, *Ck1*, is a cellular kinase that participates in the TTFL by phosphorylating PER proteins, which targets them for degradation [38], and the activity of which can alter the animals internal period[39, 40]. In addition to the core loop, an accessory loop is also driven by CLOCK/BMAL1 activation. The *Rev-erbα* gene is transcribed and produces the orphan nuclear receptor REV-ERBα, which inhibits *Bmal1* transcription via a ROR response element in its promoter. This leads to BMAL1 proteins cycling in antiphase to PER and CRY proteins. As PER and CRY interact with CLOCK/BMAL1 to inhibit transcription, *Rev-erbα* falls as well, disinhibiting *Bmal1* transcription, allowing levels to rise again[41]. This accessory loop stabilises the locomotor activity of animals, as animals lacking *Rev-erbα* have greater phase shifts in response to light [41]. Of the four core clock genes, only CLOCK is stably expressed across the circadian cycle. CLOCK/BMAL1 heterodimers also drive the transcription of a large number of other genes which contain E-box enhancers in their promoter region, termed clock controlled genes (CCGs), thus allowing the clock to influence a wide range of cellular functions[42].

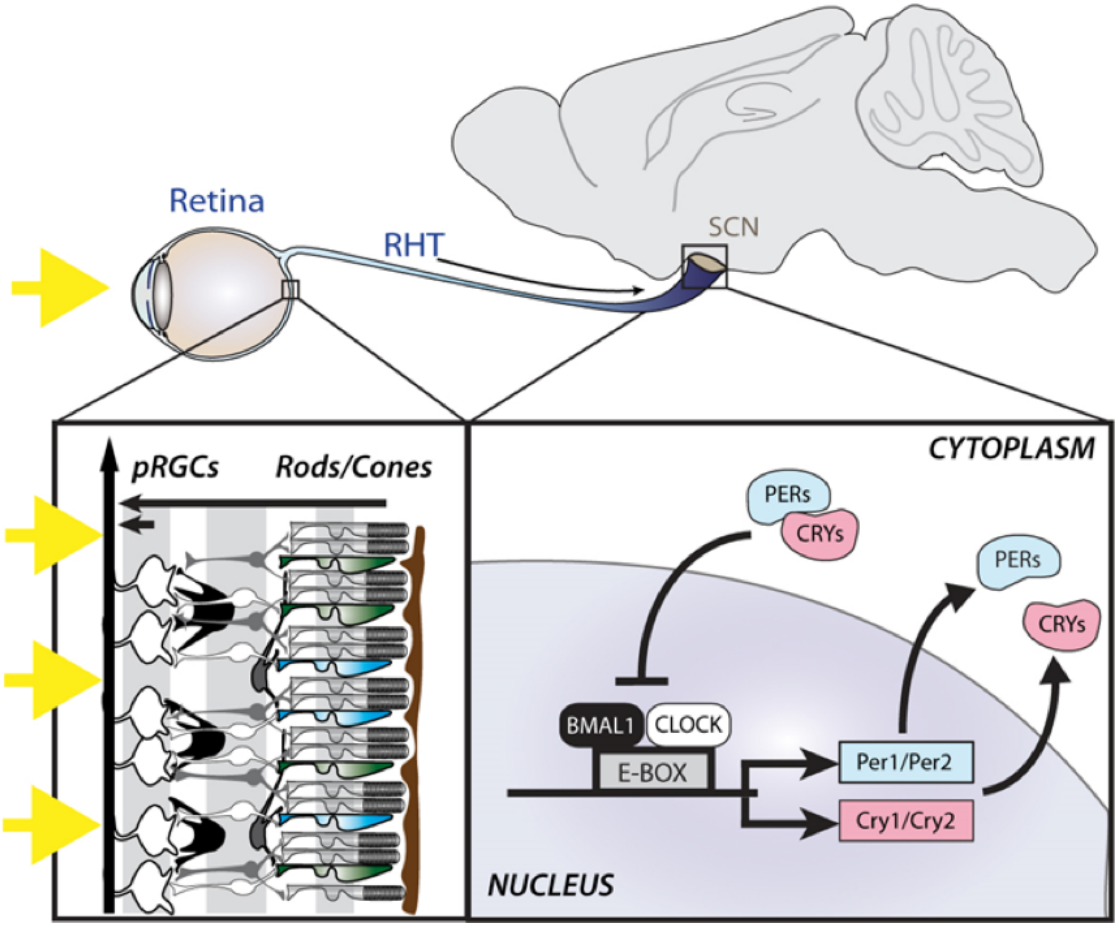


Figure 1.1: Light is detected by the photoreceptors of the retina, including the rods and cones as well as photosensitive retinal ganglion cells (pRGCs), expressing the photopigment melanopsin. Light information is relayed to the suprachiasmatic nuclei (SCN) via the retinohypothalamic tract (RHT), where it entrains an intracellular molecular clock mechanism, consisting of positive (BMAL1 and CLOCK) and negative (PER and CRY) elements.

1.2.3 Light Input the the Circadian Clock

A clock is of no use unless it can be set to the right time. In mammals, the master SCN clock is entrained to the external environment by time cues (zeitgebers), including light[43], exercise, social contacts[44], cognitive tasks[45], and food availability[46]. The most important of these is light detected by the eyes, as loss of the eyes abolishes entrainment[43, 47]. The SCN receives input from the retina via the retinohypothalamic tract (RHT)[48]. Studies on the photoreceptors mediating circadian entrainment demonstrated that mice can still phase shift their

locomotor activity rhythms and suppress pineal melatonin in response to light even in the absence of the classical rod and cone photoreceptors that mediate vision[49, 50]. These findings suggested the existence of a novel retinal photoreceptor, in addition to the well-characterized rods and cones. It was subsequently shown that a subset of photosensitive retinal ganglion cells (pRGCs) expressing the photopigment melanopsin (OPN4) are intrinsically light sensitive and form the primary projection to the SCN[51], as seen in figure1.1. However, as pRGCs receive input from the outer retina, their output via the RHT depends upon both their intrinsic responses as well as extrinsic signals from rods and cones[52, 53]. As such, mice lacking melanopsin ($Opn4^{-/-}$) display normal circadian entrainment, but attenuated phase shifting responses to light[54, 55]. However, mice in which pRGCs are genetically lesioned are unable to entrain to light[56]. Since the identification of pRGCs, it has become clear that these cells mediate more than just circadian entrainment, and are involved in a range of non-image forming (NIF) responses to light, including the pupillary light response[57], regulation of sleep–wake timing[58], light aversion[59], and cognitive function[60], as well as influencing image forming responses, such as visual adaption[61]. Melanopsin-expressing pRGCs project to multiple brain targets, including the intergeniculate leaflet, olivary pretectal nucleus, medial amygdala, lateral habenula, and superior colliculus, suggesting that different NIF responses may involve different neural projections[61, 62]. The primary neurotransmitters of the RHT are glutamate and pituitary adenylate cyclase activating polypeptide, which are released at synapses in the SCN in response to photic stimuli[63, 64]. This results in increases in calcium concentration and firing rates in SCN neurons. Increased calcium concentration activates intracellular signaling pathways [e.g., cyclic AMP (cAMP) and Protein Kinase A], converging on the phosphorylation of cAMP response element binding protein, which translocates to the nucleus, binding to cAMP response elements in the promoters of *Per1* and *Per2*, increasing their transcription. This results in the molecular clock in the SCN being either advanced or delayed[3]. Although this link between light input, membrane events, and the TTFL has been characterized, the mechanisms by which the TTFL regulates

membrane potential are poorly understood[3, 65]. While great progress has been made in understanding the molecular basis of photoentrainment, this model is almost certain to be incomplete.

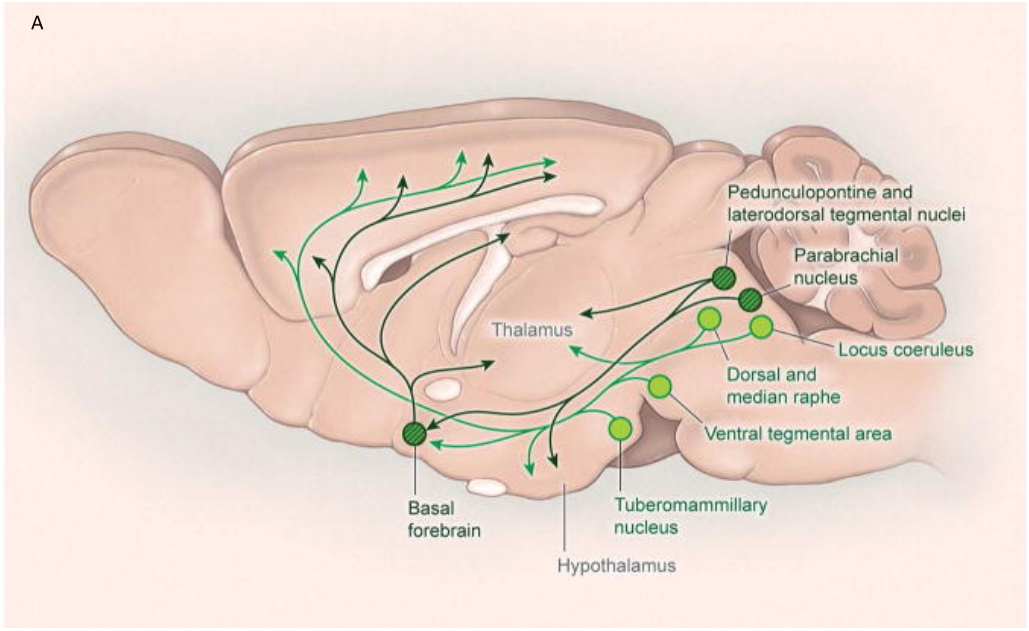
1.2.4 Clock Outputs and Peripheral Clocks

The SCN has widespread projections throughout the brain, including to the septum, the anterior paraventricular thalamus, and to multiple hypothalamic nuclei including the subparaventricular zone, ventromedial hypothalamus, dorsomedial hypothalamus, and pre-optic area[66]. The SCN also projects to the paraventricular nucleus, whereby it modulates circadian rhythms in neuroendocrine and autonomic function[67, 68]. Furthermore, molecular circadian rhythms are not confined to the SCN. Studies in the late 1990s showed that rat fibroblasts, which had been cultured for 30 years, were capable of rhythmic clock gene expression following a serum shock[69]. Furthermore, clock gene reporter studies demonstrated that many tissues throughout the body displayed rhythmic clock gene expression, including liver, lung, kidney, spleen, pancreas, heart, stomach, skeletal muscle, lung, cornea, thyroid gland, and adrenal glands[70–73]. These peripheral clocks are thought to play a key role in regulating local tissue physiology[74]. For example, in the liver, xenobiotic detoxification[75], carbohydrate[76], and lipid metabolism[77] are circadian. Neural output from the SCN is important for endocrine rhythms, as these are prevented by transplants that prevent neurite outgrowth[78], however the remaining diffusible signals are sufficient to restore circadian rhythms in locomotor activity[78]. Organisation of locomotor activity is one of the most obvious outputs of the SCN, however, locomotor activity, such as scheduled exercise in a LD cycle, can also alter the phase of peripheral clocks, including the liver, skeletal muscle, and adrenal glands[79–83]. The SCN therefore coordinates circadian timing throughout the body via a combination of neural, paracrine, hormonal, and behavioral signals.

1.3 Sleep

Sleep is a reversible behavioral state of perceptual disengagement from and unresponsiveness to the environment[84]. Sleep appears to be a universal behaviour in complex animals, as most animals show some sleep behaviour[85, 86], including elephants[87], slow-loris[88], and surprisingly simple organisms such as jellyfish[89]. While the precise function of sleep is not fully understood, it likely serves multiple functions, including metabolite clearance, memory processing, immune restoration, and other functions[90]. Deprivation of sleep has many negative consequences, including cognitive impairment[91], metabolic dysregulation[92], and following extended sleep deprivation, eventually death[93].

Sleep can be defined in both behavioral and physiological terms. Behaviorally, it involves a period of extended inactivity, with increased arousal threshold, a species-specific body posture and a typical sleep site[85]. Physiologically, sleep is defined by the EEG, which measures electrical activity at the level of the cortex. During sleep, well-characterized changes occur in the EEG, which allows classification of sleep into distinct stages, Rapid-Eye Movement sleep (REM) and Non-Rapid-Eye Movement(NREM) sleep[94, 95]. REM sleep involves low amplitude high frequency spectrum on the EEG, similar to that seen during wake, muscular atonia, and the rapid eye movements that give this stage its name. In contrast NREM sleep is characterised by high amplitude low frequency spectrum on the EEG, with no muscular atonia or rapid eye movements[96]. In humans, NREM sleep is further subdivided into stage 1-3[97]. In humans there is a characteristic cycle of these stages during the night, progressing through stages 1-3 of NREM sleep, followed by REM sleep before starting the cycle again. This cycle takes roughly 90 minutes to occur, and humans progress through several cycles per night, with higher levels of NREM sleep per cycle at the start of the night and higher levels of REM sleep per cycle at the end of the night[98]. In rodents, sleep occurs more frequently in shorter episodes, and substages of NREM sleep are not recognised[99].


A
Pedunculopontine and
laterodorsal tegmental nuclei
Parabrachial
nucleus
Thalamus
Locus coeruleus
Dorsal and
median raphe
Ventral tegmental area
Basal
forebrain
Tuberomammillary
nucleus
Hypothalamus

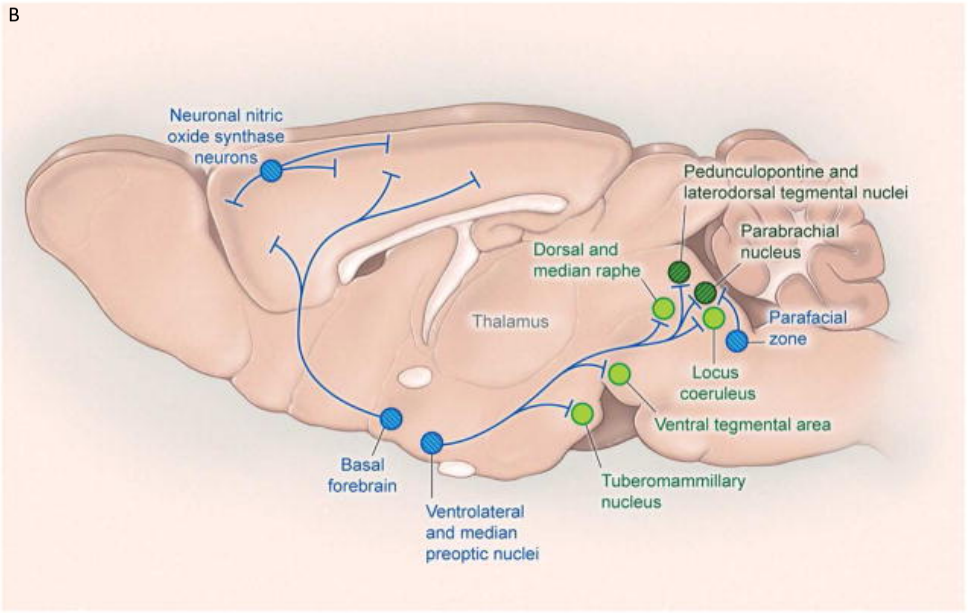

B
Neuronal nitric
oxide synthase
neurons
Pedunculopontine and
laterodorsal tegmental nuclei
Parabrachial
nucleus
Dorsal and
median raphe
Thalamus
Parafacial
zone
Locus
coeruleus
Ventral tegmental area
Basal
forebrain
Ventrolateral
and median
preoptic nuclei
Tuberomammillary
nucleus

Figure 1.2: Neural circuitry of wake and sleep. (A) shows the regions in the brainstem that project to the thalamus basal forebrain and throughout the cortex that are active during wakefulness. Cholinergic regions are dark green, monaminergic regions are light grey. (B) shows the regions associated with NREM sleep. Sleep active, dark blue, basal forebrain and ventrolateral preoptic neurons inhibit wake-promoting regions in the brainstem. Figure adapted from [100]

1.3.1 Sleep mechanisms

How are these different states created? This involves a complex process of mutually inhibiting arousal and sleep-promoting neurons which lead to either wake or sleep respectively[100–102]. Initially, it was noted that humans with lesions to the posterior hypothalamus and rostral midbrain had problems maintaining wakefulness[103]. However, von Economo made his observations before polysomnography was used to define sleep electrophysiologically, and used immobility to behaviourally define sleep. Therefore these findings must be taken carefully as some cases may be mischaracterised motor problems. This work led to the recognition of the ascending arousal system, a system of nuclei and pathways that project from the brainstem to promote wakefulness[104, 105]. This consists of two branches. The first branch projects to the thalamus, mainly from the pedunculopontine and laterodorsal tegmental (PPT/LDT) nuclei, which are both primarily acetylcholine producing cell groups[106]. These neurons fire mainly during wakefulness and REM sleep, and are much less active during NREM sleep[107]. The second branch avoids the thalamus and projects to the lateral hypothalamus, basal forebrain, and more widely throughout the cortex[108]. The source of these pathways is mostly monoaminergic nuclei in the brainstem and caudal hypothalamus, which include the noradrenergic locus coeruleus (LC), serotoninergic dorsal and median raphe nuclei (DR, MR), dopaminergic ventral periaqueductal grey matter, and histaminergic tuberomammillary neurons. These neurons interact with peptidergic neurons, containing melanin-concentrating hormone or orexin, in the hypothalamus and cholinergic and gabaergic neurons in the basal forebrain. The monoaminergic nuclei in this pathway fire fastest during wake, reduce their firing during NREM

sleep, and cease firing altogether during REM sleep[109–111]. The orexinergic hypothalamic nuclei are also most active during wake[112], while the MCH neurons are active during REM sleep[113]. The basal forebrain neurons are active during both wake and sleep[114].

By contrast, some patients with lesions in the basal ganglia and adjacent anterior hypothalamus had drastically reduced sleep[103]. Later, the ventrolateral preoptic (VLPO) nucleus was identified in this area using FOS induction, a genetic marker of neural activity, as being sleep active, and sending inhibitory outputs to the hypothalamic and brainstem arousal nuclei[115]. The VLPO contains a dense central cluster, and an extended diffuse part[104]. Lesions of the central cluster mainly reduce NREM sleep[116], anatomically explained by projections to histaminergic neurons of the tuberomamillary nucleus (TMN) that are linked to NREM/wake transitions[117]. Lesions of the extended part of the VLPO disrupt REM sleep[116]. This projects to the LC and DR, nuclei with reduced firing during NREM and absent firing in REM sleep, which are linked to gating REM sleep[118]. The VLPO is also innervated by several afferents of the arousal system[119], which inhibit the VLPO.

This mutually inhibitory network of wake and sleep promoting neurons interact to promote wake or sleep. One model for the function of this network is as a so-called "flip-flop" switch of bistable states. During wake, the monoaminergic ascending arousal nuclei inhibit the sleep-inducing VLPO, while during sleep, the VLPO fires to inhibit the same ascending arousal nuclei[107]. In this model of sleep regulation, any transitional states are quickly converted into either a wake or a sleep promoting firing pattern. In elderly humans, who have reduced VLPO neurons[120], there is an increase in sleep-wake cycling[121], and in rodents VLPO lesions lead to greater transitions between sleep and wake[116, 118]. Recent evidence suggests that this model is too simplistic, for example the same neurons can promote sleep or wake depending on their firing rate in both mice and zebrafish[122]. New models of the circuitry underlying sleep and wake have recently been proposed that challenge the idea of the 'flip-flop' switch[123]. We are still developing our understanding of this complicated system.

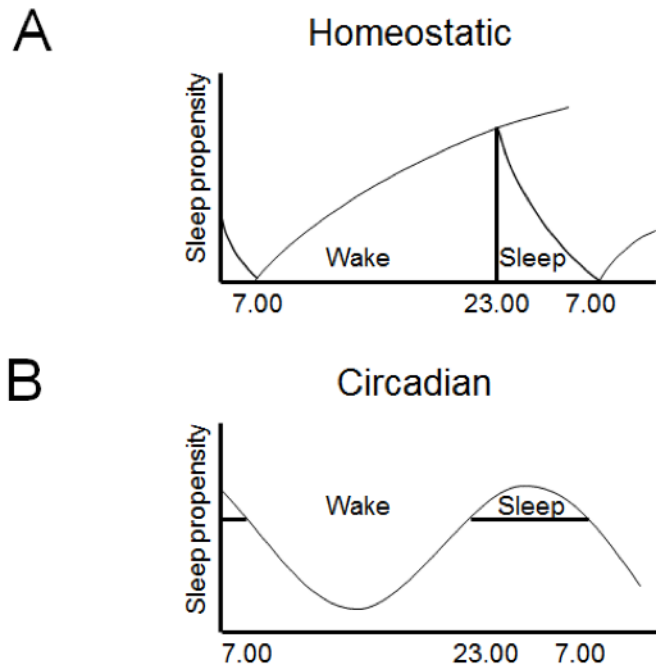


Figure 1.3: Sleep is regulated by two interacting processes. (A) A homeostatic mechanism (Process S) increases the requirement for sleep with prolonged waking and dissipates during sleep. (B) A circadian mechanism (Process C) provides a drive for wakefulness at specific phases of the 24 h cycle

1.3.2 Homeostatic and Circadian Regulation of Sleep

The sleep/wake cycle is perhaps the most familiar consequence of our circadian rhythms. While sleep is modulated by the circadian system, it is also critically regulated by a homeostatic drive that increases with extended waking. As such, sleep and wakefulness depend upon the interaction between these circadian and homeostatic processes [124, 125]. The quantity, quality, and timing of sleep and wakefulness are regulated by both a homeostatic and a circadian process (termed Process S and C, respectively) [125]. These processes interact to produce periods of wake and sleep during the day, as shown in figure 1.3 This conceptual model has been useful in interpreting disturbances of sleep/wake regulation, and has been validated by quantitative predictions [126–128].

The homeostatic regulation of sleep represents a general level of sleep that is required for an organism. This expresses itself as a set-point of the amount (intensity and duration) of sleep required over roughly 24 hours. During wake,

sleep-need gradually accumulates, which is then dissipated during sleep. When sleep is prevented, compensatory recovery sleep dissipates the extra sleep-need accumulated[129]. This process is highly correlated with the power of slow wave activity (SWA) on the EEG during NREM sleep, characterized by frequencies in the 0.5–4 Hz range[130]. The mechanisms underlying this homeostatic process are unclear. However, a number of putative sleep factors build up in the brain during prolonged wakefulness and dissipate during sleep and these may mediate the homeostatic process[129, 131]. Perhaps the best known example is adenosine, levels of which increases in the basal forebrain during wake and dissipates during sleep, and has been proposed to account for the action of adenosinergic drugs, such as caffeine, on sleep[132, 133].

The circadian process organises sleep across the day, allowing wake and sleep at alternate phases of the cycle[134]. The primary driver of the circadian process is thought to be the SCN, as following SCN lesions endogenous rhythms in rest/activity are abolished, but the homeostatic regulation of sleep remains intact[135–137]. However there is mixed evidence for the output signal of the SCN. In the above rat studies, there was no increase in total sleep time following an SCN lesion, however later studies in mice[138], squirrel monkeys[139], and siberian hamsters[140] reported increased sleep time, suggesting that the SCN provides a wake-promoting signal. The circadian control of sleep can also be dissociated from other circadian rhythms. For example, housing rats under a 22h day induces internal desynchrony, and separates rhythms of NREM sleep from REM sleep and body temperature[141]. More specifically, the circadian timing of REM sleep has been shown to be associated with clock gene expression in the dorsomedial SCN[142]. The SCN only has modest projections to hypothalamic nuclei involved in sleep and wake regulation, including VLPO and orexin neurons[143]. The majority of SCN projections go to the subparaventricular zone (SPZ) and dorsomedial nuclei of the hypothalamus (DMH), and it appears that the SPZ is the key node mediating the circadian control of sleep. Lesions of the ventral SPZ inhibit the circadian control of sleep and locomotor activity, while not affecting body temperature, while lesions of the dorsal SPZ have

the inverse effect[144]. Overall, the SCN appears to promote both wake and sleep at different phases of the circadian cycle, and may also influence sleep intensity[145].

Together, the homeostatic and circadian processes determine the timing of sleep and wake, and disturbance of either process has the potential to disrupt sleep.

1.3.3 Direct Effects of Light on Sleep

In addition to the role of homeostatic and circadian processes in the regulation of sleep, light exposure also directly modulates sleep.

Light has a range of direct effects. In diurnal species such as humans, it promotes alertness and arousal[1]. In nocturnal rodents light promotes sleep[58], alters activity, termed masking[146, 147], and affects body temperature[148]. Some studies have attempted to link light to a direct effect on mood and cognition[60, 149], however they have not ruled out the effects on sleep, arousal, or circadian rhythms so must be interpreted with caution.

pRGCs project both to the SCN and more widely throughout the brain. Different subtypes of pRGCs have different projections and electrophysiological characteristics[61]. There is emerging evidence that different subtypes of pRGCs have different physiological effects[148, 150]. One of the functions that appears to be differentially affected by pRGC subtypes is sleep[148], where those that project to the SCN appear to have a primarily circadian effect on sleep and body temperature, while those that don't project directly to the SCN appear to have a more acute effect. This shows that pRGCs can directly mediate an acute effect of light on sleep separately from their circadian effects. Since pRGCs project widely throughout the brain, it is unclear currently which projections mediate the acute effects of light, but mechanistically they project to the VLPO, known to be a sleep-promoting region[151], and the SPZ, an area that mediates the circadian rhythm of sleep[144].

Interestingly, light appears to directly increase corticosterone in nocturnal rodents, via the SCN but independent of the clock[152]. This effect is also apparent when using chemogenetic activation of pRGCs[153], and when using different wavelengths of light to stimulate melanopsin[154]. Given this, removing melanopsin

ought to remove the alerting/corticosterone effect of light and improve sleep, however melanopsin deficient mice show the opposite effect and have impaired sleep[58, 155–157]. This may be due to impaired light adaptation in melanopsin deficient mice, as any rod/cone sleep induction effect may become saturated[158]. It may also be due to altered homeostatic sleep pressure, as melanopsin deficient mice have reduced delta power during slow wave sleep, and altered response to sleep deprivation[157]. Therefore the light environment may contribute to homeostatic sleep need, as when light detection is impaired by removing melanopsin, homeostatic sleep need is also affected.

Altogether, light can have direct effects on many processes including sleep. Currently the mechanisms mediating the effect on sleep are unclear, it may act via the homeostatic mechanism or separately to it. The circadian system does not appear to be necessary.

1.4 Arousal

Arousal refers to the state of physiological alertness which results in increased attention and cortical activity, accompanied by changes in motivation and emotional state[159]. It is closely related to stress, which is the response to adverse external circumstances. Measuring arousal is difficult. Since it is closely related to stress, levels of circulating glucocorticoids is commonly used as a marker of arousal, however they are not the same thing and there are important caveats to doing so[160]. Other markers include locomotor behaviour, pupil diameter, and skin temperature[161, 162]. However given arousal involves many different systems all contributing to physiological alertness, each marker only provides part of the picture. Arousal is important for appropriately responding to internal and external stimuli. This is summarised in the classic inverse-u shaped curve termed the Yerkes-Dodson law[163] , where the performance on a cognitive task peaks at some arousal level, and decreases with too low and too high levels, as shown in figure 1.4.

Arousal can be altered by many factors, including acute changes in the environment, sleep, and circadian rhythms. There is a circadian control of the

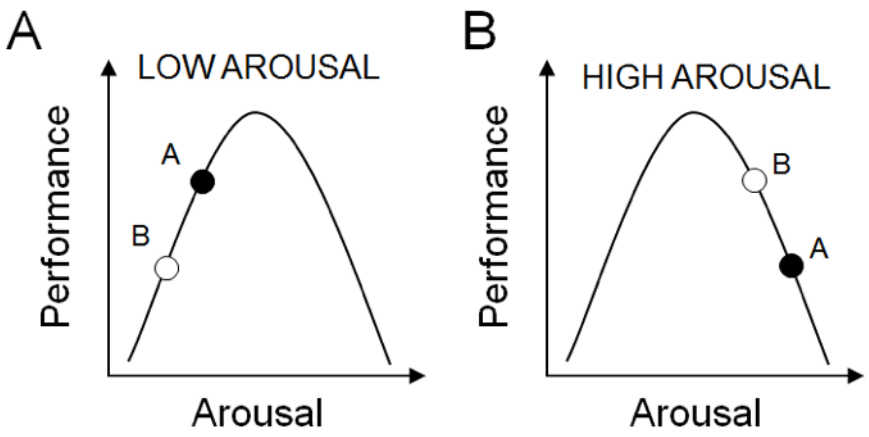


Figure 1.4: The relationship between arousal and cognitive performance. The effects of circadian time on performance may depend upon different levels of arousal. These may in turn be integrated with other arousal- promoting stimuli. (A) Under normal low arousal conditions, performance may be better at circadian time A versus circadian time B. (B) However, under conditions of high arousal, these circadian time-dependent effects may result in better performance at time B versus time A, due to excessive arousal.

levels of arousal. Performance on cognitive tasks in humans, as a marker of arousal, is modulated by circadian time independently of sleep/wake[24]. There is a circadian rhythm in the level of glucocorticoids[164], and abnormal light cycles can affect their rhythmicity[60, 165].

Arousal is also affected by sleep. Similar to the circadian control, time awake and therefore sleep pressure modulate cognitive performance independently of time awake[24]. Sleep deprivation impairs attention and working memory[166]. Sleep deprivation also increases corticosterone[167]. which is unlikely an indicator of high arousal, but a stress response required to maintain normal levels of arousal.

Light can directly modulate arousal. In humans, light exposure can influence cortical and subcortical networks involved in attention and arousal[168–173]. Furthermore, short wavelength light can reduce reaction times, suppress melatonin and increase subjective ratings of alertness[174, 175]. In animals, it is less clear. Acute light exposure can increase corticosterone levels and impair performance on cognitive tasks[152, 176], possibly by taking arousal into the decending limb of the inverted-u shaped performance curve, as shown in figure 1.4B.

These systems all interact, such that the effect of light exposure may vary by time of day, and by recent sleep/wake history. This makes it difficult to isolate the effects of any one in particular.

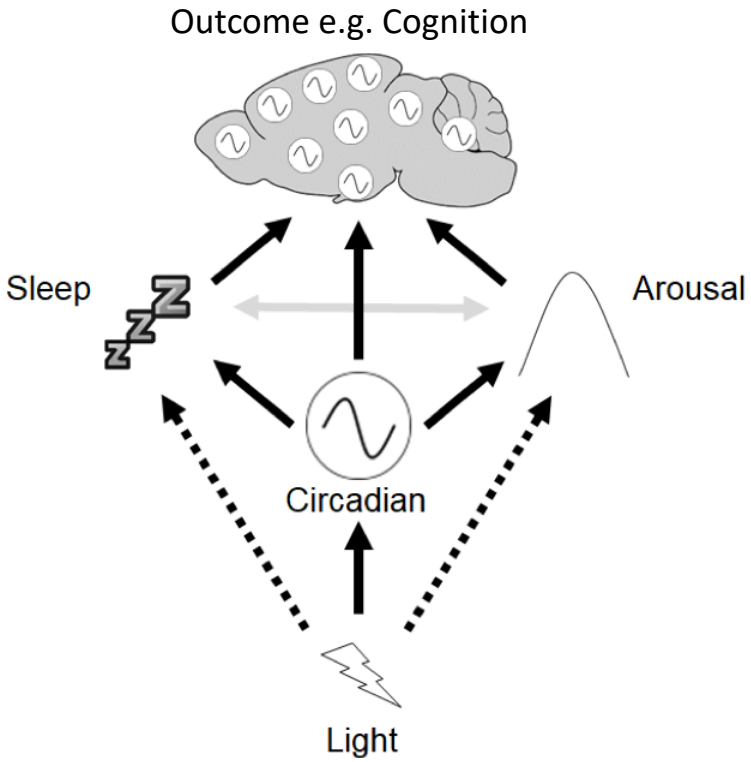


Figure 1.5: Framework to describe the interactions between circadian rhythms, sleep, and arousal. Light exerts direct effects on the circadian clock in the suprachiasmatic nuclei (SCN), which in turn modulates other rhythmic processes throughout the body, including independent oscillators found in other brain regions. The SCN clock also modulates sleep, and regulates arousal via output to hypothalamic-pituitary-adrenal axis and sympathetic nervous system. Light may also directly regulate sleep as well as arousal (dashed arrows). Reciprocal interactions between sleep and arousal also occur (gray arrows).

1.5 Circadian Disruption

The circadian system has evolved to enable organisms to anticipate and exploit predictable changes in the external environment, optimizing physiology and behavior to specific times of day. However, our modern 24/7 society produces numerous examples where lifestyle is in conflict with our internal biological clocks, including shift work and jet-lag. Moreover, artificial light results in light exposure at inappropriate times of day, including light at night as well as exposure to light

from mobile devices, such as phones, tablet, and computers. As a result, there is growing concern regarding the consequences of circadian disruption and aberrant light exposure on human health, including effects on metabolism, cardiovascular function, mental health, and even cancer risk[6, 27, 177]. Those routinely exposed to such conditions may develop problems with poor performance, insomnia, emotional disturbances, and gastrointestinal complaints. Such symptoms are thought to affect 5–10% of those involved in shift work. Treatments seek to realign the internal clock with the external environment, using scheduled light exposure (or avoidance), short naps, or use of pharmacological interventions, such as melatonin, caffeine, or even prescription drugs[178, 179]. With our increasing exposure to artificial light sources, such problems look likely to rise in the future. To investigate the mechanisms underlying the adverse health outcomes of circadian disruption, an increasing number of studies have investigated the effects of aberrant light exposure on cognitive function, using rodents housed under abnormal LD cycles. However, such abnormal LD cycles may exert their effects via different mechanisms. Some result in light exposure during the normal subjective night, whereas others result in a mismatch between internal circadian time and external environmental time, requiring a constant phase adjustment. It has been suggested that this mismatch may be the basis of the negative consequences of circadian disruption[27]. Evidence for this comes from studies in which animals show impaired health under non-24 h environmental conditions[27]. Perhaps the best evidence comes from studies on longevity, where wild-type mice show reduced lifespan under non-24 h LD cycles[180], and tau mutant hamsters show impaired longevity under 24 h conditions, but normal longevity in constant darkness[181, 182]. These studies indicate that a circadian clock is only beneficial if its period matches that of the environment. An alternative hypothesis for the adverse effects of circadian disruption is that such conditions result in internal desynchrony, where circadian clocks in different tissues (or even different brain regions) may become misaligned or even arrhythmic, resulting in impaired performance[183]. Internal desynchrony has been described as a result of scheduled feeding in mice, resulting in desynchrony between clocks in the SCN and

hippocampus and impaired learning and memory [184], as well as within neuronal subpopulations of the SCN in rats housed under 22 h T cycles, resulting in depression-like behavior [185]. The mismatch between internal and external time and internal desynchrony are not mutually exclusive hypotheses, and may both contribute to the negative effects of circadian disruption. Abnormal LD cycle protocols used to study circadian disruption include constant light (LL), jet-lag, T cycles, dim light at night and disruptive phase shifts (DPSs). The effects of these protocols are summarised in table 1.1 and described in detail below with regard to their known effects on circadian physiology, sleep, and arousal.

1.5.1 Constant Light

Constant conditions are frequently used in circadian research to study free-running circadian rhythms. While constant darkness allows animals to organize their behavior exclusively according to their internal circadian clock, constant light provides constant input to the circadian system and has been used to study circadian disruption.

Circadian

Constant light increase the period of nocturnal animals, based on the intensity, and can cause complete arrhythmia [186, 187]. Both the molecular and electrophysiological timekeeping of the SCN is altered. Long-term constant light exposure leads to constitutively higher levels of PER2 [188], and clock gene rhythms become gradually desynchronized [189]. At an electrophysiological level, as well as period lengthening, the amplitude of SCN firing is reduced and firing rate is more variable [190]. Peripheral clocks have also been shown to be affected, resulting in dampened amplitude and broadened peak phases [191], comparable with SCN lesions [192].

Sleep

Since constant light alters circadian activity, it also therefore alters distribution of sleep according to the change in period [193]. Constant light increases sleep, primarily due to increased amount of NREM and REM sleep with a subsequent

decrease in wake[194, 195]. Conflicting evidence also reports increased NREM sleep but decreased REM sleep[196, 197], while another study reported decreased sleep, REM and NREM sleep[198]. Differences in lighting may explain the different effects of constant light as the different spectra provide different stimulation of rods, cones, and melanopsin. Fishman et al[198] used incandescent lighting, which is heavily red shifted, but has power across many frequencies, while Mitler et al[196] used fluorescent lighting which has a few peaks but reduced power at most wavelengths, and finally Borbely et al[194], used neon light, which has very little power at the blue end of the spectrum. Other papers do not report the type of light used so comparison is limited. Overall it appears that constant light increases NREM sleep, at the expense of wake, but the picture for REM sleep is less clear.

Arousal

Constant light effects on arousal are not clear. It appears to alter the circulating glucocorticoid levels. Studies providing three weeks or less show reduced corticosterone[190, 199, 200], while those using more than three weeks report increased corticosterone[201–203]. One study reported no change in faecal corticosterone after two weeks[204]. Corticosterone is secreted in a pulsatile manner[205], which is maintained under constant light[165], and this may complicate the results of blood sampling for corticosterone. Constant light also reduces activity and body temperature[206]. Overall it is not clear what this indicates about arousal under constant light. It is likely altered in some way, even if just due to the changed circadian period.

1.5.2 Jet Lag

Shifting the LD cycle under which animals are housed has been used to mimic the sudden shift in time-zones produced by jet-lag. Both acute, with a single shift in the LD cycle, and chronic, with repeated shifts of the LD cycle, have been used to model circadian disruption.

Circadian

In response to acute jet-lag, rodents typically shift their activity over several days to re-entrain to the new LD cycle. Usually this involves an advance of the LD cycle so that activity onset can be easily determined. Delaying the LD cycle results in suppression of activity by light (negative masking), making the activity onset more difficult to determine. In response to a 6 h advance of the LD cycle, mice shift their activity by 1 h per day, typically taking 5–6 days to re-entrain. However, patterns of gene expression in the SCN may change more rapidly [72]. Peripheral clocks shift at different rates, potentially leading to a differing phase relationship with the SCN while they re-align [72, 207]. Chronic jet-lag results in the animal having to repeatedly re-entrain to the shifting LD cycle, and will likely alter the relationship between the SCN and other peripheral circadian oscillators.

Sleep

Acute jet-lag has been reported to result in no change in total sleep time, but mild changes to the distribution of sleep [20]. However, under chronic jet-lag, sleep is both fragmented and reduced by $\tilde{1}0\%$ per week compared with baseline conditions. While no increase in slow-wave activity was reported following chronic jet-lag, an increase in REM sleep and brief arousals was described [208, 209].

Arousal

The stress axis is markedly affected by acute jet-lag, enhancing the magnitude of the stress-evoked corticosterone response [20]. With chronic jet-lag, baseline corticosterone as well as anxiety and depression-like behaviors are unaffected [209].

1.5.3 T-Cycles

The circadian clock is not exactly 24 hours and adjusts every day to the environmental LD cycle. This entrainment process can be challenged using LD cycles whose length differs from 24 h – termed “T cycles”. A range of different T cycles

have been used, where T refers to the day length. For example, an 11 h light, 11 h dark LD cycle is referred to as T22.

Circadian

The process of entrainment is limited and can only occur over a relatively narrow range—typically 23–25 h in mammals. Within this range, under short T cycles, the animal has to accelerate its internal clock; while under long T cycles, the internal clock must be decelerated. As a result, the phasing of activity relative to the LD cycle also changes[210]. Outside the range of entrainment animals will show a non-entrained period of 24 h despite the prevailing LD cycle. In addition to the non-entrained circadian period, activity tends to also show a period corresponding to that of the LD cycle. Rhythms in different aspects of physiology and behavior may correspond to the period of the LD cycle or the $\tilde{2}4$ h period[211]. T cycles have been used to study the negative consequences of circadian disruption on healthy physiology in humans, including effects on cognitive performance[24, 212]. However, T cycle studies performed in nocturnal rodents often differ from forced desynchrony in human subjects as humans are assigned activities for the relevant time (e.g. time in bed), while rodent studies solely use the LD cycle. Animals are therefore periodically exposed to relatively bright stimuli. This results in repeated phase shifting, and as the phase response curve of nocturnal rodents largely results in delays, the non-entrained period this produces is slightly longer than the normal free-running period[213, 214].

Peripheral clocks likely respond differently to the SCN and activity. Within the range of entrainment, they alter their phase relationship with the environment[210], however outside the range of entrainment it is unknown what the effect on peripheral clocks are. They may either follow the SCN, free run with their own independent period, or follow the light cycle.

Sleep

Studies on rats housed under 22 h days result in desynchrony with animals showing both 22 and >24 h rhythms in activity, sleep/wake, and NREM sleep. However,

rhythms of core body temperature and REM sleep were desynchronized and predominantly cycled with a period >24 h[141]. Studies using extreme T7 cycles report that total sleep levels and sleep distribution were unaffected[60]. However, recent studies using spectral analysis of EEG signals from mice under 20- to 22 h T cycles show that these conditions result in higher SWA during sleep, as well as changes in the power of theta and gamma frequencies during waking[215].

Arousal

It is not clear what the effect of T-cycles are on arousal, but circulating glucocorticoids are affected. Plasma corticosterone is rhythmic but elevated under T7 cycles[60], while it is normally rhythmic but has an altered phase relationship under T22[210].

1.5.4 Dim Light at Night

Due to the widespread adoption of artificial light, nocturnal light exposure is increasingly common. To study the physiological consequences of light exposure during the dark phase, protocols have been used in which animals are exposed to light during their normal dark (active) phase.

Circadian

Dim light at night reduces locomotor activity levels, with no change in daytime activity[216, 217]. Periodogram power is reduced, and rhythms of PER1 and PER2 immunoreactivity in the SCN are blunted[216]. Peripherally, the amplitude of gene expression in the liver is reduced[218]

Sleep

Dim light at night does not affect sleep in mice, with no changes in either sleep timing or SWA[219]. However, studies in rats report a decreased amplitude of daily rhythms of REM and NREM sleep as well as specific changes in the NREM EEG spectra around 16–19 Hz[220].

Arousal

Rhythms of corticosterone are blunted under dim light at night conditions[216]. Motivation appears to be lower resulting in a depression like phenotype[200, 218].

1.5.5 Disruptive Phase Shift

A final protocol that has been used to study circadian disruption in Siberian hamsters (*Phodopus sungorus*) is the use of DPSs.

Circadian

Hamsters show phase shifting responses similar to other nocturnal rodents, with light exposure during the early subjective night giving rise to phase delays in activity and light exposure during the late subjective night producing phase advances. However, when Siberian hamsters are housed under 16:8 LD cycles then exposed to a combination of two 15-min light pulses, the first advancing and a second delaying light pulse the following day, this leads to a compression of activity and long-term arrhythmicity in the majority of animals. Rhythms in activity, body temperature, and melatonin are all affected, and hamsters remain arrhythmic even when subsequently exposed to normal LD cycles[221]. Studies using a 2 h advancing light pulse followed by a 3 h phase delay in the LD cycle produced similar irreversible arrhythmia within a few days, and these effects were associated with reductions in clock gene expression in the SCN[222].

Sleep

Sleep has been studied in DPS hamsters to investigate sleep homeostasis in the absence of circadian input. In arrhythmic hamsters, the usual difference between sleep during the light and dark was no longer apparent, with high levels of daytime sleep. Arrhythmic animals also show an increase of around 1.5 h of sleep per day compared with rhythmic controls. No differences in sleep homeostasis were detected[140].

Arousal

While we were unable to find any studies on adrenal glucocorticoids under these conditions, given the effects of DPS protocols on other aspects of circadian and neuroendocrine function, it is expected that normal rhythmic glucocorticoid rhythms will be abolished.

Condition	**Circadian**	**Sleep**	**Arousal**
Constant light	↑ Internal period length (nocturnal) ↑ mPER2 expression in SCN ↓ SCN neuronal firing ↓ Amplitude in peripheral tissues Behavioural arrythmia	?	↑ or ↓ glucocorticoid (e.g. CORT) levels
Jetlag	↓ Locomotor/exploratory activity Alter phase relationships between SCN and peripheral tissues	↓ Total sleep ↑ REM sleep Fragmented sleep	↑ CORT response to aversive stimuli
Non-24h T-cycles	↑ Internal period length (nocturnal)	Desynchronise core body temperature and REM sleep ↑ Slow-wave activity (sleep) Alter θ and γ power (wake)	↑ CORT level
Dim Light at night	↓ Locomotor/exploratory activity ↓ Amplitude of activity rhythm ↓ Amplitude of mPER1/2 rhythms	↓ Amplitude in REM and NREM rhythms	↓ CORT rhythm
Disruptive Phase Shift, hamsters only	↓ Clock gene expression in SCN Arrhythmia (activity, core body temperature, melatonin)	↑ Daytime sleep	?

Table 1.1: Effects of different abnormal light–dark cycles on circadian rhythm, sleep, and arousal.

1.6 Conclusion

Light exerts direct effects on the circadian clock in the SCN, which in turn modulates other rhythmic processes throughout the body, including independent oscillators found in multiple different brain regions. The SCN clock also modulates sleep, and regulates arousal via output to the HPA axis and sympathetic nervous system. Light may also directly regulate sleep as well as arousal. Sleep and arousal are also reciprocally linked. Finally, acute and chronic changes in lighting conditions may exert different effects. It is unlikely that any single mechanism mediates the effects of light, and instead light may exert its effects on a network of interacting processes. This includes the direct effects of light on physiology and behavior as well as the modulation of circadian rhythms. Despite the widespread use of different abnormal light exposure protocols to study the effects of circadian disruption, such protocols have very different effects on circadian rhythms, sleep, and arousal, with subtly different consequences. As a result, we propose that any studies addressing the effects of light should account for their effects on circadian rhythms, sleep, and arousal if we are to properly understand the physiological basis of these effects. Together, the experiments presented in this thesis aim to investigate the complex interractions that light has upon sleep and circadian rhythms.

1.7 Aims

Advances in our understanding of the photoreceptors mediating the effects of light on physiology and behavior have provided a greater appreciation of how different systems may interact to regulate complex downstream processes. However, this highlights the need for multiple physiological systems, including circadian rhythms, sleep, and arousal, all to be considered when interpreting the effects of any intervention on such complex behavioral outcomes. Our understanding of the mechanisms by which light modulates sleep, circadian rhythms, and arousal, and how abnormal light exposure disrupts these processes, is limited. We therefore aim to investigate the effects of light on the regulation of sleep and circadian rhythms.

1.7.1 Aim 1: Compare circadian disruption induced by different abnormal light cycles

Multiple types of abnormal light cycles have been used to study the effects of circadian disruption in the literature, and their effect is assumed to be equivalent. We therefore will compare the circadian disruption induced by different commonly used light cycles. This aim is addressed in chapter 2.

1.7.2 Aim 2: Investigate the effect of abnormal light cycles on sleep

Given what we know about the tightly linked regulation of sleep and circadian rhythms, we propose to use abnormal light cycles to investigate the regulation of sleep. We will use EEG to provide us with spectral information of sleep so that we can investigate both duration and intensity of sleep, for a closer understanding of how light and circadian rhythms affect sleep. This aim is addressed in chapter 3.

1.7.3 Aim 3: Investigate the long term effects of circadian disruption

The circadian system is widespread and crucial to many aspects of physiology and behaviour. The circadian system also appears to break down during ageing. We therefore will ask what the consequences are of long term housing under abnormal light cycles, as a way to evaluate the output of this complex system. This aim is addressed in chapter 4.

2

Not all circadian disruption protocols are created equal

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2.1 Introduction

2.1.1 Circadian Rhythms

The circadian system is highly important for proper functioning of almost all organisms. Most organisms have evolved circadian rhythms in behaviour and physiology, which co-ordinate a wide range of processes[19]. Disrupting these rhythms is detrimental to fitness [25, 26]. Not only is having a circadian system important, it must also be synchronised to the environmental light dark cycle, evidence in cyanobacteria[26], mice[180], and hamsters[181] shows desynchrony with the environmental light cycle reduces fitness.

Virtually every cell in the body has its own molecular circadian clock, which are organised into a heirarchy of tissue clocks, with the paired suprachiasmatic nuclei acting as the master clock[18]. Light is the most important factor entraining the circadian system to the external environment. Light information is passed to the SCN via the retina to synchronise the circadian system to the external LD cycle[48]. The amount that light shifts the circadian clock at different times of day varies, described in the phase response curve[18].

2.1.2 Circadian disruption

Given the importance of the circadian system, many studies have investigated the effects of disrupting the circadian system using the light dark cycle, on many systems, including cognition[223], metabolism [14], and immune function[224]. However, the methods used to induce circadian disruption are highly variable. In studies that use light, a variety of different cycles are used, including repeated shifts of the light/dark cycle, by advancing or delaying the light or the dark phase (jetlag)[20], constant light[225], dim light at night[200], and T cycles with non-24 hour periods[60]. It is unknown whether the circadian disruption induced by these different light cycles is comparable. However, it is reasonable to speculate that they are different. Firstly, the different protocols will stimulate different parts of the phase response curve, and therefore will likely cumulatively differ in their effects on the circadian system.

Second, the effects on locomotor activity are qualitatively different. Constant light can cause complete arrhythmia and suppression of activity[187], while T cycles can lead to either entrained activity with altered phase relationship or free-running activity depending on the length of T cycle used[210]. It is reasonable to assume that the circadian system is differentially affected given these differences.

A major difficulty is that the term circadian disruption is not well defined[226]. It is currently used to describe a variety of different states of the circadian system that may or may not be equivalent[14, 203, 208, 227, 228]. Circadian rhythms exist at many levels, from individual cells, to tissue clocks, to whole organism rhythms. When describing circadian disruption, it is important to define the level at which you are interested, in the majority of cases the whole organism is the relevant level. Circadian disruption generally encompasses two distinct concepts, circadian misalignment, and circadian dysfunction[226, 229, 230]. Misalignment occurs when an otherwise intact rhythm occurs at an abnormal phase of the reference cycle, e.g. feeding at an unusual time of day[184]. Depending on the level being looked at, the reference cycle may be the external LD cycle, the SCN time, or the tissue clock time. The extent to which a 'pure' misalignment can occur, where a rhythm is only shifted and the resultant misalignment causes no changes to the system, is debatable. A blind animal will have no light input to the circadian system, and so will free run and be misaligned to the environment, however holding rodents under constant dark has several effects[231], which may occur in a blind animal too. By contrast, dysfunction occurs when a rhythm is functioning abnormally, e.g. arrhythmic locomotor activity in a clock gene knockout[232]. This may be due to misalignment at lower levels, for example at the organism level this may be due to internal peripheral clock misalignment, such as in jetlag. Alternatively, it may be due to a pathology in a clock system, such as a genetic knockout. At any given time point, an impaired measurement may be due to either misalignment or dysnfunction. In order to show dysfunction, impairment needs to be demonstrated across the entire cycle, however evidence of some pathology in the circadian system, e.g. internal misalignment or a genetic alteration, would be strongly supportive.

2.1.3 Measuring Circadian disruption

The circadian system is an oscillatory system composed of a heirachichal network of oscillators, with different coupling strengths between them, which together creates a rhythmic system. Oscillatory systems are defined by their phase, amplitude, and period[233]. To accurately measure the circadian system, these would need to be measured for the system as a whole, as well as each level of the system, e.g. each peripheral clock. Despite recent advances in real time *in vivo* imaging[234, 235], it is not feasible to accurately measure all clocks. Instead, a marker of the system as a whole which is easier to measure is used, commonly locomotor activity or body temperature[148, 236]. Various markers are then calculated based upon this[237].

Circadian Phase

Phase is a marker of how far through the period the system is. Circadian phase is commonly calculated as the relationship between the circadian system and the environment. Most commonly, the start of the main activity period is related to the start of the light cycle. This is easy to measure and provides quantifiable measurement of how the system is affected by perturbation, for example it is used to construct a phase response curve from the shift in phase in response to light pulses[18].

Amplitude

The amplitude is a marker of how high and how low the system goes in each cycle. The circadian amplitude is the difference between the most active and the least active part of the rhythm. This is calculated using different time periods based upon the species typical activity patterns, for example in humans the most active 10 hours and least active 5 hours are used to reduce noise, while in rodents the most and least active hour suffice.

Period

The period of the system is the the length of time before the system repeats itself. The circadian period is the duration of a 'day' under the given conditions. The endogenous circadian period is a property of the organism, and is measured under constant conditions which do not affect the clock, e.g. constant darkness. The circadian period is plastic and can entrain to a range of periods, 22-27hours in mice[210], and represents a combination of the endogenous circadian period and the effect of the environment upon the circadian system. The period can be calculated using a Fourier transform, Enright periodogram, Lomb-Scarlge periodogram, and others[238]. The maximum value of the periodogram can be used as a marker of the signal-to-noise ratio of the data, and therefore the strength of the rhythmic component of the data[237].

The relationship between locomotor activity and the underlying circadian system is complex and not fully understood. While changes in the circadian system contribute to changes in the locomotor system, e.g. arrhythmia in clock gene knockouts, the circadian system doesn't soley explain locomotor activity. For example in nocturnal mice locomotor activity can be induced during the day by feeding, separately from SCN rhythms[184]. Locomotor activity is therefore a combination of the circadian system, known short-term environmental effects such as masking, and other unknown influences. The following markers are calculated on locomotor activity, however the extent of these different contributions is unknown, so care must be taken in interpreting any changes.

Visual inspection

Double-plotted actograms are useful to present an overview of the data and highlight and changes running through the time presented, e.g. shifts in period. These provide a primarily qualitative overview of the data that requires further quantification.

Daily rhythm of activity

Over a given time period, the activity at different times of day can be calculated over all days. This can highlight specific times of day where animals are more or less active, and can be used to classify animals into different groups[236].

Intraday Variability

Intraday Variability is the ratio of the variance from time-point to time-point over the entire variance in the data, as shown in equation 2.2, and as such provides a marker of how smooth the data is[239]. It is mostly useful for detecting whether the data consits of frequent large changes.

Interday Stability

Interday Stability is the maximum periodogram value, corrected for the number of cycles, as shown in equation 2.9, allowing comparison of different length data[239]. Similarly to the periodogram it comes from, it provides a marker of the ratio of the variance of the data around the given period, to the entire variance of the data, as shown in 2.5. As such it does not directly measure the stability of the rhythm, but rather a global measure of how strong the period is.

Light phase activity

This is the amount of activity that occurs in the light phase as a percentage of activity in the dark phase. Thus it provides an insight into the relative importance of masking and circadian contributions to locomotor activity This is most useful under abnormally entrained conditions where the contribution of the circadian system is altered, e.g. under entrained T cycles. Under non-entrained conditions (e.g. constant light, very short T cycles), interpretation of this marker is difficult. Non-environmental disruptions to the circadian system can also alter this[240].

Episodes

Activity is organised into episodes, which vary throughout the day, with longer activity episodes during the day and shorter at night. Fragmented activity involves more, shorter episodes of activity and is associated with worse dementia and ageing[239, 241]. This can be measured using the number and duration of activity episodes.

Each one of these markers provides an insight into the circadian system. By combining information from several different markers we gain a greater insight into the underlying state of the system.

2.1.4 Aim

Our hypothesis is that different environmental light cycles will have different effects on activity and sleep, and this may be able to explain the different cognitive effects reported [223]. We set out to investigate the effects of different environmental light cycles on the circadian system. We housed mice under four commonly used light cycles, 6 hour light advance every 7 days (jetlag), constant light (LL), 10:10 light/dark cycle (T20), and dim light at night (DLAN). We measured locomotor activity as a marker of the circadian system. We then compared the effects of these different light cycles on commonly used markers of locomotor activity, including periodogram power, amplitude, episodes, IS, IV, and light phase activity. This allows direct comparison of the effect of these light cycles on locomotor activity, and therefore provides some insight into the state of the circadian system.

2.2 Material and Methods

2.2.1 Animals

All procedures were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986, under project licence 30/3371 and personal licence I14C9C2DE. All procedures complied with the University of Oxford Policy on the

use of animals in research. Young male C57BL/6N were obtained from Envigo (Alconbury UK), with experiments started at 10 weeks of age. A total of 24 animals were used in groups of 6. Groups of 6 were used for practical reasons as this was the maximum capacity for each chamber with PIR monitoring capabilities, though this is in line with what has previously been reported in similar experiments[60]. Animals were split into groups of 6 by the experimenter without any formal randomisation process, then singly housed with *ad libitum* food and water in light tight chambers (LTC) for the duration of the experiment, 6 animals per chamber. Lights were standard white LED at a brightness of 100-120 lux at cage bottom during the light phase. The room temperature was maintained at 22+/-2C.

2.2.2 Experimental design

Animals were held under baseline conditions (12h light: 12h dark, LD) for at least 7 days. The groups were then exposed to disrupted light cycles for at least 28 days, adjusted based upon the light cycle, for example T20 had 32 days of disrupted conditions as this aligned the lights on with the baseline lights on time. The conditions used were as follows; Jetlag, 6 hr advance of the 12:12LD cycle every 7 days, with the final 12h dark period cut short each advance; T20, 10hL:10hD; Dim Light At Night (DLAN), 12hL:12hDimlight (5 lux:100 lux); and Constant Light (LL). Animals were then returned to 12:12 LD post baseline conditions for 14 days.

2.2.3 Activity monitoring

Each animal was monitored with the COMPASS system as described in Brown et al[236]. Briefly, each cage had a passive infra-red (PIR) sensor (model: Panasonic EW AMN32111) placed 20cm above the cage floor. Every 10 seconds, the output from the PIRs in each LTC were collated by an Arduino Uno (Rev3) using a custom-written sketch, and transmitted to Raspberry Pi, which collected the data and wrote it into a csv file using a Node-Red custom flow. Each cage was fitted with a Perspex block below the food and water to ensure the animal could not escape the range of the PIR.

2.2.4 Data analysis

All analysis was written using custom python scripts available online https://github.com/A-Fisk/P2_circ_disruption, https://github.com/A-Fisk/actiPy. Initially, the data were downloaded from the raspberry Pis and cleaned using a custom written quality control pipeline to check for labelling errors and corruption. Next the data were visualised and split into separate files for each experiment, then the appropriate sections were annotated for further analysis. Finally, the data was analysed using custom written functions and periodogram functions from AstroPy https://www.astropy.org/. Figures were produced using the Matplotlib https://matplotlib.org/ and seaborn https://seaborn.pydata.org/ plotting libraries. Statistics were calculated using the pingouin package https://pingouin-stats.org/api.html. Data were analysed using mixed methods ANOVA, followed by post-hoc pairwise tukey testing as stated in results. Results are presented as mean +/- SEM unless otherwise stated.

Intraday Variability

Intraday Variability is the ratio of the first derivative squared to the variance of the entire data, as shown in equations 2.1 2.2. This provides a marker of whether the data consists of smooth changes or sudden jumps.

$$IV = \frac{\sum d_{x1}^2}{S^2} \tag{2.1}$$

$$IV = \frac{n \sum_{i=2}^{n} (x_i - x_{i-1})^2}{(n-1) \sum_{i=1}^{n} (x_i - \bar{x})^2} \tag{2.2}$$

Interdaily Stability

Periodogram Power is the amount of variance in the data that is explained by the given period. One of the most common equations for calculating this is the Enright periodogram as shown in 2.3.

$$Qp = \frac{KN \sum_{h=1}^{p} (M_h - M)^2}{\sum_{i=1}^{N} (x_i - M)^2} \tag{2.3}$$

Where p is the given period being tested, K is the number of periods in the data, N is the total number of data points, h is the hour of the period, M_h is the mean at that hour across all periods, M is the total mean of the data, and x_i is the current data point. Therefore it is a marker of the strength of the rhythm in the given data. The power of the periodogram is affected by the length of the data, with longer data, the number of cycles is higher and therefore the period will explain a larger proportion of the variance. To get around this, Interdaily Stability corrects the periodogram power for the number of data points, as shown in 2.4.

$$IS = \frac{QP}{N} \tag{2.4}$$

Conceptually this equates to the ratio of the variance of the data across the period to the total variance of the data, as shown in equations 2.5 2.6 2.7 2.8 2.9.

$$IS = \frac{S_h^2}{S^2} \tag{2.5}$$

$$S_h^2 = \frac{\sum_{h=1}^{P}(x_h - \bar{x})^2}{P} \tag{2.6}$$

$$S_=^2 \frac{\sum_{i=1}^{N}(x_i - \bar{x})^2}{N} \tag{2.7}$$

$$IS = \frac{\frac{\sum_{h=1}^{P}(x_h - \bar{x})^2}{P}}{\frac{\sum_{i=1}^{N}(x_i - \bar{x})^2}{N}} \tag{2.8}$$

$$IS = \frac{N\sum_{h=1}^{p}(M_h - M)^2}{p\sum_{i=1}^{N}(x_i - M)^2} \tag{2.9}$$

Interdaily Stability was calculated using the equation 2.9, as previously used 239.

2.3 Results

2.3.1 Overview of activity and sleep

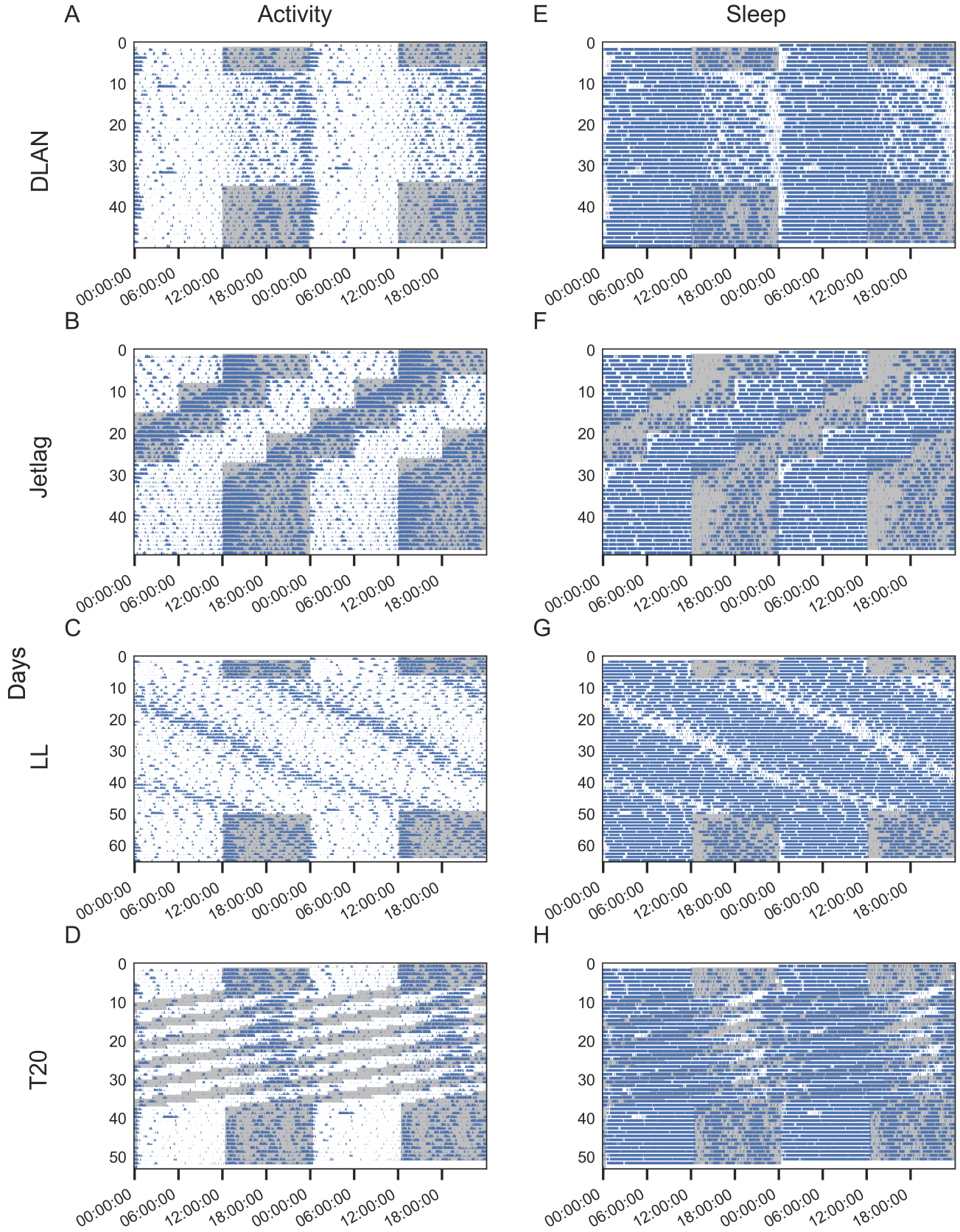
Double plotted actograms of activity and sleep
A
Activity
E
Sleep
DLAN
B
F
Jetlag
C
G
LL
D
H
T20
Days
0
10
20
30
40
50
60
00:00:00
06:00:00
12:00:00
18:00:00
Time, ZT

Figure 2.1: Double plotted actograms of activity and sleep under different light cycles. Animals were housed under baseline 12:12 LD for 7 days, before being housed under the disrupted light cycles for 28-32 days, depending on the cycle. Animals were then returned to recovery 12:12h LD for a further 14 days. A-D show activity data. A: DLAN has reduced activity and a shift of the majority of the activity to the end of the dark period. B: Jetlag gradually re-entrains in response to each shift. C: LL suppresses activity, and free runs with a long period. D: T20 free runs through the light cycle, with a long period and masking. E-H As in A-D but showing sleep data. These show similar effects to activity, with the majority of sleep occuring during the light/inactive phase of the circadian cycle.

Animals were held under 7 days of baseline 12:12 LD conditions, before being disrupted (DLAN, Jetlag, LL, T20) for 28-32 days, and then returned to 12:12 LD recovery as shown in figure2.1. In the activity data, we see the major changes expected for each protocol. DLAN shows a delay in activity from the start of the dark phase to the end of the dark phase, but otherwise normal entrainment, which persists into the recovery phase. During the disrupted section the period remains close to 24 hours (24.03hr +/-0.01). During Jetlag activity repeatedly shifts in response to the change in the light cycle, which returns to normal just before the next shift. There is a shortened period during the disrupted section (23.08hr+/-0.01). In LL, there is a reduction in activity, and a long free running period (24:53hr+/-0.06) during the disrupted section. Finally in T20, activity is not-entrained to the light cycle, and runs through the light cycles with a slightly prolonged period (24.19hr+/-0.04), however the activity rhythm shows masking in response to light/dark at different times of day. In figure2.1 E-H, we see the same data as in A-D, but processed to show behaviourally scored sleep instead of activity. We see a similar pattern in the sleep data as in the activity data, with the majority of sleep occuring in the light, and subjective light phase for free-running protocols.

2.3.2 Mean activity and sleep across the circadian cycle

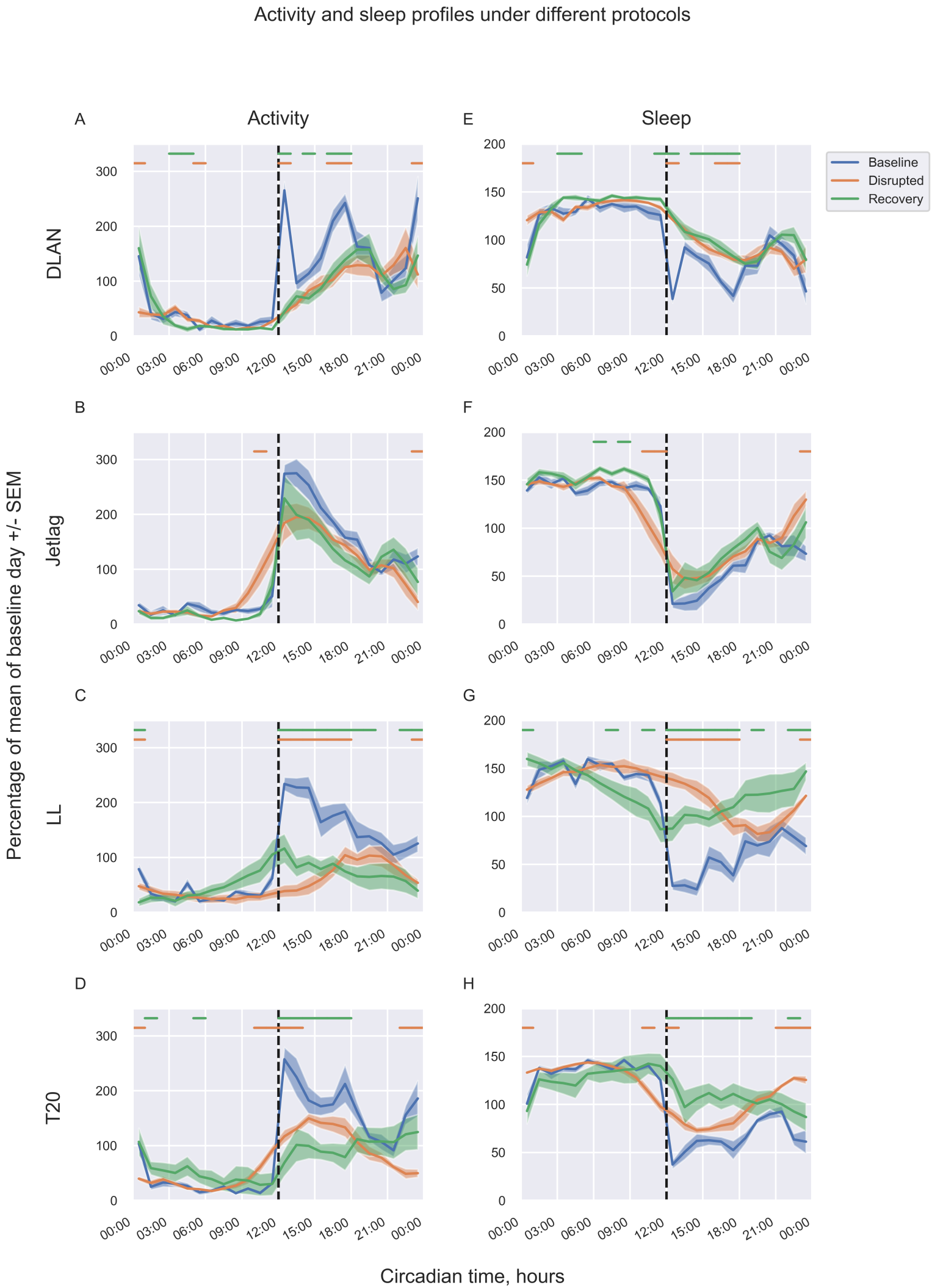
Activity and sleep profiles under different protocols
A
Activity
E
Sleep
DLAN
B
Jetlag
F
C
LL
G
D
T20
H
Baseline
Disrupted
Recovery
Percentage of mean of baseline day +/- SEM
Circadian time, hours
00:00
03:00
06:00
09:00
12:00
15:00
18:00
21:00
00:00

Figure 2.2: Activity and sleep under different light protocols. We show the profile of activity (A-D), and sleep (E-H) across the circadian day for baseline (blue), disruption (orange), and recovery (green) for DLAN, Jetlag, LL , and T20. Times where tukeys post-hoc testing showed differences with $p<0.05$ from baseline are shown at the top of each plot, orange for disrupted, green for recovery. All values are expressed as a within subjects percentage of the mean activity across the baseline period for that animal. Lines indicate mean value for each hour, shaded regions indicate +/-SEM.

Next, we calculated activity and sleep at each circadian hour as seen in figure2.2. We compared the effect of Time(hour of day), Protocol, and Section, with a three-way ANOVA on activity and sleep independently. For both activity and sleep, the effect of the Time differed between Protocols (Three way ANOVA interaction term: Activity $F(138, 1440)=2.32$, $p=2.39e-14$, Sleep: $F(138, 1440)=3.27$, $p=7.57e-29$). DLAN showed an interaction of Section x Hour of day on both activity and sleep (Two-way ANOVA: Activity $F(46, 360)=5.07$, $p=1.95e-19$, Sleep $F(46, 360)=4.18$, $p=5.06e-15$). The main change is during the first half of the dark phase, where activity is reduced and sleep increased, across both disrupted and recovery sections. Jetlag showed a weaker interaction of Section x Hour of day on both activity and sleep (Two-way ANOVA: Activity $F(46, 360)=1.83$ $p=0.001$, Sleep $F(46, 360)=2.31$, $p=1.02e-5$). In activity, only the disrupted section was affected, and the only differences were at the end of both the subjective light and dark phases. This is likely to the gradual re-entrainment altering the light exposure at these times of day. In sleep, the disrupted section changes were similar to activity, though there were some changes in recovrey in the middle of the subjective light phase. LL shows an interaction of Section x Hour of day on both activity and sleep (Two-way ANOVA: Activity $F(46, 360)=6.49$, $p=2.83e-26$, Sleep $F(46, 360)=6.88$, $p=4.51e-28$). There is a general reduction of activity during both disrupted and recovery sections, due to elimination of the main activity peak at the start of the subjective dark phase. The main peak of activity during disruption is shifted to the second half of the subjective dark phase. For sleep, the changes are similar, with increased sleep primarily at the start of the subjective dark phase during both disruption and recovery. There are some changes during the subjective light phase during

recovery too. T20 shows an interaction of Time period x Hour of day on both activity and sleep (Two-way ANOVA: Activity F(46, 360)=4.79, p=4.62e-18, Sleep F(46, 360)=4.96, p=6.43e-19). In activity, once again the main peak of activity at the start of the subjective dark phase is affected during disruption. During recovery, the activity rhythm appears heavily flattened and reduced activity during the subjective dark phase. In sleep, similar changes occur, with increased sleep during the subjective dark phase during disruption, and increased sleep throughout the subjetive dark phase for the recovery time period.

Together, DLAN, LL, and T20 reduce activity and increase sleep during the first half of the subjective dark phase.

2.3.3 Markers of circadian locomotor activity

We calculated commonly used measures of circadian disruption. The data are shown in figure 2.3 and summarised in table 2.1.

Intraday Variability

Intradaily Variability of activity is differently affected by the different light protocols (Two-way ANOVA interaction: Activity F(6, 40)=3.29, p=0.01), likely due to an increase in IV in LL during disruption (Tukeys post-hoc p=0.056). However when calculated on sleep we do not see evidence of the protocols having different effects (Two-way ANOVA interaction: Sleep F(6, 40)=1.87, p=0.11).

Periodogram power

We used the LombScargle periodogram to calculated periodogram power. We find evidence that the protocols have different effects when periodogram power is calculated both on activity and sleep (Two way ANOVA interaction: Activity F (6, 40)=2.48, p=0.039, Sleep F(6, 40)=2.92, p=0.018). These are mainly due to LL being reduced in the disrupted (activity p=0.001, sleep p=0.001), and recovery (activity p=0.001, sleep p=0.001) sections. T20 also shows reduction in periodogram power of sleep only in the disrupted section (p=0.027).

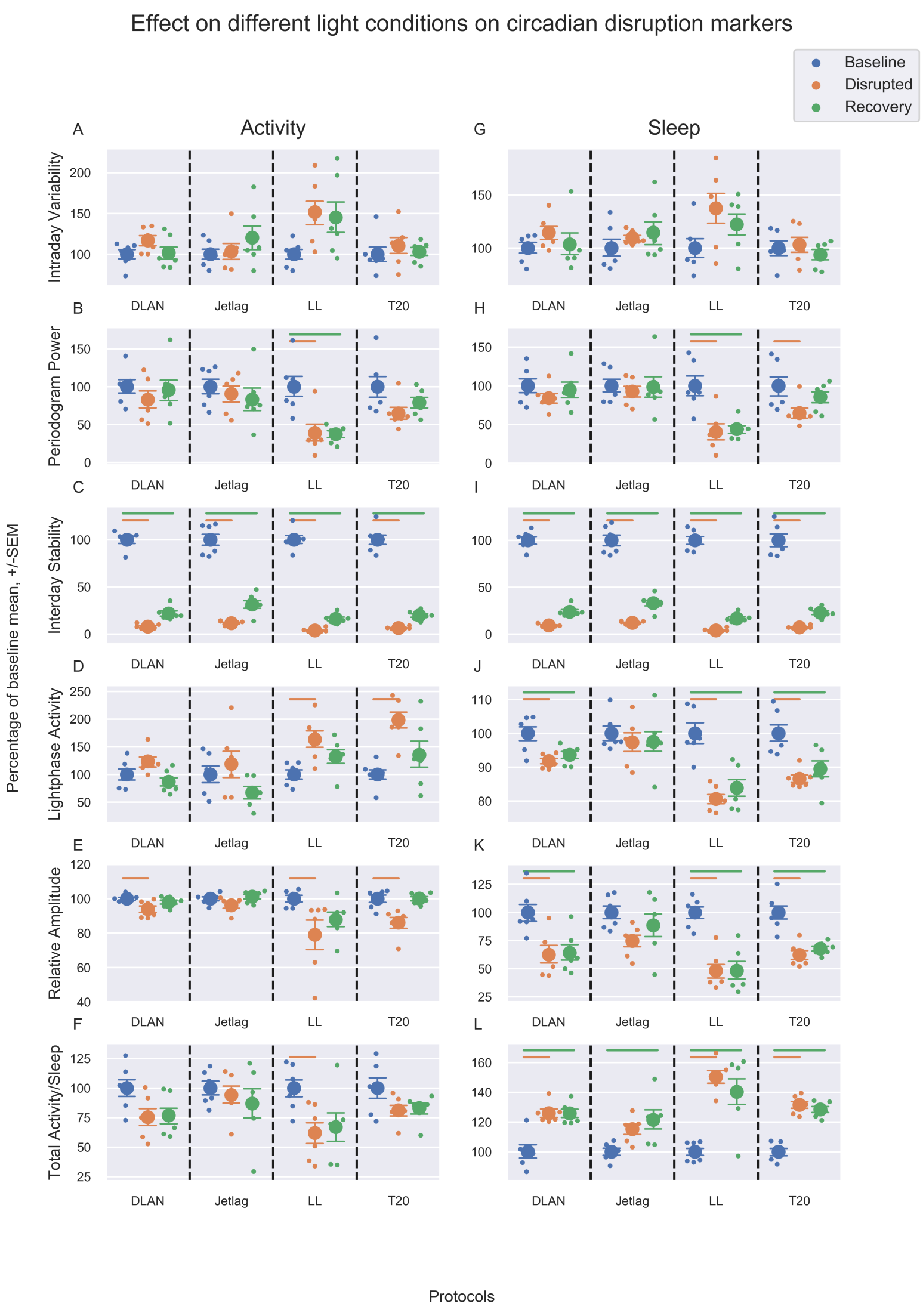
Effect on different light conditions on circadian disruption markers
Baseline
Disrupted
Recovery
Activity
Sleep
A
G
B
H
C
I
D
J
E
K
F
L
Intraday Variability
Periodogram Power
Interday Stability
Lightphase Activity
Relative Amplitude
Total Activity/Sleep
DLAN
Jetlag
LL
T20
Percentage of baseline mean, +/-SEM
Protocols

Figure 2.3: The effect of different light conditions on markers of circadian disruption. A-F show the markers calculated on activity data, G-L show markers calculated on sleep data. All markers are normalised to the group mean of the baseline section, data are presented as mean +/- SEM, with individualdata points shown too. Differences of $p<0.05$ on tukeys post-hoc testing from baseline are indicated with a line above the data, disruption in orange, and recovery in green.

Interdaily Stability

We find Interdaily Stability is reduced in the disrupted and recovery sections for all protocols similarly (Two-way ANOVA: Activity $F(2, 40)=917.18$, $p=3.84e-34$, Sleep $F(2, 40)=881.14$, $p=8.41e-34$). There are no interaction effects ($p>0.05$), indicating that we do not find evidence for the protocols affecting IS differently. All protocols show reduced IS in the disruption and recovery time periods ($p<0.001$ all).

Lightphase Activity

Lightphase activity is calculated as the amount of activity in the light phase as a percentage of activity in the dark phase. However, for LL there is no dark phase, so this marker is meaningless in this form. For the free-running conditions (LL and T20), instead we calculated this as the activity during the subjective light phase as a percentage of activity during the subjective dark phase. For entrained conditions, we calculated this as activity during lights on as a percentage of activity during lights off. The protocols affected the lightphase activity differently when calculated on both activity and sleep data (Two-way ANOVA interaction: Activity $F(6, 40)=4.04$, $p=0.003$, Sleep $F(6, 40)=4.46$, $p=0.002$). For activity, this was due to increases in both LL and T20 during disruption ($p=0.003$, $p=0.001$). In sleep, there is reduced lightphase sleep for DLAN, LL, and T20 during both disruption and recovery (disruption, $p=0.001$ all, recovery $p=0.01$, $p=0.001$, $p=0.004$).

Relative amplitude

We calculated relative amplitude as the difference between most and least active hours over the sum of the most and least active hours of the mean activity [237]. This indicates the difference between the most and least active part of the day. The

Activity

	disrupted				recovery			
	DLAN	Jetlag	LL	T20	DLAN	Jetlag	LL	T20
IV	–	–	–	–	–	–	–	–
Power	–	–	↓	–	–	–	↓	–
IS	↓	↓	↓	↓	↓	↓	↓	↓
Lightphase Activity	–	–	↑	↑	–	–	–	–
Relative Amplitude	↓	–	↓	↓	–	–	–	–
Total Activity	–	–	↓	–	–	–	–	–

Sleep

	disrupted				recovery			
	DLAN	Jetlag	LL	T20	DLAN	Jetlag	LL	T20
IV	–	–	–	–	–	–	–	–
Power	–	–	↓	↓	–	–	↓	–
IS	↓	↓	↓	↓	↓	↓	↓	↓
Lightphase Activity	↓	–	↓	↓	↓	–	↓	↓
Relative Amplitude	↓	–	↓	↓	↓	–	↓	↓
Total Sleep	↑	–	↑	↑	↑	↑	↑	↑

Table 2.1: Table summarising the effects of the different light protocols used on markers of the circadian system.

protocols had different effects on relative amplitude when calculated on activity, but not on sleep (Two-way ANOVA interaction: Activity F(6, 40)=3.75, p=0 .0047, Sleep F(6, 40)=2, p=0.088). Relative amplitude is decreased in the disrupted section for DLAN, LL, and T20 for activity (p=0.014, p=0.034, p=0.001). For sleep there is an effect of both Protocol and Section separately (Protocol F(3, 20)=3.29, p=0.042, Section F(2, 40)=47.41, p=2.8e-11). There is a decrease of sleep relative amplitude during disruption and recovery for DLAN, LL, and T20 (disruption:

p=0.0035, p=0.001, p=0.001, recovery: p=0.0056, p=0 .001, p=0.001, respectively).

Total activity/sleep

Total activity/sleep sums up the total amount of either per circadian day. We find evidence that the protocols have different effects on the total sleep per day, but not for activity. (Two-way ANOVA: Activity F(6, 40) =1.85, p=0.11, Sleep F(6, 40)=4.89, p=0.0008). Total activity does have a main effect of Section but not Protocol (Section F(2, 40)=24.78, p=9.99e-8, Protocol F(3, 20)=0.91, p=0.45) Post hoc testing shows that this is due to a decrease of activity for LL during disruption (p=0.001). For sleep there is an increase during disruption for DLAN, LL, and T20, (p=0.001, all) which was weaker for Jetlag (p=0.054). There is an increase during recovery for all conditions (DLAN p=0.001, Jetlag p=0 .0034, LL p=0.001, T20, p=0.001).

2.3.4 Episodes of activity and sleep

We also asked whether there was any fragmentation of activity and sleep rhythms, defined as an increased number and decreased duration of episodes. We therefore looked at the number and mean duration of episodes per circadian day for both activity and sleep, shown in figure2.4

We do not find evidence of the protocols affecting the number of activity or sleep episodes differently, however for both we find that the number of episodes is affected by Section. (Two-way ANOVA: Activity, Section F(2, 40)=9.31, p=0.0005, Interaction F(6, 40)=1.17, p=0 .34, Sleep, Section F(2, 40)=5.28, p=0.009, Interaction F(6, 40)=0.91, p=0.50). None of the post-hoc tests reached $p<0.05$ however, probably due to the wide spread of the data in each group. Visually, for activity episodes there appears to be a trend to reduced number during recovery. For sleep episodes, visually the data appears to show a trend to increased number of episodes during disruption. Given a main effect with no interaction effect on the ANOVA, and no post-hoc effects, this is an estimation of where the effect lies and must be interpreted with caution.

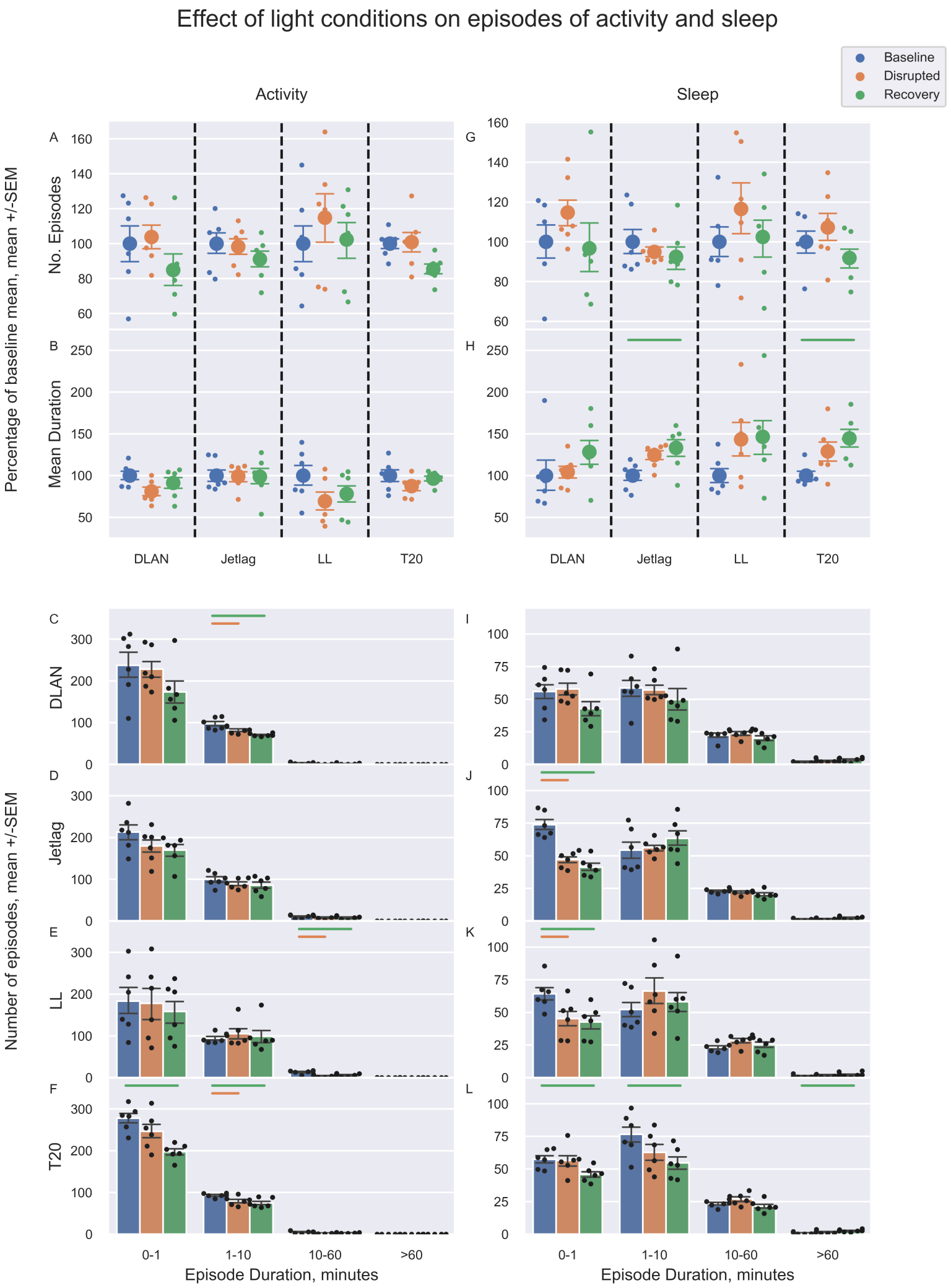
Effect of light conditions on episodes of activity and sleep
Baseline
Disrupted
Recovery
Activity
Sleep
Percentage of baseline mean, mean +/-SEM
A
No. Episodes
B
Mean Duration
G
H
DLAN
Jetlag
LL
T20
Number of episodes, mean +/-SEM
C
D
E
F
I
J
K
L
0-1
1-10
10-60
>60
Episode Duration, minutes

Figure 2.4: The effect of light conditions on episodes of activity and sleep. To examine whether any of the conditions caused fragmentation, we looked at the number and duration of episodes of sleep and activity, during baseline (blue), disruption (orange), and recovery (green). Data are normalised to the group mean during baseline, and are presented as mean +/-SEM, with individual data points highlighted. Differences from baseline on tukeys post-hoc testing are shown with a bar above the plot, orange for disruption and green for recovery. A, G. The number of episodes of activity and sleep. B, H. The mean duration of activity and sleep episodes. C-F. Histogram of activity episode duration under different protocols.

For activity episodes, we find evidence of protocols having different effects on mean duration (Two-way ANOVA interaction: $F(6, 40)=2.62$, $p=0.031$). Post-hoc testing did not provide evidence of of where the difference occured, the strongest effect was during disruption in LL ($p=0.17$). For sleep episodes however, we do not find evidence of protocols having different effects, although there is an effect of Section (Two-way ANOVA: Interaction $F(6, 40)=0.86$, $p=0.54$, Section $F(2, 40)=18.234$, p=2 .35e-6). Post-hoc testing shows an increased mean duration of sleep episodes in the recovery period for Jetlag and T20 ($p=0.01$, $p=0.0071$).

We then asked whether there was any specific duration of episodes that were affected, by examining episodes of duration 0-1, 1-10, 10-60, and >60 minutes. We treated this as post-hoc testing of mean duration, therefore performed tukeys post-hoc testing on each duration/protocol/section combination. For activity episodes, there was a decrease in 1-10 minutes episodes for DLAN for both disruption and recovery ($p=0.02$, $p=0.001$). There was no change in Jetlag activity episodes. LL showed a decrease in the number of 10-60 minute episodes for both disruption and recovery ($p=0.001$, both). T20 showed a decrease in the number of <1 minute episodes in recovery (p=0 .001), and a decrease in the number of 1-10 minute episodes in both disrupted and recovery ($p=0.038$, $p=0.0029$). For sleep episodes, there was a decrease in short sleep episodes in Jetlag in both disruption and recovery ($p=0.001$, both). In LL, there was a decrease in short sleep episodes in both disruption and recovery ($p=0.041$, $p=0.017$). In T20, there was a decrease in <1 minute episodes and 1-10 minute episodes, but an increase in episodes >60 minutes ($p=0.048$, $p=0.031$, $p=0.044$).

We therefore do not find firm evidence of fragmentation in both activity and sleep episodes. Interestingly, the mean duration of sleep episodes is increased in both Jetlag and T20, due to a reduction in short episodes.

2.4 Discussion

Here we present a comparison of the effect of several different light cycles on locomotor activity and behaviourally scored sleep. We use a variety of commonly used markers to evaluate their effects. To our knowledge, this is the first report of such a comparison.

2.4.1 Key Findings

Mean daily activity and sleep

We show that for DLAN, LL, and T20, there is a dramatic reduction in the major peak of activity at the start of the subjective dark phase. This peak of activity appears relatively intact in jetlag. A common theme between the protocols which show this reduction is that they all have some light exposure during this part of the circadian cycle, even if that is intermittent as in the case of T20. Nocturnal light exposure is well known to reduce locomotor activity and induce sleep in mice [58, 242], while exposure to darkness does the inverse. Therefore this reduction in activity in these cycles may be due to this masking effect, which can be conceptualised as either light inhibiting activity during this time period, or the usual dark exposure which promotes activity at this time not occuring. The changes seen in jetlag also support the explanation of masking due to light exposure, as the advance of the light cycle occured by turning the lights off earlier than expected, see figure 2.1, therefore during the end of the subjective day there is reduced light, and during the end of the subjective night there is increased light, which is where we see increases and reductions in activity respectively. Our behaviourally scored sleep data agrees with previous data indicating that light induces sleep [58], as we see increased sleep concurrently with reduced activity.

Markers

We show using a range of commonly used markers of locomotor activity data [237], that all the light cycles used affect one or more marker, when calculated using either locomotor activity or behaviourally scored sleep, as seen in figure 2.3 and table 2.1. Importantly, when taking all the markers as a whole, the effect of the different light cycles are not equivalent. Jetlag appears to be the least disruptive, only affecting IS, with DLAN being the next most disruptive affecting IS and relative amplitude, followed by T20 also affecting lightphase activity and finally LL affecting periodogram power as well, making it the most disruptive of the light cycles used. This is the first time such markers have been reported comprehensively for locomotor activity data under these different light cycles. The only marker we know of calculated on similar data showed that periodogram power was reduced under DLAN in siberian hamsters [216], which we did not replicate in mice. Another interesting aspect of our findings, is that when we calculated these markers on behaviourally scored sleep, the findings were not equivalent to when calculated on activity. The effects of these light cycles on sleep appeared to be greater, with DLAN having reduced lightphase sleep, and increased total sleep, while T20 had reduced periodogram power. For DLAN, this fits with previous reports indicating reduced amplitude of sleep rhythms [220], but conflicts with reports of no changing in sleep timing [219]. For Jetlag, the only change we found was decreased IS, which conflicts with previous reports of decreased sleep under chronic jetlag [208], however the previous data only found changes on specific days post-shift, while we took the amount of sleep over the entire period so may obscure differences on specific days. A previous report [243] indicates that under T20 there is no change in the amount of sleep, but changes in the spectrum, using EEG defined sleep. Using behaviourally defined sleep, we find an increase in the amount of sleep per circadian day, and an altered distribution of that sleep shown by a decreased lightphase sleep and relative amplitude. LL has been reported to increase total sleep due to increased NREM sleep [194, 195], which our data agrees with as we find an

increase in the total amount of sleep, along with altered distribution with reduced lightphase sleep and relative amplitude.

We see many of these changes persist into the recovery section. For activity data, IS is reduced under all protocols, and LL has reduced periodogram power. For sleep data, the changes reflect the changes seen during disruption, the only changes being jetlag has increased total sleep during recovery and T20 no longer has reduced periodogram power. Interpreting the changes into the recovery section is difficult and will be expanded further below.

Fragmentation

Finally we used episodes of both locomotor activity and behaviourally scored sleep to see whether the light cycles induced fragmentation in activity or sleep. Here the evidence was mixed, as we found effects of section on the number of both activity and sleep episodes, though there was no clear direction of the effect visually, as seen in figure 2.4. We found evidence that the effect of light cycles on the duration of activity episodes was not equivalent, which appeared to be driven by shorter episodes under disruption in LL. While for sleep episodes we find an effect of section driven by a trend to increased duration of episodes under disruption and recovery. When looking at different length episodes, we found reductions in shorter activity episodes for DLAN and T20, and a reduction in longer episodes for LL. For sleep, we found a reduction in shorter episodes for jetlag, LL, and T20. Together, this does not show clear evidence of fragmentation of activity episodes under any light conditions. There appears to be a trend to fragmented activity episodes during disruption in LL, but this is a trend and we found no effects on post-hoc testing, so must be interpreted with caution. For sleep, previously we have seen increases in the total amount of sleep under disruption and recovery, which fits with the increased duration of episodes under these sections. Alongside the reductions in short episodes for jetlag, LL, and T20, this shows there is no fragmentation of sleep under these conditions.

LL can induce behavioural arrhythmia under prolonged exposure[187]. We show that there is no obvious fragmentation under our conditions, and we do not see behavioural arrhythmia. We are unaware of whether arrhythmia is due to fragmentation of prolonged episodes of activity that occur during the subjective dark phase, or whether it is due to reorganisation of the otherwise normal episodes throughout the day. Altering our constant light protocol to induce behavioural arrhythmia would allow us to investigate this question.

A previous report[208] showed fragmentation of NREM and REM episodes under chronic jetlag. Our data do not agree with this, which may be due to the differences from using behaviourally scored sleep instead of EEG defined sleep.

Overall, we see changes in the distribution of locomotor activity and behaviourally defined sleep, changes on several commonly used markers, and no clear evidence of fragmentation. Importantly, the effects of these different light cycles are not equivalent.

2.4.2 Potential mechanisms

Both locomotor activity and sleep are controlled by an interaction of the circadian system, the direct effect of light, arousal, as well as other more or less understood factors, e.g. homeostatic pressure for sleep. To what extent could each of these mechanisms explain the findings we present here?

Circadian mechanisms

We know that the circadian system is important for orchestrating activity[232], but the mechanisms by which the circadian system does this are unclear[244]. We know the SCN is of crucial importance as lesions to the SCN produce arrhythmia[29], and the SCN can organise activity according to its period, ignoring the period of the rest of the body[31]. The main evidence we have for alterations in the circadian system is that we find LL has the greatest effect; LL disrupted the most markers and appears to have the largest effects, and had the largest alteration of mean daily

activity, as seen in figures 2.3 and 2.2 respectively. In contrast jetlag had minimal effect on any markers or mean daily activity. This fits with previous work indicating that LL disrupts molecular and electrophysiological rhythms in the SCN[188–190]. while jetlag appears to shift the SCN but does not impair its rhythmicity[72, 207]. LL impairs the function of the SCN, which then impairs its ability to orchestrate downstream behaviours, including activity and sleep, either via desynchronisation of peripheral clocks (including brain clocks), or directly. While other light cycles which do not impair SCN function, such as jetlag, have smaller effects.

Masking/Direct effects of light

In nocturnal rodents, light exposure can induce sleep and inhibit activity via pRGCs[58, 146, 148]. We find changes in both activity and sleep under abnormal light cycles. The main evidence we have of the direct effects of light is that in cycles where there is light exposure at the start of the subjective dark phase, DLAN, LL, and T20, the main peak of activity is not present, and in cycles where there is no light at this time, jetlag, the main peak of activity is mostly intact, as seen in figure 2.2. In addition, we see this reduced activity replaced with sleep, resulting in greater sleep over the entire day, as shown in table 2.1, suggesting that light is inducing sleep at this time. LL has previously been reported to suppress activity and increase total sleep, by removing the main peak of activity and inducing sleep instead[194, 195, 206]. T20 has been reported to cause no changes in the total amount of sleep, or the distribution while aligned to the circadian rhythm[243], but a shorter T7 cycle was unable to consistenly induce sleep in reponse to light exposure, but was able to inhibit wheel running[155]. While an even shorter T2 cycle does induce sleep but only during the first half of the subjective dark phase[156]. The difference between T7 and T2 may be due to how animals entrain to the different cycles. T2 multiplies to provides a 24 hour signal that animals can entrain to, while T7 provides either a 21 or a 28 hour day, which are outside the range of entrainment so animals free run, as seen in the actograms in [155]. This difference in entrainment under T cycles may explain the difference in light respone, however that is speculation

at this point. DLAN has been reported to impair the onset of activity at the start of the subjective dark phase previously[216], however there is no indication whether this is a circadian or direct effect of light. No firm conclusions about the contribution of the direct effects of light on increasing sleep and decreasing activity at the start of the subjective dark phase can be drawn from our data, however it is reasonable to assume that the well known effect of light on these two processes is contributing to the effects we see.

Arousal

Another major contributor to both activity and sleep is the level of arousal. Some recent previous work has attempted to model levels of arousal from home cage activity[245], however this used 2 dimensional activity and our current recordings only have 1 dimension. It is unclear whether changes in arousal contribute to the changes we see under the different light cycles here. This is because we have only recorded activity and sleep, and we do not have CORT measurements to assess the hypothalamic-pituitary axis (HPA). It is not clear how activity and sleep are affected by arousal over prolonged periods. LL has been reported to increase or decrease CORT[190, 203], jetlag does not alter baseline CORT but increases stress responses[20, 209], T7 increases CORT[60], and DLAN blunts their rhythms[216]. Since CORT can either reflect changes in arousal or stress responses to maintain levels of arousal it is not clear how these are affected by these different light cycles, furthermore they do not clearly reflect our findings. Comparative measurement of CORT under the different light cycles is required to draw firmer conclusions.

All together it is likely some combination of circadian effects, direct effects of light, changes to arousal levels, are responsible for the changes we see here. The specific mix of factors for each light cycle is not clear. Taking our data as a whole though, there does appear to be a gradient of severity. Jetlag is the least disruptive, followed by DLAN and T20, with LL being the most disruptive. This correlates well with the amount of light exposure during the subjective dark phase of each cycle.

Therefore our tentative interpretation of our data is that the crucial differentiation of different light cycles used to induce circadian disruption is the amount of light exposure during the subjective dark phase. This may be modified by the total amount of dark during a circadian cycle, and by whether an animal is able to entrain to the cycle or not. This requires further investigation. This may provide insight into a report suggesting that compression of activity due to longer photoperiods, i.e. longer light phase and shorter dark phase, impaired memory[246].

2.4.3 Implications for cognition

The implications of our findings are unfortunately not clear cut. We aimed to find an explanatory reason for differences seen in the effects of different light cycles on cognition[223]. Unfortunately our results do not provide an easy answer. Previous studies have shown that; LL impairs spatial memory, fear memory, passive avoidance[225, 247–250], interval timing[251], recognition memory[252], but not plus-maze discriminative avoidance task[253]; Jetlag impairs conditioned place preference[254, 255], a radial arm task[256], spatial memory[227, 256], and fear memory[20, 227]; DLAN impairs spatial memory in diurnal rats[257], but not in mice[218]; and finally T cycles impair passive avoidance[258], spatial memory, and recognition memory[60]. This does not fit neatly with our findings of increasing disruption of activity and sleep with increasing light at night. One reason for this may be due to the heterogeneity of tasks used to assess cognition. Therefore we will focus on two commonly used tasks for hippocampal-dependent spatial memory, the morris water maze and recognition memory[259, 260]. Using just the water maze, LL impaired learning the platform location in rats [225, 250], jetlag impaired initial and reversal learning in rats[227], T20 did not impair initial learning but impaired reversal learning in mice[14], while T7 impairs both initial learning and reversal learning in mice[60], and we could not find any reports of the effects of DLAN on water maze performance. This is difficult to explain using our data where we find a smaller effect of jetlag than of T20 and LL on activity and sleep as we would have expected to see T20 have a greater effect than jetlag. Using just object recognition

memory, LL has no effect[252], T22 also shows no effect on recognition memory[258], but T7 does impair it[60]. We could find no reports of the effect of DLAN and jetlag on recognition memory. This also does not fit with our hypothesis as we would expect LL to have a larger effect than T cycles. However there does appear to be an effect of shorter T cycles causing worse memory deficits, which agrees with our suggestion that more light exposure during the subjective dark phase is worse.

How to explain these discrepancies? First, many of these reports do not include the time of day that the test was conducted at[60]. We know that there is a daily rhythm in performance on some cognitive tasks[252, 261]. This makes it difficult to assess whether there is a global impairment, or a shift in the rhythm, and the impairment may be due to sampling at the nadir of performance instead of the peak. More thorough assessments of different times of day would help clarify this. Secondly, the different cognitive reports are from a variety of different labs. The specifics of the experimental protocol for each cognitive test can have suprisingly large effects, for example handling[262]. There may be some unreported factors that may explain the difference between these results, e.g. light levels, cleaning procedures, type of stimuli used, etc, which together make the tasks difficult to compare directly. Performing a comparison of the effects of these light cycles on cognitive tests within the same lab using the same protocols and equipment would resolve this. Next, this may also be due to convergence of different mechanisms, for example jetlag increased CORT responses but not the baseline levels[20], in contrast to other light cycles which altered baseline levels[60, 190, 216]. Given an inverted-u shaped curve of cognitive performance as a function of arousal, as shown in figure1.4, this may explain the findings, as T cycles and LL impair performance due to too high CORT levels, DLAN impairs performance due to too low CORT levels, and jetlag impairs performance due to exacerbated CORT responses which push performance into the impaired region too. Comparison of CORT baseline and responses at different times of day, and investigating the effects of CORT blocking drugs, would help resolve this. Finally, the differences we see in activity and sleep may not be relevant. This may be due to a ceiling effect where any sufficiently large change in the light

cycle, forcing the circadian system to adapt, is enough to induce a cognitive deficit, though this does not explain instances tasks where no deficits are noted. While we know local clocks can be important for specific local functions, for example olfactory clock regulating olfactory recognition memory[252], even jetlag causes a shift in the phase relationship of local clocks to the SCN[72]. This may be enough to impair performance on these cognitive tasks, and we would need simpler, or more specifically designed, tasks to detect the differences between these light cycles.

2.4.4 Strengths and Limitations

Here we show the effect of different light cycles on locomotor activity and behaviourally scored sleep. The main strength of our data is that we used the same set-up, the same facility, mouse strain, and analysis methods, for all our groups, facilitating comparison between the different cycles. Unlike previous reports, which vary by method of measurement location, and even species[216, 256, 258], our report controls these, and other unknown and unreported factors, which may influence the results, as far as is reasonably practicable. There is a question of whether standardisation is a positive goal[263, 264], in that any effect size of interest should generalise across different conditions to reduce the likelihood of finding false positives. The data we are working with is high dimensional, allowing high degrees of researcher degrees of freedom when choosing their analysis plans, which may bias the literature[265, 266]. We calculated a range of commonly used markers for this type of data[237], and analysed all different conditions similarly, allowing a stronger comparison between the different conditions. Another strength of our data is that we used immobility defined sleep[236] to assess the effects of these cycles on sleep in addition to activity. Furthermore, we found differences between the two, as seen in table 2.1. This allows some insight into the different effects on these two processes.

There are several limitations of the data we presented here. Firstly, we presented data on the effects of the light cycles both during disruption, and during a two-week recovery period. We defined the recovery period as two weeks after the end of the disruption period. This means different things for different conditions, as seen in

figure 2.1. For example, for jetlag, the recovery period started 7 days after the final shift in the lights, as that 7 days were included in the disruption section, therefore animals were fully entrained. While for LL and T20, the recovery started as soon as animals were released back into a standard 12:12 LD cycle. The animals were therefore re-entraining to the lights during part of the recovery section, and were fully entrained for the remainder. Furthermore, animals re-entrained at different rates, leading to different mixes of entrained and entraining during the recovery period. Since we corrected for internal circadian time for the majority of markers we calculated, the period we corrected for was a composite of the shifting, abnormal period, and the entrained, 24 hour period. This limits our findings for the recovery section as we cannot be sure how much of the findings are due to real effects, and how much are due to artefacts from the way we processed and lumped the data. For example, it is likely this contributes to the flat profile of mean daily activity seen in recovery under T20 in figure 2.2. To fix this problem, we would separate out the two phases, and analyse the data from the recovery section after all animals were re-entrained separately from while animals were re-entraining. We did not perform this as we did not have adequate days of data for the recovery section for all conditions. This does not affect our conclusions from the differences during disruption.

Secondly, in figure 2.2, we see marked variation in the profile of baseline activity between groups. DLAN has several major peaks of activity throughout the subjective dark phase, while jetlag has one major peak and then subsides slowly. Mice can have markedly different profiles of activity 236, and our data agrees with this high variance. To account for these differences, we normalised our data to the mean of the baseline section for each group, which removes between groups variance, and allows us to look at the effect of each condition within each group. Our sample sizes are clearly not capturing the variation in baseline activity, but does it accurately capture the response to the light conditions? Our sample sizes of 6 per group are adequate to provide 80% power to detect a 50% change with 5% alpha level. When looking at our data for the markers shown in figure 2.3, a 50% change seems a reasonable assumption across the board for the effects we are seeing. We are assumimng that

the baseline level does not influence the effects we are looking at. To investigate this we could specifically group animals based upon their baseline activity levels to see whether there are differences in their response, or we could increase our sample sizes.

We used Interdaily Stability as one of our markers, based on its previous use[237]. However, when looking at figure 2.3, the effect appears suspiciously large and homogenous across all groups. IS is calculated as the ratio of the variance explained by the period to the entire variance of the data as shown in equation 2.5. Originally, it was described as a normalisation of the value of the chi-square periodogram to the length of the data[239, 267], which should therefore allow comparison of different lengths of data. However, IS values are sensitive to the length of the data being analysed[268], therefore comparison of different lengths of data is not possible using IS. This fits with our data, where the baseline period is the shortest duration, disruption the longest, and recovery between the two, which is precisely the pattern we see in figure 2.3. It is not clear what IS provides over and above periodogram power. Furthermore, it is also not clear how IS purports to measure stability specifically. We would propose a different equation for IS, which captures the variation at each time point as opposed to the variance explained by the period, as outlined in equations 2.10 2.11 2.12.

$$IS = \frac{\sum_{h=1}^{P} \frac{S_h^2}{P}}{S^2} \tag{2.10}$$

$$IS = \frac{\sum_{h=1}^{P} \frac{\sum_{x=1}^{N} (x_i - x_h)^2}{N}}{P \frac{\sum_{i=1}^{N} (x_i - \bar{x})^2}{N}} \tag{2.11}$$

$$IS = \frac{\sum_{h=1}^{P} \sum_{x=1}^{N} (x_i - x_h)^2}{P \sum_{i=1}^{N} (x_i - \bar{x})^2} \tag{2.12}$$

Finally, we have used behaviourally scored sleep from our passive infrared sensors as our marker of sleep, namely anything over 40 seconds of immobility we scored as sleep. Given our measure of sleep is derived purely from activity, are we looking at anything other than the inverse of activity by analysing sleep effects? This is unlikely as we see differences in the effects of the light cycles on activity and

sleep, as seen in table 2.1. Furthermore, while the effects we see in figure 2.2 do appear to be closely related, this is to be expected of sleep as it is by definition an abscensce of activity. Our method of scoring sleep has previously been validated as having >95% to EEG scored sleep[236], which is within the range our lab sees in inter-rater reliability [unpublished observations]. Our method of scoring sleep relies upon a stable relationship between inactivity and sleep, such that any increase in immobility reflects an increase in sleep, as opposed to an increase in immobile wake. Under abnormal light conditions whether this assumption remains true is unknown. Previous work has shown that this assumption can be altered by genetic knockout of *retinoic acid induced-1*, a causative mutation in Smith-Magenis syndrome[269].

2.4.5 Future work

We have demonstrated the effects of different light dark cycles on locomotor activity and behaviourally scored sleep. We are limited by the type of data we have collected. Important future work is required to investigate the larger circadian system, so we can assess the comparable effects of these different conditions on central and peripheral clocks. For example looking at the expression of key clock genes across a range of important tissues under these conditions would provide useful insights, similarly to as previously done under jetlag and T22[72, 210]. It is also important to know the effects of these different conditions on CORT levels. This is complicated by the pulsatility of CORT secretion, which is maintained under abnormal light conditions[165], however useful information is still gained from less intense sampling[60]. Finally further investigation of sleep under these conditions is important. We know sleep has both a duration and an intensity component[125], and in order to understand the changes to sleep amount and distribution we see here, intensity must also be measured. This requires EEG recording from mice under these conditions.

2.4.6 Conclusion

Here we show that different light cycles commonly used to investigate circadian disruption, have different effects on locomotor activity and sleep. The literature on the cognitive effects of these different light cycles is not clear and does not easily fit with our findings. The conclusions that can be drawn from our data are limited by the fact that we have only looked at locomotor activity and behaviourally scored sleep. Further inferences as to the effect of these different light cycles requires other techniques to assess other aspects of circadian, arousal, and sleep systems.

3

The effect of constant light on sleep

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3.1 Introduction

Sleep is a reversible behavioral state of perceptual disengagement from and unresponsiveness to the environment, that appears universally required for complex organisms [84, 86], and extended sleep deprivation can eventually lead to death [93]. Sleep is regulated by both a homeostatic and a circadian system [125].

3.1.1 Homeostatic regulation

The homeostatic regulation of sleep represents a general level of sleep that is required for an organism. This expresses itself as a set-point of sleep that is required over a roughly 24 hour period. When deprived of sleep, there is a subsequent increase in sleep to compensate [129]. Importantly, sleep has both a duration and an intensity dimension [270]. The intensity of sleep is conceptualised as sleep depth, which is behaviourally expressed as an increased arousal threshold, and is well correlated with power of slow waves during NREM sleep [271], which are increased after sleep deprivation, such that despite changes in the duration and timing of sleep, the total amount of slow waves accumulated remains the same [270, 272]. The mechanisms behind this are currently unclear. A variety of putative sleep factors build up in the brain during sleep and dissipate during wake, such as levels of adenosine [129, 131], which may modulate the network of sleep/wake centres in the brain. Another potential mechanism is via changes in synaptic strength that occur during wakefulness and are re-normalised during sleep, termed the synaptic homeostasis hypothesis [90, 273]. Importantly, the accumulation of homeostatic sleep need can be affected by waking experience [274], and there is evidence that the slow wave activity (SWA) increase in response to sleep deprivation is larger in darkness than light in nocturnal rodents [275, 276], however this may be due to differences in waking behaviour rather than a direct effect of light [275]. While light can induce sleep in nocturnal rodents [58], it is unknown whether this alters its homeostatic regulation.

3.1.2 Circadian regulation

The circadian regulation of sleep promotes waking and sleep at different parts of the day. The signal primarily comes from the SCN, as lesions of the SCN lead to arrhythmic sleep[137]. What signal does the SCN provide to segregate sleeping across the day this way? This is currently unknown. The SCN is a group of about 20,000 neurons that each have their own molecular transcription based clocks, and together produce rhythms in electrical firing across the day[190, 277], though how these molecular rhythms translate to differences in neuronal firing is also currently unclear[65]. The SCN can provide a timing signal via both anatomical connections, as well as secreted factors, as a transplanted SCN isolated from anatomical connections can still orchestrate timing in the recipient[78]. Lesions of the SCN can increase the total time asleep in mice, squirrel monkeys, and Siberian hamsters[138–140], but not in rats[137]. Data in humans shows a drive for wake when sleep pressure is highest[278]. Some interpret this as the SCN providing a primarily wake-promoting signal, while others suggest the SCN promotes wake and sleep at different times of day[100, 145]. Desynchrony of different parts of the SCN under abnormal T cycles show that rhythms of REM sleep are associated with clock gene expression in the dorsomedial SCN[142]. Whether this is due to changes in firing or other factors is unknown. The SCN projects to several hypothalamic nuclei, including the sleep regulating VLPO[143, 279]. The main hypothalamic projection of the SCN is to the subparaventricular zone and dorsomedial nuclei of the hypothalamus. These are key nodes mediating the circadian control of sleep as lesions of the ventral SPZ eliminate circadian rhythms in sleep and locomotor activity[144], as do lesions of the DMH[280].

3.1.3 Direct effects of light

Light can directly induce sleep in nocturnal rodents[58], though this only occurs at certain circadian phases[156]. How light does this is currently unclear. pRGCs in the retina can regulate the direct effects of light on sleep separately from their circadian effects[148]. Although they also appear to regulate baseline levels of

homeostatic sleep pressure and response to sleep deprivation[157]. pRGCs project widely throughout the brain, but importantly project to both the VLPO and SPZ, areas important for sleep regulation[144, 151].

3.1.4 Constant light and sleep

Constant light alters sleep timing and distribution, in line with the changes in their internal period[193]. The literature on constant light is currently mixed. Some reports show increased total sleep, NREM and REM sleep, and decreased wake in rats[194, 195], others show increased NREM but decreased REM sleep in rats[197], another report shows a trend to similar findings in mice but this is not consistent across strains[196], and others show decreased total sleep, NREM and REM in rats[198]. A final study failed to show any changes in duration of NREM or REM sleep in rats, but used very low light levels[281]. Differences in the lights used for housing may explain the differences reported, with some using incandescent[198], others fluorescent[196], and others neon lights[194], which all produce different spectra, and therefore lead to different activation of melanopsin even at the same lux levels[282]. The most consistent finding is an increase in NREM sleep, whereas the effect on REM sleep is less clear. Furthermore, while these reports have all investigated the time in each state, less focus has been paid to the spectrum. Sleep has both a time and an intensity component, therefore it is crucial to investigate the effect of constant light on the spectrum of sleep.

3.1.5 Aim

Overall, light regulates sleep through a complex interaction of homeostatic, circadian, and direct effects of light, which are difficult to disentangle. Constant light causes disruption of the circadian system, see chapter 2, as well as providing light at all times of the circadian cycle. We therefore set out to examine the effects of constant light on the regulation of both sleep timing and intensity.

3.2 Methods

3.2.1 Animals

All procedures were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986, under project licence P828B64BC, and personal licence I14C9C2DE. All procedures complied with the University of Oxford Policy on the use of animals in research. Young male C57BL/6N mice were obtained from Envigo (Alconbury UK). All mice were 8 weeks old at the time of surgery. A total of 10 animals were surgically implanted with EEG/EMG recording equipment, with the aim of obtaining recordings from 8 mice, which was the limit of what could be obtained using the equipment available. Unfortunately due to technical issues, only 7 animals were recorded for the experiment. Animals were group housed until they underwent surgery, when they were singly housed in individual ventilated cages, with *ad libitum* access to food and water. 2 weeks after surgery animals were transferred to custom made individual plexiglass experimental cages (20.3 x 32 x 35 cm) where they were individually housed throughout the experiment with *ad libitum* access to food and water. Experimental cages were kept in a ventilated sound-attenuated Faraday chamber (campden instruments, 2 cages per chamber), under white LED lighting at 100–120 lux at cage bottom height while the lights were switched on. Room temperature and relative humidity were maintained at 22 +/-1C and 50+/-20% respectively.

3.2.2 Experimental design

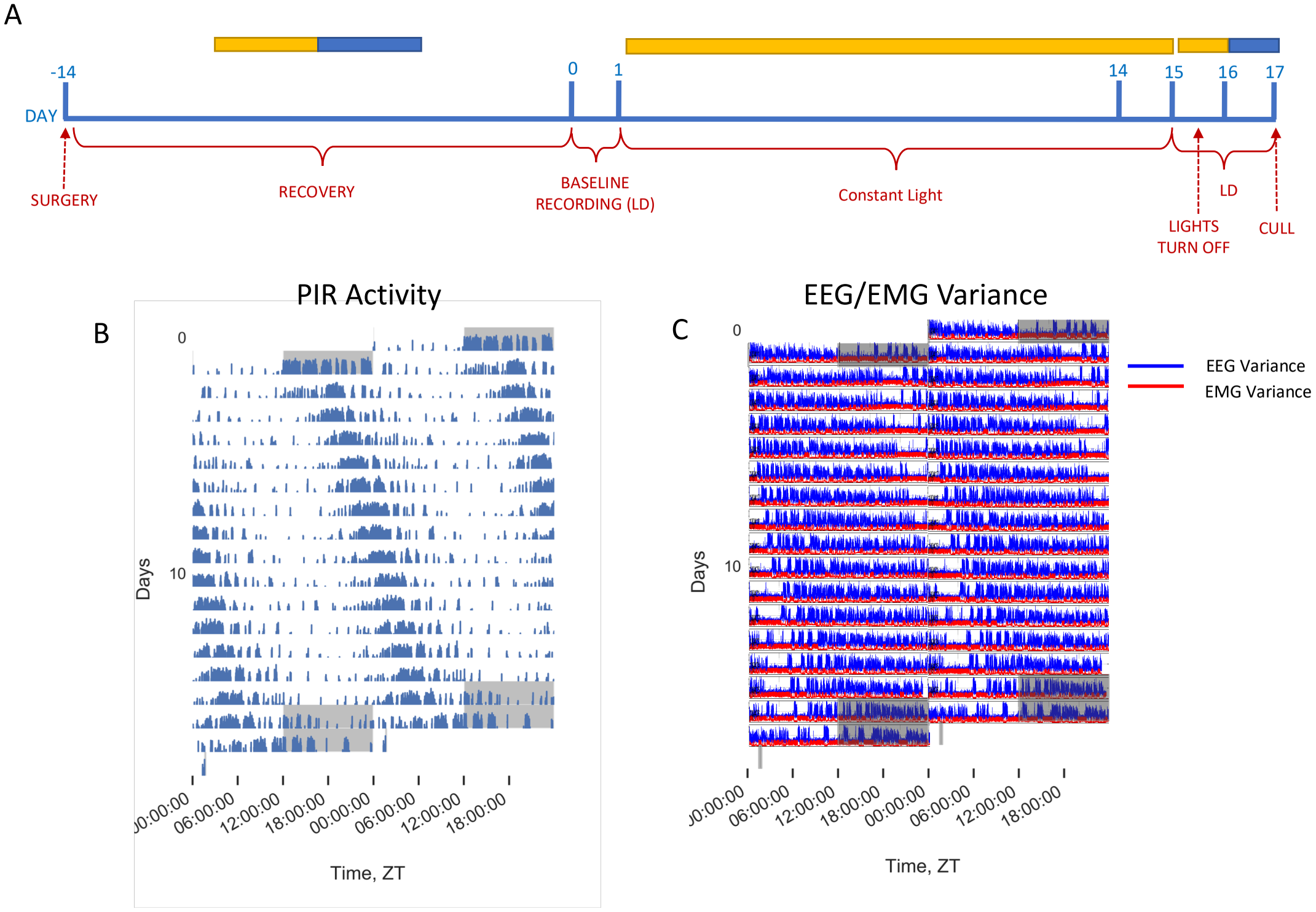
A
-14
0
1
14
15
16
17
DAY
SURGERY
RECOVERY
BASELINE
RECORDING (LD)
Constant Light
LD
LIGHTS
TURN OFF
CULL
B
PIR Activity
0
10
Days
06:00:00
12:00:00
18:00:00
00:00:00
06:00:00
12:00:00
18:00:00
Time, ZT
C
EEG/EMG Variance
0
10
Days
EEG Variance
EMG Variance
06:00:00
12:00:00
18:00:00
00:00:00
06:00:00
12:00:00
18:00:00
Time, ZT

Figure 3.1: Experimental Design of the study. A. A timeline of the experiment. We surgically implanted the EEG electrodes, before allowing the animals to recover at least 14 days in standard 12:12 conditions. We then moved the animals to the experimental chambers, and after 2 days of habituation, started baseline recordings. We then turned on the lights constantly for 14 days. We then returned the animals to 12:12 LD afterwards, by turning the lights off halfway through day 15. We recorded two days of 12:12 LD, before sacrificing the animals. B. Shows the PIR based activity for the time EEG was recorded for a single animal. Shown as a double plotted actogram where blue indicates activity, grey indicates lights off. We see a prolonged period free running in constant light as we expected, indicating that the LL worked and the animals did not entrain to any alternative zeitgebers. C. EEG/EMG variance double plotted similarly to B for a single animal. This shows a crude overview of sleep/wake behaviour, when EEG variance is high and EMG variance is low, this indicates probable sleep states, when EEG variance is low and EMG variance is high, this indicates probable wake states. Here we see that the animals also free run in their patterns on the EEG/EMG. Furthermore, the EEG variance is high during times when the PIR activity is low, indicating that the animals are likely asleep not just immobile.

The experimental design is outlined in figure 3.1. Animals underwent surgical implantation of recording devices, then were allowed to recover for 2 weeks before being transferred to recording chambers. Initially, animals were housed under a standard 12:12h LD cycle for habituation and baseline recordings. Animals were housed in the recording chambers for 2 days to habituate to the recording cables before 2 days of baseline recordings were obtained. After the baseline recordings, animals underwent 14 days of constant light (LL), before being transferred back to 2 days of 12:12 LD. All animals were culled at the end of the experiment. EEG and EMG were recorded during baseline, LL, and return to LD. The locomotor activity of the animals was also recorded using the COMPASS system[236]. Mice were checked at randomly selected times during the working day (0700h to 1600) to ensure there was no signal they could entrain to.

3.2.3 Electroencephalogram recordings

Surgical recordings were performed as previously described[274]. Surgical procedures were performed under aseptic conditions under isofluorane anaesthesia (3-5% induction, 1-2% maintenance). Before surgery, animals were provided with analgesia (meloxicam 1–2mg/kg SC, buprenorphine 0.08mg/kg SC). EEG screw

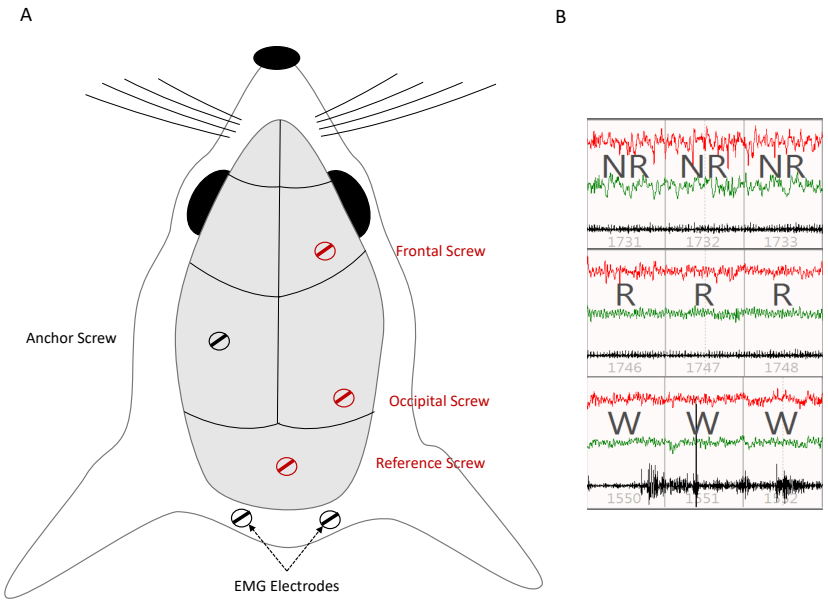

Figure 3.2: Methods used for recording EEG. A. Schematic representation of a mouse skull and electrode placement. B. Representative traces of NREM sleep, REM sleep and Wake, which were used for manual scoring of vigilance states.

electrodes were implanted unilaterally in the right frontal (anterior-posterior 2mm, mediolateral 2mm), and occipital (anteroposterior -3.5mm, mediolateral 2.5mm) cortices, coordinates given relative to bregma. A reference electrode was implanted above the cerebellum in the midline, and a ground screw was placed contralaterally to the occipital electrode. Single stranded stainless steel wires were implanted on either side of the nuchal muscle to record electromyogram. All electrodes were attached to a custom-made head-mount (Pinnacle Technology), and attached to the skull using dental acrylic. Post-surgery mice were monitored closely and provided with analgesia as necessary (meloxicam 1–2mg/kg sid for 3 days, buprenorphine 0.08mg/kg PRN), until they returned to normal.

3.2.4 Signal processing

A Tucker-Davis technologies Multichannel Neurophysiology Recording System was used for data acquisition. Cortical EEG was recorded from both the frontal and occipital derivations. EEG and EMG were filtered between 0.1Hz and 100Hz, amplified (PZ5 NeuroDigitizer preamplifier, Tucker-Davis Technologies), and stored

on a local computer at a sampling rate of 256.9 Hz. EEG and EMG were resampled offline at 256Hz, and converted into European Data Fomat using custom MATLAB scripts available at https://github.com/A-Fisk/VVlab_Scripts, and the open source Neurotraces software. Recordings were split into 4 second epochs, and vigilance states scored offline by manual inspection using SleepSign for animals http://www.sleepsign.com/. Vigilance states were classified as wake (low-voltage, high-frequency EEG, with high or phasic EMG activity), NREM (EEG slow waves with high amplitude and low frequency), or REM sleep (low-voltage, high-frequency EEG with low EMG activity), based upon visual inspection of the two EEG channels and the EMG channel. Artifacts, caused by movement in any channel, were also scored to facilitate analysis. The data were then exported as power spectra per epoch, computed by FFT using a Hanning window.

3.2.5 Data analysis

All analysis was written using custom scripts available online at https://github.com/A-Fisk/sleepPy, https://github.com/A-Fisk/03_lleeg, using a combination of MATLAB and Python. Baseline days were the last day of normal 12:12 LD, defined from the start of lights on (ZT0), then at least the first two days of LL were scored for all animals. Statistics were performed using the pingouin package https://pingouin-stats.org/api.html. Mixed ANOVAs were performed, followed by post-hoc tukey testing as stated in results.

3.3 Results

3.3.1 Overview of constant light effects on activity and sleep

We recorded EEG from 7 animals under a baseline day of 12:12 LD, followed by 14 days of continous light exposure (LL), and then a further 48 hours of LD. We also recorded PIR based activity from animals at the same time. A representative example of both is shown in figure 3.1. In the PIR activity, we see a prolonged period as the animals free run under constant light, and a reduced level of activity,

which is not rescued by the return to LD. In figure 3.1 C, we see EEG and EMG variance as a proxy marker of sleep and wake. When EEG variance is high and EMG variance is low, the animal is likely to be in NREM sleep due to the presence of slow waves and behavioural immobility; when EEG variance is low and EMG variance is high, the animal is likely to be in active wake. We see a similar pattern in the variance profile, with a shorter activity period that free runs throughout the LL period. In this example at least, when the PIR activity is reduced, the EEG variance stays high, indicating that this animal is likely asleep instead of awake but inactive.

3.3.2 Constant light increases sleep

We next looked in more detail at the sleep by manually scoring the baseline day and the first two days of LL for vigilance states. Time constraints prevented us from scoring further days under LL. To see an overview of the effect on sleep distribution and intensity, we plooted a hypnogram of SWA over the first two days of LL, coloured by vigilance state, an example of which is shown in figure 3.3 A, B, and C. In the baseline day we see the expected reduction in SWA during the light phase as the animal is asleep, followed by long activity episodes once the lights turn off, and increased SWA in the subsequent sleep episodes. In both B and C, we see a similar pattern in the subjective light phase, with reducing SWA during NREM sleep in the subjective light phase, however when the lights fail to turn off the animals then remain intermittently asleep for the next 6 hours, before having a major activity episode followed by increased SWA.

To quantify this increase in sleep, we calculated the time spent in each state, sleep, NREM sleep, REM sleep, and Wake, across each 24 hours, as well as during the subjective light and dark phases, shown in figure 3.3 D, E, F, G. We asked whether the amount of time in each stage was affected by the day using a repeated measure ANOVA, followed by pairwise post-hoc tukey testing, for the total 24 hours, subjective light and subjective dark phase.

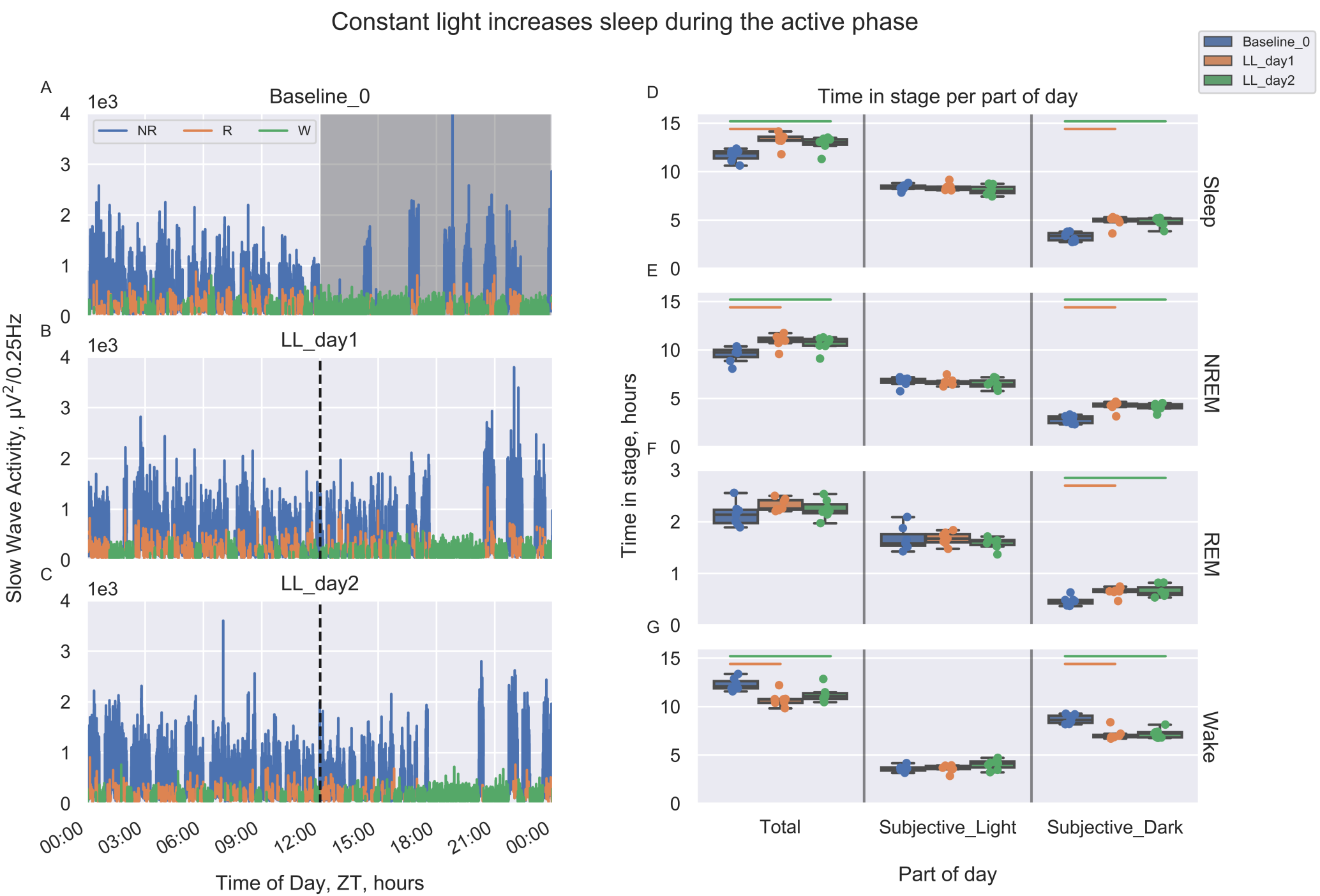
Constant light increases sleep during the active phase
A
Baseline_0
1e3
NR
R
W
B
LL_day1
C
LL_day2
Slow Wave Activity, μV²/0.25Hz
00:00
03:00
06:00
09:00
12:00
15:00
18:00
21:00
00:00
Time of Day, ZT, hours
D
Time in stage per part of day
Baseline_0
LL_day1
LL_day2
Sleep
NREM
REM
Wake
Time in stage, hours
Total
Subjective_Light
Subjective_Dark
Part of day

Figure 3.3: Hypnograms and time in different stages of sleep in the first two days in LL. A, B, C. Hypnograms showing slow wave activity for the Baseline, Day 1, and day 2 in LL respectively, colour coded for vigilance states NREM (blue), REM (orange), wake (green). Grey indicates the dark phase, and the dashed line indicates the start of the subjective dark period. D, E, F, G. Total amount of Sleep, NREM, REM, and wake respectively, split into total for 24 hours, and the subjective light and subjective dark phases. Differences of $p<0.05$ from the baseline day are indicated with a line above the plot, orange for day 1, green for day 2.

For total sleep, we find an increase on day 1 and day 2 of 1.57 and 1.19 hours respectively (ANOVA, $F(2, 12)=16.418$, $p=0.00037$, post-hoc tukey difference from baseline $p<0.05$ both). In the subjective light phase, we do not see a difference between the days (ANOVA, $F(2, 12)=3.55$, $p=0.06$), however in the subjective dark phase, there is a difference, with day 1 and day 2 increased by 1.57 and 1.48 hours respectivly (ANOVA, $F(2, 12)=26.31$, p=4.1e-5, post-hoc tukey, $p<0.05$ both).

For NREM sleep we see a similar pattern, with increased across 24 hours of 1 .39 and 1.09 hours for day 1 and 2 respectively (ANOVA, $F(2, 12)=11.02$, p=0 .002, post-hoc $p<0.05$ both), no change during the subjective light (ANOVA, F (2, $12)=1.27$, $p=0.32$), and an increase of 1.39 and 1.29 for day 1 and day 2 in the subjective dark phase (ANOVA, $F(2, 12)=26.15$, p=4.22e-5, post-hoc p<0 .05 both).

When looking just at REM sleep, we do not detect an increase across 24 hours (ANOVA, $F(2, 12)=3.57$, $p=0.061$), which is also true for the subjective light phase (ANOVA, $F(2, 12)=1.04$, $p=0.38$), however we do see an increase of 0.18 and 0.19 hours on day 1 and day 2 when looking just at the subjective dark phase (ANOVA, $F(2, 12)=8.74$, $p=0.0046$, post-hoc $p<0.05$ both).

Finally we looked at total time spent awake, which as expected, shows the inverse pattern to total sleep time, with decreases of 1.57 and 1.05 hours across the whole 24 hours (ANOVA, $F(2, 12)=14.84$, $p=0.00057$, post-hoc $p<0.05$ both). We do detect a difference in the subjective light period (ANOVA, F(2, 12)=6 .99, $p=0.0097$), but post-hoc testing failed to detect differences between the days ($p>0.05$ both days). In the subjective dark period, we see decreases of 1.58 and 1.47 hours on day 1 and 2 respectively (ANOVA, $F(2, 12)=26.26$, p=4.14e-5, post-hoc $p<0.05$ both).

We therefore find increases in total sleep, NREM sleep, and REM sleep, and a decrease in time spent awake in response short term exposure to constant light, mainly localised to the subjective dark period when the light exposure is abnormal.

3.3.3 Increased sleep is regulated homeostatically

We have so far shown that constant light induces sleep in the first two days of exposure. Next we asked how this sleep is regulated. Sleep is regulated in a circadian and a homeostatic manner, the circadian system leads to enhanced tendency to sleep at certain times of day, and the homeostatic regulation leads to a build up of sleep pressure which is dissipated by sleep. Homeostatic regulation incorporates two aspects of sleep, sleep time and sleep intensity. Sleep time is easily measured by scoring of the EEG, sleep intensity is measured by the power in the delta frequency band (0.5-4Hz), termed slow wave activity as this is mainly compromised of the characteristic slow waves seen in NREM sleep.

We therefore asked about the sleep timing and sleep intensity. Figure 3.4 A looks at total sleep duration. We asked whether the percentage of each hour spent asleep across each hour, was different for each day, using a two-way repeated measures ANOVA of sleep % h̃our*day, taking into account each animal, with pairwise post-hoc tukey testing for each hour. We find that there is a difference between the days and hours (ANOVA, interaction $F(48, 288)=5.51$, $p=9.71e-21$). Post-hoc testing shows that this is due to day 1 differing from baseline at hours 12-17, and day 2 at hours 11-17. Day 1 had isolated differences at hours 2 and 22, while day 2 was different at hour 7 (post-hoc tukey $p<0.05$ all). The majority of the difference therefore occurs at the transition to the subjective dark phase.

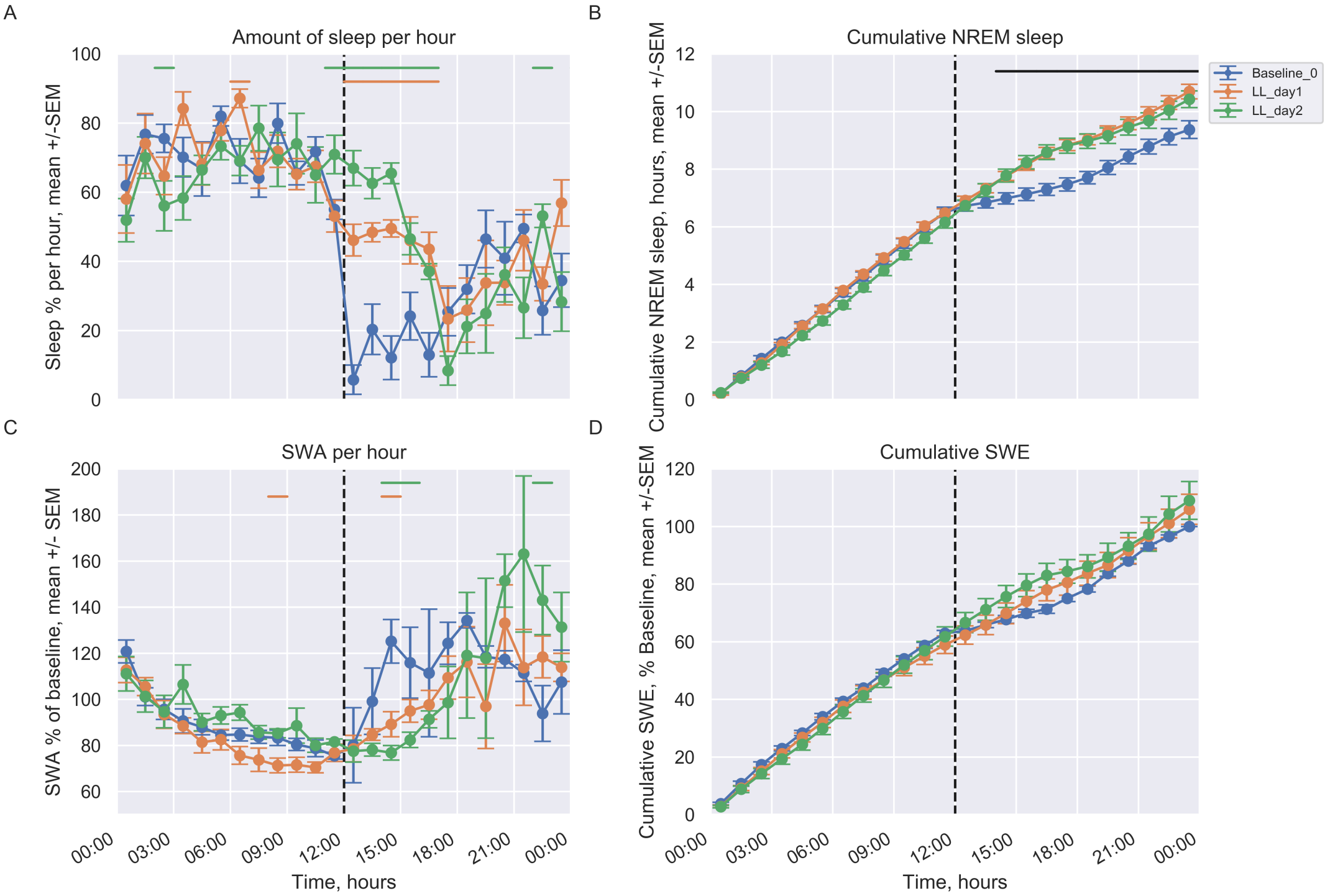
Constant light increases sleep
A
Amount of sleep per hour
Sleep % per hour, mean +/-SEM
B
Cumulative NREM sleep
Cumulative NREM sleep, hours, mean +/-SEM
Baseline_0
LL_day1
LL_day2
C
SWA per hour
SWA % of baseline, mean +/- SEM
D
Cumulative SWE
Cumulative SWE, % Baseline, mean +/-SEM
00:00
03:00
06:00
09:00
12:00
15:00
18:00
21:00
00:00
Time, hours

Figure 3.4: Constant light increases sleep and slow wave energy in the first 5 hours of the subjective dark phase. Baseline day in blue, day one in orange, day 2 in green. Lines above indicate post-hoc testing differences where $p<0.05$ from baseline for day 1, orange, and day 2, green, unless otherwise stated.
A. Total sleep per hour. B. Cumulative NREM sleep, black line above indicates where day 1 and day 2 are different from baseline $p<0.05$. C. SWA per hour. D. Cumulative SWE.

Given that sleep pressure is dissipated during NREM sleep, we therefore plotted the cumulative NREM sleep time over each day, as shown in figure 3.4 B. We have already shown in figure 3.3 an increase in NREM sleep over 24 hours, and we see that here too as the total NREM sleep is increased. However, when we look at the timecourse, we see that the animals continue to gain NREM sleep at the start of the subjective dark period, and continue to do so throughout the subjective dark to lead to the higher total amount of sleep. This leads to differences from baseline for both day 1 and day 2 at hours 14-23 (pairwise post-hoc tukey $p<0.05$ all).

We next looked at the intensity of sleep, by looking at relative SWA. The timecourse of SWA is shown in figure 3.4 C. We calculated the mean SWA during NREM as a % of the mean SWA for the baseline day for each animal, excluding any hours which had <5 minutes of NREM sleep. On the baseline day, we see the expected pattern of a decrease during the light phase as animals sleep and dissipate sleep pressure, with a rise at the start of the dark phase, which is maintained during the dark phase as animals are awake and accumulating sleep pressure. On day 1 and day 2, we also see a normal decrease in SWA during the subjective light phase, however at the start of the subjective dark phase the SWA remains low, increasing on day 1 faster than on day 2, then increasing once animals wake up and start accumulating sleep pressure again. We then asked whether the SWA was different for each hour and day, using a two-way ANOVA followed by pairwise tukey post-hoc testing. We were unable to use a repeated measures ANOVA, as removing hours with <5 minutes of NREM sleep led to missing values. We find a difference between the days and hours (ANOVA, interaction $F(2, 48) = 1.82$, $p=0.0013$), indicating the time-course of SWA is not the same for each day. Post-hoc testing showed that on day 1 there are differences from baseline at hour 8, and 14, and on day 2

there are differences at hour 14, 15, and 22 (post-hoc tukey $p<0.05$ all). The large error bars indicate high variance at each time point, but the consistent finding is that at the start of the subjective dark phase, while animals are still asleep under LL, their SWA is lower than during the baseline day.

Finally we asked whether the SWA was regulated homeostatically. If it is homeostatically regulated then there is a set amount of slow waves it should get across the 24 hours, and despite increases in the duration of NREM sleep the decreased intensity of that sleep means they return to the same set point. We therefore plotted the total SWA during NREM sleep accumulated over the day, termed slow wave energy (SWE), as shown in figure 3.4D, as a percentage of the maximum value each animal reached at the end of the baseline day. We asked whether the total amount of SWE the animals experienced across 24 hours, was altered by the days, using a repeated measures one-way ANOVA on the maximum values for each day. We find that there is no difference between the maximum SWE across the days (ANOVA, $F(2, 10)=1.77$, $p=0.22$). When looking at the cumulative graph of SWE in figure 3.4 D, we see that the intensity changes compensated for the increased duration of NREM sleep during the subjective dark phase.

We therefore find that constant light induces sleep in the first 5 hours of the subjective dark phase, but there is no alteration to the homeostatic regulation of sleep across 24 hours.

3.3.4 Architecture of light induced sleep

Finally, we asked whether we could detect any changes in the sleep that was induced between hours 12-17.

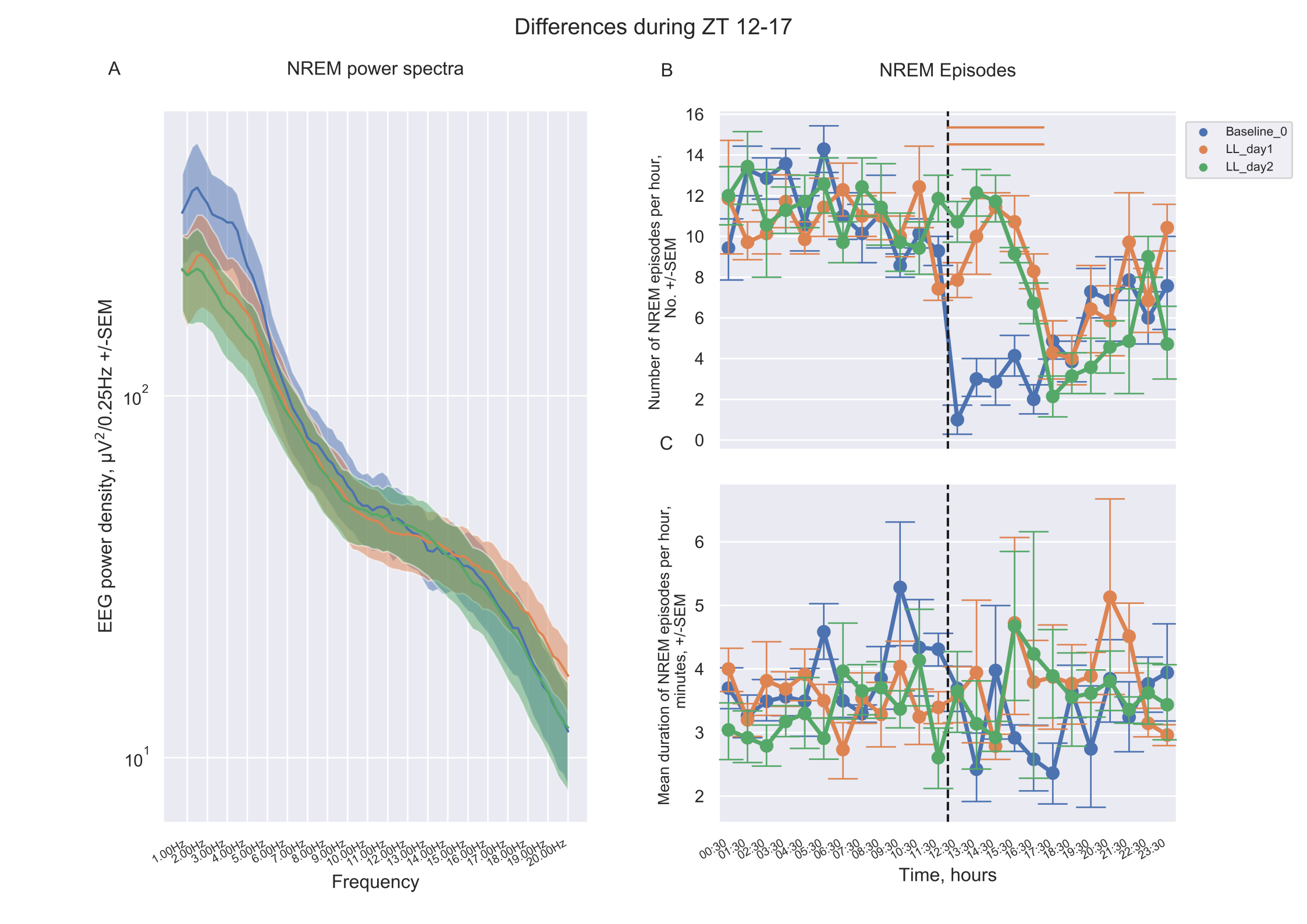
Differences during ZT 12-17
A
NREM power spectra
EEG power density, μV²/0.25Hz +/-SEM
10²
10¹
1.00Hz
2.00Hz
3.00Hz
4.00Hz
5.00Hz
6.00Hz
7.00Hz
8.00Hz
9.00Hz
10.00Hz
11.00Hz
12.00Hz
13.00Hz
14.00Hz
15.00Hz
16.00Hz
17.00Hz
18.00Hz
19.00Hz
20.00Hz
Frequency
B
NREM Episodes
Number of NREM episodes per hour, No. +/-SEM
0
2
4
6
8
10
12
14
16
Baseline_0
LL_day1
LL_day2
C
Mean duration of NREM episodes per hour, minutes, +/-SEM
2
3
4
5
6
00:30
01:30
02:30
03:30
04:30
05:30
06:30
07:30
08:30
09:30
10:30
11:30
12:30
13:30
14:30
15:30
16:30
17:30
18:30
19:30
20:30
21:30
22:30
23:30
Time, hours

Figure 3.5: Effect of LL on NREM power spectra and architectrue of sleep. Baseline day in blue, day one in orange, day 2 in green. Lines above indicate post-hoc testing differences where $p<0.05$ from baseline for day 1, orange, and day 2, green, unless otherwise stated. A. Spectrum of NREM sleep during CT 12-17. B. Number of NREM episodes per hour. C. Mean duration of NREM episodes per hour.

First, we looked at the spectrum of NREM sleep during that time period, as shown in figure 3.5A. We used a two-way repeated measures ANOVA to ask if the power was affected by the day at each frequency bin. We found no difference between the different days across frequencies (ANOVA, interaction $F(160, 810)=-5.07$, $p=1$). While in figure3.4C, we found differences in relative SWA at this time, here across the whole absolute spectrum of NREM sleep during the 6 hours where sleep was induced, we do not see a difference. Referring to figure3.5A, there is a trend to decreased power in the SWA frequencies (0.5-4Hz), but we failed to see an effect here, likely due to no change in the remaining frequencies.

Next we looked to see if the sleep was fragmented, by looking at the number and duration of NREM episodes, as shown in figure 3.5 B and C respectively. We asked whether the number and duration was affected by day at different times using a two-way repeated measures ANOVA, with pairwise post-hoc tukey testing at each time. We find that the number of NREM episodes is affected (ANOVA, interaction $F(46, 276)=2.84$, $p=7.78e-8$), on day 1 and 2, and the hours 12-16 are increased compared to baseline (post-hoc tukey $p<0.05$ all). We find that the mean duration of NREM episodes is not affected differently by day, or by hour (ANOVA, interaction $F(46, 230)=0.90$, $p=0.65$, time $F(23, 115)=0.43$, $p=0.99$).

We therefore find that light induces sleep by increasing the number of NREM episodes during the first 5 hours of the subjective dark period, but they are of similar duration to during the subjective light period, and the overall spectrum is also comparable.

3.4 Discussion

3.4.1 Key findings

Here we show that housing mice under constant light induces both NREM and REM sleep over the first two days, but that sleep is regulated homeostatically and appears similar to sleep during the subjetive light phase.

Sleep induction

We show in figure 3.3, that there is an increase of 1.5 hours of total sleep in the first two days of LL, composed of increases in both NREM and REM sleep during the subjective dark phase. This agrees with previous findings reporting increases in both NREM and REM sleep in rats[194, 195], however it contradicts findings of decreased REM sleep in rats and mice[196, 197], and decreased NREM and REM sleep in rats[198]. We did not find an effect on REM sleep when looking across the entire 24 hours, but only found an increase in REM sleep during the subjective dark phase. We used LED lights at 100-120 lux, while previous reports have used a variety of different light sources and not reported their light levels. Furthermore, previous reports have used different rat strains, and this is the first report of the effect of constant light on sleep in mice to show increased sleep.

Regulation of induced sleep

In figure 3.4, we show that although the duration of NREM sleep is increased during the first two days under LL, the intensity of sleep compensates for the increased duration. This leads to the total amount of SWE accumulated over 24 hours staying constant. This is similar to other reports where altering the daily organisation of sleep-wake behaviours did not alter the total amount of SWE accumulated over the day in mice[272], although interestingly that report showed that total SWE was altered for the first few days under restricted feeding, and only demonstrated equivalence to baseline after 7 days of habituation. This is in contrast to our findings where we see immediate changes in the first two days of exposure to the light cycle.

Architecture of induced sleep

Since we found LL induced sleep between CT 12 and CT 17, but this was regulated homeostatically to not alter the total amount of SWE accumulated over the day, we then asked if there was any difference in the sleep induced during this time period.

We compared the spectra of NREM sleep between the baseline day, and the subsequent days during CT 12-17. We found no differences in the absolute spectrum across days. This is different to the findings of figure 3.4, which found changes in relative SWA at this time. We are comparing the entire absolute spectrum in figure 3.5 A, instead of just the subset which makes up SWA. Indeed when looking figure 3.5A, there appears to lower levels around the SWA frequency band under LL, but when combined with the rest of the frequencies in the spectrum we did find evidence of an effect. We took CT 12-17 for the baseline day as the time to compare against, however this is a time when there is little sleep during the baseline day, while the first two days in LL are predominantly sleep. This answers the question of what the difference is in sleep between the three days at this time. A future comparison that may be useful would be the last hour of the light period on the baseline day, as this is the equivalent of a sleep-dominated, but low sleep-pressure period of sleep.

Finally, we looked at the number and duration of NREM sleep episodes. We see increased number of NREM episodes compared to baseline at CT 12-16, but this is roughly the same number of episodes as during the subjective light phase. We see no change in the mean duration NREM episodes under LL. There is no change in the mean duration of NREM episodes across the day either, indicating the change in time in NREM sleep is due to changes in the number of episodes, not in their duration.

Overall, we show that constant light induces 1.5 hours extra sleep across the first 5 hours of the subjective dark period. This extra sleep is homeostatically compensated to allow a normal amount of SWE accumulation throughout the day, and has similar architecture to sleep during the subjective light phase.

3.4.2 Potential mechanisms

Sleep is affected by circadian regulation, homeostatic regulation, and direct effects of light. Here we outline the evidence of the involvement of each of these on the effects we see under constant light.

Circadian mechanisms

The locus of the circadian control of sleep and wake, similar to most other functions, is the SCN[31, 100, 145]. An important output signal is the electrophysiological firing rate, as optogenetically altering SCN firing rate using dopamine receptor[283], or vasoactive intestinal peptide[284] driven ion channels is enough to entrain locomotor activity rhythms. The SCN projects to the VLPO, as well as SPZ and DMH which are also crucial for the circadian rhythms of sleep and wake[143, 144, 279, 280]. Under constant light, the rhythms of SCN firing become flattened in mice[190], staying above baseline dark-period levels, due to reducd synchrony among SCN neurons[189, 285]. However, it is known that behavioural state can alter the firing rate of the SCN[286], therefore we do not know if the alterations in firing rate seen in[190] are cause or consequence of changes in sleep. Two optogenetic studies caused behavioural delays by increasing SCN neuronal firing during the subjective night, and one caused activity masking similar to light [283, 284]. Therefore the presence of extra light during the start of the subjective dark phase may be increasing the SCN firing rate which therefore increases sleep, possibly through increased stimulation of the VLPO, or reduced wake-promotion through other projections. This model is simplified as there is heterogeneity in the firing patterns of neurons within the SCN[284], and there are also other non-neuronal signals which can entrain rhythms of sleep and wake[78], so it may be more appropriate to say that constant light pushes the SCN to a more 'day-like' state, characterised by increased firing rates, which promotes sleep.

Direct effects of light

Light directly induces sleep in nocturnal animals via melanopsin expressing pRGCs [58, 155–157]. This effect is present during the subjective light and the first half of the subjective dark phase[58, 156, 157], although Altimus et al[155] failed to show any sleep induction effect in mice, but they used a T7 cycle, as opposed to T2 or light pulses. The effects of prior sleep-wake history between these different light cycles may confound these results[151]. This sleep induction also appears to be capable of lasting up to 3 hours[155], and 6 hours of light at ZT 13-19 induces 1 hour of extra NREM sleep[156]. The mechanisms by which light induces sleep are currently unclear. pRGCs project both to the SCN and to extra-SCN areas important for sleep and wake, including the VLPO and lateral hypothalamus (LH)[145, 151]. These extra-SCN projections appear to be neccesary for the acute light-induced sleep[148], and light pulses induce FOS in the VLPO[58, 157]. This suggests that the increase in sleep we see in the first 6 hours of the subjective dark period could be due to light activating melanopsin expressing pRGCs which stimulate the VLPO and induce sleep.

Homeostatic regulation of increased sleep

Sleep is regulated homeostatically, by both time and intensity. This is represented by SWE, which maintains the same levels under LL over 24 hours despite changes in the time asleep[272]. It is unlikely that changes in homeostatic sleep need are causing the increased sleep time, as that sleep still has low SWA, as seen in figure 3.4. Therefore we do not find increased sleep need being the cause of increased sleep time.

Together, the changes we see are most likely due to a combination of direct induction of sleep, with a small contribution of an altered circadian sleep/wake signal, as we see an immediate effect. This is primarily because the increase in NREM sleep that we see during the subjective night in the first two days (1.39 and 1.29 hours respectively), is slightly larger than the 1 hour induced by a 6 hour light pulse during the subjective dark[156]. We are assuming the difference

between these two values is due to both the increase in light duration, and changes in the circadian sleep/wake signal.

3.4.3 Implications

Here we show that constant light induces sleep at the start of the subjective dark phase. We assume this is due to a direct light induced effect, with a small contribution of a change in the circadian slee/wake signal. We see no changes in homeostatic sleep pressure and conclude that the extra sleep we see is not due to any change in sleep homeostasis.

Sleep regulation is modelled by by a two-process model[125], where the homeostatic process S builds up as a function of prior wake and dissipates during sleep, while the circadian process C varies to favour wake and sleep throughout the day. While originally formulated for humans, lowering the upper threshold for process S accumulation models the polyphasic sleep seen in rodents[126]. Application of this model to SWA in rats suggested that there were different dynamics to SWA accumulation during light and dark periods[276]. Furthermore there appears to be a circadian modulation of the SWA dynamics in constant darkness[287]. Two elaborations of the two process model have been published recently. The first elaborates the parameters predicting SWA to incorporate recent history, and provides quantitative predictions of SWA across light, dark, and different waking states [288]. This model provides quantitative predictions of SWA, which are highly accurate to empirical observations, however it does not directly predict state or transitions. Another recent model attempted to address this limitation by implementing a stochastic markov-chain-model to predict duration and transition between episodes of different states[289]. This also provided accurate predictions, however required parameter tuning based on the waking state. This latter model had varied state transition and durations based upon Process S and Process C. Our interpretation of our findings, is that process S is not affected and alterations in process C provide a minimal input to the changes we see. This second point is a major assumption, but fits with the previously published literature[190]. Furthermore how

light induced sleep fits with the two-process model is still an important outstanding question, given recent work showing that this requires extra-SCN projections of pRGCs, i.e. the SCN does not directly mediate the direct sleep induction by light[148]. Assuming that our results indicate differences beyond changes that can be modelled by process S or process C, where does this leave our mathematical models? While the sleep induction effect of light has been studied well, how it fits into a wider sleep regulation framework is less clear[151]. The Rempe et al model[289] provides a possible formalisation of the changes we see. The state transitions and episode durations are already affected by Process C and Process S, and therefore alterations of this stochastic process may be able to model the prolongation of sleep we see under LL. Indeed, in agreement with this, we see that episode duration of NREM sleep stays similar to during the subjective light phase during the first few hours of constant light, as seen in figure 3.5. In the future, we hope to see whether incorporating light environment in some way into these models can capture the changes we see here.

3.4.4 Limitations

Circadian vs Direct effects

The major limitation of the data presented here is we are unable to determine whether the effects are due to changes in the circadian system or due to the direct effects of light. We are assuming that they are mainly due to the direct effects of light as we see similar sized changes as previously reported[156]. In order to address this, we would want to alter one of these processes while not affecting the other, which is difficult given their interrelated nature. Since extra-SCN projections of pRGCs were found to be neccessary and sufficient for the direct effects of light on sleep[148], performing our LL protocol in mice with either only SCN or extra-SCN pRGC projections would indicate the extent of the direct effect of light in our findings. Though this may be complicated by feedback onto the circadian system from extra-SCN areas regulated by light. On the other hand, our hypothesis that LL converts the SCN to a more day-like functional state partially due to desynchrony

between SCN neurons, suggests that if we could induce this state separately to light input then we would observe the SCN contribution to our findings. This may be approached by using optogenetic approaches to alter the functional state of the SCN in constant darkness[283, 284]. Alternatively, *Vipr2*$^{-/-}$ mice have arrhythmic SCNs similar to those seen under constant light[189, 285], so their sleep under DD would provide an insight into the effects of an arrhythmic SCN on sleep. Our hypothesis suggests that either of these conditions would only marginally alter the amount of sleep per day. This assumes that the disruption of the circadian system is constant and does not alter over time, which may not hold true [190]. Therefore over time, as the circadian system becomes more impaired its effect may become more pronounced. We can address this by looking at the effect of LL on sleep after an extended period. We currently have the data to look at this after 14 days of LL, however due to practical limitations of manual sleep scoring, we were only able to look at the first 48 hours under LL. To bolster this, we would need to perform this experiment while measuring the circadian system, such as the firing rate of the SCN.

Sleep homeostasis

Our findings rest upon our interpretation that sleep homeostasis is unchanged by our constant light protocol, as the levels of SWA remain low during the induced sleep at the start of the subjective dark phase. This is observational data, and so does not unequivocally show that sleep homeostasis is unaffected. Sleep deprivation is commonly used to investigate changes in sleep homeostasis[276]. To investigate we would need to ask two separate questions; What is the effect of constant light on the accumulation of homeostatic sleep pressure? and what is the effect on the dissipation of homeostatic sleep pressure? For the first question, we would sleep deprive animals during ZT 12-18, the start of the subjective dark phase, and look at the SWA accumulation. Light environment is a contributor to homeostatic sleep need [157, 276], therefore we may see changes in the build up of SWA. For the second question, we would sleep deprive animals during ZT 6-12, the last 6 hours of the subjective light phase, as we could then examine the dissipation of the artificially high SWA.

Rhythmicity under LL

Constant light is well known to cause behavioural arrhythmicity, in proportion to light intensity [186, 187], due to desynchronisation of neurons in the SCN [189]. We used 100-120 lux of white LED light, which did not induce behavioural arrhythmicity. This light level is in line with some previous studies looking at jetlag [20], but below previous studies that induced arrhythmicity in constant light [187, 189]. While other studies induced arrhythmicity using even lower light levels than we used here [188, 290], they used different strains of mice to us, which are known to affect light perception and circadian behaviour [282]. Therefore we may be seeing minor circadian effects as we have not fully disrupted the SCN using LL. Arguing against this, are our findings from chapter 2, which demonstrate that LL still causes major changes to circadian activity. We would have to perform this experiment again under light levels that do induce arrhythmicity to address this.

Data processing

We have measured all our markers against clock time, and have not corrected for internal circadian time, despite constant light inducing free-running. We did this due to time constraints of our data analysis. Since we only examined the first 48 hours of constant light, we do not believe this is majorly affecting our findings. The main evidence is from figure 3.4 which shows that even on the second day in LL, there are only minor deviations from baseline during the subjective light phase. As we examine further into LL, we will have to correct for circadian phase.

3.4.5 Future studies

Here we show that constant light induces sleep during the subjective dark phase in the first two days, and that sleep is homeostatically compensated. We assume that the majority of that sleep is due to direct effects of light, with some contribution of alterations of the circadian system. There are several future studies we suggest.

First, sleep at the end of an extended period of LL, when we assume the circadian system is more disrupted, will provide information about the relative contribution

of direct effects of light and the circadian system. We have this data and are working on analysing it. Second, modelling how the changes we see fit in with both SWA, and state prediction models [288, 289], will provide some quantitative insight into how our findings fit with our current understanding of sleep regulation. Third, performing sleep deprivation experiments to confirm that the build up and dissipation of homeostatic sleep pressure is normal under constant light. Finally, I have outlined a range of suggestions for investigating the relative contribution of circadian and direct effects of light in our protocol in the above sections. The most productive is likely to be repeating these experiments using animals where the direct effect or the circadian effect of sleep induction by light is removed [148]. This should be followed by performing this experiment under a range of light levels to induce behavioural arrhythmicity, and therefore see if there is a gradient in the effect of the circadian contribution.

3.5 Conclusions

Here we show that constant light induces sleep during the subjective dark phase in the first two days, and that sleep is homeostatically compensated. We suggest that this is primarily due to a direct effect of light on sleep, with a small contribution from an impaired circadian system. This effect is not captured by the quantitative models we currenlty employ of sleep regulation, and it will be of interest to see how they can be adapted to fit our findings. Our findings rely on a number of assumptions that we hope to be able to address with future studies.

4

Circadian disruption across the lifespan

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4.1 Introduction

Ageing involves an inevitable breakdown of most complex organisms[291, 292]. The circadian system is no exception, with the input[293–295], master pacemaker[296–298], and outputs such as sleep[299] all impaired in aged humans and rodents. The biggest circadian impairment in ageing is alterations to the daily sleep-wake cycle. In humans, sleep is earlier[300, 301] , fragmented[302], and there is more daytime sleep[84]. Similar effects are seen in rodent models, with reduced day-night differences in sleep mainly due to increased sleep during the active phase[298, 303, 304]. Furthermore, reduced amplitude and fragmented rhythms of locomotor activity are also found in humans[305], rodents[303, 306], and non-human primates[307]. Finally the intrinsic period of the circadian clock also is affected, shortening in hamsters[308], primates[309], and rats[310], but lengthening in mice[298], but does not appear altered in humans[311]. In animal models this match between the internal period and the light/dark cycle is important for longevity[312] in cyanobacteria[26], mice[180, 313], and hamsters[181, 314]. Ageing also has a range of other effects[315], which are suggested to be due to a range of cellular pathologies, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication [316]. The circadian clock is important for multiple cellular mechanisms, which overlap with those affected by ageing[317].

Disruption of the circadian system also causes several pathologies similar to those seen in ageing. Chronic shiftwork increases mortality in humans[318], while chronic disruption of the circadian system by changing the light/dark cycle causes increased mortality in mice[319]. Mismatch between the internal circadian period and the environment is also a form of circadian disruption, and as shown above impacts mortality[312]. In humans, epidemiological evidence shows shift work associated with increased cancer risk[320–322], while in rodent models chronic jetlag enhances tumourigenesis[323], and tumour progression[324–326]. In rodents, a disrupted circadian system can increase the incidence of age-related cataracts[327]. Shift work increases cardiovascular disease risk in humans[328], while in rodents disrupted

circadian rhythms also cause cardiovascular disease[329]. Finally, shift work is associated with cognitive deficits and increased risk of dementia in humans[12, 330], while circadian disruption in rodents leads to a range of cognitive deficits[223].

Given the similarities we see between circadian disruption and ageing, the question is whether the circadian breakdown in ageing is causative to the changes seen in ageing, or coincidental. To address this we caused circadian disruption by housing animals under long-term abnormal light cycles, and compared the changes we see with those seen in similarly aged animals. We assume that if this disruption exacerbates the same changes seen with age, then they are due to the same process. Here we evaluate the effects on locomotor activity as our marker of ageing related change.

4.2 Methods

4.2.1 Animals

All procedures were performed in accordance with the United Kindgom Animals (Scientific Procedures) Act 1986, under project licence 30/3371 and personal licence I14C9C2DE. All procedures complied with the University of Oxford Policy on the use of animals in research. Young male C57BL6/J were obtained obtained from MRC Harwell. All mice were 14 weeks of age when the experiments started. A total of 83 mice were used for this experiment. They were split into groups of 27-28, determined by practical limitations of cage accommodation available, by putting whole cages into each group, without any formal randomisation process. The animals were housed in Light Tight Chambers (LTCs) in groups of 4-6 with food and water provided *ad libitum*. The lights were white LEDS.

4.2.2 Experimental Design

Three groups of animals were held under different lighting conditions for 1 year. The three groups were: 12:12h normal Light Dark cycle (100:0lux), 12:12h Light Dim Light at Night (100:5 lux), and Social Jetlag (5 days of 8:16h LD, then 2 days of 16:8 LD, 100:0lux). After being housed for a year under these conditions, all

groups were returned to a 12:12h LD cycle for 4 weeks. Animals were then split into further groups. 12 animals from each group were singly housed in LTCs for a further 8 weeks under LD and 8 weeks under DD.

4.2.3 Activity Monitoring

Activity was monitored using the COMPASS system as described in Brown et al [236], similarly to Chapter 2, with the following differences. Activity was monitored for each cage (containing 4-6 animals) during the year long housing under experimental conditions, and then for each individually housed animal once they had been split up. While group housed, the animals had free access to the entire cage, including under the food and water hopper away from the range of the PIR sensors.

4.2.4 Data Analysis

All scripts are available at `https://github.com/A-Fisk/04_ageing` and `https://github.com/A-Fisk/actiPy`. The activity data were cleaned and analysed in a similar manner to chapter 2. The original output files were quality checked by a custom written python script, before being annotated. The annotated data was then used for further analysis. Statistics were performed using the pingouin package `https://pingouin-stats.org/api.html`, figures produced using seaborn `http://seaborn.pydata.org/`, and matplotlib`https://matplotlib.org/`. One-way, and Mixed ANOVAs were performed, with follow up post-hoc Tukey testing as stated in the results.

4.3 Results

4.3.1 The effect of 1 year of abnormal light cycles

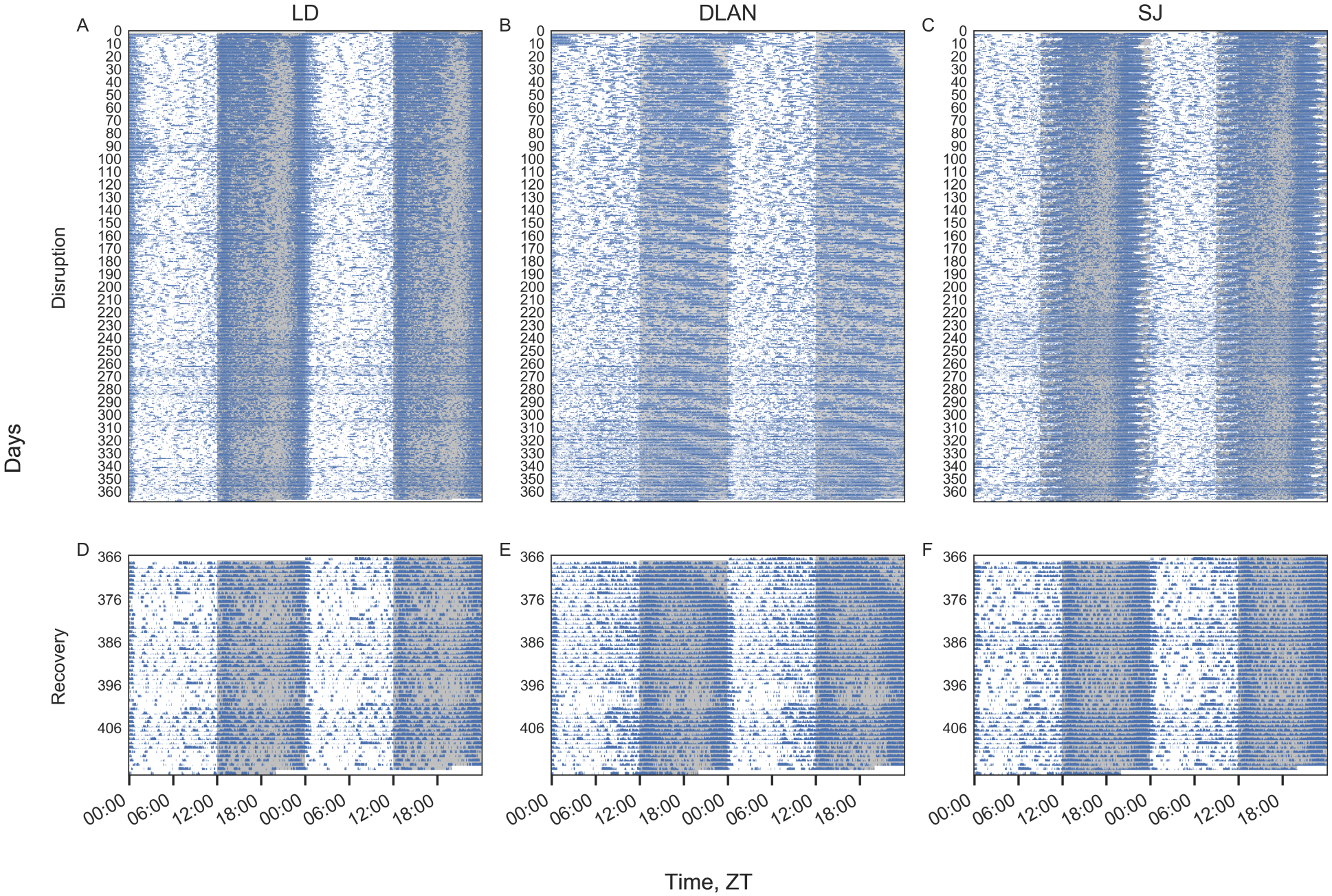
A
LD
B
DLAN
C
SJ
Days
Disruption
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
280
290
300
310
320
330
340
350
360
D
E
F
Recovery
366
376
386
396
406
00:00
06:00
12:00
18:00
Time, ZT

Figure 4.1: Double plotted actograms. A, B, C show a representative example of group housed mice under LD, DLAN, and SJ respectively. D, E, F. show the same groups of mice once they are returned to a standard 12:12 LD cycle.

Animals were housed in groups of 4-6 under different light cycles for 1 year. During that time, their activity was monitored by the COMPASS system [236]. Representative actograms of the year of abnormal light cycles are shown in figure 4.1. In the control LD group, we see a general reduction of activity over time, however the animals remain stably entrained to the light cycle. In the DLAN group, in addition to the general reduction in activity over time, 3/6 of the groups showed the pattern seen in figure 4.1B, of a free running period longer than 24 hours running through the light cycle. Finally in the SJ group, the animals also demonstrate the general reduction in activity, however they are not stably entrained. The activity onset and offset are continually shifting to the light onset and offset respectively.

4.3.2 Comparison of groups when returned to 12:12LD

After the year of abnormal light cycles, animals were returned to 12:12LD cycles, as seen in figure 4.1D-F. DLAN required 14 days to re-entrain to the LD cycle, but after this initial period, there are no obvious differences visible on the actograms.

We next compared the groups using a range of circadian parameters, similar to as in Chapter 2. We excluded the first 14 days of this section to remove the effects of re-entraining, and compare the circadian systems in an entrained environment. The results of the circadian parameters that we measured in the group housed animals are shown in figure 4.2. The only marker to show an effect was Intraday Variability, with DLAN reduced compared to LD (One-way ANOVA, $F(2, 13)=4.02$, $p=0.04$, posthoc Tukeys $p=0.03$). We did not find evidence of difference on any remaining markers ($p>0.05$). We then looked at the mean activity over the day for each group. Again, we found no evidence of difference between the groups (Two-way ANOVA, activity hour*conditions $p>0.05$ interaction term) on the mean activity over the day.

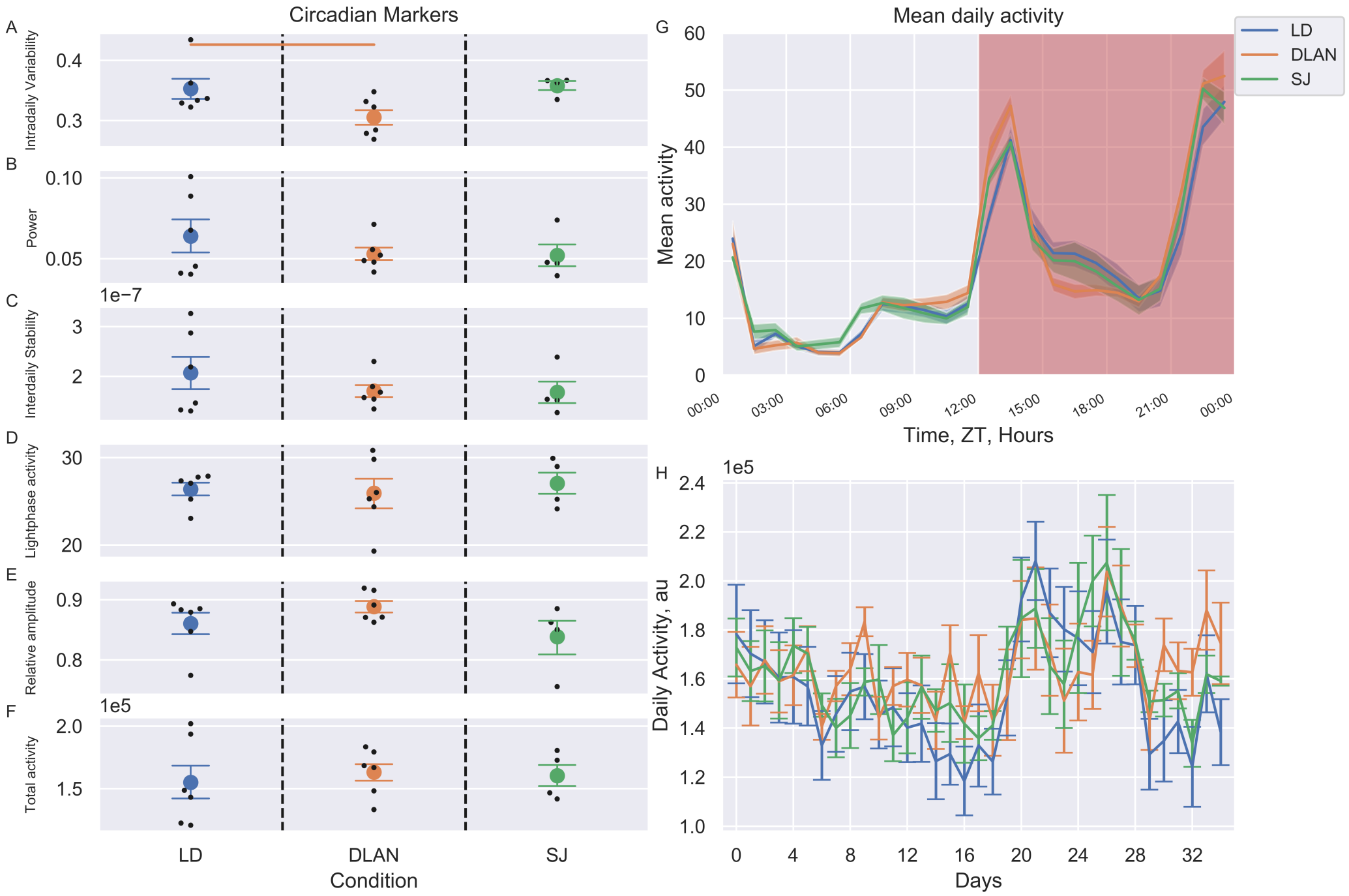
Group Housed Circadian Parameters
Circadian Markers
A
Intradaily Variability
0.4
0.3
B
Power
0.10
0.05
C
1e−7
Interdaily Stability
3
2
D
Lightphase activity
30
20
E
Relative amplitude
0.9
0.8
F
1e5
Total activity
2.0
1.5
LD
DLAN
SJ
Condition
G
Mean daily activity
Mean activity
60
50
40
30
20
10
0
00:00
03:00
06:00
09:00
12:00
15:00
18:00
21:00
00:00
Time, ZT, Hours
LD
DLAN
SJ
H
1e5
Daily Activity, au
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0
4
8
12
16
20
24
28
32
Days

Figure 4.2: Recovery circadian parameters. Once groups of mice were returned to a standard 12:12 LD cycle, we looked at their circadian system while still group housed. LD are shown in blue, DLAN in orange and SJ in green. Any changes where $p<0.05$ on Tukeys post-hoc test from baseline are indicated above the plot. A-F show circadian markers calculated on group housed mice. G shows mean activity throughout the day for all three groups. H shows total activity per day for each group. We only see a difference in Intradaily Variability between DLAN and LD.

Finally, given the general reduction in activity we see during ageing, we compared the timecourse of total activity per day to see if there was an effect on this as a marker of ageing. Again, we find no evidence of differences between the groups (two-way ANOVA, activity day*conditions, interaction $p>0.05$).

4.3.3 Circadian parameters of singly housed animals

Since animals have entraining cues from all other animals within the cage while they are group housed, we next singly housed animals to see if this uncovered greater differences in their circadian system. We first housed animals in LD, and the resulting circadian parameters are shown in figure 4.3. In the different circadian markers, we see no evidence of an effect of condition on any of the markers (One-way ANOVA, $p>0.05$). In mean activity over the day, we do see a difference between the groups (two-way ANOVA, activity hour*condition, interaction $F(46, 598)=2.033$, $p=0.00011$), with post-hoc testing showing that DLAN differed from LD immediately after dark onset, and SJ differed from LD at several points during the light phase, and at a single timepoint in the middle of the dark phase(Tukeys post-hoc $p<0.05$). However, given the similarity of the mean activity graphs, we do not conclude that there are major differences between the groups. For the timecourse of total activity per day, we find a difference between the groups across time, mainly driven by the differences between the days, across the entire section analysed the groups did not differ (Two-way ANOVA, activity day*condition, interaction $F(110, 1430)=1.517$, $p=6.9e\text{-}5$, day $F(55, 1430)=7.498$, $p=4.85e\text{-}48$, condition $F(2, 26)=0.096$, $p=0.91$).

Singly Housed Circadian Parameters LD

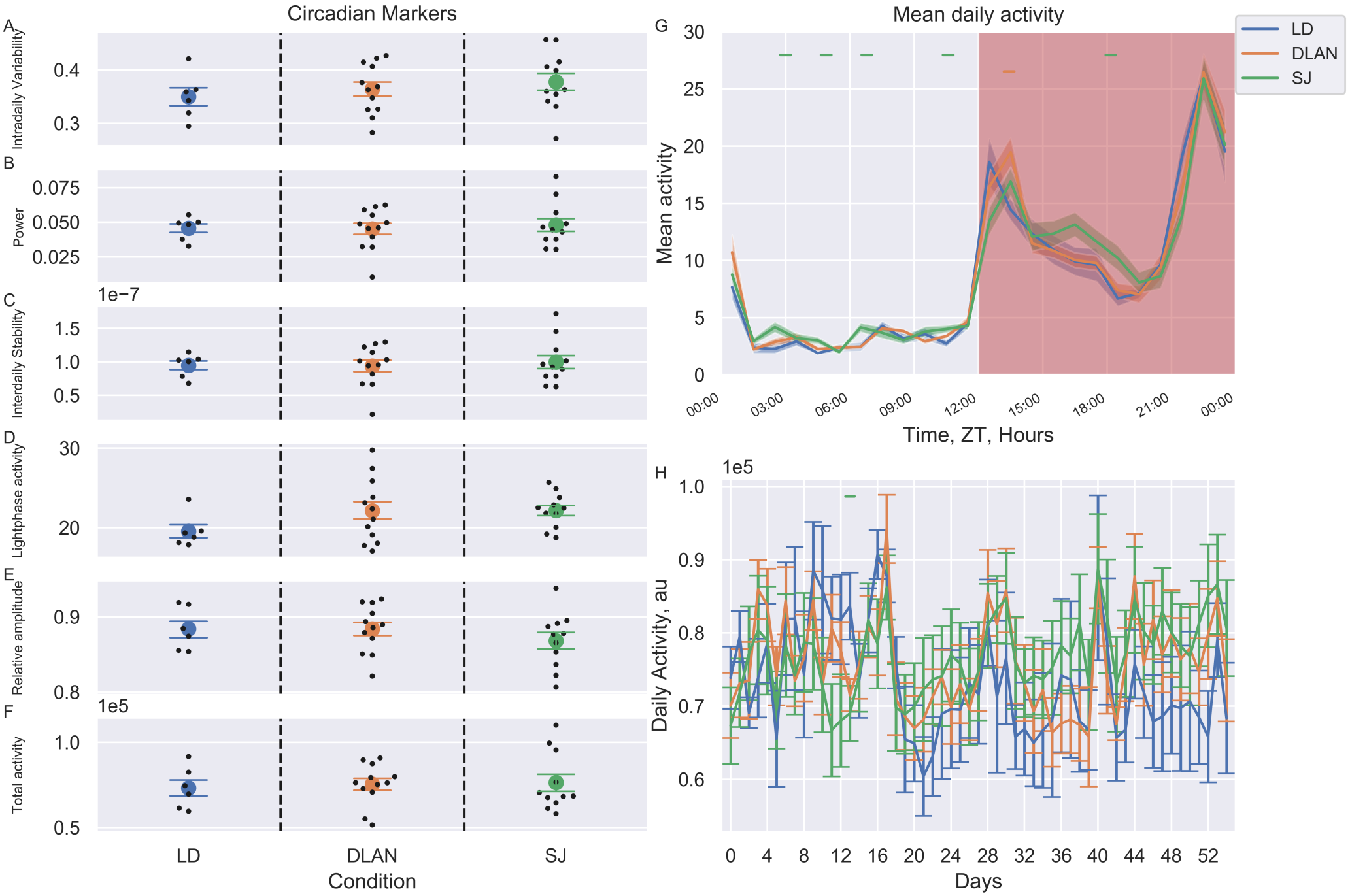

Figure 4.3: Circadian parameters of singly housed mice under LD. After group housing under LD, the groups of animals were split and animals were singly housed under LD. LD are shown in blue, DLAN in orange and SJ in green. Any changes where $p<0.05$ on Tukeys post-hoc test from baseline are indicated above the plot. A-F show circadian markers calculated on single housed mice in LD. We do not see any differences in the markers. G shows mean activity throughout the day for all three groups. In the mean daily activity we see small differences during the light phase. H shows total activity per day for each group. We only find a single day where there are differences between the groups.

There was only a single day where Tukeys post hoc testing showed an effect between SJ and LD. From looking at the graph, this appears to be driven by LD lowering over the time course given relative to the other groups. Therefore while we do find statistical differences, given the distribution of the data we do not interpret this as a large effect of interest.

Finally, in order to examine the circadian system in isolation, we then transferred animals to constant darkness (DD). Once more, we find no differences on any of the circadian markers (One-way ANOVA, $p>0.05$). For the mean daily activity, we corrected each animal to its own internal circadian period, therefore the graphs represent each point in the circadian day. We do not find evidence of any difference between the conditions on the daily activity profile (Two-way ANOVA, activity hour*condition, interaction $F(46, 552)$, $p>0.05$). Finally for the timecourse of total activity per day, we see a difference between the groups by time, and a difference between the days, but no difference between the groups taken over the whole time (Two-way ANOVA, activity day*condition, interaction $F(92, 1104)=1.846$, $p=5.46e\text{-}6$, days $F(46, 1104)=10.188$, $p=1.32e\text{-}57$, conditions $F(2, 24)=0.85$, $p=0.44$). While only two days showed a post-hoc Tukeys effect of $p<0.05$ where SJ is higher than baseline, LD appears to be more consistently lower.

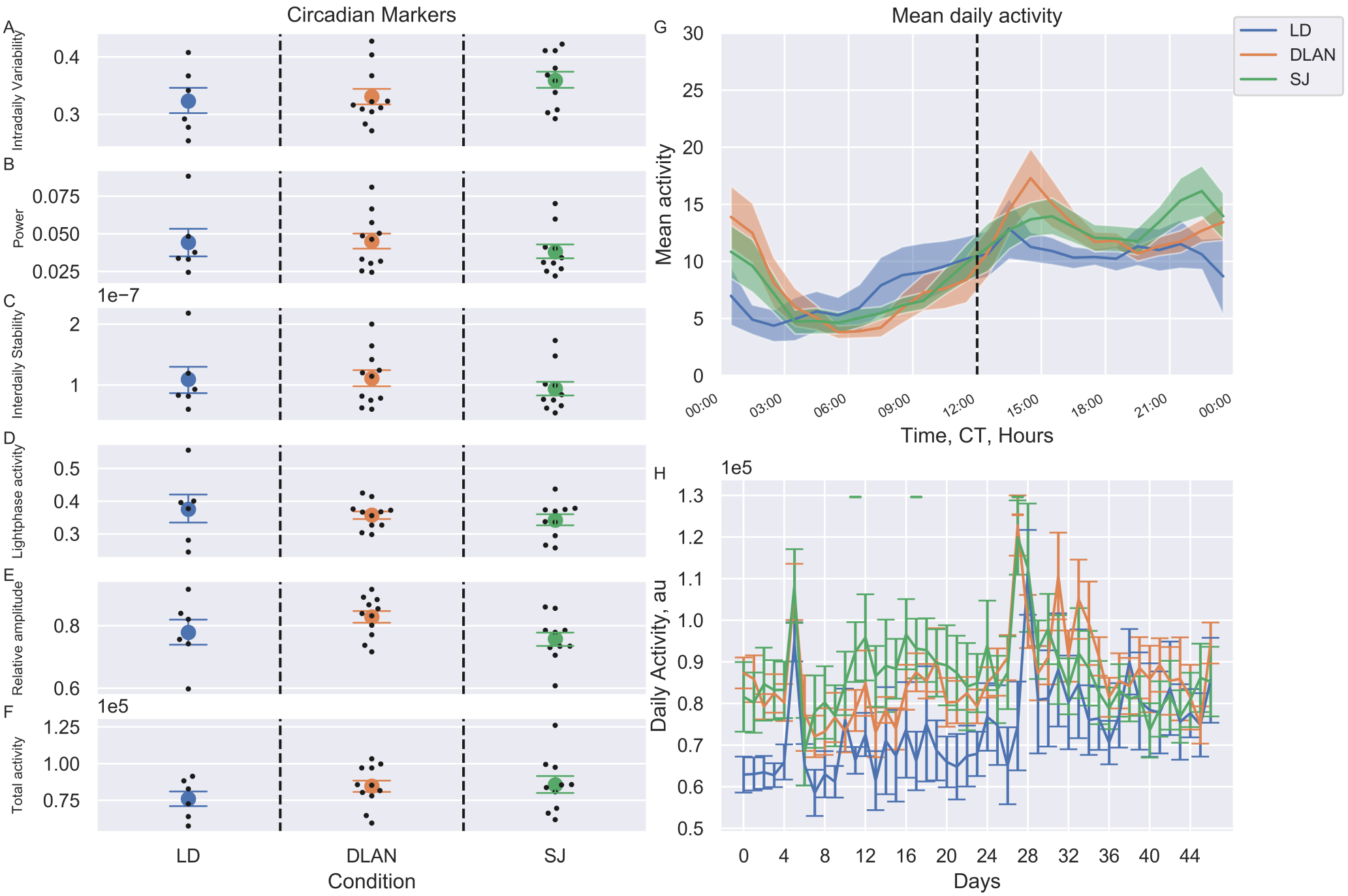
Singly Housed Circadian Parameters DD
Circadian Markers
A
Intradaily Variability
0.4
0.3
B
Power
0.075
0.050
0.025
C
1e−7
Interdaily Stability
2
1
D
Lightphase activity
0.5
0.4
0.3
E
Relative amplitude
0.8
0.6
F
1e5
Total activity
1.25
1.00
0.75
LD
DLAN
SJ
Condition
G
Mean daily activity
Mean activity
30
25
20
15
10
5
0
00:00
03:00
06:00
09:00
12:00
15:00
18:00
21:00
00:00
Time, CT, Hours
LD
DLAN
SJ
H
1e5
Daily Activity, au
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0
4
8
12
16
20
24
28
32
36
40
44
Days

Figure 4.4: Circadian parameters of singly housed mice under DD. After single housing in LD, the single housed animals were released into DD. LD are shown in blue, DLAN in orange and SJ in green. Any changes where $p<0.05$ on Tukeys post-hoc test from baseline are indicated above the plot. A-F show circadian markers calculated on single housed mice in DD. We do not see any differences in the markers. G shows mean activity throughout the circadian day for all three groups. In the mean daily activity we see no differences. H shows total activity per day for each group. We only find two days where there are differences between the groups.

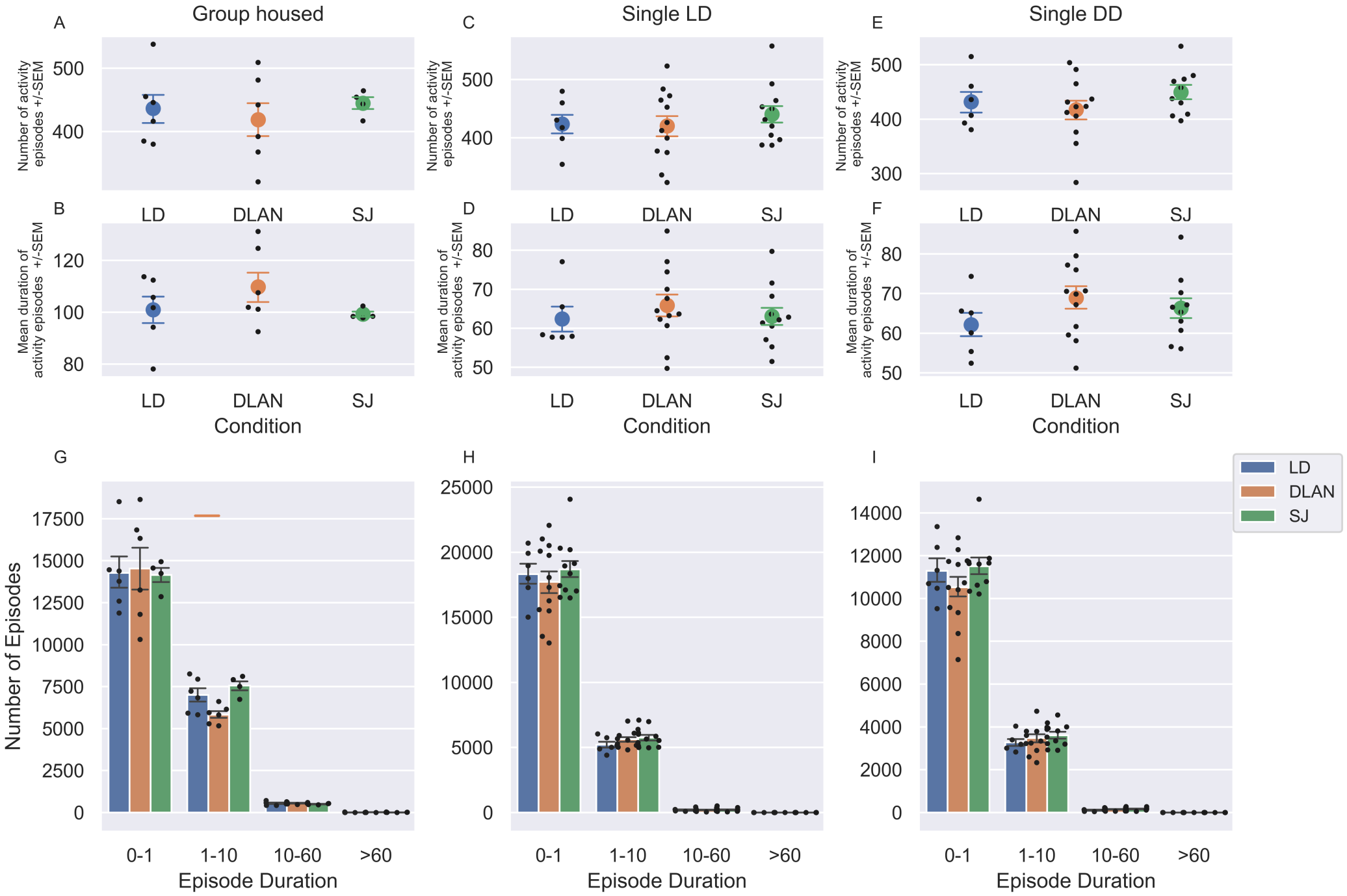
A
Group housed
Number of activity episodes +/-SEM
500
400
B
Mean duration of activity episodes +/-SEM
120
100
80
LD
DLAN
SJ
Condition
C
Single LD
D
70
60
50
E
Single DD
300
F
G
Number of Episodes
17500
15000
12500
10000
7500
5000
2500
0
0-1
1-10
10-60
>60
Episode Duration
H
25000
20000
I
14000
12000
8000
6000
4000
2000
LD
DLAN
SJ

Figure 4.5: Episodes of activity for mice that are group housed, singly housed under LD, and singly housed under DD. We looked for fragmentation by looking at the number, mean duration, and duration histogram of activity episodes of the different conditions under the three different housing conditions. A,C,E shows number of episodes B,D,F shows mean duration of episodes G-I shows histogram of duration of episodes. LD are in blue, DLAN are in orange, and SJ are green. Differences where Tukeys post-hoc $p<0.05$ from baseline are indicated above each plot.

4.3.4 Episodes of activity

Finally, we looked to see whether activity was fragmented by any of the conditions, in any of the housing situations. Fragmentation is a increase in the number, and reduction in the duration of episodes, therefore we looked at the number, and duration of activity episodes under these different conditions. We find no evidence of a change in the number or duration of activity episodes in any of the conditions under any housing situation (One-way ANOVA, $p>0.05$). Similarly to Chapter 2, we also asked whether any specific durations were altered by the conditions. We see a reduction in the number of episodes of 1-10 minutes duration in DLAN compared to LD when group housed (Tukeys post-hoc $p=0.027$). Given this is an isolated difference found among many tests, we do not conclude that there is a major effect of condition on activity episodes.

4.4 Discussion

4.4.1 Key findings

Here we housed animals under abnormal light cycles for 1 year then measured their locomotor activity to see what effects this long-term circadian disruption had. We hypothesised that this would exacerbate ageing related changes. However, we find no effect of our long term circadian disruption on locomotor activity.

Ciradian markers

We analysed the activity of animals while they were group housed and singly housed under a normal LD cycle, and while they were singly housed under constant darkness.

Across all these conditions, we found no consistent differences between the groups. The only marker where we found an effect was a decreased IV in DLAN compared to LD mice when group housed under LD. Given that this effect does not persist when animals are taken out into singly housed environments, we do not interpret this as a consistent increase in the variability of locomotor activity in response to DLAN. We used a range of markers that have previously been used to see differences between the effects of abnormal light cycles [237], and see chapter 2.

Mean daily activity

We find no changes between groups in the mean daily pattern of activity under the different conditions. Previously, we showed in chapter 2 that multiple abnormal light cycles removed the main peak of activity at the start of the subjective dark phase. Under standard LD cycles, we see a normal peak of activity at the start of the dark phase, however when singly housed under DD, we see a flattened profile of activity. This is similar to previous findings of a flattened activity profile in aged mice[331]. The fact that aged and disrupted mean daily activity profiles are both flattened and have no main peak of activity could indicate that they are both a similar state. However, given we find normal activity profiles while housed under normal LD, the flattened profiles under DD may be due to changes in masking in aged mice. Young mice under DD do not appear to have such a flattened activity profile[236, 332] , unpublished observations, therefore it is unlikely to be an entirely masking mediated change.

Total activity per day

Next, in addition to the marker of total activity per day, we looked at the timecourse of activity per day to see if there were any differences between the groups. While we do see statistical effects with the timecourse of activity per day being different between the groups when singly housed under both LD and DD, it is difficult to interpret this. The data are widely variable, with the SEM range on any given day encompassing most of the range of the data over the entire time period. Furthermore, there appear to be consistent changes across the days, for instance days 20–28 while

group housed are higher than the rest of the time, and there appears to be peaks on individual days while singly housed under both LD and DD. The individual day peaks are assumed to be days when the animals changed cages, which is known to alter the amount of time awake[333]. The deviation across all groups on the group housed data are more concerning. Guide rails were in place to ensure a consistent position of each cage relative to the sensor, in line with that described by Brown et al[236], and given the deviation is across all groups it is unlikely due to a poorly placed cage in each group. We suspect this is due to some change in the animal facility that we were unaware of while this was occuring, affecting unknown factors that influence the animals activity, e.g. change in noise levels in the room[334]. While this is an unfortunate source of variability in our data, it appears to have affected all groups similarly so our inferences about group differences are intact.

Fragmentation

Finally, we asked whether we could see any evidence of fragmentation of locomotor activity under LD, as fragmentation of sleep occurs during ageing in mice[303, 335], and models with circadian disruption show fragmentation of their activity[240]. We looked at the number, and mean duration of activity episodes per day in LD while group and singly housed, and under DD while singly housed. We did not see any changes in the number or mean duration of activity episodes between the groups under any conditions. We therefore looked at a histogram of episode duration to see if we could see any effects on specific episode durations. We treated this as a post-hoc test of the mean duration of activity episodes, and found an effect of reduced episodes of 1-10 minute duration in DLAN compared to LD when group housed under LD. As this effect did not persist under the different lighting and housing conditions, we do not interpret this as a major effect of DLAN.

Overall, we do not find any effect of housing mice under abnormal light cycles for a year on locomotor activity. Whether group housd under LD, singly houesd under LD, or singly housed in DD, we see no effects on various markers of the circadian system, daily mean activity, and episodes of activity.

4.4.2 Potential Mechanisms

Here we investigated what the effect of long term circadian disruption on the ageing process. We used a year of abnormal light cycles to induce circadian disruption, and measured locomotor activity as our measurement of ageing. We see no effect. This could be due to problems with our intervention, with the effect we are looking for, or with our measurement.

Intervention

We used dim light at night and social jetlag as our abnormal light cycles. DLAN causes a variety of negative health consequences [231]. DLAN reduces locomotor activity levels with reduced periodogram power in hamsters[216], however in rats and both C57BL/6 and Swiss Webster mice it causes no changes in the locomotor activity [202, 257, 336]. Social jetlag has been studied in humans for its effects on depression[337], obesity and metabolic disease [338]. While to our knowledge, a social jetlag lighting regime has not been used in mice. However, there is evidence that altering the photoperiod, and therefore the relationship of activity onset and offset to the light cycle, does induce cognitive deficits in nocturnal siberian hamsters[246], and prolonged photoperiods can destabilise the SCN in mice[339]. Our social jetlag light cycle induced regular changes of photoperiod so animals were regularly re-entraining their activity onset and offset to the light cycle. However given the lack of any available evidence on the effect of this light cycle on locomotor activity we cannot conclude this caused major circadian disruption. Therefore, our findings fit with the literature, or abscence of, on the effect of these light cycles on locomotor activity.

In chapter 2 we showed an effect of DLAN on locomotor activity in mice, whereas here we do not see any differences. This may be because of the time period we are looking at; In chapter 2, we looked at the effect of locomotor activity while under these abnormal light cycles, while here we are examining the effect on activity after several weeks to re-entrain to a normal LD cycle. In addition, during this experiment we group housed our mice, due to space constraints of housing large numbers of mice for a prolonged duration, while in chapter 2 they were singly

housed. Single housing increases locomotor activity, mainly at the start of the dark phase[333], but its effects on response to abnormal light cycles is unknown. Given the other animals in the cage would constitute another daily signal, it is reasonable to assume that a group of animals is more robust against circadian disruption than individual animals. Therefore, group housing may have minimised the circadian disruption induced by our light cycles.

We used light levels of 100 lux of white LED light at cage bottom for our light cycles. As discussed in chapter 3, the light levels used to induce disruption can affect the extent of disruption seen[187]. Our levels are slightly lower than that published previously where 150/5 lux is used for DLAN[216, 257]. Our light levels could have been insufficient to induce enough disruption to detect the effects we see here. Given we see disruption in singly housed animals in chapter 2, we do not expect this to be the only factor involved.

Effect

We may be seeing no effect due to the circadian disruption not causing any long lasting effects. We assumed that due to the similarity of the effects of circadian disruption and ageing, that we could exacerbate these effects by housing animals under abnormal light cycles. However this similarity may be obscuring our results. We know that ageing induces changes in locomotor activity[297], as does circadian disruption, therefore we may be seeing a floor effect, where locomotor activity is already affected so much at this age, that we are unable to induce any further defects in locomotor activity. To address this, we would need to monitor individual animals across the lifespan[303], but this requires lots of time and resources so we were unable to this for this study. We may also be seeing no effect as the aged circadian system is more robuts than we assumed. This seems unusual as SCN synchrony is reduced as animals age[298, 340], and synchrony of the SCN gives rise to behavioural rhythmicity[285]. While the relationship of SCN synchrony to recovery from prolonged circadian disruption is unknown, it seems reasonable to assume that disruption would impair synchrony and impair recovery. In very young

mice, constant light leads to long term disruption of several key rhythms[341], but how the recovery of these rhythms from disruption changes with age is unknown. Hughes et al[285] show that in animals with reduced SCN coupling, constant light can enhance SCN synchrony and behavioural rhythmicity, which rapidly dissapears when removed from constant light. This raises the possibility that our abnormal light cycles may be buffering the effects of ageing to a certain extent potentially due to remodelling of the SCN, and thereby showing no impairment. However this is unlikely as the previous report used constant light which is very different from DLAN and SJ we used here.

Outcomes

Here we used locomotor activity as our marker of ageing of the circadian system as this was easy to collect and analyse, and is an important output of the circadian system. While we know locomotor activity is related to SCN timekeeping[31], and SCN synchrony[285], and both ageing and circadian disruption can affect SCN synchrony[285, 298], this is only one output of the circadian system. The circadian system consists of a heirachy of tissue oscillators that are synchronised by the SCN, which is in turn synchronised to the external environment by light through the eyes[68], which are important for a variety of cellular processes[317]. While we show no effect of long term circadian disruption on locomotor activity, this does not rule out an effect on other important areas. First, the input to the clock, which is known to be altered in ageing[303], and could be assessed by looking at pupillary light responses, or cFOS or clock gene induction in the SCN. Next, the SCN itself. Previously it has been shown that aged SCN firing is more variable[342], due to desynchronised individual cells[296, 298]. In addition, clock gene expression appears affected[296]. To address this, we could look at the rhythm of SCN gene expression, both clock and non-clock genes. We would ideally also look at electrophysiological firing to inform us directly about SCN output, but this is a difficult experiment to perform during ageing in a study of this kind. We could also look at peripheral oscillators to assess the circadian system. Important aspects are the amplitude

and phase of peripheral clocks, which we could assess using rhythms of clock gene expression in these tissues. To assess these further, we would need to look at the strength of coupling between central and peripheral clocks, which could be done using a shift of the light dark cycle and looking at the rate of re-entrainment. This is known to be impaired in aged rats and mice[343, 344]. Our results of intact locomotor activity in aged mice after long term circadian disruption do not show that the circadian system is entirely intact and there are many more aspects to examine.

On the other hand, we may want to look for markers of ageing instead of the circadian system. Aging involves changes to many body systems[316], and discussing the merits of particular markers of ageing is beyond the scope of this thesis, but for example we could look at the effect of our abnormal light cycles on DNA methylation[345], or double-stranded DNA breaks[346].

4.4.3 Ageing Implications

Here we show that after a year of abnormal light dark cycles, there was no effect on locomotor activity of mice, when returned to normal LD cycles or in constant darkness. We aimed to test whether long term circadian disruption altered the ageing process, given the similarity between circadian disruption and ageing, we assumed that they shared a common mechanism, so by worsening the disruption we would worsen the ageing phenotype. If our results hold up to further analysis, i.e. beyond looking only at the locomotor system, what could this mean for our hypothesis? Our results would suggest that instead of sharing a common mechanism, circadian disruption is downstream from ageing, therefore worsening circadian disruption does not affect the ageing process. This seems surprising given the similarities between the two, for example on mortality and cancer[319, 323], but would fit with a model where the circadian disruption seen in ageing mediates some of its downstream consequences.

The other major possible interpretation is that our animals have recovered from the circadian disruption fully by the time we examined their activity. In humans, it takes roughly 1 day to reentrain activity to each hour shifted in jetlag, and the

effects on peripheral clocks are unkonwn[347]. In rodents, locomotor activity also gradually re-entrains, and peripheral clocks shift differently, muscle rapidly and liver slowly, but are re-aligned 6 days after a phase advance[72]. It takes at least 5 years to recover from long term shift-work in humans[12], while in rodents the only evidence shows that memories formed shortly after jetlag are still impaired at least 17 days following the shift[348], but the ability to form new memories is unknown. In humans, the effect of shift work is cumulative[12], while in rodents there is less evidence of the cumulative effect of abnormal light cycles. Acute and chronic jetlag both cause impairments in multiple forms of memory[223, 227], however repeated shifts of the lights appears to be protective of fear memory[20]. This highlights the difference between humans and rodent models. Shift work involves active, directed behaviour in conflict with the circadian and sleep pressures, while rodents are free to organise their behaviour in accordance to the circadian and sleep pressures. Therefore abnormal light cycles in rodents may be a poor model of the effect of shiftwork, a more realistic model may be cognitive training at an abnormal time of day which requires more engagement in conflict with their internal pressures[349].

4.4.4 Limitations

Our results indicate no effect of prolonged circadian disruption on locomotor activity of aged mice. There are two major limitations to our study. Firstly, we have only looked at locomotor activity as our outcome measure. Locomotor activity is known to be disrupted by both ageing[303] and circadian disruption, see chapter 2, and we hoped that this would increase our chances of seeing any differences. Both the circadian system and ageing have far more effects than just on locomotor activity[68, 316], therefore we may be missing important changes induced by our abnormal light cycles by looking at the wrong place.

The second major limitation of our study is a lack of a similarly treated and housed young group of animals to compare to. While the effects of ageing on the circadian system have been described previously[303, 331, 335], we are relying on the published literature to provide our comparison. There are inter-laboratory

effects which can affect the results of experiments in unexpected ways[264], although meaningful effects should be robust to such variations [263]. However, previous reports have not analysed their data exactly comparably to our, which can lead to selective reporting of results[266]. Therefore, to have an accurate picture of how our light cycles affect ageing induced changes in locomotor activity, we need a control group of young animals to compare against.

4.4.5 Future studies

Our results suggest several avenues of future investigation. Firstly, we would want to extend the results we present here. To start with, we would look at individual animals across the lifespan under different types of circadian disruption to look at the timecourse of changes within subjects. We would also examine further aspects of both the circadian system and ageing effects. For the circadian system, while ideallly having electrophysiological recordings of the SCN under different conditions would show the effect on central circadian output[342], this is a difficult experiment to perform. We could look at the circadian rhythm of gene expression in the central and a range of peripheral oscillators[210], to see any changes in organisation of the circadian system. For aging, we could look at the effect of our light cycles on DNA methylation [345], double-stranded DNA breaks[346], or number of senescent cells[350].

Next, we would want to investigate our findings by trying to worsen the circadian disruption and seeing its effects on ageing and circadian breakdown. We could do this using brighter light levels during the day[282]. We could also induce circadian disruption using a different paradigm, for example cognitive training during the day[349]. The circadian system is important for many aspects of behaviour and physiology, therefore it stands to reason that arrhythmic genetic mutants, such as *cry1/2* $^{-/-}$, would have a worse ageing phenotype due to the loss of rhythmicity.

Finally, to show that ageing and circadian disruption are mechanistically similar, we would want to rescue any deficits we find. We could give treatments which have shown to reduce the effects of ageing, such as SIRT1[351] and calorie restriction[352],

during circadian disruption and see if it alleviates its effects. Conversely, we could apply interventions that improve the circadian system, such as wheel running[353] and nobiletin[354], during ageing and see if it mitigated the effects of ageing. Some evidence indicates that wheel access mitigates the effects of ageing already[355], though this is complicated by the known effects of exercise on ageing as well[356]. Whether these effects require a circadian system could be examined by using an arrhythmic animal, such as *cry1/2* $^{-/-}$ mutants.

4.5 Conclusions

Here we show no effect of long term abnormal light cycles on locomotor activity in mice. We suggest that, though we see no effect on the parameters that we looked at, this study does not represent an exhaustive examination of circadian and ageing related markers, and these need to be explored before concluding our intervention did not have any effects. In the future we hope to examine these other aspects, and investigate mechanistically whether we can alter the ageing process by manipulating the circadian system.

5 Discussion

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5.1 Main Findings

The main aim of this thesis is to investigate the role of light in the regulation of sleep and circadian rhythms. We used abnormal light cycles to induce circadian disruption and then measured its effects on sleep and circadian rhythms, using both behaviourally and EEG/EMG defined sleep.

5.1.1 Aim 1: Compare circadian disruption induced by different abnormal light cycles

Disruption of the circadian system is a commonly used method of investigating the importance of circadian rhythms in any given system. Circadian disruption has been used to study cognition, immunity, metabolism, cardiovascular function and cancer[6, 177, 210, 223, 224, 324]. However, the light cycles used to induce circadian disruption vary widely[223]. We therefore set out to compare the effect of commonly used light cycles on locomotor activity. We used Jetlag, constant light, dim light at night, and T20 light cycles, and we found that they differed on key markers of the circadian system. We found that the different light cycles differentially affected the distribution of activity and sleep across the day, and also differentially affected several commonly used markers of sleep and wake, but there was no clear evidence of fragmentation of either sleep or wake under any of these conditions. We suggest the effects we see are due to a combination of changes in the circadian system, the direct effects of light, and changes in arousal levels. Overall, constant light appeared the most disruptive, followed by T20, then DLAN, and Jetlag was the least disruptive. This mirrors the amount of light present during the subjective dark phase under each protocol which may be the key factor determining severity of disruption. Future studies could investigate the effect of nocturnal light, specifically at the start of the subjective dark phase, CT12-18, to test this hypothesis.

5.1.2 Aim 2: Investigate the effect of abnormal light cycles on sleep

Sleep is regulated through a complex interplay of circadian and homeostatic processes, as well as the direct effect of light. As constant light provides light at all phases of the circadian cycle, and we see it disrupts the circadian system, we asked how constant light affects the regulation of sleep. We showed that there is an increase of 1.5 hours of sleep per day in the first 2 days of exposure to constant light, comprised primarily of NREM sleep. This increased sleep was regulated homeostatically so animals dissipated the same total amount of sleep pressure over the day, and it had normal architecture. We suggest that given the size of the effect, the majority of the effect is likely due to a direct effect of light inducing sleep, similar to previous reports[156], while the remainder is likely due to disrupted SCN functioning[283, 284]. This effect does not fit in our current models of sleep wake regulation[288, 289]. Indeed, in both human and animal models sleep wake regulation has been described using a conceptual three-process model which incorporates the direct effects of light[357, 358], however they have not been incorporated into quantitative predictive models. This represents an interesting challenge for how to adapt our models in the future to capture the effects we see here.

5.1.3 Aim 3: Investigate the long term effects of circadian disruption

Ageing and circadian disruption share several common features, such as affecting cancer[324, 359], cardiovascular disease[328, 360], and mortality[312, 361]. Furthermore, ageing involves a breakdown in the circadian system[296]. Given this, we asked whether circadian disruption was causative to the changes seen in ageing. We looked at the organisation of locomotor activity of mice after prolonged abnormal light cycles, and saw no effect of long term circadian disruption. This is likely due to our choice of looking at locomotor activity, which while a useful marker of both the circadian system and ageing is not a comprehensive assessment of either system. The effects of age on circadian activity are highly variable between

individual animals. We group housed our mice during ageing due to practical limitations, however this meant we were unable to trace individual trajectories of ageing changes, limiting our findings. Our results do not prove that these light cycles do not have any effect, and a more thorough investigation of both circadian and ageing systems is required before we can draw firm conclusions on the effect of long term abnormal light cycles on ageing.

5.2 The importance of the circadian system

The rotation of our planet on its axis provides a rhythmic 24 hour environment for the majority of life on Earth[362]. The central question of circadian science is to understand how organisms cope with this rhythmic environment. Circadian clocks have been demonstrated in both prokaryotes and eukaryotes, which consist of a molecular negative feedback loop that takes 24 hours to complete[17, 363]. In mammals, the circadian system is organised into a heirachy of tissue oscillators, orchestrated by the master pacemaker in the SCN, which is entrained to the environment by light from the eyes[68]. A circadian system appropriately aligned to the environment gives greater fitness to the organism[25, 26, 181]. However what is the point of having a heirachy of oscillators instead of a single clock that entraines the entire body? It is likely that it allows integration of tissue-specific signals while maintaining stability of the network against sudden changes[68, 364].

Most aspects of physiology show a circadian rhythm[19]. Moreover, one of the most fundamental aspects of the human experience follows a circadian rhythm, that is daily patterns of sleep and wakefulness that supports our conscious experience. The daily cycle of sleep and wakefulness may even be a fundamental feature of any sufficiently complex organism[86, 89]. Beyond these basic scientific questions, this is an important field to study as it has been subject to major changes in the last few hundred years. Invention of electric lighting extended light from being just during the solar day to all times of day, and this has had major effects on the organisation of our social and work lives[365, 366]. This has led to widespread circadian disruption, with many negative effects including metabolic

and cardiovascular disease [6], as well as increased risk of cancer[321]. Furthermore, ageing also leads to breakdown of circadian rhythms[305], and given the dramatic increase in the aged population[367, 368], this will be an increasingly important area in the future. Therefore, developing strategies to mitigate the negative effects of disrupted circadian systems is crucially important.

There has been widespread interest in the circadian system, and many studies have tried to disrupt it to investigate the mechanisms and effect it has. Since light is the main input to the circadian clock, many studies use abnormal light cycles to induce circadian disruption[223]. We therefore used a variety of abnormal light cycles to study the effects of these disruptive light cycles on sleep and circadian rhythms. Our results showed that the extent of disruption to locomotor activity was related to the amount of light at night, that sleep is induced likely directly by light, and that locomotor activity is normal after a short recovery from prolonged circadian disruption. The effects of any abnormal light cycle are due to a combination of their effects on the circadian system, and the direct effect of light at abnormal times of day. Our results suggest that the contribution of the circadian system to the changes seen under these abnormal light cycles are smaller than previously thought, and the direct of effects of light are more important.

Previous studies have shown that abnormal light cycles disrupt the circadian system[70, 190, 210]. We have shown that these abnormal light cycles also alter locomotor activity and sleep. The combination of direct effects of light and circadian effects on this are unkown. What is known about the effect on activity and sleep of disrupting the circadian system directly, without any light confounds? *Cry1/2* $^{-/-}$ and *Bmal1*$^{-/-}$ deficient mice are completely arrhythmic under constant conditions, and show increased sleep of 1.5hrs NREM per day[369, 370]. A disruptive phase shift in hamsters also renders them completely arrhythmic regardless of the light environment, and they get 1.5hrs more NREM sleep over 24 hours as well, though the quantification in the report came from LD conditions, they show no difference in DD conditions but do not quantify sleep under DD[140]. SCN lesions produce arrhytmicity in nocturnal rodents, but do not appear to increase time asleep[145].

All of these conditions come from animals that are completely arrhythmic, which models a loss of circadian rhythms, rather than a disruption of them. Other mouse models of altered circadian genes, which maintain rhythmicity, show either normal or reduced levels of sleep over 24 hours in constant conditions[134]. Interestingly, when tissue specific genetic techniques were used to create animals with a different period in the SCN to the rest of the body, there appeared to be minimal effects on the profile of mean daily activity[371]. We conclude that there is not good evidence currently available on the effect of directly disrupting the circadian system on locomotor activity and sleep, separately to the effects of light. Conversely, there is a history of evidence on the direct effects of light [147, 151], and recent evidence indicates the acute and circadian effects are mediated by separate pathways[148].

The effect of long-term circadian disruption in rodents is unknown, with a single report demonstrating cumulative shifts of the lights were protective against memory deficits, but provided no data on the effect on activity and sleep[20]. This is in contrast to humans where the negative effects of shift work are cumulative[12]. We are unaware of any detailed study on the recovery of locomotor activity and sleep from circadian disruption in rodents. What does this say about the resilience of the circadian system? Our results indicate that 14 days of a 12:12 LD cycle is sufficient to recover from a year of abnormal light cycles. In hamsters, two sequential light pulses are enough to cause permanent arrhythmia[221], though this effect has not been replicated in mice, suggesting the murine circadian system is more robust. In animals with a different period in parts of the SCN, the majority of animals were able to entrain to a 12:12 LD cycle [371], indicating the flexibility of the circadian system to disruption of a single component. Indeed, in animals with SCN lesions, circadian rhythms can be observed in response to both timed food availability[372, 373], and timed methamphetamine injection[374]. Furthermore, presence of *Bmal1* in skeletal muscle is sufficient to restore circadian rhythms of sleep and wakefulness[375]. Together, these show that the circadian system is robust to several major insults, and suggest that the network of peripheral oscillators provides

protection against these insults. Our results agree with this by suggesting that even after long-term abnormal light cycles, locomotor activity rhythms are not disrupted.

5.3 Future directions

The major challenge facing work in the circadian field is the close interaction between the direct and circadian effects of light. This complicates the interpretation of almost any study performed using light. To advance the field, we need to address this interrelation. This has historically been difficult given that our main method of disrupting the circadian system was using light. However, new techniques are now available which allow tissue and time restricted expression of genetic manipulations[376]. This has already been used to insert mutations in circadian genes into specific tissues such as the master clock [371]. Furthermore, these techniques also allow expression of optogenetic and chemogenetic ion channels which can be used to modify the behaviour of cells directly[153, 377]. This has been used to investigate the effects of manipulating SCN firing directly on activity[283, 284].

Here we suggest ways of addressing the interrelation of lights effects. Firstly, we can hold the effects of light constant while disrupting the circadian system to see what the contribution of the circadian system is to these effects. We would want to investigate the effects of disruptive input, the disrupted system itself, and disrupted outputs. To look at inputs, we could stimulate the retinal circuitry which only mediates the circadian effects of light in constant dark at different times of day, mimicing abnormal light cycles used elsewhere, to see what the circadian contribution are to these effects [148]. To examine the effects of a disrupted SCN, we could use optogenetics to mimic the effect of LL on the SCN under constant darkness[190, 283, 284]. To examine the outputs of a disrupted SCN system is more difficult, as these are more diffuse[68]. Instead, one approach to this question would be to disrupt important peripheral clocks, either optogenetically or via circadian gene effects, and observe the effects[375].

Next, we would aim to hold the circadian system constant while altering the effects of light. This is more difficult as holding the circadian system constant is

more of a challenge than removing light. Furthermore, the pathways mediating the various direct effects of light are not well defined. We would aim to stimulate the pathways that mediate the acute effects of light, in constant darkness, such as by using *Brn3b* mice[148]. We could use optogenetics to stimulate them in constant darkness, or use mice that have the remaining photoreceptor pathways removed, such as crossing with *rd/rd cl* mice, and use light.

These experiments are still prone to some interaction of the direct and circadian effects. For example, firing in the SCN is also dependent on the behavioural state of the animal[286], therefore light directly inducing sleep via circadian independent pathways will still alter SCN firing if it induces sleep. However, the experiments suggested here would help clarify some of the differences between circadian and direct effects of light.

Finally, we need further modelling studies to quantify the effects of light on sleep and circadian rhythms. The phase response curve of nocturnal mice is well described[282], and the effect of light on inducing sleep has also been described previously[151]. It would be of interest to combine these two effects to see if the effects of abnormal light cycles fit within our current understanding of phase shifts and masking. Any residual errors not explained by this model may reasonably be ascribed to circadian effects. Furthermore, while we have highly accurate quantitative predictions of slow wave activity during sleep from two-process models[288], these models do not predict state or state transitions, though work has moved to incorporate this[289]. These models do not capture the effects of light on inducing sleep [151], and it would be of major interest to see whether we could incorporate these effects into these models.

5.4 Overall conclusions

Here we use a range of abnormal light cycles to examine the effect of light on locomotor activity and sleep. We show that a range of abnormal light cycles disrupt the normal rhythms of locomotor activity and sleep. Furthermore, the extent of the disruption appeared related to the amount of light occuring during the subjective

dark phase. Our results also indicate that constant light induces sleep, likely mostly due to direct effects of light. Finally our results show that there were no long-term effects of prolonged circadian disruption on locomotor activity. We suggest that although abnormal light cycles all have important effects on the circadian system, most of the effects we see here are due to direct effects of light. We suggest a range of experiments to hold either the circadian effect or light effect constant to investigate the extent to which either of these processes contribute to the effects we see. We suggest that we model the effects we see here using our knowledge of phase shifts, masking, and sleep regulation, to investigate where these models fail as an indicator of where we are missing information. Finally, we suggest that, as a field, circadian biology needs to take more account of direct effects of light on the effects we see, and ascribing the effects of abnormal light cycles to the circadian system without further investigation of the contribution of direct effects of light may be premature. This is crucial as we develop strategies to mitigate the effects of circadian disruption in widespread conditions that we face as a society today, including shift work, social jetlag, and ageing.

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