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The Interplay of Serotonin and Clock Genes in Mediating the Interaction Between Clinical Depression and Chronotypes

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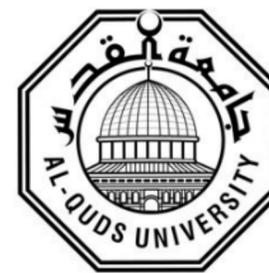
Thesis Approval

The Interplay of Serotonin and Clock Genes in Mediating the Interaction

Between Clinical Depression and Chronotypes

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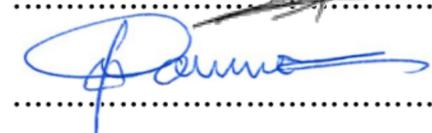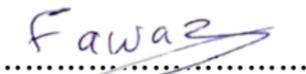
Jerusalem – Palestine

1440 – 2019

Dedication

To my family, who supported me all the way.

To my friends, and people I met in my journey,

You were a great help and source of inspiration,

To you I dedicate my thesis.

Abdelrahman Salah Jabr Sawalma

Declaration

I certify that this thesis submitted for the degree of master, is the result of my own research, except where otherwise acknowledged, and that this study has not been submitted for a higher degree to any other university or institution.

Signed: ....

Abdelrahman Salah Jabr Sawalma

Date: 5 / 8 / 2019

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Abdelrahman Salah Jabr Sawalma

Abstract

The biological clock regulates a myriad of physiological functions. It is synchronized by various environmental cues and follows a 24-hour cycle with variable start and end points that are referred to as "chronotypes". The suprachiasmatic nucleus (SCN) in the brain orchestrates the circadian rhythm as the central clock of the body, with oscillating expression of biological clock genes, including the *PERIOD* genes. The biological rhythm is modulated by serotonergic neurotransmission, with the largest afferent projection to the SCN coming from the serotonergic median raphe nucleus. Also, disruptions of the biological rhythm contribute to the pathophysiology of various psychiatric disorders. For instance, in clinical depression (a hypo-serotonergic state), patients exhibit a generalized disruption of their biological rhythm in the form of disturbances in sleep, hormonal, mood and temperature rhythms.

The main aim of our study is to investigate the intercorrelations between four factors: genotype, depression symptoms, chronotype and brain functionality. We examined the interaction between serotonin neurotransmission, electroencephalography (EEG) brain oscillations, chronotype, and the expression of depression symptomatology in healthy subjects. In particular, we examined naturally-occurring genetic polymorphisms in the 5-HT1A receptor gene and the *PER2* clock gene as indirect measures of serotonergic neurotransmission and the circadian clock, respectively.

We recruited Sixty-three healthy subjects who underwent evaluations for biological clock phase (chronotype), clinical depression symptomatology. A subgroup of the subjects underwent EEG testing to measure their baseline brain activity and response to various stimuli. A proportion of the subjects were genotyped for *5-HT1A* receptor (28 subjects) and the *PER2* polymorphisms (41 subjects).

Our results confirmed the interaction between the four studied factors. We found that subjects with the later chronotype express higher level of depression symptoms. The power of brain's theta oscillation was positively correlated with chronotype. We found that females had an earlier chronotype than males, which can be explained by the differences we found in their genotypes. This can be considered an indirect evidence for the correlation between genotype and chronotype, but a direct link that confirms these results is still lacking.

This study provides preliminary evidence for the molecular underpinnings of the interaction of biological clock functioning with serotonin as a potential mechanism for the development of clinical depression. Future studies should include a larger sample size and patients with clinical depression to clearly find the differences between healthy and clinical states in terms of chronotypes, genotypes and brain funcionality. This will help build a comprehensive overview of the underlying pathophysiology of clinical depression. Ultimately, this can inform the development of novel treatment modalities that take into account not just the symptomatology of clinical depression, but also genetic, physiological, and cognitive correlates.

Table of Contents

List of Tables

List of Figures

Definitions

Chronotype: The individual variations in the biological clock, it appears as a personal preference of sleep and wake timing, activity timing, meal timing … etc. Some people tend to be later chronotypes, who sleep later and wake up later, become more active at a later-than-average time. Some are early people, who tend to wake up earlier, sleep earlier and become more active at an earlier time.

Zeitgebers: German for "Time givers", are the environmental cues that drive our biological clock, such as sunlight, which is the main zeitgeber. It also includes some social cues and behavioral cues.

Abbreviations

5-HT1A: Serotonin 1A

BMAL1: Brain and Muscle ARNT-like Protein 1

CLOCK: Circadian Locomotor Output Cycles Kaput Protein

CRY1: Cryptochrome 1

CSK-3β: Glycogen Synthase Kinase 3β

EEG: Electroencephalography

MDD: Major Depressive Disorder

PCR: Polymerase Chain Reaction

PER1: Period 1 protein

RFLP: Restriction Fragment Length Polymorphism

SCN: Suprachiasmatic Nucleus

SSRI: Selective Serotonin Reuptake Inhibitor

ZT: Zeitgebers (Time Givers)

1. Chapter One:

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Introduction and Literature Review

1.1 Molecular Underpinnings of the Biological Clock

There are multiple physiological processes in the body that follow a circadian rhythm, meaning that they follow an endogenously-controlled regular rhythm that repeats every 24 hours. These include sleep-wake cycle, temperature control, fat and glucose metabolism, among others (Cardinali & Pandi-Perumal, 2011). These biological processes are governed by three main clocks. These are the biological clock, the social clock and the solar clock. The biological clock is the one endogenous to the body. It is a clock that can run free from outside environmental factors, and we feel it mostly when we experience jet lag or change work shifts. The social clock is defined by regular social events, such as work times, sleep times, and other social habits. The solar clock is the main clock that follows a 24-hour rhythm in accordance with the 24-hour cycle of the day. It is controlled by light and is the main clock that entrains (synchronizes) the other two clocks with it. In real life, these three clocks are usually in synchrony (Roenneberg, Kumar, & Merrow, 2007; Roenneberg, Wirz-Justice, & Merrow, 2003).

People can be divided in terms of their circadian rhythm variability into different chronotypes. A Chronotype is defined as the personal preference in terms of the daily rhythm of sleep, wake and activities, with earlier chronotypes having earlier rhythms, and later chronotypes having later rhythms (Nováková, Sládek, & Sumová, 2013; Roenneberg et al., 2003). These different chronotypes have basis in the genetic expression level, with

circadian clock genes varying in expression according to each person's chronotype (Nováková et al., 2013).

Zeitgebers (German for "time-givers") are daily environmental or external signals that entrain the biological clock and thus the entire circadian rhythm (Grandin, Alloy, & Abramson, 2006; Roenneberg & Merrow, 2016). The most prominent zeitgeber is light which affects the solar clock, which in turn affects the biological clock. The biological clock functions to entrain all peripheral clocks in the body using "internal zeitgebers", which are basically hormonal or neuronal inputs to peripheral organs (Curtis, Bellet, Sassone-Corsi, & O'Neill, 2014; Germain & Kupfer, 2008; Roenneberg et al., 2007). External zeitgebers include specific behavioral factors as well, such as sleep time, nicotine and alcohol consumption, sleep deprivation … etc, which might affect the biological clock directly or indirectly (Roenneberg & Merrow, 2016).

The main circadian pacemaker in mammals is found in the suprachiasmatic nucleus (SCN) in the anterior hypothalamus. It contains an autonomous clock that maintains rhythmicity, and can be considered as an entrainable oscillator that functions even before birth (Weaver, 1998). Although this structure was recognized anatomically, it wasn't until the 1970s that its function as a temporal regulator was recognized (Moore, Speh, & Leak, 2002).

There are three major inputs to the SCN. The first is the retinohypothalamic tract, which originates from retinal ganglion cells, and sends photic (light) signals by releasing glutamate in the SCN. The other input is from the median raphe nucleus, which release serotonin at target neurons in the SCN. A third input comes from the intergeniculate leaflet, which is controlled by the dorsal raphe nucleus. It conveys its signals to the SCN through the release of neuropeptide Y. The two main pathways from the retina and the

median raphe inhibit the function of each other, and both of them terminate in the middle of SCN (Ciarleglio, Resuehr, & McMahon, 2011; Germain & Kupfer, 2008).

Major output targets of SCN include the paraventricular nucleus of the hypothalamus, which affects melatonin secretion, pituitary hormones and the sympathetic and parasympathetic nervous systems. The dorsomedial hypothalamic nucleus is another major output target. It affects the circadian rhythm of cortisol and other hormones, and sleep. SCN then synchronizes peripheral tissues by both hormonal and neuronal signals. Cells of particular organs such as the kidneys and liver, can synchronize with SCN through the hormonal signal only, while others, such as the heart and skeletal muscles, require neuronal input (Germain & Kupfer, 2008).

Cells of the SCN as well as the peripheral tissues, follow a common mechanism of achieving circadian rhythmicity of gene expression (Fig. 1.1). The biological clock at the molecular level includes genes that are controlled by proteins that are able to inhibit their own function in rhythmic manner. The activation of the biological clock genes is initiated by two transcription factors of the helix-loop-helix PAS-domain containing family of transcription factors. They control transcription of other genes, including the *PER1,2* and *3*, and *CRY1* and *2* genes. The products of these genes form PER-CRY dimers and translocate back from the cytoplasm to the nucleus to inhibit *Bmal1* and *Clock* gene expression. The main controller for the rhythmicity of the biological clock is the delayed translation/transcription feedback loop, that is controlled by phosphorylation of clock proteins by casien kinase epsilon and delta (CKIε/δ) (Cardinali & Pandi-Perumal, 2011; Dibner, Schibler, & Albrecht, 2010).

The suggested mechanism of PER2- and CRY1 in inhibiting the actions of BMAL1 involves two steps. The first step occurs at the beginning of the circadian cycle (at dawn), where CRY1 binds to BMAL-CLOCK-E-Box complex and inhibits transcription by

3

blocking it. At night, both PER2 and CRY1 cause dissociation of CLOCK-BMAL1 from the promoter, causing transcription repression (Ye et al., 2014).

PER2 contains a coding-region polymorphism, the rs934945. This polymorphism results in G to A substitution (Englund et al., 2009; Lee et al., 2011). Diurnal preference was found to be associated with this polymorphism, with the G-variant being associated with morningness phenotype in specific (Lee et al., 2011; Song et al., 2016) The authors of (Lee et al., 2011) suggest that this might indicate that G-variant genotype results in a less functional variant of PER2, thus leading to more morningness. However, other studies failed to show an effect of this polymorphism on morningness (Carpen, Archer, Skene, Smits, & von Schantz, 2005).
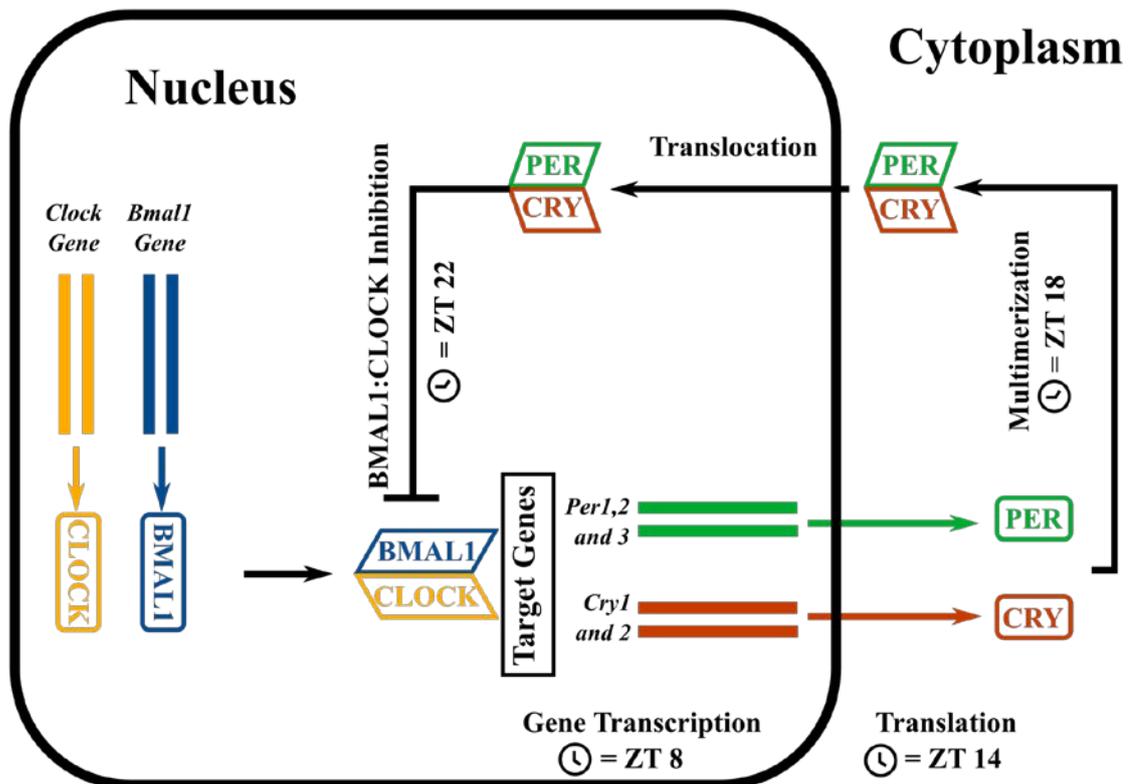
Figure 1.1: Simplified schematic diagram showing the cycle of mammalian clock genes. BMAL1 and CLOCK form a heterodimer that binds to the EBox elements of target clock genes, including PER1, 2 and 3, and CRY1 and 2, activating their transcription. The resulting proteins in the cytoplasm multimerize, enter the nucleus and inhibit the action of BMAL1 and CLOCK. ZT: Zeitgeber time, which represents time in a 12:12 light-dark cycle, with ZT12 being dusk and ZT24 being dawn.

1.2 Molecular Biology of Clinical Depression

Tryptophan is an essential amino acid that plays a major role in the brain-immune-endocrine systems interaction. It is metabolized in the liver, the periphery (macrophages) and the central nervous system. There are two main pathways for Tryptophan, the kynurenine and serotonin pathways (Fig. 1.2). The Kynurenine pathway is the dominant pathway, and 99% of tryptophan is metabolized in the pathway. It involves the opening of the indole ring of tryptophan through the action of one of two enzymes, the 2,3-dioxy-genase, which is found only in the liver, and the indoleamine 2,3-dioxygenase, which is found in the periphery and the central nervous system. One of the products of the kynurenine pathway is 3-hydroxykynurenine which is known to be harmful for neurons, as it causes neuronal death from free radicals (de Jong, Smit, Bakker, de Vries, & Kema, 2009; Miura et al., 2008).

The other pathway is the production of serotonin from tryptophan. After tryptophan enters the brain through a carrier, it is converted to 5-HTP (5-Hydroxytryptophan) by the action of tryptophan hydroxylase, and is then converted to serotonin (5-HT; 5-Hydroxytryptamine). When a serotonergic neuron in the brain releases serotonin in the synaptic terminal, serotonin needs to be retaken to the same terminal again, and is either metabolized to 5-HIAA (5-Hydroxyindoleacetic acid) or repackaged in vesicles for further release (Miura et al., 2008; Owens & Nemeroff, 1994).
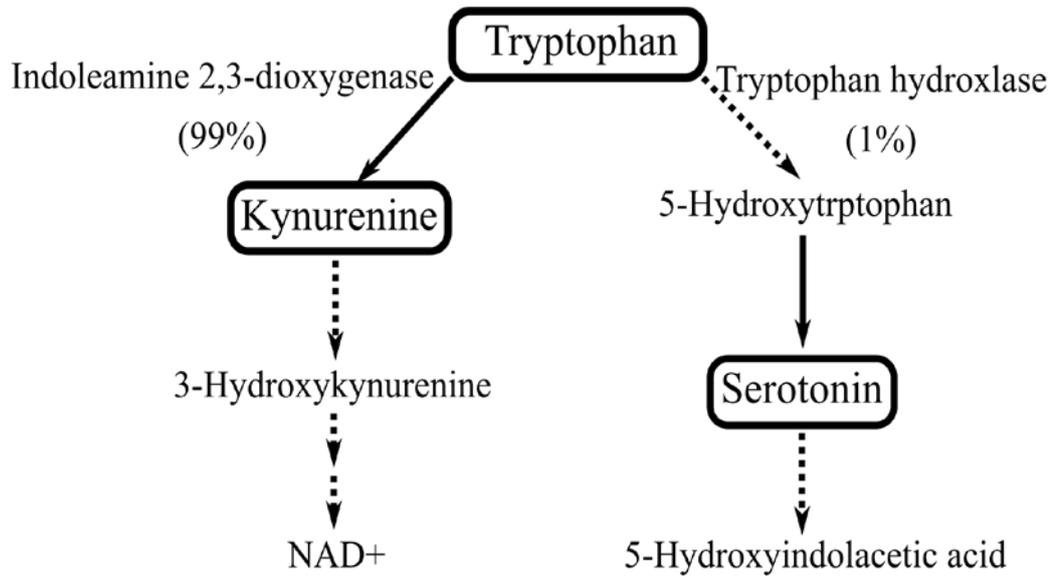
Figure 1.2: Pathways of tryptophan in producing serotonin or kynurenine. Less production of serotonin in favor of kynurenine might be harmful for neurons. The kynurenine pathway metabolizes 99% of dietry intake of tryptophan, and the serotonin pathway metabolizes

It has been postulated that serotonin levels in the brain directly control the expression of depression symptomatology. This is known as the monoamine hypothesis of clinical depression. It states that the low levels of monoamines, serotonin and norepinephrine, is related to the onset and symptoms of clinical depression (Miura et al., 2008). This hypothesis is one of the most well-established theories of clinical depression (Hindmarch, 2001). There are findings supporting the monoamine hypothesis of clinical depression, and these include the relapse of depressive symptoms after tryptophan depletion or inhibition of tryptophan hydroxylase, mood lowering after tryptophan depletion in recovered patients and vulnerable healthy controls, decreased sensitivity in 5-HT1A receptors and 5-HT1B receptor malfunctioning (Belmaker & Agam, 2008; Miura et al., 2008).

Serotonin is released mainly from the raphe nucleus in the brain stem. It sends serotonergic input to various areas in the brain (Savitz, Lucki, & Drevets, 2009). There are

14 subtypes of serotonin neurotransmitters that belong to 7 main families (Blier & Abbott, 2001). Polymorphic changes in these receptors' genes can affect some psychiatric illnesses. For example, a polymorphism affecting the 5-HT2A receptor is correlated with clinical depression and response to treatment, and 5-HT3 is correlated with schizophrenia and bipolar disorder (Savitz et al., 2009).

5-HT1A receptor is the best characterized of these receptors, it is found both postsynaptically in neurons of target areas, or presynaptically. The presynaptic 5-HT1A is an autoreceptor that, when activated, reduces the firing rate, firing amount and neurotransmitter synthesis in the presynaptic neuron. Thus, it has the ability to modulate serotonergic function in the brain, such as cognition and emotion. Furthermore, it can affect neuronal migration, axonal and dendritic outgrowth and synapse formation. This makes the 5HT1A receptor a good candidate to play a role in affective disorders (Albert & Lemonde, 2004; Savitz et al., 2009).

5-HT1A is associated with clinical depression. It is hypothesized that there are alterations (increase) in basal expression of 5-HT1A autoreceptors in clinical depression. In general, there are multiple mechanisms that reduce 5-HT transmission can predispose to clinical depression (Albert & Lemonde, 2004). On the other hand, there are different lines of evidence suggesting that antidepressants exert their effects through this receptor. Selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors cause desensitization of presynaptic 5-HT1A, while tricyclic antidepressants increase sensitization of postsynaptic 5-HT1A (Savitz et al., 2009).

5-HT1A receptor promoter contains a functional polymorphism under the name C(-1019)G (rs6295). The G variant was found to increase autoreceptor activity in raphe neurons, which causes these neurons to become less active in terms of serotonin firing rate and amplitude, which in turn increases the risk for developing clinical depression. It was

also found to decrease resilience to stressful events (Benedetti et al., 2011). Patients with clinical depression were found to have the G/G variant of the polymorphism twice as much as the non-depressed. This variant was also associated with suicide, with 4 fold increase in those who committed suicide (Lemonde et al., 2003). However, there are studies done on healthy controls that did not support this, in which there was no difference in serotonin binding in either genotype of 5HT1A receptor, which suggests that it expresses its effect in the disease state only (David et al., 2011). The suggested underlying mechanism involves the nuclear-deformed epidermal autoregulatory factor (DEAF)-1-related protein (NUDR). NUDR is a transcription repressor that can bind the C variant of the polymorphism and not the G variant. This means that NUDR only represses the activity of the C variant, which decreases autoreceptors activity in this variant. This is not the case in G variant, and this increases the activity of autoreceptors in this variant more, increasing the risk of clinical depression (Lemonde et al., 2003; Watanabe et al., 2017).

1.3 Clinical Depression and the Disruption of the Biological Clock

One of the most prominent features of clinical depression is the disruption of the circadian clock in the form of sleep disturbances. It can manifest in the form of inability to initiate sleep, inability to maintain sleep, early morning waking, excessive sleepiness … etc. This has been reported in 70-80% of patients with clinical depression (American Psychiatric Association, 2013; Bunney & Potkin, 2008). Other abnormalities include hormonal dysregulation of melatonin and cortisol. Disruption of the diurnal regulation of temperature, which appears as an increase in core body temperature at night, has been reported. Finally, many patients with clinical depression report having melancholic mood early during the day, with almost euthymic state at evening (Bunney & Potkin, 2008).

Symptoms of clinical depression might be related to chronotype. In patients with clinical depression, eveningness is associated with more suicidal thoughts, work impairment and anxiety symptoms (Gaspar-Barba et al., 2009). This correlation has also been reported in healthy subjects, in which healthy controls with later chronotype were found to show more severe depression symptoms (Hidalgo et al., 2009; Levandovski et al., 2011).

Clock genes can affect mood-related behaviors. For example, patients with clinical depression showed lower CRY2 baseline levels, and no upregulation of CRY2 expression following sleep deprivation (Lavebratt et al., 2010). In another study, chronic unpredictable stress was shown to have depressive properties. It showed that the oscillation of PER2 expression in specific, and not PER1, was reduced in the SCN. This effect of chronic stress in reducing PER2 oscillation was removed after the introduction of desipramine, an antidepressant (Jiang et al., 2011). These results suggest that biological clock changes are present on the molecular level in patients with clinical depression.

1.4 Interplay Between Serotonin and the Circadian Clock

There are three main inputs to the SCN. The first is the retinohypothalamic tract, which relays light signals to the SCN. The second input is from the median raphe nucleus, which affects the SCN by releasing serotonin at the target cells. The third pathway starts from the dorsal raphe nucleus, which sends serotonergic signals to the IGL, which in turn affects the SCN through the release of neuropeptide Y (Ciarleglio et al., 2011; Germain & Kupfer, 2008).

Serotonin is a main neurotransmitter that plays a role in non-photic entrainment of the circadian clock. Serotonergic agonists can cause phase advance in the circadian rhythm, and this is suggested to be through the reduction of both *PER1* and *PER2* mRNA

levels (K Horikawa et al., 2000). Activation of 5-HT1A and $5HT_7$ receptors mediate non-photic signals to the SCN. This happens through both receptors functioning in synchrony and not separately (Kazumasa Horikawa, Fuji, Fukazawa, & Shibata, 2013).

A suggested correlation between PER2 and monoamines comes from the theory of Hampp & Albrecht (2008). This theory is based on findings in mammals, and it states that phosphorylation of PER2 by GSK-3β increases PER2 accumulation in the nucleus, which enhances transcription of monoamine oxidase A (MAO-A) by NPAS/BMAL1. MAO-A degrades dopamine and serotonin (Owens & Nemeroff, 1994). This indicates that higher PER2 levels increase serotonin degradation, increasing the chance of depression.
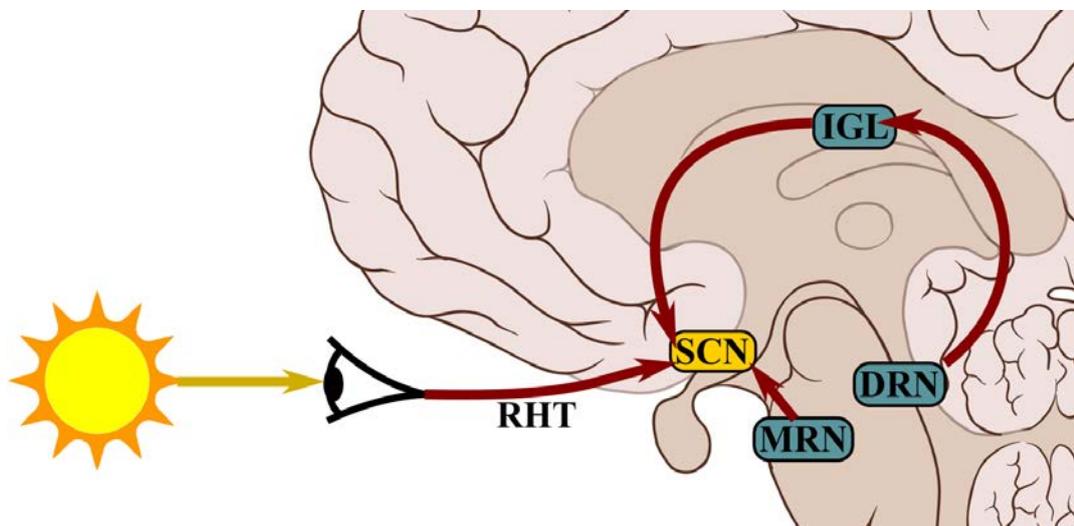
Figure 1.3: Graphical representation of the input pathways to the SCN. The main input is from light, transmitted to the SCN through the retinohypothalamic tract. The MRN gives direct serotonergic input to the SCN. DRN sends serotonergic projection to IGL, which in turn controls the SCN through neuropeptide Y.

DRN = Dorsal Raphe Nucleus, IGL = Intergeniculate Leaflet, MRN = Median Raphe Nucleus, RHT = Retinohypothalamic Tract and SCN = Suprachiasmatic Nucleus.

The figure used the following figures from the web: ("File:Brain bulbar region.svg - Wikimedia Commons," n.d.; "The Sun 4 Free Stock Photo - Public Domain Pictures,").

1.5 Electroencephalography, Clinical Depression and the Circadian Clock

1.5.1 EEG and Clinical Depression:

Studying brain oscillations can provide us with valuable information about the correlation between clinical depression,sleep patterns, and other neurophysiological signals that can be collected via EEG. It has been reported that EEG pattern during sleep is different in patients with clinical depression than healthy controls. These differences include reduced sleep efficiency, reduced power of the entire oscillation spectrum, especially the delta waves, reduced rapid eye movement (REM) latency and changes in REM density (Kupfer et al., 1980; Mendelson et al., 1987).

Previous studies introduced valuable information about mood and how it correlates with brain oscillations. The brain's asymmetric activity was correlated to response to positive and negative stimuli, with higher left brain activation being associated with more positive mood in response to positive stimuli. Conversely,activation of the right brain was associated with more negative responsiveness to negative stimuli (Kupfer, Spiker, Coble, & Neil, 1998). These findings were replicated in patients with clinical depression, where Henriques and Davidson found that there was less activation of the left side of the brain, as evident from the higher alpha activity in that side (Henriques & Davidson, 1991).

These findings, including changes in brain function and laterality in alpha activity, might be challenged by the fact that such changes do not regress after treatment of clinical depression (Fingelkurts et al., 2006). However, this doesn't change the fact that clinical depression is associated with changes in brain physiology. These changes might be evident in these patients, regardless of the expression of symptoms. Furthermore, it was reported

that patients with clinical depression have higher alpha and beta, and lower delta power. EEG power changes in patients with clinical depression were found to happen across nearly the whole cortex and it showed changes in frequency composition over a wide range of frequencies (Fingelkurts et al., 2006).

1.5.2 EEG and the Circadian Clock

EEG can be used to identify and analyse various stages of sleep (Hori et al., 2001; Rechtschaffen & Kales, 1968). Advancements in EEG computation helpeded in studying contributions of various frequency components to sleepiness and wakefulness (De Gennaro, Ferrara, & Bertini, 2001)

EEG spectral analysis in various studies found that alpha power decreases during wakefulness while theta power increases. These power changes can be used as an indicator of sleepiness, and might be a representation of the homeostatic process (Arcady A Putilov, Donskaya, & Verevkin, 2012), which is the process in which the drive for sleep increases during wakefulness (Borbély & Achermann, 1999). The same study also suggests that the theta to alpha power change ratio is a better predictor than any of them alone.

Chronotype naturally affects sleepiness and wakefulness patterns. EEG studies performed during wakefulness show that chronotype can affect EEG signal of wakefulness and sleepiness (A A Putilov, Donskaya, & Verevkin, 2009). An EEG sleep study done on morning- and evening-types of people found that REM latency was the only variable different between morning people and evening people, which they attributed to other factors such as personality factors or anxiety (Ishihara, Miyasita, Inugami, Fukuda, & Miyata, 1987)

1.6 Problem Statement:

The effects of biological clock variations on clinical depression are well established. However, the interaction between the genotype, brain oscillations, chronotype, and clinical depression symptomatology levels warrants further investigation in a more coherent way. This can be done by assembling a theoretical framework for the relationship between these different factors to evaluate the interaction between all of these levels to shed light on the pathophysiology of clinical depression.

1.7 Study Justification

Since the effects of genotypes in general are complex and likely multidimensional, studying multiple aspects of genotype effects will give a more comprehensive overview of the effects of genotype. This study will allow us to search for the interaction effect between the four factors of study, which will lead us to formulate a comprehensive overview on how these factors participate in producing clinical depression.

1.8 Study Goals

To examine the gene-oscillation-chronotype-symptom axis by examining the interaction between serotonin and the circadian clock at the genetic level to infer effects on brain oscillations, chronotypes, and depression symptomatology.

1.9 Significance of the Study

- Mapping the neural basis of circadian rhythms as well as the effect of selected genes onto the symptoms of clinical depression.

- Inform the design of future treatment modalities for clinical depression that capitalize on circadian rhythms.

1.10 Study Questions

- Is there an effect of *5-HT1A* and *PER2* genotypes on brain oscillations,chronotype, and depression symptom severity?
- Is there a correlation between chronotype and the severity of depression symptoms?
- Is there an interaction between chronotype and the severity of depression symptoms with brain oscillations?

1.11 Predictions

We predict that the G variant of 5-HT1A polymorphism (rs6295) is associated with eveningness. This is based on the finding that the G variant is associated with less serotonin activity (Benedetti et al., 2011), and the notion that higher serotonin leads to phase advance (K Horikawa et al., 2000), which means that lower serotonin (G variant) will lead to phase delay, and thus more eveningness. For *PER2*, we expect that the G-variant is associated with morningness, which is what was found in (Lee et al., 2011).

We predict that the G-variant of 5-HT1A and the A-variant of PER2 are associated with less serotonin levels and later chronotype, and that the C-variant of 5-HT1A and the G-variant of PER2 are associated with higher serotonin and earlier chronotype. The high-serotonin group is predicted to be associated with less depression symptoms than the low-serotonin group.

Depression scores are predicted to change according to 5-HT1A polymorphism. The G-variant is expected to give more tendency towards depression, as it is associated with lower serotonin levels (Benedetti et al., 2011).

We predict that later chronotype is associated with more depression. This is based on the findings of (Hidalgo et al., 2009; Levandovski et al., 2011)

We also predict a negative association between chronotype and theta power in EEG. The hypothesis is based on findings reported by Putilov and colleagues (Arcady A Putilov et al., 2012). The authors found that theta power increases during wakefulness. This indicates that the more the wakefulness period, the more the theta power. Since earlier chronotype have larger wakefulness period at any particular point in the day, earlier chronotype is expected to be associated with more theta power.

Finally, I predict to have higher theta power in subjects with more morningness-genotype (the G variant in *PER2*, and the C variant in *5-HT1A*).

2. Chapter Two:

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Materials and Methods

2.1 Subjects

Sixty-three subjects were randomly recruited from Al-Quds university students and the general population in the West Bank, Palestine. Demographic data are presented in (Table 2.1). All subjects underwent the mini international neuropsychiatric interview (Amorim, Lecrubier, Weiller, Hergueta, & Sheehan, 1998). All subjects were psychologically healthy at the time of testing according to the MINI. Five subjects had a history of clinical depression previously.

Table 2.1: Demographic data for the participants

Demographic	Value
Age (Mean ± SD)	20.93 ± 2.07 years
Gender (Female %)	63.2 %
Education (Mean ± SD)	14.61 ± 2 years

2.2 Ethical considerations

This research was conducted based on the Declaration of Helsinki and was approved by the Al-Quds University Research Ethics Committee. A clear description of the procedure was given to subjects prior to testing. A written informed consent was

obtained from all subjects prior to starting the testing protocol. The signed consent forms are available upon request.

2.3 Psychometric and Chronotype Questionnaires

2.3.1 The Beck Depression Inventory II (BDI-II)

The BDI-II (Beck, Steer, & Brown, 1996) is a 21-item questionnaire that assesses cognitive, affective and somatic symptoms of depression. Each question has choices that range from 0 (no symptoms) to 3 (severe symptoms), with a final score between 0-63 (Levandovski et al., 2011).

2.3.2 The Munich ChronoType Questionnaire (MCTQ)

The MCTQ (Roenneberg et al., 2003) is a questionnaire that assesses personal chronotype in a quantitative manner. It does so by asking about personal preferences of sleeping and waking during free and work days. Chronotype is calculated based on mid-sleep time in free days. Mid-sleep time is expected to change because of the sleep debt the subjects accumulate during the week. That is why, chronotype is calculated based on a corrected mid-sleep time that takes into account this sleep debt (Allebrandt & Roenneberg, 2008).

2.4 Blood Collection and DNA Extraction:

About 5 ml of blood were drawn from each subject. The samples were centrifuged at 1000g for 10 minutes. The buffy coat was then taken and DNA was extracted using MasterPure DNA Purification Kit for Blood Version II ©.

2.5 Primer Design

Primers were designed using Prime 3.0 software for both 5-HT1A receptor and *PER2* polymorphisms.

The first primer was for 5-HT1A receptor polymorphism (rs6295). The primers are displayed in (Table 2.2). For the 5-HT1A receptor polymorphism, there was no enzyme that can cut at the site of polymorphism. The reverse primer was designed so that it overlaps the polymorphism site, and we introduced a mismatch at the nucleotide second to last in the reverse primer, thereby creating an artificial polymorphic restriction site that can be cut using the HpyCH4IV restriction enzyme (Beste, Heil, Domschke, & Konrad, 2010; Hong, Chen, Yu, & Tsai, 2006).

The second primer was for the *PER2* polymorphism. The primers are shown in (Table 2.2), and the cutting enzyme was BamHI.

Table 2.2: Primers used, their sequences, and their cuttings enzymes

Polymorphism	Direction	Primer	Restriction Enzyme
5-HT1A (rs6295)	Forward	5'-TTCTCCCTGAGGGAGTAAGGCTGG-3'	HpyCH4IV
	Reverse (mismatched)	5'-TGGAAGAAGACCGAGTGTGTCT**A**C-3'	
PER2 (rs934945)	Forward	5'-TGGGACTCAGCGAAGTGTC-3'	BamHI
	Reverse	5'-GGAAACAGCCATGAAGATCTTTC-3'	

2.6 Polymerase Chain Reaction (PCR):

We used Syntezza tubes (Syntezza Bioscience Ltd., Jerusalem, Israel) for preparing the PCR mix. The mix for both polymorphisms was similar in proportions. For each sample, the mix consisted of 18 µl of ultrapure water, 0.5 µl of the forward primer (which is equivalent to one unit), 0.5 µl of the reverse primer (which is equivalent to one unit) and 1.5 µl of DNA, with a final volume of 20.5 µl.

Twenty-eight subjects had their PCR done for 5-HT1AR polymorphism. The PCR protocol followed for 5-HT1AR polymorphism consisted of an initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of 95 °C for 30 seconds, 58 °C for 30 seconds and 72 for 30 seconds. This was followed by 7 minutes of 72 °C (**Fig. 2.1 - right**).

Forty-one subjects had their PCR done for *PER2* polymorphism. The PCR protocol for the *PER2* consisted of an initial denaturation at 95 °C for 2 minutes, followed by 35 cycles of 95 °C for 30 seconds, 58 °C for 30 seconds and 72 for 45 seconds. This was followed by 5 minutes of final elongation at 72 °C (**Fig. 2.1 - left**).

2.7 Restriction Fragment Length Polymorphism (RFLP) Analysis

The Nebcutter tool ("NEBcutter V2.0 | LabTools," n.d.). For 5-HT1A, the enzyme HpyCH4IV was used, which cuts at the palindromic sequence A$^\vee$CGT. The length of PCR product is 182 nucleotides. For the genotype that has the C variant, the enzyme will cut and produce two bands with lengths of 157 and 25. For the G-variant, the enzyme will not cut, and the product will be of length 182 (**Fig. 2.2 - left**).

The enzyme digestion mix consisted of 13 µl ultrapure water, 0.1 µl of the cutting enzyme, 2 µl of the buffer and 5-7 µl of the pcr product. The incubation period for the enzyme digestion mix was 30-60 minutes at 37 °C.

For *PER2*, the enzyme BamHI was used, which cuts at the sequence G$^\vee$GATCC. The length of PCR product is 163 nucleotides. For the genotype that has the G variant, the enzyme will cut and produce two bands with lengths of 121 and 42. For the A-variant, the enzyme does not cut, and the product is of length 163 (**Fig. 2.2 - right**).

The enzyme digestion mix consisted of 13 µl ultrapure water, 0.1 µl of the cutting enzyme, 2 µl of the buffer and 5 µl of the pcr product. The incubation period for the enzyme digestion mix was 60 minutes at 37 °C.

2.8 Measure of DNA Concentration and Purity

DNA concentrations were measured using a NanoDrop Spectrophotometer. 10 Samples were chosen to check DNA concentration and A260/A280. Mean concentration was 629.46 ± 464.27 ng/µL, and values ranged from 28 - 1560).

A260/A280 is a measure of DNA purity, it may indicate the presence of a protein, phenol or other contaminants in the sample. A measure of more than 1.80 is considered an acceptable number for DNA. For our samples, mean A260/A280 was 1.913 and SD was 0.054. No sample fell below 1.80.

PER2

163 pb

5HT1A

182 bp
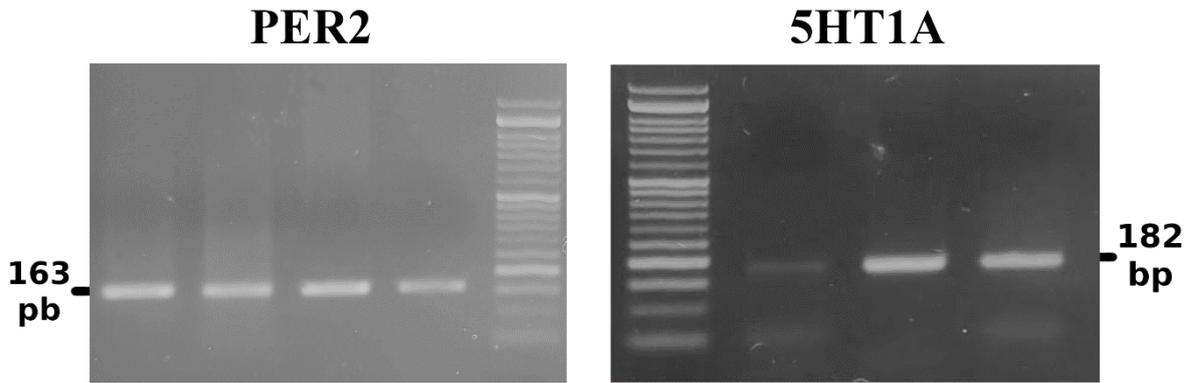
Figure 2.1: Gel electrophoresis of PCR product for PER2 (left) and 5-HT1A (right). Band lengths are 163 for PER2, and 182 for 5-HT1A. 50-bp ladder is shown in each of the pictures.

PER2

GG GA   AA GG

163
121
bp

5HT1A

GC GG GG CC

182
157
bp
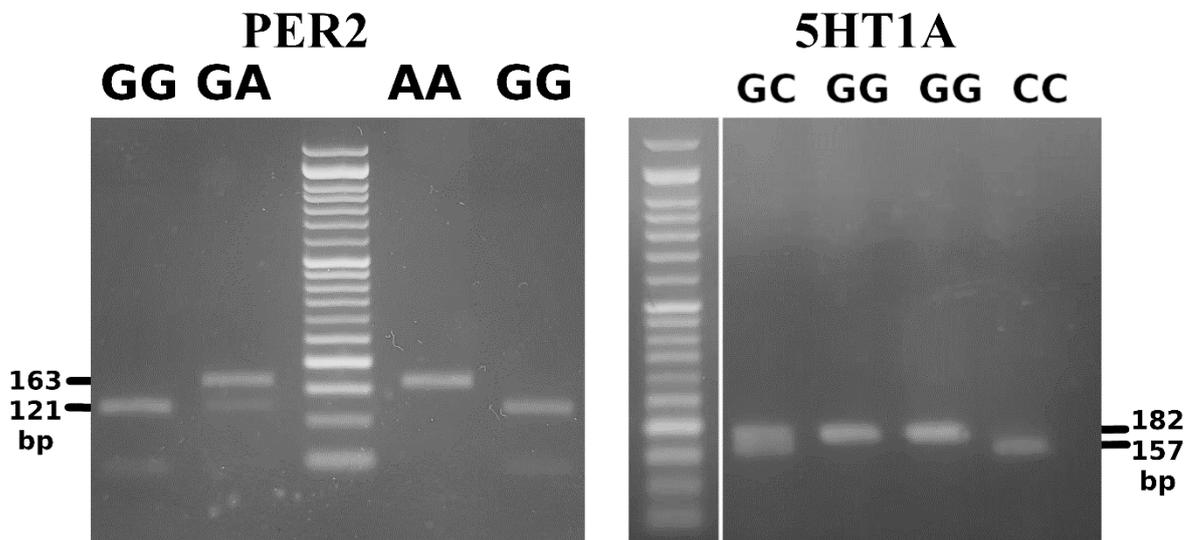
Figure 2.2: Enzyme digestion product for PER2 (left) and 5-HT1A (right). Products of enzyme digestion for the G-variant of PER2 are 121+42, and for 5-HT1A the digestion product for the C allele are 157+25. The ladder used is 50 bp in length in both pictures.

22

2.9 Electroencephalography (EEG)

A subset of our subjects underwent EEG recording in Al-Quds university. Electrical signals of the brain were acquired on scalp using a 23-channel recording system (Virgo 32, Allengers Global Healthcare Pvt. Ltd.). EEG was recorded according to the international 10/20 system. A recording cap was used for the recording consisting of 21 electrodes. Two additional electrodes were added manually on the frontal (FPz) and occipital (Oz) areas. Eye blinks and horizontal eye movements were recorded in synchrony using two electrodes attached above and below the right and left eye, respectively.

Data analysis, including noise and artifact rejection, were done using the MNE-Python toolbox (Gramfort et al., 2014). All data were bandpass filtered between 1-40 Hz.

Artifacts were removed using independent component analysis (ICA). After segmenting the data into 5-minutes segments, ICA was applied to each segment separately. An automatic identification scheme was applied to search for ocular activity using either both ocular or two frontal channels (FP1 and FP2). The identification of cardiac activity was done by visual inspection. In some segments an additional artifact was identified that resulted from the stimulus computer that is used to play the task. This artifact was also removed by means of the corresponding components Finally, bad segments of the data (i.e., EEG signals exceeding untypical large magnitudes) were identified and annotated by using a threshold of 270 µV. These data segments were discarded from analysis.

EEG resting state recordings ranging from 5-20 minutes were recorded with eyes open (Mean ± SD = 10.7 ± 5.8) and 5-20 minutes with the eyes closed (Mean ± SD= 15.5 ± 5.55) in a comfortable sitting position in a quiet room. For the eyes open study, the subjects were asked to fixate on a crosshair placed in the middle of a white A4 paper with 50 cm distance in order to limit horizontal eye movement. For the eyes closed study, the

subjects were asked to close their eyes for 20 minutes and try not to sleep. the tester monitored the subjects to make sure they do not fall asleep during the session.

The subjects were then asked to play a probabilistic feedback-based learning and categorization task (Bódi et al., 2009). This task includes two types of stimuli, the rewarding stimuli and the punishing stimuli. The rewarding stimuli give either reward (smiling face) or no feedback when the subject answers correctly or incorrectly, respectively. The punishing stimuli give either no feedback or a punishing feedback (sad face) when the subject answers correctly or incorrectly, respectively. The computer screen was placed about 50 cm in front of the subject in a comfortable sitting position. The task consisted of 280 trials, divided between rewarding stimuli and punishing stimuli. The stimulus is presented to the subject and the task waits for the response of the subject. After the subject responds to the stimulus, the answer is presented for 1 second, then a cross sign is presented for a variable time between 0.74 and 0.84 seconds, then the feedback is presented for a variable time between 0.72 - 0.82 seconds.

2.10 Statistical Analysis

2.10.1 Inferential Statistics:

We used R programming language version 3.5.2 and SPSS V20 to conduct the analyses.

In order to test the interaction between genotypes in affecting depression symptoms and chronotypes, we divided the subjects according to their genotypes to two groups per genotype. For PER2, subjects who were homozygous for the G-allele were put in the group "G-Homo", the rest were named "A-Carrier". For 5-HT1A, subjects homozygous for the G-allele were named "G-Homo", the rest were put under the name "C-Carrier". These groups were entered as two independent variables in two-way MANOVA., with

24

chronotype and BDI-II scores as the dependent variables. Gender was included in this ANOVA, to see the effect of gender on the chronotype.

Finally, The correlation between chronotype and depression was tested using two methods. The first is a t-test between BDI-II score of early chronotypes, and BDI-II score of later chronotypes. Then a correlational analysis was conducted between mid-sleep time in free days, as an additional measure of chronotype, and BDI-II scores.

2.10.2 EEG Analysis

In this part of the analysis, we tested a subgroup of our sample using EEG, as an additional way of looking at brain functionality and connecting it with the underlying aspects of the biological clock, genotype and depression symptomatology.

As a first step in this analysis, we identified the most prominent channel that reflects most of the signal changes during the task. For this, we need to measure brain activity after giving stimuli that are known to activate the brain at specific time points.

To do that, we introduced a feedback-based learning task to look at brain activation at specific time periods. The task asks the subject to categorize objects into two groups of categories, and based on that it gives a reward or a punishment. The content of the task and the results obtained from it are not relevant to our study. We will use the task only for analyzing the brain's response to reward and punishment.

To identify the most relevant electrode position for our study, a Monte Carlo cluster permutation test was applied based on the method introduced by (Maris & Oostenveld, 2007) and implemented in MNE-Python (Gramfort et al., 2014) using EEG signals from all subjects averaged upon stimulus onset. For testing variations within the spatio-temporal dynamics, t-scores were computed over a time window ranging from 0 to 600 ms following stimulus onset, using a large amount of permutations (10^4). To identify a

25

significant increase in the EEG signal changes a critical alpha level of 0.05 was used. As the cluster level permutation test addresses the multiple comparison problem at the same time (Maris & Oostenveld, 2007), the increased signal changes reflect a significance level of $p \leq 0.05$.

The second step was to compare brain alpha and theta waves in different groups of subjects. First, chronotype correlation with brain waves was calculated. Second, alpha and theta waves were correlated with BDI-II using the same test.

The effects of genotypes on alpha and theta content was also tested using independent samples t-test. The number of subjects did not allow us to perform more complicated tests on alpha and theta content.

3. Chapter Three

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Results

3.1 Genotyping

The results of genotyping are provided in (Table 3.1). Hardy-Weinberg equilibrium was done using chi-square-test revealed a non-significant deviation from the Hardy-Weinberg equilibrium for both genotypes (5-HT1A: χ^2 = 0.03541, p-value = 1, PER2: χ^2 = 0.17778, p-value = 1). Linkage disequilibrium analysis revealed no linkage disequilibrium between the two genotypes (D' = 0.1771, r^2 = 0.02, χ^2 = 1.099, p = 0.294), See (Table 3.1) for details.

Differences between males and females in terms of genotype distribution showed a significant effect of gender in the PER2 gene but not in 5-HT1A. Female:Male ratio in the G/G group was higher than Female:Male ratio in the A/A and G/A groups (Table 3.2).

Table 3.1: 5-HT1A and PER2 haplotypes. Shown are the frequencies for 5-HT1A and PER2. D' and p-value for linkage disequilibrium analysis are also shown.

5HT$_{1A}$R / PER2	GG	GC	CC	D'	p
GG	7	6	1	0.1771	0.294
GA	3	6	2		
AA	1	1	0		

Table 3.2: Comparing genotype frequencies of males and females for both 5-HT1A and PER2 polymorphisms

Polymorphism	Genotype	Female	Male	Total	χ^2	P value
5-HT1A	**C/C**	1	2	3	0.41	0.815
	G/C	7	6	13		
	G/G	6	6	12		
PER2	**A/A**	0	2	2	8.152	0.017
	G/A	6	9	15		
	G/G	17	5	22		

3.2 Effect of Genotype on Chronotype and BDI-II

The effect of 5-HT1A and PER2 groups on depression symptoms and chronotype was tested using MANOVA. Results show the absence of significant difference for both 5-HT1A and PER2, with no interaction between them. The effect for gender was significant in this ANOVA. See (Table 3.3) and (Figure 3.1) for MANOVA details.

Following on this with between-subjects tests, The effect of gender was shown to be affecting the chronotype (F-value = 14.258, p=0.004). With the chronotype of females (mean =4.2, SD =1.62) than the chronotype of males (mean = 5.68, SD = 0.96).

Table 3.3: MANOVA results for the effect of genotypes and gender or depression symptoms and chronotypes. Significant effects are marked with a star.

| Variable (s)	F	df between	df within	P value
5-HT1A	0.455	2	8	0.650
PER2	0.586	2	8	0.579
Gender	6.686	2	8	0.020*
5-HT1A*PER2	0.190	2	8	0.830
5-HT1A*Gender	0.311	2	8	0.741
PER2*Gender	0.084	2	8	0.921
5-HT1A*PER2*Gender	0.229	2	8	0.800
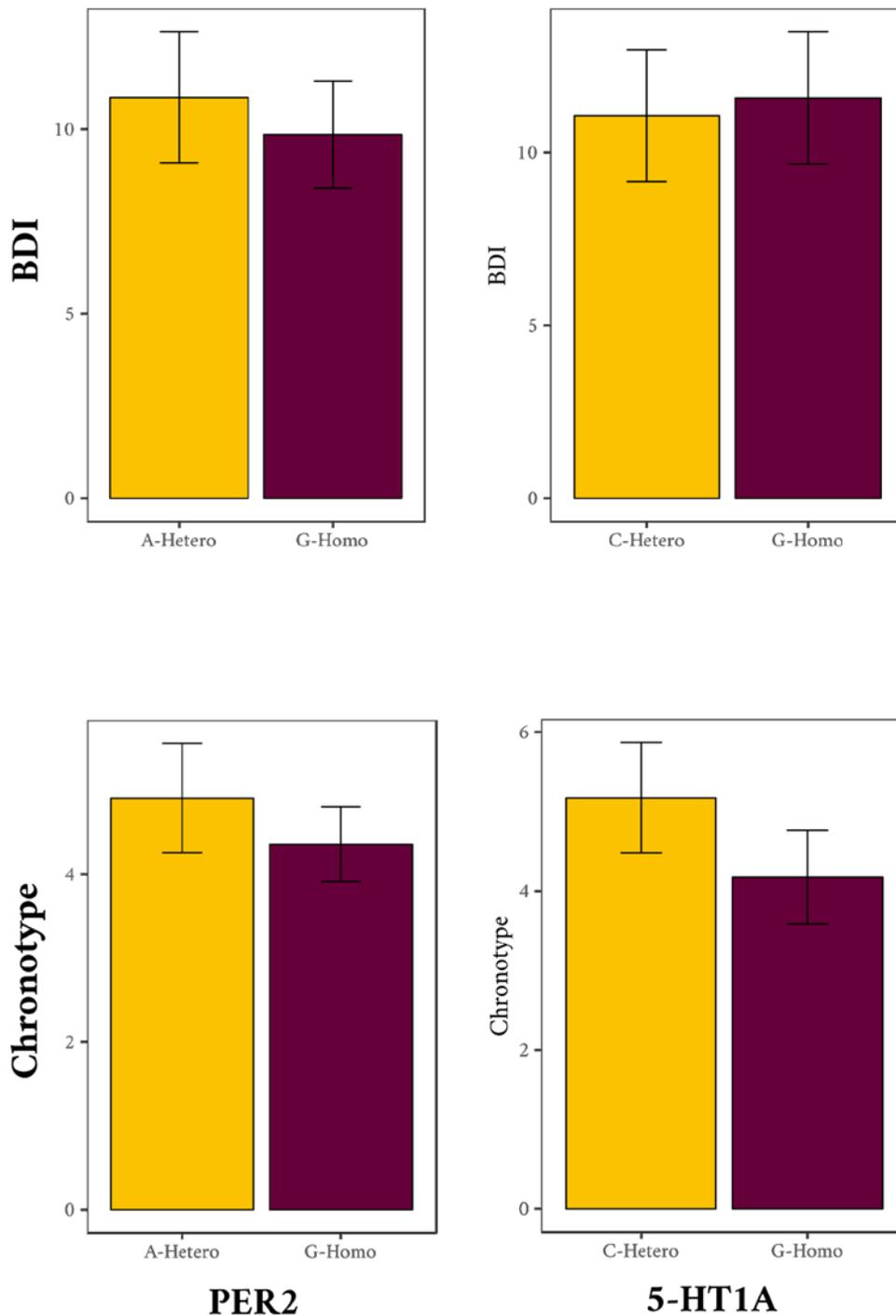
Figure 3.1: General look at the interaction effects of 5-HT1A and PER2 as the independent variables, on chronotype and BDI-II as the dependent variables, with. There was no significant effect for either genotype on either measure.

3.3 Comparing Chronotype and BDI-II

Next, I looked at the effect of chronotype on BDI-II. The mean BDI-II value for early chronotype subjects (mean =8.4, SD = 4.2) was not significantly different than the mean BDI-II value for subjects with late chronotype (mean = 11.2, SD = 7.5). T-test shows a non-significant effect ($t(29.31) = 1.387$, p-value = 0.176). See (Figure 3.2) for details.

This test was followed by a correlation test between mid-sleep time in free days, as a simple measure of chronotype (Figure 3.3). Pearson's correlation analysis showed that there was a significant correlation between BDI-II and mid-sleep in free days ($r = 0.428$, n = 45, p = 0.003). A partial correlation followed this test, we tested the correlation between BDI-II and mid-sleep time taking into account the weekly sleep loss. The partial correlation was also significant ($r=0.402$, n=45, p = 0.007). This indicates that mid-sleep time was significantly correlated with BDI-II symptoms even when taking the weekly sleep loss into account.

Figure 3.2: Comparing BDI scores according to chronotype. Later chronotype did not differ significantly from earlier chronotype in terms of depression symptom scores.

Midsleep VS BDI Score
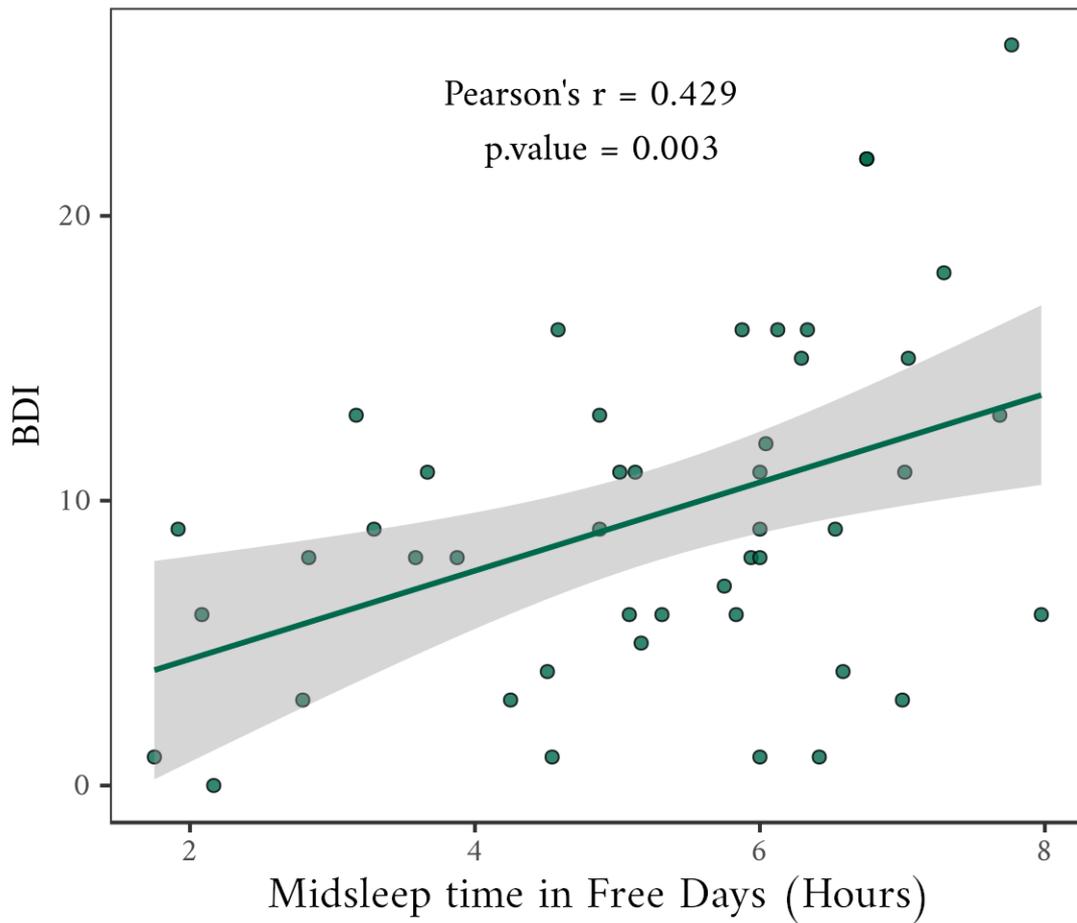
Figure 3.3: The correlation between midsleep time and depression scores as measured by the BDI-II. A significant correlation was found.

3.4 EEG Data Analysis

3.4.1 Identifying the most relevant electrode position

The first step in this EEG analysis was to determine the electrode that reflects most of the signal changes due to probabilistic learning. This was done using a spatio-temporal cluster permutation test. To find the most prominent cluster that picked up most of the brain signal, we looked at brain signals after giving feedback to the subjects. We used three comparisons: Reward Vs no feedback, Punishment VS no feedback and reward VS punishment. In all three comparisons the area at the middle of the brain was activated the most. This includes electrode Cz, which is the electrode located in the center of the cluster with the largest magnitude. Hence, the electrode Cz was used for subsequent analysis. See (Figure 3.4) for details.

A. Punishment VS No Feedback

B. Reward VS NoFeedback

C. Reward VS Punishment
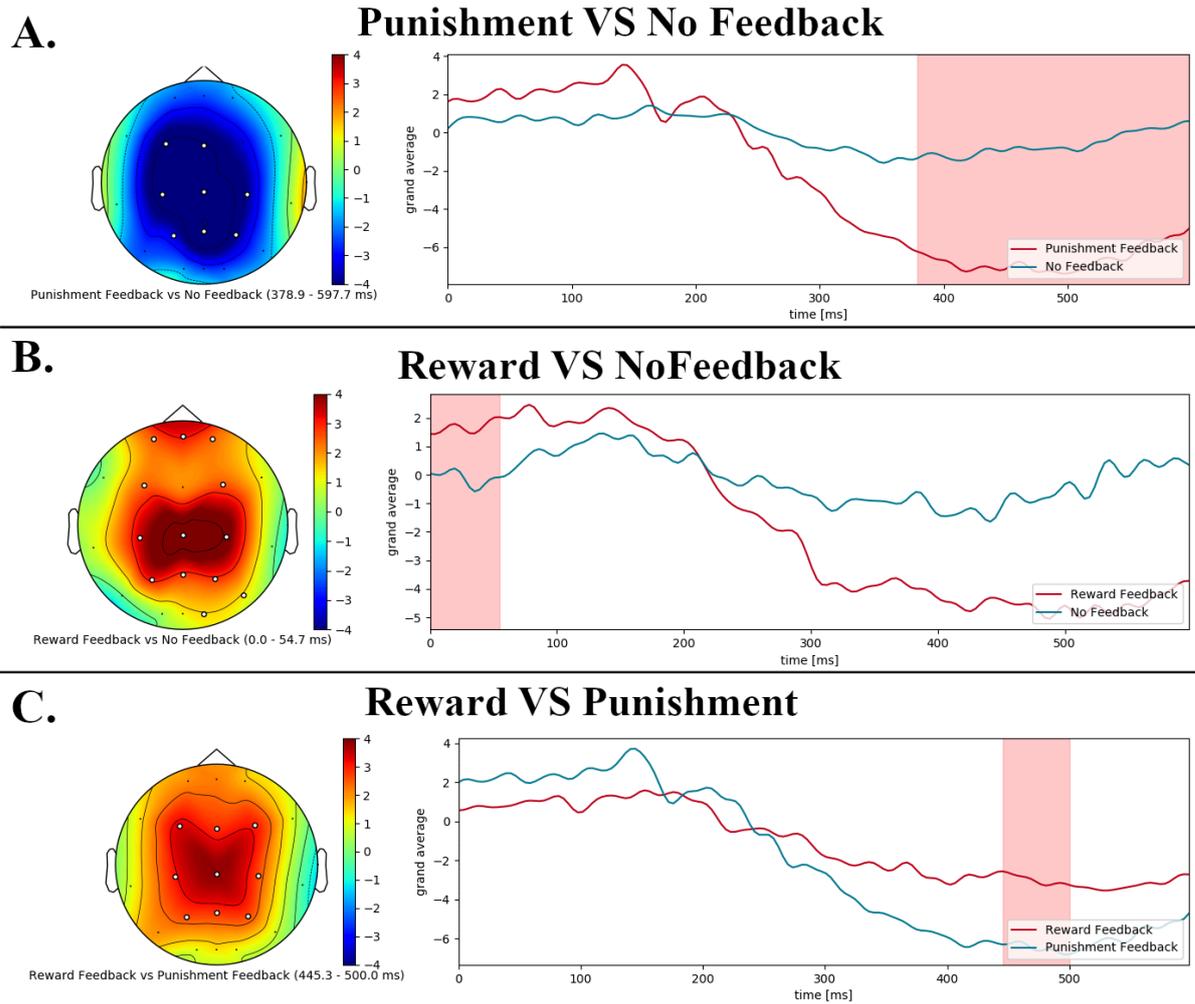
Figure 3.4: Three feedback comparisons were done to identify the most prominent channel. The first is punishment signal VS no feedback signal, the second is the reward signal VS no feedback signal, and the third is the reward signal VS the punishment signal. The area of most activation is around Cz electrode in the middle.

3.4.2 EEG Findings

Theta wave power was calculated for the channel Cz as the most prominent channel. The interaction between theta and chronotype was calculated. A significant interaction was found between the two factors. Pearson's correlation ($r(5)=0.9$, $p = 0.006$) showed there is a significant interaction effect with large size. (Figure 3.5) gives more details.

Finally, testing the differences in theta power in terms of the expected serotonin content (based on genotype) showed that subject group with higher expected serotonin (Mean = 3253, SD = 3298) did not differ significantly from the group of lower expected serotonin (Mean = 3954, SD = 2954). T-test result showed the absence of significant effect ($t(5)$ = 0.285, p= 0.785).
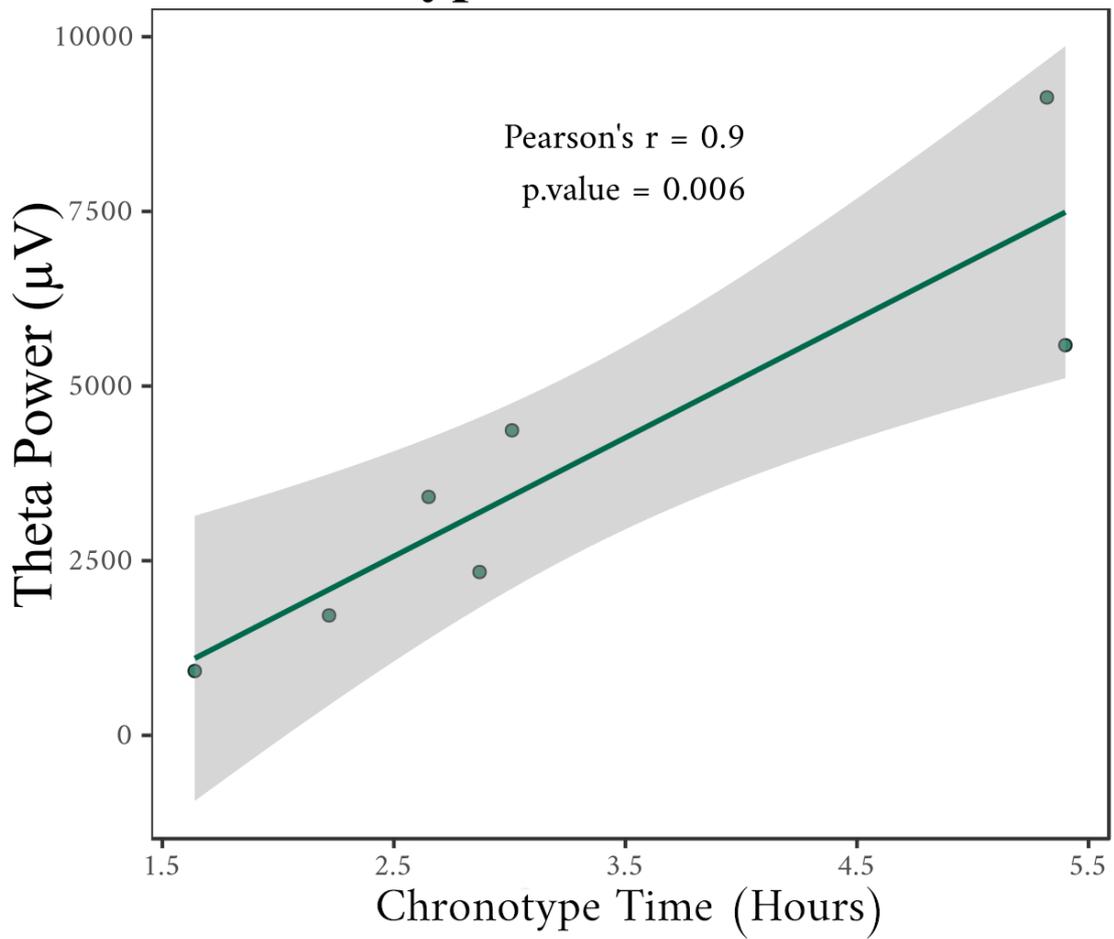
Figure 3.5: The interaction between chronotype and theta power.

4. Chapter Four

Discussion

4.1 Findings Summary

There was neither an effect of the 5-HT1A or PER2 genotypes nor an interaction between the genotypes on depression symptoms and chronotype. There was a significant effect for gender on chronotype, with females having earlier chronotypes. Depression symptoms did not correlate with chronotype, but it correlated significantly with mid-sleep time (another measure of chronotype). Theta activity in EEG was significantly correlated with chronotype.

4.2 Chronotype, Depression Symptoms and Genotype

We based our genotype prediction of serotonin levels on previous findings. (Benedetti et al., 2011) found that the G-variant of 5-HT1A is associated with more autoreceptor activity, which leads to less serotonin activity indicating that the C variant is associated with more serotonin activity. As for (Lee et al., 2011), they suggest that the G-variant of *PER2* polymorphism leads to less functional protein and more morningness. We combined this with the suggested hypothesis of Hampp & Albrecht (2008), which states that lower PER2 levels decrease serotonin breakdown. This indicates that the G-variant of *PER2* is associated with higher levels of serotonin. We searched for these expected differences between genotypes in terms of chronotype and depression symptoms.

We divided the subjects in terms of their 5-HT1A receptor genotype into G-Homozygous and C-Carriers, and according to PER2 into G-Homozygous and A-Carriers. We expected to find an interaction between the two genotypes in which the C-Carriers of 5-HTA1 and the G-Homozygous of PER2 will be associated with higher serotonin levels, and thus more morningness and lower depression symptoms. We did not find an effect of either of the genotype nor there was an interaction between them.

The interactions between the genotype-predicted serotonin level and the chronotype- and BDI-II-predicted serotonin levels were explored. No effect of genotype-predicted serotonin level on chronotype of BDI-II scores was observed. Notably in our sample, we only included 11 subjects who had their chronotype, BDI-II, 5-HT1A genotype and *PER2* genotype completed. Such a low number is hard to be used to find group differences that are as subtle as the effects of serotonin changes. However, we found find an indirect correlation between the genotype and phenotype. Females had higher G/G frequency as compared to males. The female subjects also had earlier chronotypes.

4.3 Chronotype and Depression Symptomatology

Previous studies show that later chronotypes are associated with higher severity of depressive symptoms (Hidalgo et al., 2009; Levandovski et al., 2011). In the present study, a similar effect for later chronotypes could not be detected in our subjects. The calculation of chronotype was originally based on mid-sleep time (Roenneberg et al., 2003), and was modified later to take the weekly sleep debt into account. The currently used method in calculating chronotype according to Roenneberg et al. (2004) involves calculating sleep duration difference between free days and the average weekly sleep duration, then subtracting half of this amount from the mid-sleep time in free days. While this method takes into account the sleep debt accumulating during work days, it does that with no

regard to the amount of sleep loss itself. Rather, it uses an indicator of sleep loss. In our case, we proposed a new method of calculating the effects of chronotype that takes sleep loss directly into account. We used the mid-sleep time as the chronotype, but when performing correlations, we took the calculated weekly sleep loss into account. However, it might not be usable to compare chronotype as a solid number in other tests, such as the ANOVA.

We did a partial correlation between mid-sleep time and BDI-II, taking into account sleep loss. The results showed that mid-sleep time is positively correlated with depression symptoms. This falls in line with findings in previous studies (Gaspar-Barba et al., 2009; Hidalgo et al., 2009; Levandovski et al., 2011).

4.4 Brain Oscillations and Chronotype

The first step was to determine the channel that picks up the most of neural processing involved in picking up the rewarding and punishing feedbacks. To do this, we introduced a probabilistic feedback-based learning task for the mere purpose of measuring brain signal in response to reward and punishment stimuli. For identification of the most relevant electrode we analyzed three types of brain response signals (brain signal in response to reward, punishment, and no-feedback) using a spatio-temporal cluster permutation test. We found that the channel Cz picked most of the activity involved in processing probabilistic learning. This channel was used for further analysis.

Theta activity is considered as a measure of wakefulness and an indicator of the homeostatic process (Arcady A Putilov et al., 2012; Strijkstra, Beersma, Drayer, Halbesma, & Daan, 2003) that builds up during the day. Earlier chronotypes are expected to have higher sleepiness scores since they have had been awake for a longer time period at the time of testing session, and thus they are expected to have a higher theta activity. This

was not the case in our sample. In fact, we found a strong positive correlation between chronotype and theta power, with later chronotypes having stronger theta power. This can be explained by that subjects with later chronotypes have more sleepiness during working days as a result of having less sleep time, as indicated by later biological clock. Accordingly, the main factor here is sleepiness that results from sleep loss, and not from the longer awake time.

4.5 Limitations

The major limitation was the small sample size in terms of genotyping. Only 28 subjects undergone 5-HT1A genotyping and 41 for PER2. Our study measured the interaction between many factors, and this requires a larger sample size in general given the subtle changes, such as the interplay of serotonin and behavior, that we are investigating.

In addition, the confined range of depression symptoms given the recruitment of healthy subjects was another limiting step. Including patients with clinical depression will provide a new aspect to our study.

4.6 Conclusions

The results of this study provide further evidence about the correlation between genotype and phenotype in terms of molecular correlates, brain oscillations, chronotype, and clinical depression. Females had a higher frequency of the G allele in both 5-HT1AR and PER2 alongside early chronotype. There was a positive interaction between chronotype and clinical depression, with later chronotypes having higher severity of depression symptoms, especially when using a chronotype calculation that takes sleep loss into account. Chronotype was also associated with a change in brain oscillations in terms of higher theta waves for later chronotypes. Based on our findings, serotonin and clock

genes could play a role in controlling chronotype, thus affecting brain oscillations and depression symptomatology. However, a direct connection between them is still lacking. This research provides the basis for further experiments in the future. More subjects will be included in our study, and more polymorphisms will be examined in clock and serotonin genes. This will go hand in hand with analysis of brain oscillations in EEG that provides valuable a physiological measure of brain function.

التفاعل بين جينات السيروتونين والساعة البيولوجية وتواسطها في التفاعل بين الاكتئاب السريري والأنماط الزمنية

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المشرف المشارك: د. يورغن دامرز

الملخص

تتحكم الساعة البيولوجية بعدد كبير من الوظائف الفيزيولوجية، وتعمل العديد من الإشارات البيئية المحيطة على مزامنة هذه الساعة، بحيث تتبع دورة مدتها 24 ساعة تتباين في نقطة بدايتها ونقطة نهايتها، وهذا التباين هو ما يشار إليه "بالنمط الزمني". تتحكم النواة فوق التصالبة (واختصارًا SCN) في الدماغ بالنظم اليوماوي، وتشكل بذلك الساعة المركزية للجسم، وما يتبعها من تذبذب في التعبير عن جينات الساعة البيولوجية، والتي تشتمل على جينات PERIOD. كما يتأثر النظم الحيوي بالنقل العصبي السيروتونيني. بل إن المسارات الواردة إلى الـSCN تأتي من النواة الرفائية الإنسية التي تستخدم السيروتونين كناقل عصبي. وتساهم الاختلالات التي تحدث في النظم البيولوجي في الفيزيولوجيا المرضية بالعديد من الاضطرابات النفسية؛ فعلى سبيل المثال، يعاني مرضى الاكتئاب (والذي يمكن اعتباره كحالة نقص في السيروتونين) من خلال عام في النظم البيولوجي لديهم على شكل اختلالات في نظم النوم والهرمونات ودرجات الحرارة.

الهدف الرئيس لدراستنا هو إيجاد العلاقة التبادلية بين أربعة عوامل؛ وهي النمط الجيني، وأعراض الاكتئاب، والنمط الزمني، والوظيفية الدماغية. وقد بحثنا في هذه الدراسة في العلاقة المتبادلة بين النقل العصبي السيروتونيني، والتذبذبات الدماغية في تخطيط كهربية الدماغ (اختصارًا EEG)، والنمط الزمني، والتعبير عن أعراض الاكتئاب عند الأشخاص الأصحاء. بالتحديد، فحصنا اثنين من التعددات

الشكلية التي تحدث بصورة طبيعية في جين مستقبلة السيروتونين من النمط A1 (اختصارًا

5HT1A)، وجين PER2، باعتبارهما مقياسين غير مباشرين للنقل العصبي السيروتونيني ولعمل

الساعة اليوماوية، على الترتيب.

فحصنا ثلاثة وستين شخصًا، وأخضعناهم لتقييم لطور الساعة البيولوجية لديهم (نمطهم الزمني)،

وفحصنا أعراض الاكتئاب السريري لديهم. كما فحصنا مجموعة جزئية من الأشخاص باستخدام

تخطيط كهربية الدماغ من أجل فحص نشاط دماغهم الأساسي واستجابتهم لمختلف المحفزات. فحصنا

النمط الجيني لأغلب الأشخاص، وذلك للنمطين الشكليين للجينَيْن 5HT1A وPER2.

أكدت نتائجنا وجود علاقة تبادلية بين هذه العوامل الأربعة. وبالتحديد، وجدنا أن الأشخاص الذين

يتميزون بنمط زمني متأخر يظهرون نسبة أعلى من أعراض الاكتئاب، ووجدنا أن قوة موجات ثيتا

كانت مرتبطة بشكل إيجابي بالنمط الزمني. كما وجدنا دليلًا غير مباشر على علاقة النمط الجيني

بالنمط الزمني، ولكنا لم نحصل على علاقة مباشرة تؤكد هذه النتيجة.

تزودنا هذه الدراسة بأدلة مبدئية على الأسس الجزيئية للعلاقة بين وظيفية الساعة البيولوجية مع

السيروتونين على أنهما آليتان مقترحتان لحدوث الاكتئاب السريري. يجب أن تشتمل الدراسات

المستقبلية على عينات بحجم أكبر وعلى أشخاص مصابين بالاكتئاب السريري من أجل العثور على

فرق واضح بين الحالات السليمة والحالات المرضية، وهذا سيساعد في تكوين صورة أكثر شمولًا

للفيزيولوجيا المرضية الكامنة وراء الاكتئاب. وفي النهاية، يمكن لذلك أن يرفد عمليات تطوير

العلاجات الحديثة بحيث لا تقتصر على أعراض المرض، بل تأخذ بالاعتبار العلاقات الجينية،

والفيزيولوجية، والمعرفية.

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