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IT'S ABOUT A DAY

The Effect of Glucocorticoids on Shifting and Re-entraining the Circadian Rhythm in Peripheral Cells: A Review and Meta-Analysis

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Anton Degerfeldt

Supervisor: Oskar MacGregor
Examiner: Katja Valli

Abstract

The circadian rhythm is a rhythm which permeates all aspects of biological life and follows the hours of the sun. The pace of the rhythm is controlled by a collection of neurons in the hypothalamus, called the suprachiasmatic nucleus (SCN), whose signals affect rhythms throughout the body as can be seen in aspects of life from behavior down to oscillations of proteins in the cells. A disruption of this rhythm such as what happens during jet lag, where the rhythm of the SCN is out of synch with the rhythm of the rest of the body, is something that can have adverse effects on mental and physical health. To realign the SCN and the rhythm of the body, different methods and be implemented. This thesis investigated the effectiveness of glucocorticoids on re-aligning the rhythms of the body following a disruption through a meta-analysis and a qualitative review. The meta-analysis and review incorporated experiments from six articles investigating the hours of circadian rhythm shifts in the mouse model, after administering glucocorticoids. What was found was that the individual experiments presented results with high effect sizes; however, the direction of said effects was not uniform as the rhythms shifted in different directions. The lack of uniform direction caused no significant combined effect size to be found by this meta-analysis (MES=0.11 ± 0.06), showing that a statistical analysis based on hours shifted could not find a significant combined effect. The qualitative review, however, indicates that the administration of glucocorticoids shows an effect in re-entraining the rhythm of the peripheral parts of the body to that of the environmental cues and the SCN. Though no significant statistical effect was found in this analysis, the effect of glucocorticoids should not be discounted and could still prove a promising treatment for circadian disruptions, such as jet lag.

Keywords: *Circadian rhythm, glucocorticoids, endocrine system, circadian disruption, jet lag*

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1. Introduction

Most people have at some point in their life experienced the side effects which follow, for example, staying up too late one night finishing a paper which is due the next day. These side effects often make one feel physically weak and slow and can make one easily irritated and in a generally bad mood. Perhaps also leading to the feeling of having a hard time concentrating or more easily forget to do simple but essential things such as attaching the paper to the submission email. During these dreadful days, these symptoms are often combated through the use of things like coffee or other stimulants, which may increase alertness to some degree and perhaps make it slightly easier to keep through the day. But why do these side effects appear following events like a late night or a long flight, for example, and what are the effects on the biological and psychological processes? Furthermore, are there perhaps better ways to combat the side effects and perhaps even return to a state of normal functioning after shifting the diurnal rhythm other than drinking large amounts of coffee?

Biological life is constantly adapting and evolving alongside the environmental factors surrounding it. One such environmental factor is the rotation of the earth, creating the cycles of day and night. This environmental factor is something both prokaryotic and eukaryotic organisms have been found to adapt to as they seem to be following a cyclical behavioral pattern attuned to the rotation of the earth. This cyclical rhythm is called the *circadian rhythm* (meaning "approximately a day" in Latin).

The circadian rhythm permeates all aspects of daily life, in a way it defines daily life, as without it "daily" would not exist. It controls things such as the sleep-wake cycle, fasting-feeding rhythm, as well as the immune system and the best and worst hours of cognitive

performance. The disruption of this rhythm through, for example, jet lag, shift work or working late nights in order to finish a thesis can have detrimental effects on various aspects of physical and mental health, as well as increased mortality rates in populations with disrupted rhythms (Karatsoreos, 2012). Reliable treatments for re-alignment of the circadian rhythm in those with disrupted rhythms are few and far between. However, the need for one is great and increasing, as society shifts from following sun hours to follow social hours instead, keeping activity up during naturally dark hours through the use of artificial lights (Roenneberg, Kumar, & Merrow, 2007).

Current treatments and different methods used, following a circadian disruption, whether it is acute circadian disruption such as jet lag after flying long distances, or perhaps chronic disruption such as what a shift worker may experience, often only focus on treating the behavioral symptoms. These are, for example, coffee or other stimulants such as modafinil and armodafinil which combat the drowsiness by directly stimulating and increasing arousal (Rosenberg et al., 2010; Zee & Goldstein, 2010). However, these methods only combat some of the symptoms and not the underlying causes that follow due to the rhythm disruption. Other methods also show promising results, such as mediating the sleep-wake cycle through specific wavelengths of light and time-dependent melatonin administration. These methods can then shift the sleep-wake cycle to follow the new rhythm more quickly and therefore may aid in re-aligning the rhythm (Laste et al., 2013; Lockley, 2000; Lockley, Brainard, & Czeisler, 2003).

However, methods focusing on the treatment of circadian disruption by re-aligning the rhythms of the body directly are not yet too common. There are some methods found to affect the rhythms of the body directly, such as modulation of the hormones involved with the circadian rhythms in some way (Morris, Aeschbach, & Scheer, 2012), for example, melatonin (Laste et al., 2013; Lockley, 2000). The hormone glucocorticoid has also been found to be a

promising candidate for this sort of treatment as animal studies have found that the hormone can influence the body's rhythms by shifting it without altering the length of the rhythm (Balsalobre, 2000; Cheon, Park, Cho, & Kim, 2013).

The subject under review in this thesis is, the current understanding of the system maintaining and regulating the circadian rhythms throughout different parts of the body. The consequences of disrupting these rhythms on behavior, physiology and mental health will also be outlined. Furthermore, though the research surrounding re-alignment of the circadian rhythm throughout the body after a disruption has progressed and research on the topic is being conducted, there is a lack of reviews and analyses on the topic. Therefore, a text presenting an overview of the subject would be beneficial in order to investigate whether the different studies produce similar results on the re-alignment of the circadian rhythm. In that vein, a meta-analysis of current research looking into methods for re-aligning the disrupted circadian rhythm throughout the body will be conducted. The meta-analysis will analyze the current research in the field of circadian rhythms and the effects of circadian re-alignment methods. Specifically investigating the effect modulating the hormonal system, particularly, the hormone glucocorticoid has on the re-alignment of the circadian rhythm throughout the body and the possible use of glucocorticoids for treatment of the circadian disruption.

2. The Circadian Rhythm

The circadian rhythms of biological organisms were known of for a long time as it was recorded around the fourth century BC that Androsthenes, a sea captain of Alexander the great, observed daily movements of the tamarind tree. Similar findings were also reported in human physiology as Galen and Hippocrates reported observing a 24-hour rhythm in fever (Foster & Kreitzman, 2013). However, what these rhythms were and how important and pervading they were in all aspects of life was for a long time poorly understood. That is until Jean Jacques d'Ortous de Mairan, a French astronomer, performed studies on plants in 1729

(Braunwald, 2012; Foster & Kreitzman, 2013). He noticed that the Mimosa plant, which opened its leaves when exposed to sunlight, also opened and closed with the cycle of each day, opening in the morning and closing it at night. He then placed these plants in a cupboard, keeping them in constant darkness and found that they still exhibited these rhythms of the leaves opening and closing, in the absence of sunlight. What he uncovered was an internal mechanism which anticipates the rhythm of the sun even in the absence of sunlight (Braunwald, 2012; Foster & Kreitzman, 2013).

Research in more recent years have come further in understanding the underlying mechanisms of these rhythms, what cues entrain these rhythms, what could lead to a disruption of the circadian rhythms and what the effects of disrupting the rhythms could have on things like physical health, well-being and mental illness (Karatsoreos, 2012; Moore, 1997; Scheiermann, Kunisaki, & Frenette, 2013).

This research is conducted on several levels such as the analyses of behavior such as sleep and wake cycles, food-seeking behavior in animals as well as cognitive performance (Blatter & Cajochen, 2007; Dijk, Duffy, & Czeisler, 1992). Physiological changes at different times a day such as body temperature (Moore, 1997; Roenneberg et al., 2007) and metabolic activity (Bass, 2012; Karatsoreos, Bhagat, Bloss, Morrison, & McEwen, 2011) are also being studied. Studies are also being performed at the cellular level, analyzing the active neural structures such as the neurons in the *suprachiasmatic nucleus* (SCN) which are seemingly responsible for aligning the cells throughout the body to the circadian rhythms (Hurd & Ralph, 1998; Johnson, Moore, & Morin, 1988). Furthermore, how and why these structures activate and how they react to varying environmental cues is also being investigated (Dibner, Schibler, & Albrecht, 2010; Duffy & Wright, 2005). Studies are also investigating how the neurons in SCN vary in their spontaneous firing patterns between day and night (Nakamura, Honma, Shirakawa, & Honma, 2002). The mechanisms within the cells such as

the protein oscillations and gene transcriptions have also been thoroughly studied, unveiling a self-regulating timekeeping system within the cells, which creates a system of *cell clocks* throughout the body that subserves the circadian rhythm (Darlington, 1998).

The research on the underlying genetic and cellular mechanisms, mainly conducted on the fruit fly *Drosophila melanogaster* have made substantial progress in understanding the mechanisms of the circadian rhythms. The research on the fruit fly's cellular oscillations has even awarded the researchers, Jeffery Hall, Michael Rosbash, and Michael Young the Nobel Prize in Physiology and Medicine in 2017 for their findings of this self-regulating system within the cells and its mechanisms, active genes, and proteins, which will be outlined further below.

The combination of the different levels of research has yielded promising results as our understanding of the workings of the circadian system and the consequences following disruption of the rhythms. However, not everything regarding the circadian rhythms has been understood, for example, how and why the spontaneous firing of SCN neurons follows the circadian rhythm so reliably (Colwell, 2011), and how the cells throughout the body are realigned to the circadian rhythm after it being disrupted (Karatsoreos, 2012).

3. Entrainment and Disruption

All the cells throughout the body are mainly aligned to one single rhythm, following the rhythm of sun hours and rotation of the earth (Roenneberg et al., 2007), the way the body aligns to these rhythms is through information from external cues. These external cues entraining the body to the circadian rhythm are called *zeitgebers*, and include factors such as, light, temperature and feeding timings (Duffy & Wright, 2005; Husse, Eichele, & Oster, 2015; Lockley et al., 2003; Moore, 1997; Tahara, Aoyama, & Shibata, 2017).

It was long thought that social cues were the primary zeitgeber affecting human circadian rhythms. The social cues thought enough for entrainment being things like simply

knowledge of the time of day and social communication. This was found through studies investigating the circadian rhythms of participants in environments without the natural light-dark cycles. This was investigated by keeping participants in a time isolated environment for four days. Time isolated environment meaning, they were either kept in constant darkness for four days or an artificial light-dark cycle. Participants were, in both conditions, still deprived of natural sunlight but participants in the light-dark cycle condition were kept in an environment with artificial lights turned on from 7:30 to 23:30, thereby simulating the natural light cycle. (Aschoff et al., 1971). It was then found that the circadian rhythm was largely maintained despite the absence of sunlight and a natural light-dark cycle. As the participants were not isolated from social cues as they still received meals at set times brought in by the experimenters and in the artificial lighting experiment, the time was also indicated through the lighting hours. These findings led to the conclusion that social cues were the primary zeitgeber and as such contributed to that theory of the importance of social cues in entrainment (Aschoff et al., 1971; Moore, 1997).

However, the theory that social cues are the primary zeitgeber has since been refuted (Duffy & Wright, 2005; Husse, Leliavski, Tsang, Oster, & Eichele, 2014; Johnson et al., 1988; Roenneberg et al., 2007). Though social cues do affect entrainment to the circadian rhythm to some degree, it has been found that the circadian rhythm is maintained mainly through exposure to natural light cycles (Johnson et al., 1988; S. W. Lockley et al., 2003; Roenneberg et al., 2007). The importance of natural light can be seen in studies where adjusting the light cycle for animals by administering light during the natural dark hours resulted in a shift of their circadian rhythm as if it was a lengthening of the day (Kiessling, Sollars, & Pickard, 2014; Moore, 1997). It can also be seen when observing circadian rhythm in relation to the social time society follow through social cues, as well as the natural light-dark cycles. When looking at this, studies have found that the social cues are not enough to

entrain our circadian rhythm and we instead tend to follow our free-running rhythm entrained to the sun time of the area, meaning it also varies slightly depending on the part of the world one is in as sun hours differ (Roenneberg et al., 2007).

The importance of the sun time and light can be seen when observing the disruption of the circadian rhythm during jet lag or shift work, and the effects of a disrupted circadian rhythm on psychology and physiology. During jet lag, you are exposed to similar external factors as the animals studied during adjusted light cycles, as you are exposed to light during your subjective dark cycle. This shift in light exposure disrupts the circadian rhythm of the body due to the difference between the central and peripheral clocks when resetting to a new rhythm following a shift in external factors, such as the shift in light exposure when traveling to new time zones (Karatsoreos, 2012). The adverse effects of jet lag occur because the central pacemaker, SCN, adjusts instantly to new conditions as it is entrained by the zeitgebers, but the peripheral clocks take longer to adjust (Karatsoreos, 2012), causing your body to follow a different rhythm than the external cues and the central pacemaker.

A somewhat different process to the one outlined above is underlying the effects on an individual during shift work. During shift work, workers are not following the rhythm of the natural sun hours and are most likely often shifting your sleep-wake cycles to fit the required working hours. The circadian rhythm is therefore, constantly disrupted by not following the entraining cues of sun hours and natural light exposure. These disruptions of the circadian rhythm and misalignment of the biological clocks has been found to have possibly detrimental effects on physical and mental health as well as things like cognitive performance and life span (Blatter & Cajochen, 2007; Cho, 2001; Cho, Ennaceur, Cole, & Suh, 2000; Dijk et al., 1992; Hurd & Ralph, 1998; Reinberg & Ashkenazi, 2008).

Studies investigating the physiological effects of circadian rhythm disruption have found the circadian rhythm to be strongly connected to the fasting-feeding rhythm (Bass,

2012). It has been found that disruption of the rhythm strongly impacts metabolism as studies investigating the health of shift workers have found that they have an increased risk of obesity (Reinberg & Ashkenazi, 2008). It has also been found to be connected to metabolism linked diseases such as diabetes (Bass, 2012) as the circadian rhythm has also been found to vary the insulin levels in the body (Karatsoreos, 2012). Further studies on mice have found that disruption of circadian rhythm through genetic manipulation, sleep deprivation and entrainment disruption has led to increased weight gain, both diet dependent as well as non-diet dependent weight gain (Karatsoreos, 2012; Landgraf et al., 2016).

The detrimental effects of circadian disruption can also be seen in relation to cognitive performance (Cho et al., 2000; Dijk et al., 1992; Moore, Speh, & Leak, 2002). The effects of circadian disruption on cognitive performance have most likely been experienced by most people at some point. When experiencing jet lag, working shifts, or have a few sleepless nights for some other reason, simple and essential things such as maintaining focus on a task, for example, may be more difficult (Moore, 1997). The effects on work performance due to the supposed drop in cognitive performance can also be seen when looking at accidents during shift work. As many of the major industrial disasters such as the nuclear powerplant accident in Chernobyl and the Exxon Valdez catastrophe in Alaska, USA have been found to have occurred during the night shift (Karatsoreos, 2012), there seems to be an adverse effect of shift work which is largely overlooked. However, cognitive performance during circadian disruption is largely dependent on the demands of the task performed (Karatsoreos, 2012) as well as the difference between individuals' tolerance to shift work (Reinberg & Ashkenazi, 2008).

Findings of the effects of circadian rhythm disruption have also been made in airline staff as they suffer chronic jet lag due to their constant travels (Cho, 2001; Cho et al., 2000). A study conducted by Cho (2001) investigated the neurological effects of chronic jet

lag in airline staff. The study found the airline staff to have impaired hippocampal neurogenesis as the body clocks were realigning themselves during jet lag. It also found this impaired neurogenesis to continue to be impaired well after the immediate effects of jet lag had seceded. This impairment could lead to deficits in special memory and cognition for both long-term and short-term tests. These lasting effects after the circadian disruption have seceded also found support in a recent study which found that catching up with sleep retroactively after insufficient sleep does not negate the adverse effects of the sleep-wake disruption in the first place (Depner et al., 2019). This study found that metabolic dysregulation due to insufficient sleep during workweeks cannot be negated by recovering the sleep lost during the week in the weekend.

The circadian rhythm has also been found to have lasting effects on mental health as disruption of the rhythm has been found to be linked to mood disorders (Karatsoreos, 2012; Lockley, Dijk, Kosti, Skene, & Arendt, 2008; Monk, Fookson, Moline, & Pollak, 1985; Wirz-Justice, 2008), behaviors linked to major depressive disorder and suicide ideation (Bahk, Han, & Lee, 2014; Landgraf et al., 2016). These effects on mental health have been shown in animal studies through genetic disruption of the circadian rhythm which showed an increase in behavior associated with depressive and other mood disorders such as helplessness and anxious behavior (Bahk et al., 2014; Landgraf et al., 2016). Similar findings have been made in studies of humans which showed an increased risk of mood disorders in shift workers (Karatsoreos, 2012; Karatsoreos et al., 2011; Lockley et al., 2008). Depression and major depressive disorder also seem to have a two-way causal relationship with circadian rhythm disruption (Karatsoreos, 2012). Disruption of the circadian rhythm has been found to lead to an increased risk of mood disorders and depression (Karatsoreos et al., 2011; Landgraf et al., 2016; Lockley et al., 2008). Furthermore, the inverse relationship has been shown as mood disorders and depressive disorders are often accompanied by disruptions to the

biological rhythms such as sleep-wake cycle and fasting-feeding rhythm (Bahk et al., 2014; Wirz-Justice, 2008).

4. The central pacemaker

The entraining elements or, zeitgebers are external elements which aligns the cells and their oscillating clock-mechanisms throughout the body to one central rhythm. However, what is it that is entrained by these zeitgebers, what is the underlying mechanisms controlling the circadian rhythms of the cells throughout the body? Studies of the biology and neural correlates of the circadian rhythms have found a central mechanism for regulating the peripheral cell clocks to the circadian rhythm in mammals to be in the SCN. The SCN is a bilaterally located set of nuclei positioned just atop the optic chiasm in the anterior basal hypothalamus (Figure 1) (Inouye & Shibata, 1994). It has been found that it is in large part due to the regulating activity of the neurons in the SCN that the circadian rhythm is entrained to light cycles, environmental temperature and other zeitgebers as it receives information regarding external stimuli and regulates the cell clocks throughout the body (Colwell, 2011; Dibner et al., 2010; Moore, 1997).

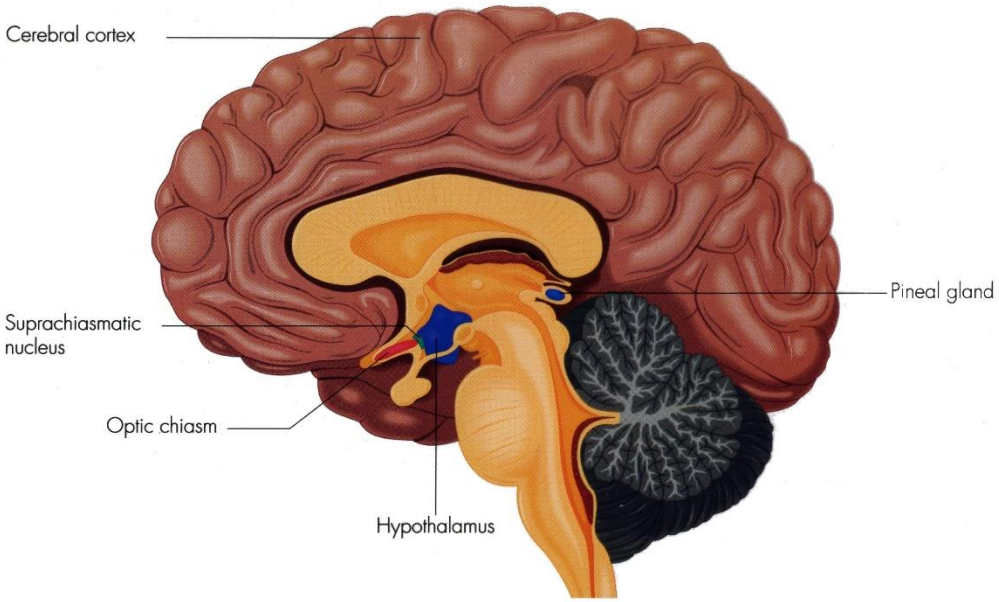

Figure 1: Image displaying the SCN located atop the optic chiasm in the hypothalamus.

From 黃雨伞 (https://commons.wikimedia.org/wiki/File:Suprachiasmatic_Nucleus.jpg), "Suprachiasmatic Nucleus", https://creativecommons.org/licenses/by-sa/3.0/legalcode

The importance of the SCN for alignment of the peripheral clocks throughout the body has been made clear in studies investigating the SCN in hamsters. In a study by Ralph, Foster, Davis, and Menaker (1990) the SCN of mutant hamsters with a short circadian rhythm was transplanted into hamsters in which the SCN had been ablated, causing them to have arrhythmic circadian oscillation. After the transplantation, the arrhythmic hamsters instead began following the short circadian rhythm of the donor hamsters (Ralph et al., 1990). These findings indicated that the SCN, which had before been thought to be a mere relay station for the zeitgebers was more like the central pacemaker of the body's circadian rhythm.

The SCN has been found to receive information of external time cues such as light and temperature primarily through three different pathways (Colwell, 2011; Dibner et al., 2010). The first of the three pathways projecting into the SCN being the geniculohypothalamic tract from the intergeniculate leaflet of the lateral geniculate body. This is a photic pathway relaying visual and photic information to the SCN, enabling entrainment to light as the lateral geniculate body from where it projects, is a place where retinal ganglion cells densely project (Colwell, 2011; Inouye & Shibata, 1994).

Another pathway is the serotonergic projections from the raphe nuclei into the SCN, which has been found to regulate locomotor activity in mammals (Fuller, Gooley, & Saper, 2006; Moore, 1997).

The third and possibly most crucial pathway that projects external info into the SCN is the retinohypothalamic tract. The retinohypothalamic tract, as the name suggests, is a direct connection from the retina into the hypothalamic areas, where the SCN are located (Colwell, 2011; Inouye & Shibata, 1994; Moore, 1997). This tract, though it runs from a few select photoreceptors in the eye, is not relevant for vision and is a separate pathway to the conscious visual information. It is however essential for circadian entrainment as shown in a study by Johnson et al. (1988) where the retinohypothalamic pathway in hamsters was

lesioned. This study found the hamsters' vision to be seemingly unchanged and normal behavior regarding vision was retained after the lesion. However, the circadian rhythm of the hamsters was disrupted, and they were unable to entrain their circadian rhythm to the environmental light cues (Johnson et al., 1988). Other studies have also found blind subjects with no residual vision and an intact retinohypothalamic pathway to still retain their circadian rhythm seemingly through entrainment from light cues (Moore, 1973).

Though the circadian system exists throughout the entire body through the peripheral clocks, and a circadian clock mechanism exists in most cells, the SCN is essential for regulating these peripheral clocks in the body. The SCN is what receives the information from the zeitgebers and projects the information to the rest of the body and modulates their oscillations (Colwell, 2011). The SCN is comprised of neurons which all exhibit circadian rhythms with different periods. However, what differs the neurons in the SCN from other peripheral clock cells is the superior stability of the SCN neurons in retaining their free-running rhythm to a rhythm of about 24 hours, which can be seen when studying SCN neurons in vitro (Nakamura et al., 2002). Even when in slice cultures, the SCN neurons exhibit these stable self-sustained electrical oscillations with peaks in spontaneous discharges during mid-day and inactivity during the subjective night (Dibner et al., 2010). These peaks always occur during mid-day, regardless of whether the animal is diurnal or nocturnal (Colwell, 2011). This firing pattern seems to be due to the SCN neurons being more depolarized during mid-day, increasing the chance of spontaneous firing.

5. The Circadian Rhythm of the Cells

Though it is not the primary focus of this paper, the molecular oscillations of the peripheral and central cells which exhibit circadian rhythmicity will now be briefly outlined. This section is simply included for the sake of understanding some concepts presented in the paper as well as understanding how the cell clocks and their oscillations work.

The research of the underlying cellular mechanisms has, as mentioned above, awarded the researchers Hall, Young and Rosbash with a Nobel Prize for their findings of the self-regulating molecular timekeeping oscillations within the cells. This research has mainly been performed on non-human subjects and cells as the model used in this research has mostly been the fruit fly. However, this research has been found to translate well into mammalian and other models; these findings were also corroborated with further studies on mammalian cells (Moore, 1973).

The circadian system within the cells throughout the body, exhibiting rhythmicity (*clock cells*), is a self-regulating time-keeping system which follows a rhythm of approximately 24h. The system relies upon the oscillations of several different proteins being expressed within the cells (Inouye & Shibata, 1994; Nakamura et al., 2002; Price et al., 1998). These proteins are gene products, transcribed (transcription is the process where information in DNA, such as in a gene is copied into a new messenger molecule, *RNA* or messenger RNA (mRNA), which then creates a product of the gene which can affect the biological system) from a group of genes, called, *clock genes*. The primary gene product oscillating in the cells is called *per* (Benedetti et al., 2007; Darlington, 1998; Inouye & Shibata, 1994). Per is transcribed and builds up in the cells during the day and clears away during sleep and specific times during the day (Darlington, 1998). Before the protein begins to be cleared from the cells the production of per stops as it reaches a certain concentration, thereby creating the self-regulating feedback loop within the cells (Darlington, 1998; Price et al., 1998).

Further studies have found several important molecules involved in the circadian oscillation of the cells. One of these is a product of Period transcription called *BMal1*; it is found to have an important role in circadian rhythmicity as it is disruption of BMal1 which has been found in many problems following circadian disruption such as

diabetes and mood disorders (Landgraf et al., 2016). It also showed an increase in major depressive disorder behaviors such as helplessness and anxiety during high-stress situations.

These genes and gene products are what will be referred to further in the thesis, specifically focusing on the gene products of period 1 and 2; BMal1, per1 and per2.

6. The Endocrine System

As mentioned above, the circadian rhythms of our bodies are entrained by external factors such as light, temperature and feeding, it aligns the mechanisms throughout the body to one central rhythm through modulation by the SCN (Colwell, 2011; Welsh, Takahashi, & Kay, 2010). The SCN has been reliably found to be the central pacemaker of the body through studies where the SCN was lesioned or transplanted which showed the SCN not just to be a relay for external stimuli but the central pacemaker (Hurd & Ralph, 1998; Johnson et al., 1988). However, how the SCN mediates the rhythms of the peripheral clocks is not entirely agreed upon (Karatsoreos, 2012). Though, the endocrine system is believed to be a primary actor as hormones under SCN control have been found to affect the entrainment of peripheral cell clocks (Morris et al., 2012).

The endocrine system has been identified as involved with the rhythmicity of the body as the hormones have been found to oscillate along with the circadian rhythm. The hormones do this, either by entrainment through light and modulation by the SCN or, by following the sleep-wake cycle and the fasting-feeding rhythm (Hastings, O'Neill, & Maywood, 2007; Morris et al., 2012). The hormones found to be involved in the circadian system are hormones found to oscillate along with the circadian rhythm of the individual, either seemingly modulating the circadian rhythm through the hormonal oscillations or hormones disrupted by circadian disruption (Morris et al., 2012). The primary hormones found to be involved with the circadian rhythms are hormones under control of the SCN and seem to be involved in controlling peripheral clocks in the body. These primary hormones which will be outlined

below are glucocorticoid and melatonin (Balsalobre, 2000; Barclay, Husse, & Oster, 2011; Laste et al., 2013; Nicolaides, Charmandari, Chrousos, & Kino, 2014).

6.1 Glucocorticoid

Glucocorticoid, which is a steroid hormone that is secreted by the adrenal gland and oscillates along with the circadian rhythm, has been found to be an integral part of the endocrine system controlling circadian rhythmicity. The role of glucocorticoids has been found through studies showing that they are important for modulation of both the central cell clocks in the SCN as well as controlling the circadian oscillations in the peripheral cell clocks (Morris et al., 2012).

A study by Le Minh, Damiola, Tronche, Schütz and Schibler (2001) investigated food-induced circadian phase-shifting in rats as they shifted their feeding schedule from their regular nocturnal feeding to daytime feeding. This shift in feeding rhythm of rats induces a slow shift in circadian rhythm, similar to the light-induced shift we can experience during jet lag. Just as during jet lag, the disruption to the circadian rhythm occurs because the SCN and the peripheral clocks are misaligned. The peripheral cell clocks take longer to realign to the new phase provided by the zeitgebers. What was found by Le Minh et al. (2001) was that the misalignment of the SCN and the peripheral clocks was in part due to the glucocorticoid signaling. They found that glucocorticoid helps modulate the circadian rhythm as stimulation of glucocorticoid receptors can induce or inhibit circadian phase-shift. The modulation of glucocorticoids causes a misalignment of the central and peripheral cell clocks as it only affects cells with glucocorticoid receptors, such as the cells in the liver. However, the neurons in SCN does not have glucocorticoid receptors. Therefore, the SCN can reset to follow the zeitgebers almost instantly while the peripheral cells take longer due to the glucocorticoids inhibiting the phase-shift (Balsalobre, 2000; Le Minh et al., 2001).

The stability provided by glucocorticoids shows how important signaling is to the circadian rhythm. Though the SCN is the central pacemaker, the peripheral cells have also been found to be able to entrain to zeitgebers without direct modulation by the SCN (Husse et al., 2015, 2014; Le Minh et al., 2001). Therefore, the inhibitory effect glucocorticoids can have on peripheral cells stabilizes the oscillations of the cells to one central circadian rhythm. However, as the stability it provides does not apply to the SCN, misalignment during zeitgeber phase-shift does occur.

Glucocorticoids have also been found to be a promising candidate for a method to help re-entrain the circadian rhythm of the peripheral cells during disruption such as jet lag to the central pacemaker. Studies have found it aided in recovery after jet lag by decreasing the number of days needed to recover from jet lag (Kiessling, Eichele, & Oster, 2010).

6.2 Melatonin

Though the glucocorticoids are an essential hormone signaling system for the circadian rhythm, it is, as mentioned, not the only important hormone. Melatonin is a hormone that modulates the sleep-wake cycles as it is the hormone that induces the feeling of drowsiness and being tired. It was thought that melatonin was built up and secreted during sleep (Hastings et al., 2007). However, it has been found that melatonin is not as closely connected to sleep as it is with the circadian rhythm (Morris et al., 2012). Although melatonin levels are high during the night, the melatonin oscillation instead follows the subjective day and night with levels peaking during the subjective night and being at its lowest during subjective day (Morris et al., 2012). Furthermore, its interaction with the SCN is also dependent on the circadian rhythm. Melatonin is secreted along with the circadian rhythm in opposite activity patterns from the SCN, melatonin peaking at night and SCN electrical oscillations peaking during midday. The availability of the receptors for melatonin in the

neurons of SCN also follows a rhythm, being at its lowest during the night (Inouye & Shibata, 1994).

Just as glucocorticoids, melatonin has been found to be an integral part of entraining the peripheral cell clocks to a central circadian rhythm as it is also controlled in part by entrainment by light and modulation of the SCN (Lockley et al., 2003) it helps entrain the body to the rhythm of the SCN. However, just as the glucocorticoids, melatonin is not merely a hormone that is maintaining the rhythm of the SCN as it has been found to mediate the peripheral rhythms without the input of the SCN. Meaning that it is also an output signal for the SCN entraining the peripheral clocks (Inouye & Shibata, 1994). This entrainment of the peripheral clocks can be seen in a study which investigated the effects of melatonin in entraining the circadian rhythm of blind subjects (Lockley, 2000). The study found that exogenous administration of melatonin caused a phase-shift in some of the participants, measured by cortisol levels and body temperature. These findings indicate that melatonin signaling is important for modulating the circadian rhythm.

Further indications of melatonin signaling involvement in circadian rhythm are found in studies of melatonin and light. These studies further show that melatonin is most likely involved in the control and resetting of the peripheral cell clocks during jet lag and other circadian disruptions (Lockley et al., 2003; Morris et al., 2012). What these studies found was that the oscillating melatonin rhythm is mediated by alternating light and wavelengths of light (Kiessling et al., 2014; Lockley et al., 2003). These findings indicate that the melatonin rhythm is entrained by light signals and subsequently modulates the peripheral clocks causing a phase shift along the rhythm of the light signals.

Though the melatonin rhythm is modulated by circadian rhythm rather than sleep, it does not mean that the melatonin signaling does not affect sleep, in fact, disruption of

the melatonin rhythm often leads to a disrupted sleep-wake cycle, and decreased sleep efficiency (Morris et al., 2012).

Many other hormones and other active agents of the endocrine system have also been found to follow the circadian rhythm to some extent, such as insulin and glucose which follow the subjective biological rhythm, peaking during the late biological night and early morning (Morris et al., 2012). That they follow the circadian rhythm can also be seen as metabolism is disrupted following circadian disruption as they are both involved in metabolism and the fasting-feeding rhythm (Bass, 2012). Other hormones which seem to follow the circadian rhythm are, for example, growth hormones which peak during sleep. Though, growth hormones seem to follow the sleep-wake cycle rather than the biological circadian rhythm as it peaks during early sleep irrespective of it being the subjective biological night (Morris et al., 2012).

6.3 Endocrinal treatments for circadian disruption

The research surrounding the endocrine systems effect on the circadian rhythm does provide a promising venture for identifying methods to alleviate the negative effects following disruption of the circadian rhythm. Treatment methods showing promise are, for example, melatonin which, as mentioned above, has been shown effective in alleviating the symptoms of circadian disruption as it can shift the sleep-wake cycle. This treatment has proven relatively risk free as melatonin is a hormone, mainly involved with modulation of the sleep-wake cycle and the melatonin presence can be modulated by ingesting melatonin pills or through light therapy (Lockley, 2000; Lockley et al., 2003). The taking of a melatonin supplement, or other application methods, are therefore recommended to, for example, shift workers in order to more easily fall asleep during the natural light cycle (Morris et al., 2012).

The alleviation of circadian disruption symptoms through modulation of glucocorticoids, however, is less researched and not commonly implemented as a treatment method in humans. Though research surrounding glucocorticoid modulation for circadian re-entrainment does show promise, the field is still young and safe and effective ways of modulating the clocks of the peripheral cells have yet to be established for human use. However, current research focusing on glucocorticoid manipulation often use *dexamethasone* (DEX) or *metyrapone* (MET) in order to manipulate the glucocorticoid levels of the subjects (Barclay et al., 2011; Nicolaides et al., 2014). Dexamethasone is a synthetic glucocorticoid which, when administered to the subject increases glucocorticoid presence of the cells. Metyrapone, on the other hand, is a medication that inhibits glucocorticoid secretion in the adrenal gland thereby controlling the glucocorticoid presence of the cells as well as the glucocorticoid rhythm (Kiessling et al., 2010).

Though there is research indicating an effect on the phase of the circadian rhythm due to manipulation of the glucocorticoids, there is a lack of any studies reviewing the combined reported effect glucocorticoids has on the circadian rhythm. Therefore, a meta-analysis investigating the combined effect reported across several different papers will be conducted in this thesis in order to identify the common effect reported across the different studies.

7. Methods

The question that will be answered in this thesis is, can the circadian rhythm of the peripheral cell clocks be re-entrained after a disruption of the circadian rhythm such as jet lag by modulation of glucocorticoids?

For this, a meta-analysis was conducted investigating the effect of manipulation of the endocrine system and specifically glucocorticoid administrations effect on shifting the circadian phase. Furthermore, a qualitative analysis and review were also conducted of the literature collected for the qualitative meta-analysis.

7.1 Literature search

A literature search was conducted using the online databases Medline, PubMed, SCOPUS and Web of Science. These databases were chosen as the works that can be found through them are relevant within the fields of neuroscience, and medical research such as chronobiology. The search was limited to studies performed on mice and rats to limit the heterogeneity of the selected studies. This limitation was implemented as there are not many studies of glucocorticoids effect on disrupting or re-entraining the circadian rhythm performed on humans. The model of the circadian system has however, been found to translate well into the mammalian and human models concerning the circadian system (Moore, 1973).

The literature search was conducted using the phrases "circadian rhythm", "circadian disruption" or "jet lag" and "glucocorticoid" or "dexamethasone" or "metyrapone" and "resynchronization" or "desynchronization" or "light". These phrases were used to investigate the effects of glucocorticoids on the circadian phase and its possible effect on resynchronizing disrupted circadian rhythms. DEX was included in the search as it is a synthetic glucocorticoid often used in both animal and human studies of circadian disruption (Fuller et al., 2006)**.** MET was included in the search as it is a medication that has been found to inhibit adrenocorticotropic hormone secretion and thereby affect glucocorticoid levels of the administered subject (Kiessling et al., 2010).

Included studies were those who investigated the phase of the circadian rhythm through measuring the oscillations of the clock genes and proteins per1, per2, per3 and BMal1. The presence of which was measured through the levels of mRNA in various peripheral cells. Exclusion of studies followed if a study did not involve the previously mentioned measures as they would not address the research question. If it was performed on animals not kept in 12-12 light day cycles prior to the experiment as this would introduce unnecessary variables concerning the non-disrupted circadian rhythm of the animal. Those

who did not manipulate glucocorticoid or the circadian rhythm were also excluded as, once again, they would not address the research question. Furthermore, studies were also excluded if they did not report or failure to fulfill the quality assessments listed below, as well as if they failed to report the required descriptive statistics to evaluate the effect size of the results of the phase shift in the study.

The inclusion and exclusion criteria of studies for the qualitative review were the same as for the quantitative review. However, the included studies will also be contrasted and discussed along with studies and theories mentioned in the review presented in the first half of this paper.

7.2 Collection and extraction of data

In order to assess the quality and relevance of the studies, a list of relevant qualities was compiled. This list included the total number of subjects used in each study, the number of animals used in each control and experimental groups. Whether the studies specified the time of intervention, meaning zeitgeber time (ZT; lights on ZT0 and lights off ZT12) when the intervention occurred and the dosage of the intervention. Furthermore, what the control group consisted of in the experiments was also listed and assessed and whether it influenced the phase of the animal's circadian rhythm as compared to their phase prior to the experiments. Examples of valid interventions for control groups of the studies included here are *saline* injections in the place of DEX or MET injections and sham surgery in place of adrenalectomy (surgical removal of the adrenal gland, causing a loss of glucocorticoid rhythmicity in the peripheral cells).

Information on the animals was also included in this list, such as age, gender, the general strain of the animals as well as any gene manipulations to knock in or knock out certain genes. Specifying these criteria for the studies were, as mentioned used to assess the quality of the studies, but also to perform sub-group analysis of the studies to investigate

possible stronger relationships and confounding variables for the meta-analysis of the included studies.

Reported statistical information was judged as adequate when effect sizes of the relevant experiments were reported or could be calculated, including the number of subjects of each group. Meaning, the mean ZT of the clock gene peak was reported as well as standard deviation (SD) or standard error of the mean (SEM). In cases where neither standard deviation or standard error mean was reported their values were extracted using the reported confidence interval, the sample size of the study and the difference between means, as recommended by Cochrane reviews (Higgins & Green, 2011).

7.3 Determinant of analysis for meta-analysis

The effect of glucocorticoids on re-entrainment of a disrupted circadian rhythm was measured through the difference between ZT of the peak oscillation of clock genes in the control groups and the experimental group's peripheral cells. As the number of included papers was low, several of the experiments reported in the studies were included. Some papers report more than one experiment, studying the phase shift of several different clock genes as well as different cell groups throughout the body. The phase shifts reported in these different experiments were included in the meta-analysis.

7.4 Statistical analysis

A meta-analysis was conducted, following Cochrane (Higgins & Green, 2011) recommendations for analysis of effects sizes in continuous data. Effect sizes of individual experiments were calculated using mean differences to calculate Cohens d and Hedges g (corrected Cohens d). Hedges g was used as it accounts for the sample size and is more reliable to use when the sample sizes are smaller (<20) and vary between experiments.

The mean difference within studies was calculated as the mean circadian phase peak of the control group M_C subtracted from the mean circadian phase peak of the

experimental group M_E ($M_E - M_C$). M_E is thereby showing a shift causing an earlier peak phase as negative and a peak phase occurring later as positive.

A combined weighted meta effect size (MES) of the included experiments was calculated through the included experiments individual effect sizes and sample size. A random-effects model was implemented for the meta-analysis due to the included studies' experimental designs varying. The MES was interpreted similarly to Cohen's d and Hedge's g where, $MES = 0.2$ is seen as a small effect size, $MES = 0.5$ is a medium effect size and $MES = 0.8$ is a large effect size (Hesser & Andersson, 2015).

A measure of the heterogeneity (Q) was calculated as well as a measure of between-study variance, tau-squared (τ^2), which indicates the spread of results between experiments. However, as substantial heterogeneity can be expected in a meta-analysis, the impact of heterogeneity on the combined effect size of the studies (I^2) was also calculated. I^2 is interpreted as an indication of how much of the variation between studies is due to heterogeneity and not just sampling error or chance. It can be interpreted as a small percentage of heterogeneity when $I^2 = 0.25$, moderate when $I^2 = 0.5$ and large when $I^2 = 0.75$ (Hesser & Andersson, 2015).

Several analyses were performed post hoc, of isolated subgroups of experiments within the sample, to investigate the possible causes for heterogeneity and combined effect sizes of the analysis. These isolated the studies which measured different clock genes by dividing studies measuring per1, per2, and BMal1 into separate groups to be analyzed. The same thing was done to investigate the intervention occurred in-phase with the endogenous glucocorticoid oscillation peak (ZT12) or antiphase with its peak (ZT1). Studies were also isolated and analyzed based on if they used gene knock-in or knock-out mice to disrupt the circadian rhythm and measure the clock genes as opposed to those that used wild-type mice or adrenalectomized animals.

8. Results

8.1 Literature search

From the literature search using the previously outlined search methods, phrases and databases and culling irrelevant and review articles, 14 relevant articles which investigated the circadian phase following glucocorticoid manipulation in mice or rats were identified. After further culling of the papers which did not present adequate statistical data in order to perform effect size calculations, six papers were included.

All six of the included articles presented more than one experiment, analyzing several different clock genes in different peripheral areas. These experiments were treated as separate studies. After these were treated as separate studies, 32 different experiments were identified and included in the analysis. The different experiments also investigated different clock genes peak phases that were specified as well. Of the included experiments, 7 investigated the phase of BMal1, 18 investigated the phase peaks of per1 and 13 investigated the peaks of per2. Furthermore, the intervention was also administered at different ZT for different experiments. Of the included experiments, 13 investigated the phase shift after administering the intervention in-phase (around ZT12) and 12 experiments administered it during the antiphase period (around ZT1).

As the included experiments investigated the effects glucocorticoids on the circadian rhythm in relation to realigning or re-entraining the rhythm to the ZT after it had been disrupted, different experiments had different methods for disrupting the animal's rhythms. Of the included experiments 11 used adrenalectomy (Soták et al., 2016; Woodruff, Chun, Hinds, & Spencer, 2016), surgical removal of the adrenal gland as the disruption of the rhythm of the peripheral cells. 12 experiments used gene knock-in or knock-out to disrupt the animals' peripheral circadian rhythm (Honma et al., 2015; Kamagata et al., 2017; Pezük, Mohawk, Wang, & Menaker, 2012) and 9 used unmodified, wild-type mice with a disrupted

rhythm through shifted light-dark cycles, simulating jet lag (Kamagata et al., 2017; Wu & Fu, 2017).

8.2 Data and means

All studies included were presented using a 95% confidence interval and the characteristics of the studies included are summarized in table 1. The included studies were all quite recent as they were published between 2012-2017. After compiling the experiments, the mean circadian phase shift post-intervention of the studies was calculated, which found that the mean circadian phase shift was ($M_{PS} = 4.25h \pm 3.23$). Seemingly showing that the effect of modulating the presence of glucocorticoids can cause a 4.25-hour shift in the individual's clock gene peak phase. However, this data of the phase shift as presented without accounting for the direction of the phase shift, whether it causes the peak to occur earlier or later than the peak of the control animals. When the direction of the phase shift is taken into account, we can see that 9 of the experiments showed a negative mean difference, indicating that the circadian peak oscillation has been shifted to occur earlier than the peak prior to the intervention.

The effect sizes of the individual experiments which were calculated using Cohens d and Hedges g can be seen in table 1. Using this information, the analysis of the combined effect of included studies found the weighted meta effect size to be MES=0.11 ± 0.06. To then investigate the impact heterogeneity of the included studies had on this result, I^2 and τ^2 were calculated, which showed I^2 of the total sample of included studies was $I^2 = 95.45\%$ and $\tau^2 = 1.91$. As these results indicate that the sample is very heterogeneous, a number of subgroup analysis was performed to investigate the heterogeneity of the sample. The tests isolated the different clock genes analyzed, ZT of administration and how the circadian rhythm of the animal was disrupted.

Article	Sample size	Studied gene product	Effect sizes	ES SD	Mean phaseshift
Honma et al., 2015 (ZT24)	25	per2	0.809	4.4	-4
Honma et al., 2015 (ZT18)	25	per2	-0.91	4.1	3.3
Kamagata et al., 2017 (Kidney WT)	8	per1 and per2	-1.786	2.2	-4
Kamagata et al., 2017 (Kidney, CLOCK)	10	per1 and per2	-2.429	4.9	12
Kamagata et al., 2017 (Liver WT)	8	per1 and per2	1.786	2.2	3
Kamagata et al., 2017 (Liver CLOCK)	6	per1 and per2	2.41	1.7	-4
Kamagata et al., 2017 (Subgla WT)	8	per1 and per2	1.786	2.2	4
Kamagata et al., 2017 (Subgla CLOCK)	10	per1 and per2	-1.59	5	-8
Pezük et al., 2012 (Lung)	11	per1	2.05	2.1	-4.31
Pezük et al., 2012 (Pineal)	10	per1	0.046	3.1	-0.14
Pezük et al., 2012 (Salivary)	10	per1	-0.387	2.4	0.88
Pezük et al., 2012 (Kidney)	11	per1	-1.69	5.3	8.48
Pezük et al., 2012 (Liver)	10	per1	-1.414	6.4	9.12
Pezük et al., 2012 (Pituitary)	11	per1	-3.42	1.3	4.19
Pezük et al., 2012 (Cornea)	10	per1	1.193	2.6	3.14
Soták et al., 2016 (Liver, Bmal1)	27	per1, per2 and BMal1	-1.26	0.5	-0.6
Soták et al., 2016 (Liver, Per1)	27	per1, per2 and BMal1	1.696	0.9	1.6
Soták et al., 2016 (Liver, Per2)	27	per1, per2 and BMal1	-0.615	0.7	-0.4
Soták et al., 2016 (Junenjum, Bmal1)	27	per1, per2 and BMal1	2.349	0.4	1
Soták et al., 2016 (Junenjum, Per1)	27	per1, per2 and BMal1	1.15	1.3	1.5
Soták et al., 2016 (Junenjum, Per2)	27	per1, per2 and BMal1	1.2	0.5	0.6
Soták et al., 2016 (Kidney, Bmal1)	27	per1, per2 and BMal1	1.766	0.9	1.6
Soták et al., 2016 (VAT, Per2)	27	per1, per2 and BMal1	-0.06	1.7	-0.1
Woodruff et al., 2016 (Experiment 1 per2)	48	per2	0.58	10.3	6
Woodruff et al., 2016 (Experiment 2 Per2)	40	per2	-0.654	9.2	-6
Woodruff et al., 2016 (Experiment 2 Bmal1)	40	BMal1	-0.56	10.7	12
Wu & Fu, 2017 (nd0, Bmal1)	8	BMal1	-1.73	2.3	-4
Wu & Fu, 2017 (nd0, per1)	8	per1	-2.188	4.6	4
Wu & Fu, 2017 (dl0, Bmal1)	8	BMal1	-2.188	3.7	8
Wu & Fu, 2017 (dl0, per1)	8	per1	-1.73	4.6	8
Wu & Fu, 2017 (dl12, Bmal1)	8	BMal1	1.73	2.3	4
Wu & Fu, 2017 (dl12, per1)	8	per1	1.094	3.7	4

Total mean phase shift	0.11	0.06	2.026875
Total mean phase shift, absolute values			4.25

Table 1: Parameters of the analysis. Sample size=total number of animals included. Mean phase shift presented as hours of phase shift. Absolute values presented refers to data without direction where all data were calculated as if it was positive.

8.3 Effect sizes and heterogeneity of subgroups

The analysis separating the sample into the different clock genes showed that, though the combined effect size BMal1 increased slightly, the heterogeneity for the isolated sample did not differ substantially from the original sample (per1 MES=0.05, $I^2 = 91.72\%$, $\tau^2 = 2.98$; per2 MES=0.02, $I^2 = 81.72\%$, $\tau^2 = 1.16$; BMal1 MES=0.26 $I^2 = 86.4\%$, $\tau^2 = 2.67$). The results of this analysis suggest that the heterogeneity found in the meta-analysis cannot be explained by the inclusion of several different clock gene products in the analysis.

The analysis of the sample as divided into in-phase or antiphase administration, similarly to the isolation by clock gene, did not affect the heterogeneity to the point where it could be regarded as acceptable for the meta-analysis (in-phase, MES= -0.24, $I^2 = 84.27\%$, $\tau^2 = 1.24$; antiphase MES= -0.15; $I^2 = 85.25\%$, $\tau^2 = 2.21$). The heterogeneity of these groups is still very high, as indicated by the $I^2 > 80\%$, the heterogeneity cannot be explained solely by the administration times in the studies either.

Finally, the analysis of the method for disruption of the circadian rhythm in the animals was conducted by dividing the sample into experiments using wild type mice (MES= -0.34, $I^2 = 77.25\%$, $\tau^2 = 2.71$), gene manipulation (MES= -0.43 $I^2 = 85.86\%$, $\tau^2 = 2.27$) or adrenalectomy (MES=0.42 $I^2 = 88.43\%$, $\tau^2 = 1.19$) to disrupt the peripheral rhythm in the animals. Though isolating the sample like this did provide slightly larger effect sizes, the samples still have very high heterogeneity.

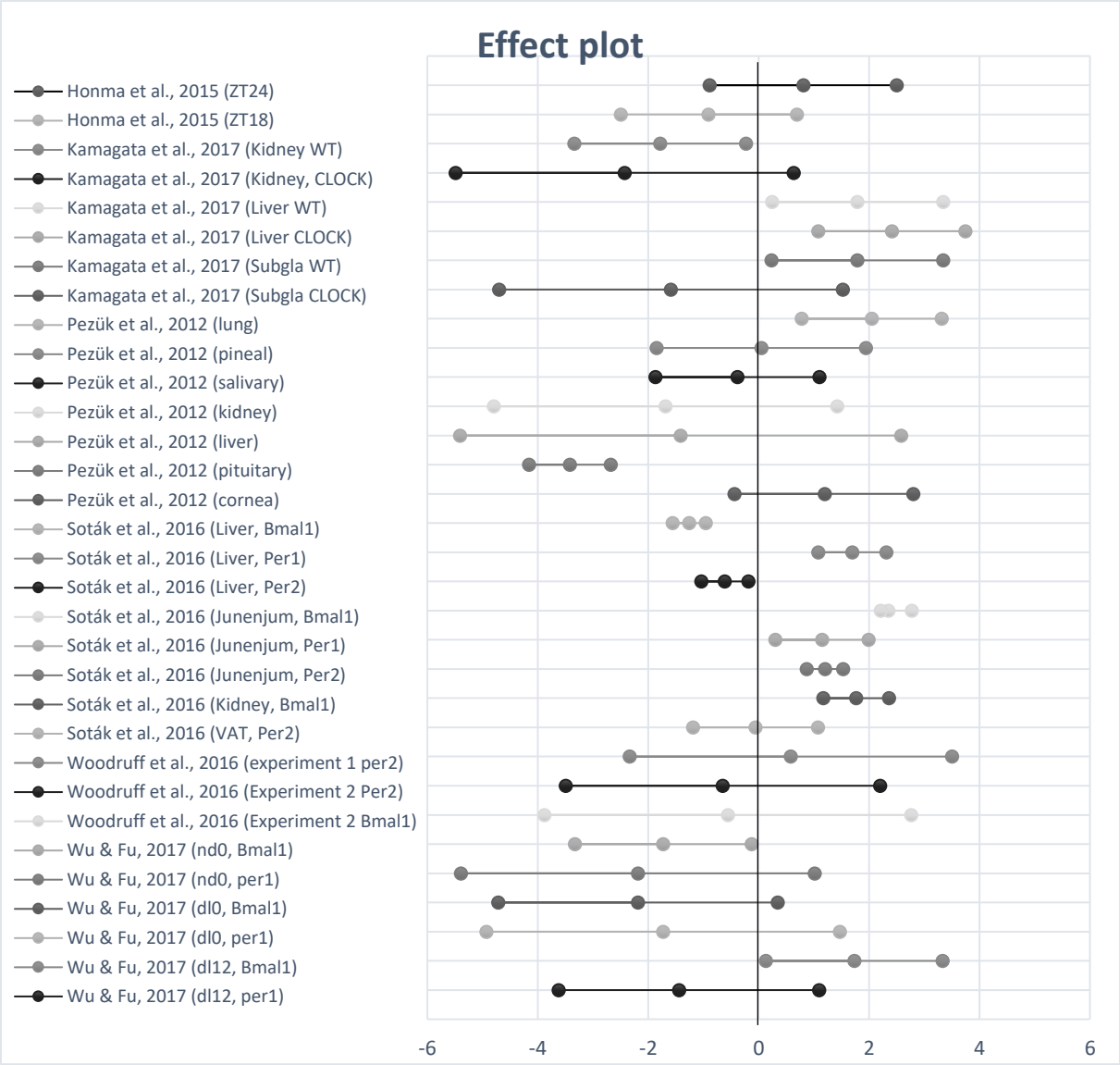

Figure 2: Figure presenting the effect size and the confidence intervals of the included studies, in a forest plot.

9. Discussion

As previously mentioned, the included studies differ some in their experimental design, even though they investigated the effect of glucocorticoids on the peripheral cells circadian rhythm.

That the body and cells of mammals follow a rhythm has been outlined above (Moore, 1997), and that disruption of this rhythm can lead to adverse long-term effects such as mood disorders (Lockley et al., 2008; Monk et al., 1985) and decreased neurogenesis in the hippocampus (Cho, 2001; Cho et al., 2000) as well as negative short-term effects such as

impaired cognitive function and metabolic issues (Karatsoreos et al., 2011; Karatsoreos, 2012).

Circadian disruption can occur in shift workers, who often change their sleep-wake cycle and in those who experience jet lag or chronic jet lag such as airline staff. This disruption of the circadian rhythm after shifting the sleep-wake cycle or the zeitgebers around you occurs because the central pacemaker, SCN, is misaligned to the rest of the peripheral cell clocks throughout the body (Duffy & Wright, 2005; Kiessling et al., 2010).

In order to alleviate the negative effects, treatments for circadian disruption have been investigated. These are treatments such as, adjusting the circadian rhythm by shifting the sleep-wake cycle and fasting-feeding rhythm prior to traveling a long distance, which may cause jet lag. Another method is taking melatonin to shift the sleep-wake cycle during jet lag or shift work (Laste et al., 2013; Zee & Goldstein, 2010).

Glucocorticoids have been found to be able to modulate the circadian rhythms of almost all cells in the body which exhibit circadian rhythmicity, aside from the cells in the SCN as these cells do not have receptors for glucocorticoids (Balsalobre, 2000; Le Minh et al., 2001). However, what is still not fully understood is the mechanisms of how glucocorticoids modulate the clock gene expression in these peripheral cells. That is what these studies investigate, the administration times, circadian disruption and the effect glucocorticoid administration has on the circadian oscillations and expression of clock genes. After reviewing these included articles results, we can start to see how glucocorticoids are thought to relate to and mediate the circadian oscillations in the peripheral cells outside of the reported hours of phase shift investigated in this analysis. We may also be able to highlight causes which could underlie the heterogeneity of the results encountered in this analysis.

9.1 Meta-analysis

In this thesis, a meta-analysis has been conducted, investigating the effect of glucocorticoids on shifting and re-entraining the circadian rhythm in the peripheral cells of the body to the zeitgebers and central pacemaker time. In order to investigate this a random model meta-analysis was conducted of the studies presenting experiments which measured the phase shift in animals with disrupted circadian rhythms in the peripheral cell clocks in animals after they received Dexamethasone or adrenalectomy. The meta-analysis included six studies which all presented several experiments which were included in this final sample totaling in 32 experiments.

An analysis of the clock gene peak in the combined total group was conducted using a random model analysis and provided a low combined effect size of the sample of MES=0.11 ± 0.06. Indicating that the experiments included in the sample did not produce similar effects across the board as the effect sizes of the included studies are not large enough or that the direction of effects is conflicting, decreasing the total effect. For this study, the latter case is most likely to apply.

As mentioned earlier, the phase shift post-intervention in 9 of the included experiments found a shift of the phase causing the peak to occur earlier than controls, whereas the other 23 experiments found a delay of the phase, causing the peak to occur later than in controls. This lack of uniform direction for the results most likely contributed to the low effect size of the meta-analysis. Furthermore, the I^2 identified in this analysis was too high to ignore $I^2 = 95.45\%$ and indicates that the pooled effect size of the entire sample should not be interpreted as meaningful. However, for exploratory purposes an investigation into whether the direction of the effects and the cause for the heterogeneity of the studies could be identified the sample was isolated into different subgroups mentioned above, this was done post hoc. These additional analyses were included in order to investigate what made the phase

shift differ between the experiments, and if it could be due to the difference in experimental designs of the included experiments and studies.

The division of the experiments by which clock gene they investigated divided the sample into three groups, each investigating either per1, per2, or BMal1. The analysis of these subgroups showed that the combined effect size of each subgroup had not increased significantly nor did I^2 of the sample show that the effect size should be viewed as significant.

The same result was found for the groups divided by the ZT of administration, though this was a division that could be expected to show decreased heterogeneity as well as increased effect size. This could be expected due to the findings in previous studies that the phase of administration has an effect on the shift of the circadian rhythm, either abolishing or re-entraining the rhythm (Morris et al., 2012; Wu & Fu, 2017).

When dividing the groups by the method of disrupting the circadian rhythm prior to intervention the effect size of the groups did increase, and the direction of the effect shifted showing only the adrenalectomized animals to have a delayed peak according to the effect of the pooled mean shift. However, the heterogeneity for the groups was still too high to consider the pooled effect size meaningful. These results indicate that the heterogeneity does not lie solely in either of the subgroups of the sample mentioned above and may instead lie in some other facet of the analysis.

These findings do not follow the expectation and data presented within the articles included as the effect does not seem to follow a uniform direction, according to this analysis at least. The reason for the heterogeneous results of the analysis is not clear as the reason is not found in the above analyses of the studies. In-phase and anti-phase administration of the intervention was the most likely to have an effect that may change the direction of results as differential ZT of administration of various medications have been found to have different

effects (Fuller et al., 2006). Similar results are also reported in relation to circadian realignment in different studies as the time of administration differently affects circadian rhythm (Woodruff et al., 2016; Wu & Fu, 2017). However, as this was not the source for the unknown variance and that variance has not been able to be identified, the results of the meta-analysis did not find a statistically significant combined effect of the included experiments.

9.2 Re-entraining the rhythms, qualitative review

Though the meta-analysis could not find meaningful results, a qualitative review of the included literature was conducted and will be discussed below, along with previously mentioned literature.

As many of the studies included did find that the modulation of glucocorticoids within the animals' peripheral cells, which exhibit circadian oscillation of clock gene expression, did affect said clock gene expression. These results can, for example, be seen in the studies performed by Pezük et al. (2012), which found that the circadian rhythm of clock gene expression in the peripheral cells of the kidney and liver were especially prone to be affected by glucocorticoids. These were the peripheral cells found most easily re-entrained to the surrounding ZT, which can also be seen in table 1 as the mean shift for the clock gene expression was the largest for the kidney and liver with $M_{ps\ kidney} = 8.48h$ and $M_{ps\ liver} = 9.12h$.

The effect on the circadian oscillations of clock gene expression can also be seen in peripheral cells more directly related to the cognitive processes such as presented in the experiments by Woodruff et al. (2016). This study investigated the effect of glucocorticoids and more specifically, on the phase, rhythm, and amplitude of the clock gene expression in peripheral cells located in the prefrontal cortex. What was found was that, the cell clocks in the prefrontal cortex more quickly re-entrained to the surrounding zeitgebers and the rhythm of the SCN after administration of glucocorticoids.

The prefrontal cortex is an area involved with several higher cognitive functions such as planning for the future, and other functions often found impaired during circadian disruption (Woodruff et al., 2016). This could therefore mean that glucocorticoids could be used in possibly alleviating the drop in cognitive performance that can come with circadian disruption (Blatter & Cajochen, 2007; Cho et al., 2000; Dijk et al., 1992; Woodruff et al., 2016).

Worth noting for the results presented by Woodruff et al. (2016) is that, similarly to what had been found in other studies (Honma et al., 2015; Wu & Fu, 2017), that the intervention was performed in-phase, or antiphase with the endogenous rhythm made a difference in how the rhythm was affected. The administration of glucocorticoids normalized the clock gene expression in the prefrontal cortex when it was administered in-phase with the endogenous glucocorticoid rhythm while when administered antiphase to the rhythm, it abolished the rhythm (Woodruff et al., 2016). This significant difference the same glucocorticoid modulator can have, depending on the ZT of administration, can be found being reported or discussed in all studies included in this analysis. Indicating that for glucocorticoid manipulation to potentially be a viable alternative for re-entraining the circadian rhythm after disruption through regulating the rhythm of peripheral cells the administration of the glucocorticoid should be administered at a specific time during the rhythm.

When the optimal time for administration fall is, however, not made entirely clear from the included studies. For example, the study mentioned above by Woodruff et al. (2016) concludes that the optimal time for administration of glucocorticoids is during in phase of endogenous glucocorticoid peak at ZT11 whilst the study by Wu and Fu (2017) suggests that the optimal time for administration is during the endogenous peak time for the clock gene expression ZT0. What the optimal time for administration of glucocorticoids to elicit the most

substantial phase shift, and re-entraining the peripheral cells could be, was not clarified by the post hoc analysis of ZT0 compared to ZT12 in this analysis either, due to the high impact heterogeneity had on the pooled effect size analysis (ZT12, $I^2 = 84.27\%$; ZT0, $I^2 = 85.25\%$).

There are still many facets of the mechanisms of how glucocorticoids affect the phase and amplitude of clock gene expression in the peripheral cells. It is still not completely understood how the hormones can affect clock gene expression and modulate the circadian rhythm. Studies have found that the per1 gene expression (Soták et al., 2016), and per2 gene expression, seem to be directly influenced by the presence of glucocorticoids (Honma et al., 2015). However, studies have also reported that the regulation of per1 and per2 transcription is not an effect of direct interaction of the glucocorticoids on per1 and per2 transcription. They instead suggest that glucocorticoids affect the circadian system by indirectly affecting the circadian system in the cells (Wu & Fu, 2017). However, the data does point towards the glucocorticoids being able to regulate the feedback loop, be it direct or indirect. Therefore, as the circadian system is a feedback loop, the presence of rhythm-altering glucocorticoids in some cells can affect the whole system (Darlington, 1998).

This effect of the glucocorticoids on the feedback loop is also thought to be able to signal back into the SCN. Therefore, affecting the circadian system, as a whole, even without the cells in the SCN having any glucocorticoid receptors for the hormone to bind to (Pezük et al., 2012; Wu & Fu, 2017). Reports claiming that the SCN can be affected by glucocorticoid signaling despite the absence of receptors further support this effect of the glucocorticoids (Pezük et al., 2012).

Though the importance of glucocorticoids has been shown above as it modulates per1 and per2 expression and the circadian rhythm in the peripheral cells, whether glucocorticoids presence in the peripheral cells is necessary for clock gene expression unclear.

The presence is mainly thought necessary, as glucocorticoids have previously been found to stabilize the circadian rhythm in peripheral cells (Husse et al., 2015, 2014; Le Minh et al., 2001) Studies involving adrenalectomized animals found that the absence of glucocorticoids in the cells does not necessarily ablate the circadian rhythm in the cells (Pezük et al., 2012; Soták et al., 2016). However, what was found was that the amplitude of the oscillations, meaning the transcription of clock genes was not as high in the animals which had an abolished glucocorticoid rhythm due to adrenalectomy (Kamagata et al., 2017; Pezük et al., 2012; Wu & Fu, 2017).

Though the circadian rhythm of the peripheral cells is not abolished by lack of glucocorticoid signaling, there are some areas where the phase is shifted. These are clocks such as the cells in the kidney, which were found to lose rhythmicity as well as the amplitude of per2 following adrenalectomy (Kamagata et al., 2017).

10. Limitations

10.1 Included studies

The experimental designs, and the limitations of the studies included in the meta-analysis will be discussed briefly here. The limitations will be addressed in order to discuss certain aspects of the studies that may have affected the final result of the meta-analysis, as well as aspects of the experimental designs, which may be subject to critique and improvement for future studies.

The included studies have investigated the effect on the circadian rhythm in peripheral cells following modulation of glucocorticoids presence in those cells and the re-alignment of cells in a disrupted circadian system. In order to study disrupted circadian systems where clock phase peaks differed from normal in the peripheral cells, the researchers used gene-manipulated animals and adrenalectomized animals to induce a phase shift. Whether these are appropriate methods or whether they should be included in the same meta-analysis could be

discussed further, as the risk of extraneous variables in the sample of meta-analysis is great. The risk for extraneous variables within the experiment itself is similarly increased by the presence of different methods of disruption. However, this meta-analysis, as well as the included studies judged method of disruption as an appropriate method for disrupting the circadian rhythm if the length of the circadian rhythm was not significantly different after the disruption and it was not accounted and corrected for. The length of the free-running rhythm should be kept in mind in the case of clock/clock mutated mice, as they can sometimes have a longer free-running rhythm than wild type mice if kept in constant darkness (Kamagata et al., 2017). Though this may not necessarily have a significant effect on the results of the studies or this analysis, it is worth to take note of as something which could prove to be a problematic aspect of this type of study.

Another aspect of the experimental design which could prove problematic is the method for administration and the method by which the mice are put down in a postmortem study. Glucocorticoids are one of the hormones which are secreted when the animal is stressed (Tahara et al., 2017). Meaning, that the way, for example, dexamethasone is administered to the animals may prove stressful and therefore affect the level of glucocorticoids present in the animal after injection and similarly with the way they are euthanized if the measurements are performed postmortem. However, this is often controlled for through control groups also going through the injection process with saline injections and if euthanizing the animals follows the ethical regulations, the stress on the animal is minimized.

10.2 Conduction of meta-analysis

The limitations and the design of this meta-analysis, as well as possible problematic inclusions, will be discussed below. They will be discussed in order to address the

shortcomings of the analysis and to shine a light on the possible reasons for the non-meaningful results of the meta-analysis.

The most prominent of the limitations encountered in this meta-analysis is the use of only one reviewer, data extractor and coder of the articles for this review, this was done due to lack of time and accessible personnel with adequate knowledge on the subject. However, it substantially increases the risk of errors in sampling, extracting and coding the data as well as increasing the risk of bias. The use of only one reviewer could, therefore, be an underlying cause for the following limitations and problems faced by this study as well.

Another prominent limitation of this analysis is the substantial impact the heterogeneity of the study sample had on the results as it far exceeded acceptable levels of variance not due to sampling error. Attempts were made to find the possible cause for the unknown variance as post hoc analyses of subgroups were also performed, isolating different aspects of the studies, previously outlined. However, no significant change in the effect heterogeneity had on the sample. Causes which were not explored could be underlying the heterogeneity, such as those mentioned above in the limitations of the included studies. A reliable way to include these in the meta-analysis was not found, and they are therefore unexplored. Furthermore, the heterogeneity could not be ignored using a fixed model in place of the random one as the studies vary too greatly in design and size for a fixed model to be statistically responsible.

Another possible issue with the construction of this analysis was the use of hours of phase shift. The use of these parameters may not have been the best for analysis of the effect of glucocorticoids on peripheral re-entrainment. A more appropriate analysis would preferably include the loss or retention of rhythmicity as well as amplitude and strength of the rhythm. However, rhythmicity being lost or retained was hard to incorporate in the analysis,

and the amplitude of phase was also difficult to incorporate, as it was sparsely reported, it was not included in this analysis.

The studies included may have varied too greatly in the experimental design as the peripheral cells measured were also found to vary between the study, therefore having a too wide scope for the analysis. A similar problem arises with the previously addressed method used to disrupt the circadian rhythm, as it also varied between studies and could have caused the study to have a too wide scope, therefore increasing the heterogeneity of the sample.

The small sample of articles can also be discussed as a limitation this analysis faced. Though the final sample of included experiments was relatively large and should not provide any limitations by themselves. However, the final sample of relevant experiments was extracted from only six articles which are a very limited number of articles and could, therefore, increase the risk of errors and bias in the final sample of experiments. The included experiments should therefore ideally have been sampled from a larger selection of articles to minimize these possible errors.

11. Conclusions

Nonexistent results of this analysis do not necessarily indicate that the modulation of the glucocorticoid presence in the peripheral cells is an inappropriate alternative to re-entrain the circadian rhythm. However, it should instead reflect on the execution of this quantitative review.

As seen in the qualitative review of the included articles, glucocorticoids do seem to affect the circadian rhythmicity of the peripheral cells. A similar effect can also be seen in the circadian feedback system overall as it may also affect the SCN indirectly through its modulation of the peripheral cell loops, which then feeds back information to the SCN. However, the mechanisms underlying the effect of glucocorticoids on peripheral cells are still

unclear. Though it seems glucocorticoids affect the rhythms of the peripheral cells as they have been found to re-entrain the cells to ZT after disruption, how they do it and when the optimal time for administration of glucocorticoids for re-entrainment is still not fully understood.

Glucocorticoid modulation of the peripheral cells cannot yet, based on this meta-analysis be considered a possible relevant method for re-entrainment of the circadian rhythms following a disruption. When suffering the effects of jet lag or having stayed up late, finishing your thesis, glucocorticoids cannot yet be recommended above ingesting large amounts of stimulants such as caffeine to combat the symptoms. More research is needed to understand the underlying mechanisms of the glucocorticoids and the optimal administration times, during which it can effectively re-entrain the rhythm, and not disrupt it further.

12. References

Aschoff, J., Fatranska, M., Giedke, H., Doerr, P., Stamm, D., & Wisser, H. (1971). Human circadian rhythms in continuous darkness: Entrainment by social cues. *Science*, *171*(3967), 213–215. doi:10.1126/science.171.3967.213

Bahk, Y. C., Han, E., & Lee, S. H. (2014). Biological rhythm differences and suicidal ideation in patients with major depressive disorder. *Journal of Affective Disorders*, *168*, 294–297. doi:10.1016/j.jad.2014.07.001

Balsalobre, A. (2000). Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science*, *289*(5488), 2344–2347. doi:10.1126/science.289.5488.2344

Barclay, J., Husse, J., & Oster, H. (2011). Adrenal glucocorticoids as a target for jet lag therapies. *Expert Review of Endocrinology & Metabolism*, *6*, 673–679. doi:10.1586/eem.11.56

Bass, J. (2012). Circadian topology of metabolism. *Nature*, *491*(7424), 348–356. doi:10.1038/nature11704

Benedetti, F., Radaelli, D., Bernasconi, A., Dallaspezia, S., Falini, A., Scotti, G., … Smeraldi, E. (2007). Clock genes beyond the clock: CLOCK genotype biases neural correlates of moral valence decision in depressed patients. *Genes, Brain and Behavior*, *7*(1), 20-25. doi:10.1111/j.1601-183X.2007.00312.x

Blatter, K., & Cajochen, C. (2007). Circadian rhythms in cognitive performance: Methodological constraints, protocols, theoretical underpinnings. *Physiology & Behavior*, *90*(2–3), 196–208. doi:10.1016/j.physbeh.2006.09.009

Braunwald, E. (2012). On circadian variation of myocardial reperfusion injury. *Circulation Research*, *110*(1), 6–7. doi:10.1161/CIRCRESAHA.111.260265

Cheon, S., Park, N., Cho, S., & Kim, K. (2013). Glucocorticoid-mediated Period2 induction delays the phase of circadian rhythm. *Nucleic Acids Research*, *41*(12), 6161–6174. doi:10.1093/nar/gkt307

Cho, K. (2001). Chronic 'jet lag' produces temporal lobe atrophy and spatial cognitive deficits. *Nature Neuroscience*, *4*(6), 567–568. doi:10.1038/88384

Cho, K., Ennaceur, A., Cole, J. C., & Suh, C. K. (2000). Chronic jet lag produces cognitive deficits. *The Journal of Neuroscience*, *20*(6), RC66. doi:10.1523/JNEUROSCI.20-06-j0005.2000

Colwell, C. S. (2011). Linking neural activity and molecular oscillations in the SCN. *Nature Reviews. Neuroscience*, *12*(10), 553–569. doi:10.1038/nrn3086

Darlington, T. K. (1998). Closing the circadian loop: CLOCK-induced transcription of its own inhibitors per and tim. *Science*, *280*(5369), 1599–1603. doi:10.1126/science.280.5369.1599

Depner, C. M., Melanson, E. L., Eckel, R. H., Snell-Bergeon, J. K., Perreault, L., Bergman, B. C., … Wright, K. P. (2019). Ad libitum weekend recovery sleep fails to prevent metabolic dysregulation during a repeating pattern of insufficient sleep and weekend recovery sleep. *Current Biology*. doi:10.1016/j.cub.2019.01.069

Dibner, C., Schibler, U., & Albrecht, U. (2010). The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. *Annual Review of Physiology*, *72*(1), 517–549. doi:10.1146/annurev-physiol-021909-135821

Dijk, D. J., Duffy, J. F., & Czeisler, C. A. (1992). Circadian and sleep/wake dependent aspects of subjective alertness and cognitive performance. *Journal of Sleep Research*, *1*(2), 112–117. doi:10.1111/j.1365-2869.1992.tb00021.x

Duffy, J. F., & Wright, K. P. (2005). Entrainment of the human circadian system by light. *Journal of Biological Rhythms*, *20*(4), 326–338. doi:10.1177/0748730405277983

Foster, R. G., & Kreitzman, L. (2013). The rhythms of life: What your body clock means to you!. *Experimental Physiology*, *99*(4), 599–606. doi:10.1113/expphysiol.2012.071118

Fuller, P. M., Gooley, J. J., & Saper, C. B. (2006). Neurobiology of the sleep-wake cycle: Sleep architecture, circadian regulation, and regulatory feedback. *Journal of Biological Rhythms*, *21*(6), 482–493. doi:10.1177/0748730406294627

Hastings, M., O'Neill, J. S., & Maywood, E. S. (2007). Circadian clocks: Regulators of endocrine and metabolic rhythms. *Journal of Endocrinology*, *195*(2), 187–198. doi:10.1677/JOE-07-0378

Hesser, H., & Andersson, G. (2015). *Introduktion till metaanalys och systematiska översikter*. Lund: Studentlitteratur AB.

Higgins, J. P. T., & Green, S. (Ed.). (2011). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0. Retrieved from www.handbook.cochrane.org

Honma, A., Yamada, Y., Nakamaru, Y., Fukuda, S., Honma, K. I., & Honma, S. (2015). Glucocorticoids reset the nasal circadian clock in mice. *Endocrinology*, *156*(11), 4302-4311. doi:10.1210/en.2015-1490

Hurd, M. W., & Ralph, M. R. (1998). The significance of circadian organization for longevity in the golden hamster. *Journal of Biological Rhythms*, *13*(5), 430–436. doi:10.1177/074873098129000255

Husse, J., Eichele, G., & Oster, H. (2015). Synchronization of the mammalian circadian timing system: Light can control peripheral clocks independently of the SCN clock. *BioEssays*, *37*(10), 1119–1128. doi:10.1002/bies.201500026

Husse, J., Leliavski, A., Tsang, A. H., Oster, H., & Eichele, G. (2014). The light-dark cycle controls peripheral rhythmicity in mice with a genetically ablated suprachiasmatic nucleus clock. *The FASEB Journal*, *28*(11), 4950–4960. doi:10.1096/fj.14-256594

Inouye, S. I. T., & Shibata, S. (1994). Neurochemical organization of circadian rhythm in the suprachiasmatic nucleus. *Neuroscience Research, 20*(2), 109–130. doi:10.1016/0168-0102(94)90029-9

Johnson, R. F., Moore, R. Y., & Morin, L. P. (1988). Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. *Brain Research, 460*(2), 297–313. doi:10.1016/0006-8993(88)90374-5

Kamagata, M., Ikeda, Y., Sasaki, H., Hattori, Y., Yasuda, S., Iwami, S., ... & Tahara, Y. (2017). Potent synchronization of peripheral circadian clocks by glucocorticoid injections in PER2:: LUC-Clock/Clock mice. *Chronobiology International*, *34*(8), 1067-1082. doi:10.1080/07420528.2017.1338716

Karatsoreos, I. N. (2012). Effects of circadian disruption on mental and physical health. *Current Neurology and Neuroscience Reports*, *12*(2), 218–225. doi:10.1007/s11910-012-0252-0

Karatsoreos, I. N., Bhagat, S., Bloss, E. B., Morrison, J. H., & McEwen, B. S. (2011). Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proceedings of the National Academy of Sciences*, *108*(4), 1657–1662. doi:10.1073/pnas.1018375108

Kiessling, S., Eichele, G., & Oster, H. (2010). Adrenal glucocorticoids have a key role in circadian resynchronization in a mouse model of jet lag. *The Journal of Clinical Investigation*, *120*(7), 2600–2609. doi:10.1172/JCI41192

Kiessling, S., Sollars, P. J., & Pickard, G. E. (2014). Light stimulates the mouse adrenal through a retinohypothalamic pathway independent of an effect on the clock in the suprachiasmatic nucleus. *PLoS ONE*, *9*(3), e92959. doi:10.1371/journal.pone.0092959

Landgraf, D., Long, J. E., Proulx, C. D., Barandas, R., Malinow, R., & Welsh, D. K. (2016). Genetic disruption of circadian rhythms in the suprachiasmatic nucleus causes

helplessness, behavioral despair, and anxiety-like behavior in mice. *Biological Psychiatry, 80*(11), 827-835. doi:10.1016/j.biopsych.2016.03.1050

Laste, G., Vidor, L., de Macedo, I. C., Rozisky, J. R., Medeiros, L., de Souza, A., … Caumo, W. (2013). Melatonin treatment entrains the rest-activity circadian rhythm in rats with chronic inflammation. *Chronobiology International, 30*(9), 1077–1088. doi:10.3109/07420528.2013.800088

Le Minh, N., Damiola, F., Tronche, F., Schütz, G., & Schibler, U. (2001). Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. *The EMBO Journal, 20*(24), 7128–7136. doi:10.1093/emboj/20.24.7128

Lockley, S. (2000). Melatonin administration can entrain the free-running circadian system of blind subjects. *Journal of Endocrinology, 164*(1), R1–R6. doi:10.1677/joe.0.164R001

Lockley, S. W., Brainard, G. C., & Czeisler, C. A. (2003). High sensitivity of the human circadian melatonin rhythm to resetting by short wavelength light. *The Journal of Clinical Endocrinology & Metabolism, 88*(9), 4502–4502. doi:10.1210/jc.2003-030570

Lockley, S. W., Dijk, D. J., Kosti, O., Skene, D. J., & Arendt, J. (2008). Alertness, mood and performance rhythm disturbances associated with circadian sleep disorders in the blind. *Journal of Sleep Research, 17*(2), 207–216. doi:10.1111/j.1365-2869.2008.00656.x

Monk, T. H., Fookson, J. E., Moline, M. L., & Pollak, C. P. (1985). Diurnal variation in mood and performance in a time-isolated environment. *Chronobiology International, 2*(3), 185–193. doi:10.3109/07420528509055558

Moore, R. Y. (1973). Retinohypothalamic projection in mammals: A comparative study. *Brain Research, 49*(2), 403–409. doi:10.1016/0006-8993(73)90431-9

Moore, R. Y. (1997). Circadian rhythms: Basic neurobiology and clinical applications. *Annual Review of Medicine*, *48*(1), 253–266. doi:10.1146/annurev.med.48.1.253

Moore, R. Y., Speh, J. C., & Leak, R. K. (2002). Suprachiasmatic nucleus organization. *Cell and Tissue Research*, *309*(1), 89–98. doi:10.1007/s00441-002-0575-2

Morris, C. J., Aeschbach, D., & Scheer, F. A. J. L. (2012). Circadian system, sleep and endocrinology. *Molecular and Cellular Endocrinology*, *349*(1), 91–104. doi:10.1016/j.mce.2011.09.003

Nakamura, W., Honma, S., Shirakawa, T., & Honma, K. (2002). Clock mutation lengthens the circadian period without damping rhythms in individual SCN neurons. *Nature Neuroscience*, *5*(5), 399–400. doi:10.1038/nn843

Nicolaides, N. C., Charmandari, E., Chrousos, G. P., & Kino, T. (2014). Circadian endocrine rhythms: The hypothalamic-pituitary-adrenal axis and its actions. *Annals of The New York Academy of Sciences*, *1318*, 71–80. doi:10.1111/nyas.12464

Pezük, P., Mohawk, J. A., Wang, L. A., & Menaker, M. (2012). Glucocorticoids as entraining signals for peripheral circadian oscillators. *Endocrinology*, *153*(10), 4775-4783. doi:10.1210/en.2012-1486

Price, J. L., Blau, J., Rothenfluh, A., Abodeely, M., Kloss, B., & Young, M. W. (1998). double-time is a novel drosophila clock gene that regulates PERIOD protein accumulation. *Cell*, *94*(1), 83–95. doi:10.1016/S0092-8674(00)81224-6

Ralph, M., Foster, R., Davis, F., & Menaker, M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. *Science*, *247*(4945), 975–978. doi:10.1126/science.2305266

Reinberg, A., & Ashkenazi, I. (2008). Internal desynchronization of circadian rhythms and tolerance to shift work. *Chronobiology International*, *25*(4), 625–643. doi:10.1080/07420520802256101

Roenneberg, T., Kumar, C. J., & Merrow, M. (2007). The human circadian clock entrains to sun time. *Current Biology*, *17*(2), R44–R45. doi:10.1016/j.cub.2006.12.011

Rosenberg, R. P., Bogan, R. K., Tiller, J. M., Yang, R., Youakim, J. M., Earl, C. Q., & Roth, T. (2010). A phase 3, double-blind, randomized, placebo-controlled study of armodafinil for excessive sleepiness associated with jet lag disorder. *Mayo Clinic Proceedings*, *85*(7), 630–638. doi:10.4065/mcp.2009.0778

Scheiermann, C., Kunisaki, Y., & Frenette, P. S. (2013). Circadian control of the immune system. *Nature Reviews. Immunology*, *13*(3), 190–198. doi:10.1038/nri3386

Soták, M., Bryndová, J., Ergang, P., Vagnerová, K., Kvapilová, P., Vodička, M., ... & Sumová, A. (2016). Peripheral circadian clocks are diversely affected by adrenalectomy. *Chronobiology International*, *33*(5), 520-529. doi:10.3109/07420528.2016.1161643

Tahara, Y., Aoyama, S., & Shibata, S. (2017). The mammalian circadian clock and its entrainment by stress and exercise. *The Journal of Physiological Sciences: JPS*, *67*(1), 1–10. doi:10.1007/s12576-016-0450-7

Welsh, D. K., Takahashi, J. S., & Kay, S. A. (2010). Suprachiasmatic nucleus: Cell autonomy and network properties. *Annual Review of Physiology*, *72*, 551–577. doi:10.1146/annurev-physiol-021909-135919

Wirz-Justice, A. (2008). Diurnal variation of depressive symptoms. *Dialogues in Clinical Neuroscience*, *10*(3), 337–343. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3181887/

Woodruff, E. R., Chun, L. E., Hinds, L. R., & Spencer, R. L. (2016). Diurnal corticosterone presence and phase modulate clock gene expression in the male rat prefrontal cortex. *Endocrinology*, *157*(4), 1522-1534. doi:10.1210/en.2015-1884

Wu, T., & Fu, Z. (2017). Time-dependent glucocorticoid administration differently affects peripheral circadian rhythm in rats. *Acta Biochimica et Biophysica Sinica*, *49*(12), 1122-1128. doi:10.1093/abbs/gmx115

Zee, P. C., & Goldstein, C. A. (2010). Treatment of shift work disorder and jet lag. *Current Treatment Options in Neurology*, *12*(5), 396–411. doi:10.1007/s11940-010-0090-9