Diplomarbeit

Correlation between methylated *ARNTL* and peripheral levels of bound dopamine and norepinephrine in bipolar disorder

eingereicht von

Konstantin Bauer

zur Erlangung des akademischen Grades

Doktor(in) der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an der

Universitätsklinik für Psychiatrie und Psychotherapeutische Medizin Graz

unter der Anleitung von

Assoz. Prof.in Priv.-Doz.in Dr.in med.univ. et scient.med. Reininghaus Eva

&

Priv.-Doz. in Dr. in med.univ. et scient.med. Susanne Bengesser, Bakk.rer.nat.

Graz, 19.10.2018

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 19.10.2018 *Konstantin Bauer eh*

Danksagung

Zuerst möchte ich mich ganz besonders bei meiner Familie bedanken, die mich zu jeder Zeit unterstützt und mir mit Rat und Tat zur Seite steht. Ohne euch wäre mein Studium sicher nicht so unbeschwert verlaufen.

Mein Bruder, Max, hat mir mit seinen Fähigkeiten bei der Gestaltung der Illustrationen dieser Diplomarbeit große Dienste erwiesen und war nie zu müde jede meiner kleinen Änderungen perfekt umzusetzen.

Nicht zuletzt möchte mich beim Team der Spezialambulanz für Bipolare Störung bedanken. Die Arbeit in dieser Forschungsgruppe hat mir einerseits viel Spaß gemacht und andererseits habe ich wertvolle Erfahrungen in der Wissenschaft und im Umgang mit Patienten und Patientinnen machen dürfen, die ich in meiner zukünftigen medizinischen Karriere sicher gebrauchen werde. Hervorheben möchte ich Eva Reininghaus und Susanne Bengesser für die wunderbare Betreuung meiner Diplomarbeit, bei welcher sie stets für Korrekturen und Hilfestellungen, wenn ich an meine Grenzen kam, für mich da waren.

Zusammenfassung

Einleitung: Störungen im circadianen Rhythmus könnten einige der charakteristischen Symptome der bipolaren Störung (BD), wie zum Beispiel das veränderte Schlafbedürfnis, erklären. Die bisherige Literatur gab Anlass epigenetische Veränderungen, wie die Methylierung von *ARNTL*, in Kombination mit den peripheren Werten von Dopamin (DA) und Noradrenalin (NE) zu analysieren. Daraus ergab sich meine Haupthypothese: Ein hoher Methylierungsgrad von *ARNTL* führt zu einer verringerten Expression der Monoaminooxidase A (MAOA), die für den Abbau der Katecholamine zuständig ist. In weiterer Folge führt der verringerte Abbau zu erhöhten Plasmaspiegeln von DA und NE, die die Stimmungsänderungen in der BD erklären könnten.

Methoden: Die Daten wurden im Zuge der BIPFAT und der BIPGEN Studie mit einer Patientengruppe aus ehemals stationär oder ambulant betreuten Personen der Uniklinik für Psychiatrie, Graz, erhoben. Die Analyse des Methylierungsgrades von *ARNTL* wurde mittels DNA Isolierung durch die Aussalzmethode, Bisulfit-Behandlung, PCR und Pyrosequenzierung durchgeführt. Die Werte von DA und NE wurden mit einer Hochleistungsflüssigkeitschromatographie analysiert. Für die statistische Analyse wurde das IBM SPSS Version 23 verwendet. Um die Daten zu vergleichen wurde in Abhängigkeit der Homogenität entweder der t-Test oder der Mann-Whitney-U Test verwendet und um die Beziehung zwischen *ARNTL* und DA und NE zu analysieren wurde die Spearman-Rang-Korrelation durchgeführt.

Ergebnisse: Es wurden keine signifikanten Unterschiede in den Spiegeln von DA und NE zwischen der Patienten- und der Kontrollgruppe gefunden und auch die Korrelation zwischen dem Methylierungsgrad von *ARNTL* und den Katecholaminen war nicht signifikant.

Konklusion: Wider Erwarten konnte kein Zusammenhang zwischen dem epigenetischen Marker und den Katecholaminen gefunden werden. Gründe dafür könnten die laufende medikamentöse Behandlung der BD-Gruppe, eine zu kleine Kohorte und die Querschnittsmessung sein. Nichtsdestotrotz ist die aktuelle genetische Forschung vielversprechend und sollte sich in epigenetische Studien vertiefen, wobei allerdings multiple Messungen der Werte notwendig sein werden.

Abstract

Introduction: Disturbances in the circadian rhythm could explain some of the characteristic symptoms of bipolar disorder (BD), like alterations in sleeping behaviour. Hitherto literature gave reason to analyse epigenetic changes, such as methylation of *ARNTL,* in combination with peripheral levels of the catecholamines dopamine (DA) and norepinephrine (NE) resulting in my main hypothesis: A higher status of *ARNTL* methylation leads to a reduced expression of monoamine oxidase (MAOA), which is responsible for the catecholamine's breakdown. According to our postulated model, reduced levels of MAOA cause higher levels of DA and NE, which could explain mood swings in BD.

Methods: Data were gathered concluding the BIPFAT and the BIPGEN study. Participants were former in- or outpatients of the Department of Psychiatry and Psychotherapeutic Medicine at the Medical University of Graz. The status of methylated *ARNTL* was analysed by using DNA isolation with the salting out technique, bisulfite treatment of DNA, PCR and pyrosequencing. Peripheral levels of bound DA and bound NE were analysed by high performance liquid chromatography. Statistical analysis was performed by IBM SPSS version 23. Depending on homogeneity, t-test or Mann-Whitney-U test were used to analyse bound DA and NE. Spearman rank correlation was used to investigate the relation between methylated *ARNTL* and DA and NE.

Results: No significant differences in the peripheral levels of bound DA and bound NE were found between BD and controls. The analysis of correlation between status of methylated *ARNTL* and bound DA and bound NE was not significant either.

Conclusion: Against the expectations, I could not reveal any correlations between the epigenetic marker of *ARNTL* and DA as well as NE. This may be caused by a mood stabilizing treatment, a small sample size and the one-time measurement of the catecholamines. Nevertheless, current genetic research is promising and should focus on epigenetic studies, but multiple measurements will be necessary.

Inhaltsverzeichnis/Table of Contents

Abkürzungen/Abbreviations

5-HT…	5-hydroxytryptamine
ACTH…	Adrenocorticotropic hormone
ARNTL…	Aryl hydrocarbon receptor nuclear translocator-like
BBB…	Blood-brain-barrier
BD…	Bipolar disorder
BD I…	Bipolar disorder, type I
BD II…	Bipolar disorder, type II
BDNF…	Brain-derived neurotrophic factor
BHLHB2…	Basic helix-loop class b2
BMAL1…	Brain and Muscle ARNT-like 1
cAMP…	Cyclic adenosine monophosphate
CANMAT…	Canadian Network for Mood and Anxiety Treatment
CG…	Cytosine-Guanine
CLOCK…	Circadian Locomotor Output Cycles Kaput
CNS…	Central nervous system
COMT…	Catechol-O-methyl-transferase
COPD…	Chronic obstructive pulmonary disease
CRH…	Corticotropic-releasing-hormone
CRY1-2…	Cryptochrome gene 1-2
CSNK1 D/E…	Casein kinase 1 D/E
DA…	Dopamine
DAG…	Diacyl glycerol
DEX…	Dexamethasone
DMH…	Hypothalamic dorsomedial nucleus
DNA…	Deoxyribonucleic acid
DOPA…	Dihydroxyphenylalanine

DSM…	Diagnostic and Statistical Manual
EPI…	Epinephrine
fMRI…	Functional magnetic resonance imaging
Gsk3B…	Glycogen-synthase-kinase-3b
GWAS…	Genome wide association studies
HPA…	Hypothalamic- pituitary- adrenal
HPLC…	High performance liquid chromatography
ICH…	International Conference on Harmonization
IP3…	Inositol 1,4,5-triphosphate
kg…	Kilogram
MAO A/B…	Monoamine oxidase A/B
M…	Mean
m…	Metre
Max…	Maximum
MB…	Membrane-bound
MHPG…	3-metoxy-4-hydroxyphenylglycol
Min…	Minimum
ml…	Millilitre
MUG…	Medical University of Graz
N…	Number
NaCl…	Sodium chloride
NE…	Norepinephrine
NPAS2…	Neuronal PAS domain protein 2
NR1D1…	Nuclear receptor subfamily 1, group D, member 1
PCR…	Polymerase Chain Reaction
PER1-3…	Period genes 1-3
pg…	Picogram

PHE…	Phenylalanine
REV- ERBα…	Reverse erb receptor alpha
ROR A/B…	RAR-related orphan receptor A/B
SCID…	Structured Clinical Interview for DSM-IV Axis I Disorders
SCN…	Suprachiasmatic nuclei
SD…	Standard Deviation
SN…	Substantia nigra
SNP…	Single nucleotide polymorphism
SOP…	Standard Operation Procedure
SSRI…	Selective Serotonin Reuptake Inhibitor
TIM…	Timeless
TYR…	Tyrosine
VTA…	Ventral tegmental area

Abbildungsverzeichnis/Table of Figures

Tabellenverzeichnis/List of Tables

1. Introduction and Theoretical Background

1.1. Bipolar Disorder

1.1.1. History

Notifications in the changes of mood and level of energy date back to the Ancient Greece. In the 5th century BC the terms “melancholia” and “mania” appeared in the humoral conception “Corpus Hippocraticum”. Araeteus from Cappadocia, a Greek physician, first recognized a connection between mania and melancholia about 1st century A.D. In his opinion they were part of the same disorder and mania was the worsened form. This concept dominated until 19th century when Jean-Pierre Falret published “la folie circulaire“ 1851 and three years later Jules Baillarger published his work “folie à double forme“. Falret claimed that bipolar disorders were cyclic melancholic and manic episodes separated by symptom-free episodes. In contrast Baillarger argued that there were no symptom-free intervals at all (Pichot 2004).

Pioneering was the separation of schizophrenia and affective disorders by Emil Kraepelin in 1910. Three years later he classified mood disorders and as a result bipolar disorders were assigned within the category of “manic-depressive insanity”. Karl Leonhard was the first one to mention “bipolar” and “unipolar” in 1957. This nomenclature of “bipolar affective” and “unipolar affective disorder” is valid until date (Perris 1990).

1.1.2. Definition

The bipolar disorder (BD) is an affective disorder, which means that it is characterized by mood disturbances. These mood disturbances can occur as depressive episodes or as episodes of elevated mood called hypomania or mania. According to DSM-IV there is a specific classification that contains four subtypes of BD.

Bipolar I Disorder (BD I)

There has to be a minimum of one depressive and one manic episode during life-time for the diagnosis.

Bipolar II Disorder (BD II)

At least one depressive and one hypomanic episode during life time are required but there must not be any manic episode. A hypomanic episode does not go to the full dimension, in contrast to mania, where more distinctive symptoms occur and loss of control as well as reality is prevalent.

Cyclothymic Disorder

A cyclothymic disorder is by means of the intensity of the symptoms a milder subtype of BD but the duration of the symptoms has to be at least two years. The existence of numerous periods with hypomanic as well as numerous periods with depressive symptoms, that do not reach the full dimension of a major depressive episode define cyclothymia. There is one exemption: In children and adolescents the duration must be at least one year.

Bipolar Disorder- Not Otherwise Specified

This category subsumes disorders with bipolar feature that do not fulfill the diagnostic criteria for BD I, BD II or cyclothymia. So called “rapid cycler” belong to this group. Rapid cycler experience at least four episodes (depression, hypomania, mania) during 12 months.

1.1.3. Epidemiology

It is quite challenging to name the explicit prevalence of BD because of a high number of estimated unreported cases. Studies found a prevalence for BD I of 1% and for BD II of 1.5-3% (Akiskal et al. 2000; Angst et al. 2003). Another analysis suggested a lifetime prevalence for BD I of 1.0 %, BD II of 1.1% and for subthreshold symptoms of 2.4% (Merikangas et al. 2007).

Researches by other teams showed that a relevant number of people with diagnosed unipolar depression has developed manic or hypomanic episodes after years which impede the research of prevalence in BD (Goldberg et al. 2001; Judd

et al. 2002). The incidence of BD seems to be comparable in both sexes and the average age of onset is reported to be 25 years (Ferrari et al. 2011; Baldessarini et al. 2010).

1.1.4. Etiopathogenesis

Genetics

According to current scientific knowledge bipolar disorder is caused by the vulnerability-stress-model. This model suggests that an interplay between a genetic vulnerability, chronic biopsychosocial stressors and acute triggers lead to affective episodes.

The genetic heritability of BD has been reported for decades. Family studies have reported a heightened peril of 7-10% for BD if 1st degree relatives are already affected, in contrast to the general population with a risk of 1% (Möller et al. 2007). Furthermore, Smoller et al. (2003) have shown in twin studies that the heritability of the bipolar disease based on the concordance rates of monozygotic twins has been estimated up to 80% (Smoller et al. 2003). However, there is not a single risk-gene that influences the progress of BD, but there is an interplay between a variety of predisposing genes. An excerpt is depicted in table 1 (Schulze 2010). Recent GWAS (genome wide association studies) investigating BD compared to controls detected over 50 genome wide significant genes, but there is still missing heritability and further research in this issue will be necessary (Muhleisen et al. 2014).

Table 1 Excerpt of genes in Bipolar Disorder, created by Schulze et al. (2010).

Gene	Symbol	Evidence
Aryl hydrocarbon receptor nuclear translocator-like	*ARNTL*	+
Cadherin gene	*FAT*	+
Tryptophan hydroxylase 2	*TPH2*	++
Disrupted-in-schizophrenia-1	*DISC1*	++
Brain-derived neurotrophic factor	*BDNF*	+++
Serotonin Transporter	*SLC6A3*	+++
D-amino acid oxidase activator (G72)	DAOA	+++
Diacylglycerol kinase eta	*DGKH*	++++
alpha-1 subunit of a voltage-dependent calcium channel	*CACNA1C*	++++
Ankyrin 3	*ANK3*	++++

Evidence supported by: + … 2 studies, ++ … several studies, +++… meta-analysis of ≥ 3 samples, ++++ … genome-wide studies

Neuropathology

As mentioned earlier, the causes of BD are multimodal and beside the genetic component, structural differences were observed as well. There are several studies that show a variation of the brain structure in the magnetic resonance imaging in people with BD compared to healthy ones. Remarkable were reductions in the volume of grey matter in the prefrontal cortex (Arnone et al. 2009) and an increased volume of amygdala (Hajek et al. 2009). What is more, a wide meta-analysis showed enlarged lateral ventricles and so called “white matter hyperintensities” (Kempton et al. 2008). Interestingly, Birner et al. (2015) presented a significant relation between white matter hyperintensities and the number of depressive as well as manic episodes in males, displaying also clinically relevant consequences of structural brain changes (Birner et al. 2015). Furthermore a decreased volume of hippocampus, thalamus and amygdala were demonstrated in recent research (Hibar et al. 2016).

Environment

Latest research has focused on the environmental influence on bipolar disorder. In this context, in a study including euthymic BD I diagnosed people, 61% reported childhood abuse (Erten et al. 2014). In general, childhood abuse is considered to be a central determinant predicting the course of BD. Especially the physical abuse was linked to particular severe symptoms like aggression, impulsivity and even suicidality. Additionally, earlier onset, rapid cycling, a high number of hospitalizations and comorbidities were associated with physical abuse in childhood what underlines the impact of negative experiences in childhood BD (Daruy-Filho et al. 2011). Trauma in early years seems to influence the severity of chronic stress (Gershon et al. 2013) and the susceptibility to stressors in adulthood (Dienes et al. 2006). Furthermore, Gershon et al. (2013) demonstrated that greater chronic stressors may lead to a more distinct form of depressive symptoms at BD I but the manic symptoms were not affected. Data about negative life events were analysed at a follow-up study including 140 offsprings of parents with BD. Each negative impact increased the danger of onset about 10% (Hillegers et al. 2004). In this context it is quite interesting, that Cecil et al. (2016) investigated the epigenetic impact of childhood abuse. Individuals with any kind of childhood abuse showed a significant altered status of methylation in several gene loci, including genes with a high relation to psychiatric disorders (Cecil et al. 2016).

Neuroendocrinology

Literature reveals that there is an over-activity of the hypothalamic- pituitary-adrenal-axis (HPA-axis) in individuals affected by unipolar and bipolar depression. This is based on an increased basal secretion of cortisol and adrenocorticotropic hormone (ACTH), a decreased suppression of HPA-axis in the dexamethasone (DEX) suppression test and a down-regulation of corticotropic-releasing-hormone (CRH) receptors (Möller et al. 2007). In a combined DEX-/CRH-test, depressive people showed raised concentration of cortisol compared to physiological decreased concentrations (Rybakowski et al. 1999; Holsboer 2000).

1.1.5. Symptoms of affective episodes

Manic episode

The major characteristic of manic episodes is the pathological elevated mood state or dysphoric mood. Concerned people feel very “up” or “high” and tend to talk much and fast (=logorrhoea). They often sleep only a few hours, are full of energy and present increased activity. Sometimes it can lead to delusional ideas, particularly megalomania. Other characteristic symptoms can be a reduced appetite, flight of ideas, agitation, socially disinhibition and to be prone to risky things like spending irresponsible amounts of money or having reckless sex (Möller et al. 2007).

Depressive episode

The spectrum of depressive symptoms includes strong feelings of negativity, hopelessness, guilt and fear and the loss of their feeling for joy. Furthermore, depressed individuals with BD often have motivational problems and cognitive deficits like problems with short term memory and concentration. Sleeping disturbances are reported to be prevalent in 80-100% in a depressive episode, which underlines again the relevance of the circadian rhythms in BD-pathogenesis and the importance of a chronotherapy in a therapeutic setting. Some lose their interest in having sex and also suicidal thoughts are often present (Möller et al. 2007).

Mixed episode

It is also possible to suffer from manic as well as depressive symptoms simultaneously. This state is called “mixed episode” and is associated with a dysphoric-irritated mood, emotional lability, fear, feeling of guilt and aggressivity. Moreover, mixed episodes are particularly dangerous because of a high risk of suicidality (Möller et al. 2007).

1.1.6. Diagnostics

Diagnostic is based on DSM IV-TR (Diagnostic and Statistical Manual of Mental Disorders, 4th edition- Text Revision):

Manic episodes are characterized by:

1.
A distinct period of abnormally and constantly elevated mood (sometimes expansive or irritable mood) with a minimal duration of one week but if a hospitalization is needed for the treatment, time is irrelevant.

2.
During the manic stage, three (or more) of the following symptoms have continued (four if the mood is only irritable) and have been present to a serious intensity:

- elevated self-esteem or even grandiosity
- reduced need for sleep (only a few hours a day)
- more communicative than typical or desire to keep talking
- a higher distractibility
- flight of ideas
- increase in target-oriented activity or psychomotor agitation
- excessive involvement in activities that have a undisputable risk for negative consequences (e.g., exorbitant buying of unneeded products, cheating on wife/husband, reckless sex or absurd business investments)

A hypomanic episode is a milder form of mania in which the symptoms are not intense enough to induce serious restrictions in social or professional performance, but the symptoms can be observed by others (especially family members or close friends). The durations of the symptoms must be present for at least four following days.

Depressive episodes are characterized by:

Depressed mood and/or loss of interest/joy in life activities lasting for at least two weeks define a depressive episode. The symptoms are leading to a considerable restriction in social life or work and at least five of the following symptoms are required:

- depressed mood for most of the day
- reduced interest or joy in activities
- altered sleeping behaviour (insomnia or sleeping too much)
- agitation or psychomotor retardation
- noticeable unintentional gain or loss of weight
- strong loss of energy or fatigue
- problems in thinking or concentrating
- feelings of guilt or worthlessness
- repetitive thoughts of death

1.1.7. Course and prognosis

Judd et al. (2002, 2003) claim a median for the duration of an individual episode from four to five months and a ratio of depressive and (hypo-) manic episodes of 3:1. Nevertheless, also in symptom free periods, cognitive as well as working problems remain (Judd et al. 2002; Judd et al. 2003). Only 30% to 40% of the individuals with BD were satisfied with social and occupational function in a study. Selected parameters predicting a severe course of disease are estimated to be early onset, abnormalities in childhood, mixed episodes, frequency of episodes (e.g. rapid cycling), positive family history and psychiatric comorbidities, particularly substance misuse (MacQueen et al. 2001).

1.1.8. Psychiatric comorbidity

Merikangas et al. (2007) constructed the following table 2 linking bipolar disorder with several other psychiatric disorders.

Noticeable and clinically relevant is the fact that almost three fourth also suffer from any kind of anxiety disorder.

Table 2 Psychiatric comorbidities in BD

	Any BD, %	BD I, %	BD II, %	Subthreshold BD, %
Any anxiety disorder	74.9	86.7	89.2	63.1
Panic disorder	20.1	29.1	27.2	12.1
Post-traumatic stress disorder	24.2	30.9	34.3	16.5
Generalised anxiety disorder	29.6	38.7	37.0	22.3
Social phobia	37.8	51.6	54.6	24.1
Obsessive–compulsive disorder	13.6	25.3	20.8	4.3
Attention-deficit hyperactivity disorder	31.4	40.6	42.3	23.0
Oppositional defiant disorder	36.8	44.4	38.2	32.8
Conduct disorder	30.3	43.8	18.6	28.9
Alcohol dependence	23.2	38.0	19.0	18.9
Drug dependence	14.0	30.4	8.7	9.5
One comorbid diagnosis	12.7	8.1	7.0	17.1
Two comorbid diagnoses	9.4	3.4	2.9	14.7
Three or more comorbid diagnoses	70.1	86.2	85.8	56.7

1.1.9. Pharmacological Therapy

The pharmacological treatment of BD mainly consists of lithium, antipsychotics (predominantly atypical antipsychotics), antiepileptics and antidepressants.

A differentiation between acute or maintenance therapy and bipolar mania or depression is necessary, since the medication varies, depending on these stages.

Acute mania

Gold standard of the management of mild or moderate acute mania is lithium. Its efficacy is well established for acute mania as well as prophylactic therapy (Yatham et al. 2013). In severe forms of acute mania benzodiazepines are also often required to induce sedation but should not be used as monotherapy. Olanzapine, quetiapine, risperidone, asenapine, aripiprazole, paliperidone and ziprasidone are substances of the group of atypical antipsychotics and show good data if compared to placebo. In addition, antiepileptics like valproate and carbamazepine reveal significant results in several studies and serve as an alternative in the therapy of acute mania (Yatham et al. 2013).

CANMAT (2013) recommend following pharmacological treatment (Yatham et al. 2013):

Table 3 CANMAT recommendation of pharmacological therapy of acute mania

First line	Monotherapy: lithium, valproate, aripiprazole, olanzapine, quetiapine, risperidone, asenapine, paliperidone, ziprasidone Adjunctive therapy with lithium or valproate: aripiprazole, risperidone, quetiapine, asenapine, olanzapine
Second line	Monotherapy: haloperidole, carbamazepine Combination therapy: lithium + valproate

Atypical antipsychotics and valproate are preferred in the treatment of special forms like “rapid cycling” or mixed states (Möller et al. 2007).

Acute bipolar depression

Whereas in the United States atypical antipsychotics and mood stabilizers are often used as a monotherapy for a bipolar depression, in Europe also antidepressants are in use more frequently. It should be considered that the use of antidepressants is seen controversially because they bear the risk of a switch from a depressive to a manic state. To avoid such a switch antidepressants are combined with mood stabilizers (Möller et al. 2007).

CANMAT's recommendation (2013):

Table 4 CANMAT recommendation of pharmacological therapy of acute depression

First line	Monotherapy: lithium, quetiapine, lamotrigine Combination therapy: lithium or valproate + olanzapine, SSRI+ SSRI, lithium + valproate, lithium or valproate + bupropion
Second line	Monotherapy: valproate Combination therapy: quetiapine + lithium, SSRI or valproate + lithium, lamotrigine

Maintenance therapy:

After an acute state of bipolar disorder a maintenance therapy is required for at least six months. Lithium, valproate, olanzapine and quetiapine as monotherapy or in a combination therapy reveal notable rates of outcome. Möller et al. (2007) state a response of lithium prophylaxes of 65-80%. In case of an abrupt stop of a long time of lithium intake, the risk of "rapid cycling", relapse and suicide raises significantly. Therefore the dose of lithium should be reduced slowly over months (Möller et al. 2007).

1.2. Circadian rhythm

The circadian rhythm is an inner clock that is approximately corresponding to the day/night cycle of the rotation of the Earth (Czeisler et al. 1999). It influences several biological mechanisms such as the sleep-wake cycle, hormonal secretion (particularly melatonin and cortisol), body temperature and consequently mood (Linkowski 2003). Dysregulation may lead to physiological disturbances and especially sleeping disorders are a relevant issue in psychiatric disorders as they are a highly prevalent symptom (Charrier et al. 2017).

A superordinate central clock generates the circadian rhythm. This master pacemaker is located at the suprachiasmatic nuclei (SCN) of the hypothalamus above the optic chiasm (Moore 1997; Reppert et Weaver 2002). Although the circadian rhythm slightly varies from 24 hours (Scheer et al. 2007) external stimuli, called zeitgeber, are able to synchronize the clock to 24 hours. A fundamental external stimulus in human is light. Retinal photosensitive receptors, rods and cones, excite if light falls on the retina. Thus, the information reaches the SCN via the so called “retinohypothalamic tract” (Reppert et Weaver 2002).

Further zeitgeber are food intake, temperature, exercise and drugs (Tahara et Shibata 2013). Acosta-Galvan et al. (2011) showed in an experiment with mice an interaction between SCN and the hypothalamic dorsomedial nucleus (DMH), which is expected to be a food-entrained oscillator. In this context it is mentionable that periphery send the SCN hormonal or metabolic feedback (e.g. Leptin, Noradrenalin, FGF 21, glucose) and thus stabilize the circadian rhythm (Acosta-Galvan et al. 2011; Buijs et al. 2016). Furthermore the SCN is linked with the immune system. Lesions in the SCN induce a 10 fold increase in immune response in rodents. In a study with individuals, who do shift work, the desynchronization of the SCN could explain associations with several health problems such as metabolic and cardiovascular dysfunctions and immune dysregulation (Guerrero-Vargas et al. 2014). Anew, this underlines the influence of a disturbed circadian rhythm.

More than 20 genes are significantly involved in the control of the molecular clock. The underlying mechanism is a transcriptional translational feedback loop, where the cycle starts with a heterodimerization from ARNTL (Aryl hydrocarbon receptor

nuclear translocator-like) and CLOCK (Circadian Locomotor Output Cycles Kaput) or alternatively NPAS2 (Neuronal PAS domain protein 2) and ARNTL. As a second step the ARNTL-CLOCK (or ARNTL-NPAS2) heterodimers trigger the genetic transcription of *CRY* (Cryptochrome genes) and *PER* (Period genes) by binding to e-box-enhancer. Consecutively, PER and CRY proteins accumulate and gain their top concentrations after 12 hours in cytoplasma. Special casein kinases (Csnk1 or Csnk1e) or the glycogen-synthase-kinase-3b (Gsk3B) are able to phosphorylate the proteins. Phosphorylated PER and CRY proteins form heterodimers in cytoplasma and re-enter the nucleus, where the heterodimers supress their own transcription by binding to ARNTL-CLOCK complexes. Figure 1 demonstrates the process of the feedback loop of the molecular mechanism. Deciding for the length of the cycle is the phosphorylation of PER and CRY and thus it is an important mechanism of phase shift (Bengesser S 2013).

Additionally there is a second regulating mechanism. The ARNTL-CLOCK complex initiates the synthesis of two proteins, RORA and REV-ERBa. The proteins bind to a receptor in the promoter of *ARNTL* and activate *ARNTL's* expression (RORA) or inhibit the expression (REV-ERBa) (Bengesser S 2013).

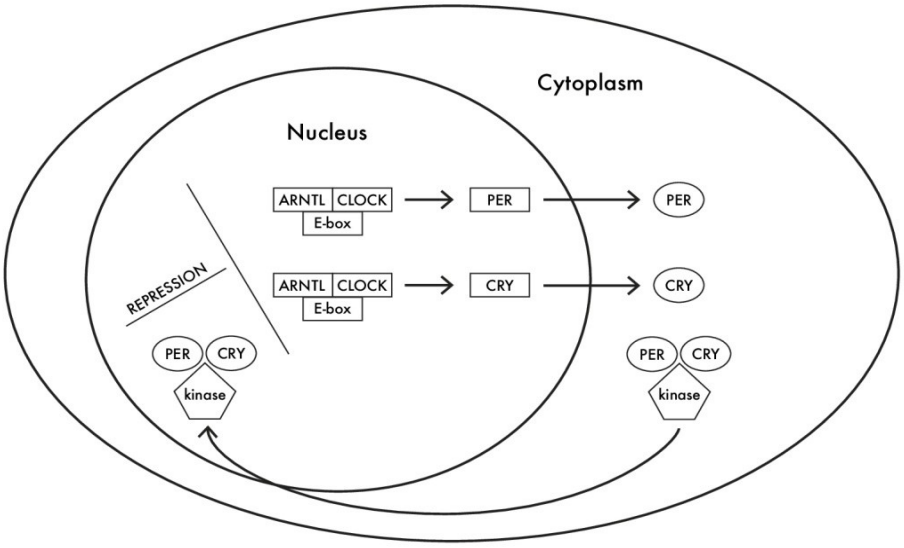


Figure 1 Mechanism of the feedback loop of the molecular clock. ARNTL-CLOCK activate PER's and CRY's expression in the nucleus. Afterwards, PER and CRY proteins accumulate in the cytoplasm, where they get phosphorylated by kinases and finally repress their own transcription.

1.2.1 Clock genes and their function

ARNTL (*BMAL1* or *MOP3*)

The *ARNTL*-(Aryl hydrocarbon receptor nuclear translocator-like) gene is located at chromosome 11p15. *BMAL1* (Brain and Muscle ARNT-like 1) is a synonym of *ARNTL* and also well established in literature. Several studies associated *ARNTL* with BD (Nievergelt et al. 2006; Mansour et al. 2006; Le-Niculescu et al. 2009; Soria et al. 2010). Focusing on BD, *ARNTL* plays a major role and has two main functions. First, it is an important factor of the feedback loop of the molecular circadian rhythm and as a heterodimer with CLOCK it is responsible for the expression of CRY1, CRY2, PER1, PER2 and PER3. Second, ARNTL builds a heterodimer with NPAS2 and after binding to specific sites (e-boxes) on the *MAOA* (monoamine oxidase A) promoter, the transcription and protein synthesis of *MAOA* starts. Subsequently, dopamine gets degraded resulting in a more depressive mood state (Bengesser S 2013). In this context Hampp et al. (2008) and Hampp et Albrecht (2008) demonstrated that elevated mood is linked with decreased expression of *ARNTL,* while in contrast, depressed mood is linked with increased expression of *ARNTL* (Hampp et Albrecht 2008; Hampp et al. 2008).

Continuing associations with *ARNTL* concern somatic diseases like diabetes mellitus II, hypertension (Woon et al. 2007) and tumorigenesis as it is a putative regulator of p53 (Mullenders et al. 2009). The metabolic correlation of *ARNTL* was displayed by Shimba et al. in 2011, when *ARNTL* knock-out mice showed an elevated respiratory quotient and increased circulating fatty acids (Shimba et al. 2011).

NPAS2

NPAS2 is a paralogue from *CLOCK* and as a heterodimer with ARNTL it participates in the feedback mechanism of the circadian rhythm. NPAS2 proteins operate as transcription factors and initiate the expression of other clock genes and *MAOA* (Bengesser S 2013; Akashi et Takumi 2005). In *CLOCK* knockout mice, *NPAS2* substitutes *CLOCK* and stabilizes the circadian clock. However, a knockout of both genes (*CLOCK* and *NPAS2*), lead to arrhythmicity (Landgraf et al. 2016). Recent studies have claimed a significant correlation between *NPAS2*

and seasonal affective disorder through a metabolic pathway (Bengesser S 2013; Kim et al. 2015). Furthermore, *NPAS2* seems to be involved in tumorigenesis of breast and colorectal cancer (Yi et al. 2010; Yuan et al. 2017).

CLOCK

Circadian Locomotor Output Cycles Kaput, *CLOCK*, is found at chromosome 4q12. Its protein is a central transcription factor in the circadian rhythm, where it activates as a heterodimer with ARNTL the genetic expression of *PER1, PER2, PER3, CRY1* and *CRY2*.

The CLOCK-ARNTL dimer activates the transcription of a further clock gene, *REV-ERBa* (reverse-erb receptor alpha), which inhibits the expression of *ARNTL* again and is a target of Lithium (Bengesser S 2013). So far, several polymorphisms in *CLOCK* were linked with abnormalities (Bengesser S 2013; Kripke et al. 2009). A single nucleotide polymorphism (SNP) in T311C was associated with increased insomnia and with a high relapse rate of BD (Serretti et al. 2003; Benedetti et al. 2003). In this context, another research observed *CLOCK* mutant mice developing manic behaviour (Roybal et al. 2007).

NR1D1 (REV-ERBa)

The *NR1D1* (nuclear receptor subfamily 1, group D, member 1) gene encodes a transcription factor that inhibits the transcription of *ARNTL* by binding on its promotor site. This inhibition leads to decreased activity of MAOA and consequently, to lower levels of dopamine, serotonin and noradrenalin with a promanic effect (Bengesser S 2013; Hampp et al. 2008). Additionally, *NR1D1* has an effect on the treatment of BD since it is sensitive for Lithium (Bengesser S 2013; Yin et al. 2006). Moreover *NR1D1* has a key function in regulating metabolic processes in liver, macrophages, muscle, brown fat and brain (Jager et al. 2016).

PER1, PER2 and PER3

PER1, PER2 and *PER3* (Period genes) are upregulated by ARNTL-CLOCK heterodimers. After being phosphorylated in the cytoplasma, they build heterodimers with CRY, resulting in a reentry of the nucleus with a following suppression of their own transcription by a negative feedback mechanism. The duration of the rhythm is depending on the process of phosphorylation of *PER* genes (Bengesser S 2013). Kripke et al. (2009) found significant associations in polymorphism of *PER1* and *PER2* and BD but not in *PER3* (Kripke et al. 2009). Contrary, several studies revealed a significant relationship between *PER3* and BD (Nievergelt et al. 2006; Mansour et al. 2006; Dallaspezia et al. 2011; Karthikeyan et al. 2014). In addition *PER2* seems to influence the transcription of MAOA. Therefore, Hampp et al. (2008) reported a reduced expression of *MAOA* in *PER2* mutant mice, leading to increased levels of dopamine and promanic behaviour (Bengesser S 2013; Hampp et al. 2008). *PER2* has also been related to Lithium, which is important in the therapy of BD (McCarthy et al. 2013).

CRY1, CRY2

Cryptochrome genes are located at chromosome 12q23-q24.1 (*CRY1*) and 11p11.2 (*CRY2*). Equal to *PER1-3, CRY1* and *CRY2* are main regulators in the SCN's feedback mechanism. Anand et al. (2013) analysed *CRY1* and *CRY2* and claimed that both lessened the circadian rhythm but *CRY1* was significantly more influential than *CRY2* (Anand et al. 2013).

Although many studies associated *CRY1* with BD (Soria et al. 2010; Ewald et al. 2002; Green et al. 2005; Glaser et al. 2005; Cassidy et al. 2007) others could not find any associations (Nievergelt et al. 2005; Mansour et al. 2006; Shi et al. 2008). *CRY2* was found to be highly linked with rapid cycling in BD (Sjoholm et al. 2010), depression and dysthymia (Kovanen et al. 2013; Lavebratt et al. 2010).

TIMELESS (TIM)

The importance of *TIMELESS* as a clock gene was shown in an experiment with *Drosophila melanogaster* but in mammals it is controversial if the impact is similar. It seems that TIM dimerizes with CRY or PER proteins (Mazzoccoli et al. 2016). However, studies associated *TIMELESS* with BD (Etain et al. 2014; Rybakowski et al. 2014) but other could not prove (Shi et al. 2008).

RORA, RORB

RAR-related orphan receptor (*ROR*) can be classified to the group of nuclear receptors and there are three forms. Considering the circadian rhythm two forms are interesting and therefore associations with bipolar disorder were found in *RORA* (Soria et al. 2010; Lai et al. 2015; Geoffroy et al. 2015) and *RORB* (Lai et al. 2015; Geoffroy et al. 2015; Mansour et al. 2009; McGrath et al. 2009). In contrast, for *RORA* no significance was found from McGrath et al. (2009).

CSNK1D, CSNK1E

Casein kinase 1D and casein kinase 1E are responsible for the phosphorylation of core clock genes. Particularly the phosphorylation of PER is determining the duration of the circadian rhythm (Bengesser S 2013). Etchegaray et al. (2009) revealed the importance of *CSNK1D* for maintaining the molecular clock in mammals (Etchegaray et al. 2009).

BHLHB2

Basic helix loop helix class b2 is a gene that encodes a protein, which can interact with ARNTL or in another mechanism bind in the promotor of *PER1* and thereby repress CLOCK/ARNTL. Shi et al. (2008) could find a significant association between a SNP (rs6442925) and BD.

Table 5 Summary of important clock genes and their association with BD

Clock gene	Function	Significantly associated with BD
ARNTL (BMAL1)	Important factor in the negative feedback loop of the molecular clock As a heterodimer with CLOCK responsible for transcription of CRY1, CRY2, PER1, PER2 and PER3 As a heterodimer with NPAS2 it starts the protein synthesis of MAOA	Nievergelt et al., 2006; Mansour et al., 2006; Le-Niculescu et al., 2009; Soria et al., 2010; Bengesser S, 2013
NPAS2	As a heterodimer with ARNTL it starts the protein synthesis of MAOA Stabilizes the circadian rhythm in CLOCK knockout mice	Bengesser S, 2013; DeBruyne et al. 2004; Landgraf et al. 2016
CLOCK	Transcription factor with ARNTL for CRY1, CRY2, PER1-3 and NR1D1	Shi et al. 2008; Kripke et al. 2009;
NR1D1 (REV-ERBa)	Inhibits transcription of ARNTL	Kripke et al. 2009 Severino et al. 2009
PER1 ***PER2*** ***PER3***	PER and CRY are upregulated by ARNTL-CLOCK. They build PER-CRY heterodimers after being phosphorylated and thus supress their own transcription	*PER1-2*:Kripke et al. 2009; *PER3*: Mansour et al. 2006; Nievergelt et al. 2006; Dallaspezia et al. 2011; Karthikeyan et al. 2014
CRY1 ***CRY2***		*CRY1*: Ewald et al. 2002; Glaser et al. 2005; Green et al. 2005; Cassidy et al. 2007; Soria et al. 2010;

TIMELESS	Dimerizes with CRY or PER proteins	Mansour et al. 2006; Etain et al. 2014; Rybakowski et al. 2014
RORA ***RORB***	ROR- related orphan receptor are nuclear receptors and are involved in the circadian rhythm	*RORA*: Soria et al. 2010; Lai et al. 2015; Geoffroy et al.2015 *RORB*: Mansour et al. 2009; McGrath et al. 2009; Lai et al. 2015; Geoffroy et al.2015
CSNK1D ***CSNK1E***	Phosphorylate core clock gene and especially the phosphorylation of PER determines the circadian rhythm	*CSNK1D*: Kripke et al. 2009; *CSNK1E*: Nievergelt et al. 2006; Shi et al. 2008;
BHLHB2	Binds to PER1 and represses CLOCK/ARNTL	Shi et al. 2008

1.3. Neurotransmitters

1.3.1 Mechanism

Neurotransmitters are chemical substances, which transfer signals from a neuron to another neuron, gland or a muscle cell via synapses. They are stored at vesicles in axons. A depolarising action potential at the end of a nerve leads to an opening of calcium channels, since they are voltage-sensitive. Subsequently, the inflow of calcium at the terminal of an axon induces a fusion between vesicles, which are full of neurotransmitters, and the presynaptic membrane. This process releases the neurotransmitters into the synaptic cleft, where they bind to receptors at the postsynaptic membrane. It has to be added, that the availability of the neurotransmitters in the synaptic cleft is only for a short time because of enzymatic metabolism or reuptake by transporters into the presynaptic synapse as well.

The effect of binding to specific receptors can only be excitatory or inhibitory. An excitatory receptor leads to a local depolarisation by an increased permeability for sodium and potassium. In contrast, an inhibitory receptor causes a hyperpolarisation (Horn 2009).

1.3.2. Receptors

Two different types of neurotransmitter receptors are known so far: the ionotropic and the G-protein coupled receptor.

The ionotropic receptor is a receptor and a transmembrane ion channel simultaneously. In response to a binding ligand the ion channel opens or closes, which influences the flow of ions in the membrane.

The second types are the G-protein coupled receptors. These receptors are the most common ones within the neurotransmitters. The mechanism of signal transduction consists of four steps. First, a ligand binds to the transmembrane receptor. This is leading to a conformational change in the G-protein, which is coupled to the receptor. Thus, the G-protein is activated and in a second step activates an enzyme. In this context, the two most important enzymes are adenylate cyclase and phospholipase C. Third, the activated enzyme releases a second messenger. Cyclic adenosine monophosphate (cAMP) as a product of an activated adenylate cyclase and inositol 1,4,5-trisphosphate (IP3) and diacyl glycerol (DAG) as a product of phospholipase C. The last step is the mediation of the intracellular effect of the neurotransmitters (Horn 2009).

1.3.3. Chemical classification

There are many known neurotransmitters and one way to classify them is by their chemistry (Monoamines, amino acids, peptides). Several prominent examples are in the following table (6).

Table 6 Chemical Classification of Neurotransmitters

Monoamines	**Amino acids**	**Peptides**
Dopamine	Glutamate	Somatostatin
Norepinephrine	Aspartate	Substance P
Epinephrine	Glycine	Neuropeptide Y
Serotonin	GABA	Enkephalin
Histamine		

1.3.4. Monoamines

In matters of psychiatric disorders, the group of monoamines seem to have an enormous impact. The “monoamine hypothesis”, developed since the 1960s, assumes, that imbalances in the neurotransmission cause mood disturbances. This hypothesis was first supported by the effect of antidepressants, which increase the levels of amines in the synaptic cleft by either inhibiting their reuptake or blocking their metabolism. Nowadays, numerous studies confirmed a dysfunction in neurotransmission in psychiatric disorders. In consideration of the bipolar disorder, exaggerated levels of monoamines were associated with mania as well as low levels were associated with a depressive state (Möller et al. 2007; Bengesser S 2013).

Monoamines can further be divided in 3 classes:

1. Catecholamines
2. Indolamines
3. Histamine

1.3.5. Biosynthesis of monoamines

Necessary for the biosynthesis of all biogenic amines is the course of action of decarboxylation. Therefore the coenzyme pyridoxal phosphate, the active form of vitamin B6, is indispensable (Horn 2009).

Dopamine (DA), norepinephrine (NE) and epinephrine (EPI) belong to the catecholamines and derive from the essential amino acids phenylalanine (PHE) and tyrosine (TYR). PHE can be converted by the phenylalanine 4-hydroxylase into TYR. The rate-limiting process in the biosynthesis of catecholamines is the hydroxylation of TYR to dihydroxyphenylalanine (DOPA). This process is catalysed by the tyrosine hydroxylase. When DOPA is formed, it is decarboxylated by aromatic l-amino acid decarboxylase and as a product, DA results. The enzyme dopamine b-hydroxylase converts DA into NE. Neurons that use EPI as transmitter, contain another enzyme, phenylethanolamine-*N*-methyl transferase, which converts NE into EPI (Fernstrom et al. 2007). The biosynthesis of DA, NE and EPI is shown in figure 2.

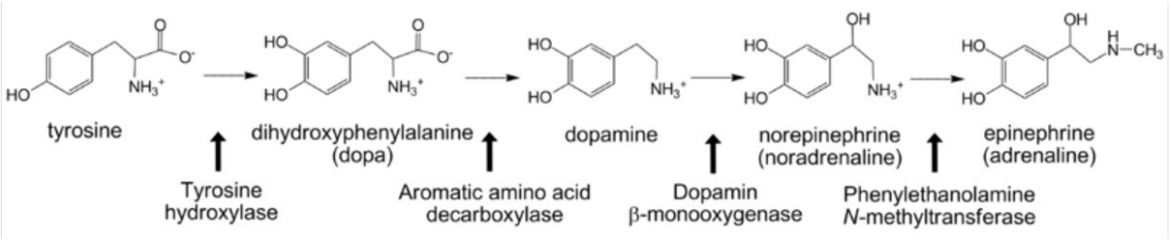


Figure 2 Biosynthesis of dopamine, norepinephrine and epinephrine (Windahl 2009)

5-hydroxytryptamine, better known as serotonin (5-HT), is chemically regarded an indolamine and it is derived from tryptophan, which is an essential amino acid. Analogue to the catecholamine biosynthesis there is a rate limiting enzyme (tryptophan hydroxylase), which converts tryptophan into 5— hydroxytryptophan. In a further step an enzyme (5-hydroxytryptophan decarboxylase) transforms 5-hydroxytryptophan into 5-HT (Gross et al. 2016). Moreover, 5-HT can be converted into N-acetylserotonin by arylalkylamine N-acetyltransferase and in a last step to melatonin by acetylserotonin O-methyltransferase (Etain et al. 2014). Figure 3 shows the biosynthesis of 5-HT and melatonin.

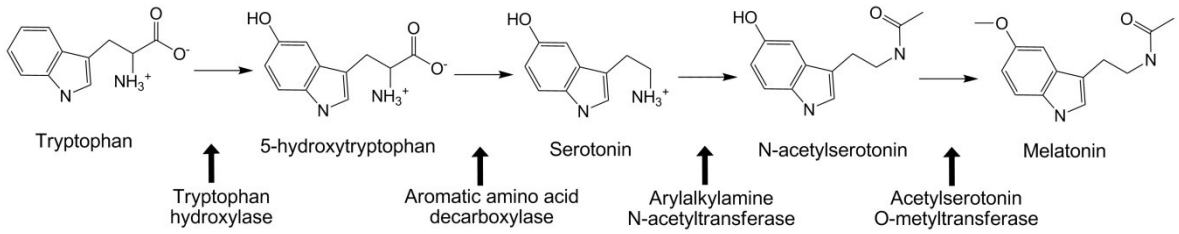


Figure 3 Biosynthesis of serotonin and melatonin (Windahl 2009)

1.3.6. Inactivation and metabolism of monoamines

Once in the synaptic cleft, monoamine neurotransmitters operate as long as specific transporter reuptake them. This reuptake brings the neurotransmitters into the nerve terminal, from which they were released before. The inhibition of monoamine reuptake is a prominent onset in the medical therapy of many psychiatric disorders.

The breakdown of monoamines mainly requires two enzymes. The first one is the monoamine oxidase (MAO). MAO is bound to the outer membrane of the mitochondria and belongs to the group of flavin-containing amine oxidoreductases. According to its name, oxygen is used to catalyse the deamination resulting in a correspondent aldehyde. MAO is classified into two subtypes: MAO-A and MAO-B. Both share about 70% of their identity but vary in the preferentially metabolism. Whilst MAO-A primarily oxidizes serotonin, norepinephrine, epinephrine, melatonin and dopamine, MAO-B prefers to metabolise dopamine, a catecholamine-related trace amine, called phenethylamine and norepinephrine. Serotonin can also be oxidised by MAOA-B, but only in a slow way. This differentiation preserves a clinically relevance, since MAO-A inhibitors are used as antidepressants and MAO-B inhibitors in the pharmacological treatment of Parkinson's disease (Möller et al. 2007; Horn 2009; Gaweska et Fitzpatrick 2011).

The second enzyme, that is important in the metabolism of monoamines, is the Catechol-O-methyl-transferase (COMT). It degrades catecholamines by methylation of the catechol-ring. COMT is also able to methylate substrates, which have been metabolised by MAO before. At the end of both breakdown pathways, metabolites are formed. These metabolites can be measured and have a significant role in clinic and research (Möller et al. 2007; Horn 2009).

1.3.7. Dopamine

Dopamine, as a neurotransmitter in the CNS, is built in cell bodies that are predominantly located in the mesencephalon. The cell bodies are confined to cluster and project to several other brain areas. Dahlström (1964) first identified areas with dopaminergic neurons (dopamine-producing cells). These areas are the substantia nigra (SN), the ventral tegmental area (VTA), the zona incerta, the arcuate nucleus the posterior hypothalamus and the periventricular nucleus. The projection of dopamine contains four major pathway systems:

- **The nigrostriatal pathway**
  Its origin is in the pars compacta of the SN and projects to the dorsal striatum with an important function in motor activity.

- **The mesolimbic pathway**
  This pathway begins in the VTA and ends in the ventral striatum, bed nucleus of the stria terminalis, hippocampus, and septum. Together with the mesocortical pathway it has a significant role in reward seeking, working memory, emotional processing, attention and impulsivity.

- **The mesocortical pathway**
  Equal to the mesolimbic pathway, the mesocortical originates in the VTA area but extends extensively more to the frontal and temporal cortices.

- **The tuberoinfundibular pathway**
  The arcuate and the periventricular nucleus form the tuberoinfundibular pathway. It reaches the pituitary gland and has an impact on prolactin's secretion.

The effect of DA depends on the type of receptor. Dopamine receptors are G-protein coupled receptors and can either stimulate or inhibit the production of cAMP. In this context you can classify the receptor in two families: D1-like (D1 and D5) and D2-like (D2, D3 and D4). The D1-family increases the cAMP-concentration and thus has an excitatory effect. Contrary the D2-family decreases the cAMP-concentration, resulting in an inhibitory effect.

The distribution of the different types of receptors is not equally within the brain. Whereas D1-receptors are the most common and are typically found in the prefrontal cortex, striatum and thalamus, D5-receptors are located in the entorhinal cortex, hippocampus and striatum. The D2-family prefers the striatum. while D3-receptors are highly prevalent in the ventral striatum, prefrontal regions (D2- and D4-receptors), hippocampus (D4-receptors) and low concentrations of D2-receptors in medial temporal regions (Möller et al. 2007; Cousins et al. 2009).

Dopamine in BD

Historically, the dopamine hypothesis claims, that a hyperdopaminergic state leads to the development of manic and even psychotic symptoms. Reversely a hypodopaminergic state induces depressive episodes. This simplified view did not clarify how a change in states arises. Nowadays, the exact mechanism still remains unclear but models propose a cyclical dysregulation in dopamine neurotransmission. Increased dopamine neurotransmission leads to manic episodes. Afterwards, the homeostatic mechanism comes into action with a secondary downregulation of receptors and key system elements, with the consequence of a reduced dopamine neurotransmission, typically for a depressive state. Anew, the feedback mechanism starts and upregulates the neurotransmission, which could lead to the next episode. The phenomena of mixed states, which could never be explained with the classic dopamine hypothesis, could be the effect of a desynchronization of different receptor types or regions within the cycle (Berk et al. 2007).

Animal models with dopamine transporter knockout rodents presented manic-like behaviours with increased locomotion and exploration. The same results were observed by stimulating the dopamine receptors with the dopamine agonist quinipirole. In matters of the circadian rhythm, a study with *CLOCK* gene knockout mice demonstrated manic-like behaviours synchronously with a raised dopaminergic activity in the VTA, an elevated DA synthesis, plus an increased tyrosine hydroxylase activity (Sidor et al. 2015; Ashok et al. 2017). A recent review by Ashok et al. (2017) summarized numerous studies in BD patients and the main results for mania were on the one hand, a pharmacologically proof, that increased concentrations of DA induce to manic symptoms. On the other hand, elevations in D2- and D3- receptor densities were noticed. Further investigations with functional magnetic resonance imaging (fMRI) showed hyperactivities of the reward cycle in mania. In bipolar depression the findings focused on an increased level of dopamine transporters.

1.3.8. Norepinephrine (Noradrenaline)

Equal to dopamine, noradrenergic areas in the brain were first identified by Dahlström (1964). These areas are located at the lateral tegmentum and most importantly at the locus coeruleus. The projections of the locus coeruleus reach almost every part of the cerebral cortex, cerebellum, brainstem and the medulla spinalis. Projections of the lateral tegmentum and smaller areas in the pons and the medulla terminate in the basal forebrain, thalamus, hypothalamus, brainstem and the medulla spinalis (Möller et al. 2007).

Two families of noradrenergic receptors are known so far: alpha and beta. The families are further subdivided in alpha1, alpha2, beta1 and beta2. All are G-proteins coupled receptor. Alpha1, beta1 and beta2 have an excitatory effect by either activation of phospholipase C (alpha1) or activation of adenylate cyclase (beta1 and beta2). Contrary, alpha2 receptors inhibit the adenylate cyclase, leading to decreased levels of the second messenger cAMP. Interestingly, alpha2 receptors are also found pre-synaptically, where they form a feedback-mechanism and inhibit the release and production of norepinephrine (Möller et al. 2007).

Norepinephrine in BD

Several abnormalities concerning norepinephrine and bipolar disorder were found in the hitherto literature. In a posthumous study of BD patients a significantly increased norepinephrine turnover was found in thalamic and cortical areas (Young et al. 1994). Furthermore, lower plasma levels of NE`s main metabolite 3-metoxy-4-hydroxyphenylglycol (MHPG) were found in bipolar depressed patients compared to unipolar depressed ones and higher levels were observed in manic BD patients than in depressed BD patients. Additionally, the urine concentration of norepinephrine and MHPG is lower in depressed than in manic BD patients (Manji et al. 2003). It is worth mentioning, that Kurita et al. (2014) claimed, that MHPG could be a candidate biomarker from the manic to remission state, since they found a weighty correlation between peripheral MHPG and the severity of mania, measured by Young Mania Rating Scale (Kurita et al. 2014).

1.3.9. Serotonin

Serotonergic neurons are mainly found in the brainstem. Especially the dorsal and the median raphe nucleus, which are part of the formation reticularis, are of prime importance as serotonergic projection systems. Their projections reach cerebellum, spinal cord, thalamus, amygdala, hippocampus, hypothalamus and cortex.

The serotonin receptors (5-HT – receptors) are mainly G-protein coupled receptors with one exception: the 5-HT3 receptor, which is an ionotropic one. Once more, the HT-receptors can be classified in families: from 5-HT1 to 5HT-7. To give a simplified view about the mechanism, one can assert, that 5-HT1 receptors inhibit the adenylate cyclase with the effect of decreased cAMP. Obversely, binding to 5-HT4,6,7 receptors lead to an activation of the adenylate cyclase with increased cAMP. 5-HT2 receptors operate with an activation of phospholipase C and consequently raised levels of IP3 and DAG. As mentioned above, 5-HT3 is an ionotropic receptor and effectuates an influx of cations (Möller et al. 2007; Horn 2009).

1.4. Aims and hypothesis

Methylation of genes is an epigenetic mechanism, which influences the expression of genes. It is a complicated and not fully known procedure but according to current knowledge the higher the methylation status of a gene, the lower the gene expression. Latest research by Bengesser et al. (2016) has already shown significant differences in the methylation status of *ARNTL* between BD patients and controls at two different sites. The first was at the CG site cg05733463 were BD patients showed a higher methylation status than controls and the second was PS2 POS1 with an inverse result (Bengesser et al. 2016).

In this thesis, I would like to widen this research and investigate if there is a correlation between methylated *ARNTL* and the peripheral levels of DA and NE in BD patients compared to healthy controls.

An illustrated summary of my main hypothesis, based on research by Bengesser et al. (2016), Hampp et al. (2008) and Hampp et Albrecht (2008) is depicted in figure 4 below.

My hypothesis:

1.) There is a significant positive correlation between the status of methylated *ARNTL* and the peripheral levels of bound DA and bound NE in BD patients.
2.) There is a significant difference in the peripheral levels of bound DA and bound NE between BD patients and controls.

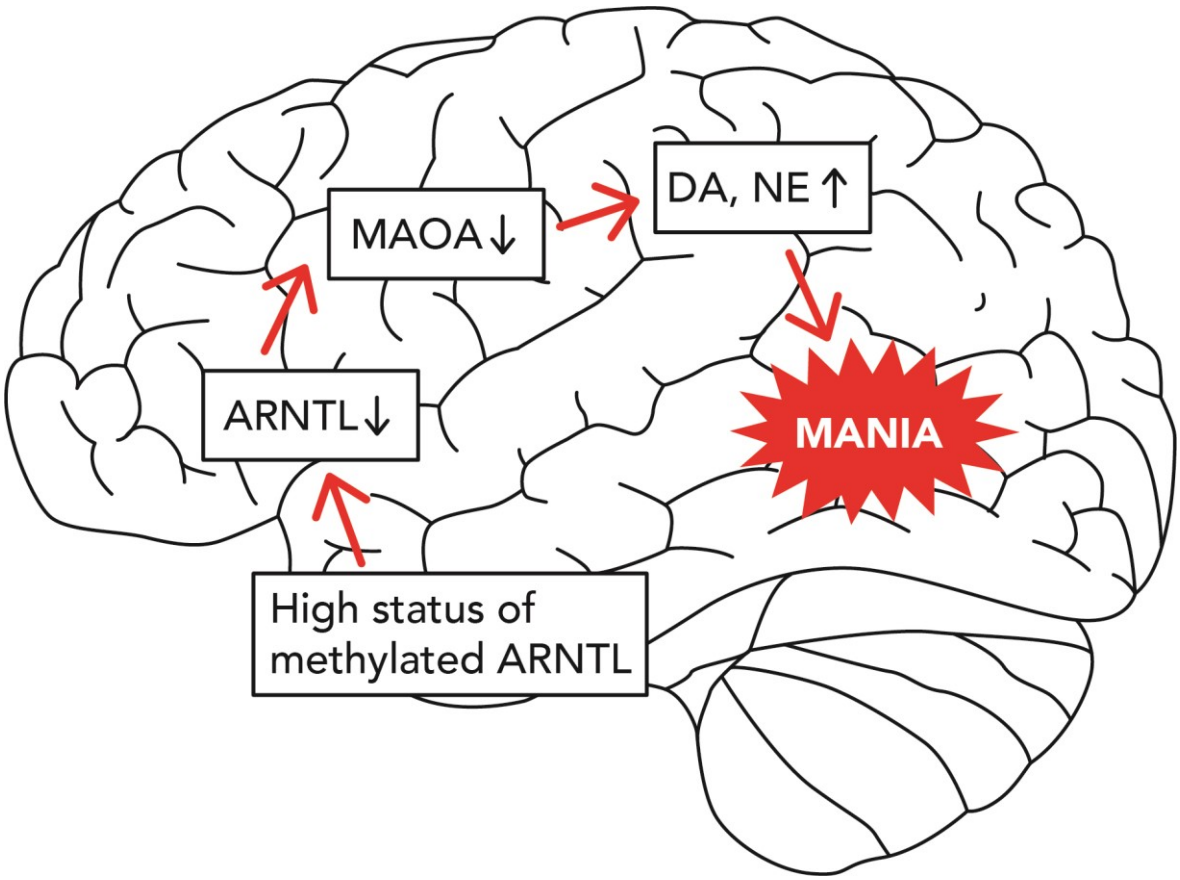


Figure 4 Illustration of the main hypothesis; A high status of methylation of *ARNTL* is leading to an decreased expression of ARNTL, which is responsible for MAOA's expression; subsequently decreased levels of MAOA cause increased catecholamines (DA, NE); elevated levels of DA and NE are highly linked with BD (more precisely with mania); MAOA… monoamine oxidase A, DA… dopamine, NE… norepinephrine

2. Material and Methods

2.1. Design of the study

Under the direction of Assoz. Prof.in Priv.-Doz.in Dr.in med.univ. et scient.med. Reininghaus Eva data of two studies (BIPFAT Follow-Up and the BIPGEN study) were gathered at the Department of Psychiatry and Psychotherapeutic Medicine at the Medical University of Graz (MUG). The primary aim of the studies was to analyse pathomechanisms of BD in association with metabolic measures.

Patients were diagnosed with BD by using the SCID-I (Structured Clinical Interview for DSM-IV Axis I Disorders) according to the DSM-IV criteria. Study participants were former in- or outpatients of the Department of Psychiatry and Psychotherapeutic Medicine. The study included a complete actual and lifetime psychiatric history, fasting blood sampling, anthropometric measures, cognitive testing, an electroencephalogram, magnetic resonance imaging of the brain and numerous lifestyle questionnaires. Exclusion criteria for the participation in the study were several chronic diseases, like Alzheimer's and Parkinson's diseases, Chorea Huntington, multiple sclerosis, lupus erythematosus, inflammatory bowel diseases, rheumatoid arthritis and chronic obstructive pulmonary disease (COPD). Test person of the healthy control group must not have any psychiatric disorder in their lifetime history. Another excluding criteria in the control group was severe psychiatric disorders in first-grade relatives. A written informed consent was demanded from all individuals (BD-patients as well as controls) before participating (Bengesser et al. 2016; Reininghaus et al. 2014).

The ethics committee of the MUG permitted both studies (BIPFAT and BIPGEN) in compliance with the current revision of the Declaration of Helsinki, the International Conference on Harmonization (ICH) guidelines for Good Clinical Practice and current regulations (Reference numbers: 24-123 ex 11/12 and 23-199 ex 10/11).

2.2. Diagnosis of Bipolar Disorder by SCID-I

As mentioned earlier, BD was diagnosed by using SCID-I. The interview started with an open exploration, in which the examined person gave a rough overview about present and past symptoms. Afterwards, the structural part began, in which the interviewer queried certain modules (A-J, see the table 7 below) in open questions. Depending on the interviewer's assessment of the answers, the interviewer found a reference to the next question. Lastly, in a procedure of exclusion, a diagnosis according to the DSM IV – criteria was established.

Table 7 Modules of SCID-I with examples

Module	Example
A - Mood Episodes	Major Depressive Episode, (Hypo-) Manic Episode
B - Psychotic Symptoms	Delusions, Hallucinations
C - Psychotic Disorders	Schizophrenia (paranoid or catatonic type)
D - Mood Disorders	BD I, BD II, Other BD
E - Substance Use Disorders	Alcohol and/or drug abuse
F - Anxiety Disorders	Panic Disorder
G - Somatoform Disorders	Pain Disorder
H - Eating Disorders	Anorexia nervosa, Bulimia nervosa
I - Adjustment Disorder	Adjustment Disorder
J - Optional Module	Acute Stress Disorder

2.3. Analysis of methylated ARNTL

The methylation status of *ARNTL* was measured by investigating several CG- rich (Cytosine and Guanine) regions. The regions were POS2 POS1-7 and cg05733463. In order to assess the methylation, numerous procedures and the cooperation of other departments were necessary. The Institute of Human Genetics at the MUG conducted the DNA-isolation from fasting blood with the salting out technique. As the name suggests, this method salted out DNA of cellular proteins by dehydration and precipitation with a saturated NaCl (sodium chloride) solution. In the next step the methylation analysis was conducted at the Institute of Neuropathology at the University of Bonn. In detail, the isolated DNA was treated with bisulfite. This treatment was performed with the Epitect-Kit® by Qiagen (Hilden, Germany). Thereby unmethylated cytosine was transformed into uracil by deamination, while methylated cytosine was protected and thus not modified. Afterwards the specialized cooperation partners performed a Polymerase Chain Reaction (PCR) and a pyrosequencing according to their SOP (Standard Operation Procedure) to detect the changes of the DNA-sequences. The primers, which were used for PCR and pyrosequencing, are given in table 8 below.

Table 8 Used primers for pyrosequencing and PCR (conceived by Prof. Andreas Waha); f… forward, r… reverse, ps… pyrosequencing, BIOT… biotinylated

Primer for cg05733473	cg05733463-f	GGGAATTGTTTTTTTGGTTGTAGT
	cg05733463-r	CCCACAACACAAAATATTAATCAT (BIOT.)
	cg05733463-ps	TGTTTTTTTGGTTGTAGTTTAA
Primer for Pos2	PS2-f	TAGGGTAGGTAGAGGTGTTGTAGG
	PS2-r	TCACTACCCCCAAAAACAAAACTAT (BIOT.)
	PS2-ps	GGTGTTGTAGGAGTTT

Afterwards, the sequences differed, depending on the methylation status of the cytosines. Finally, the methylation status was calculated and stated in percent. The summary of the workflow from a peripheral blood sample to the status of methylation is illustrated in figure 5.

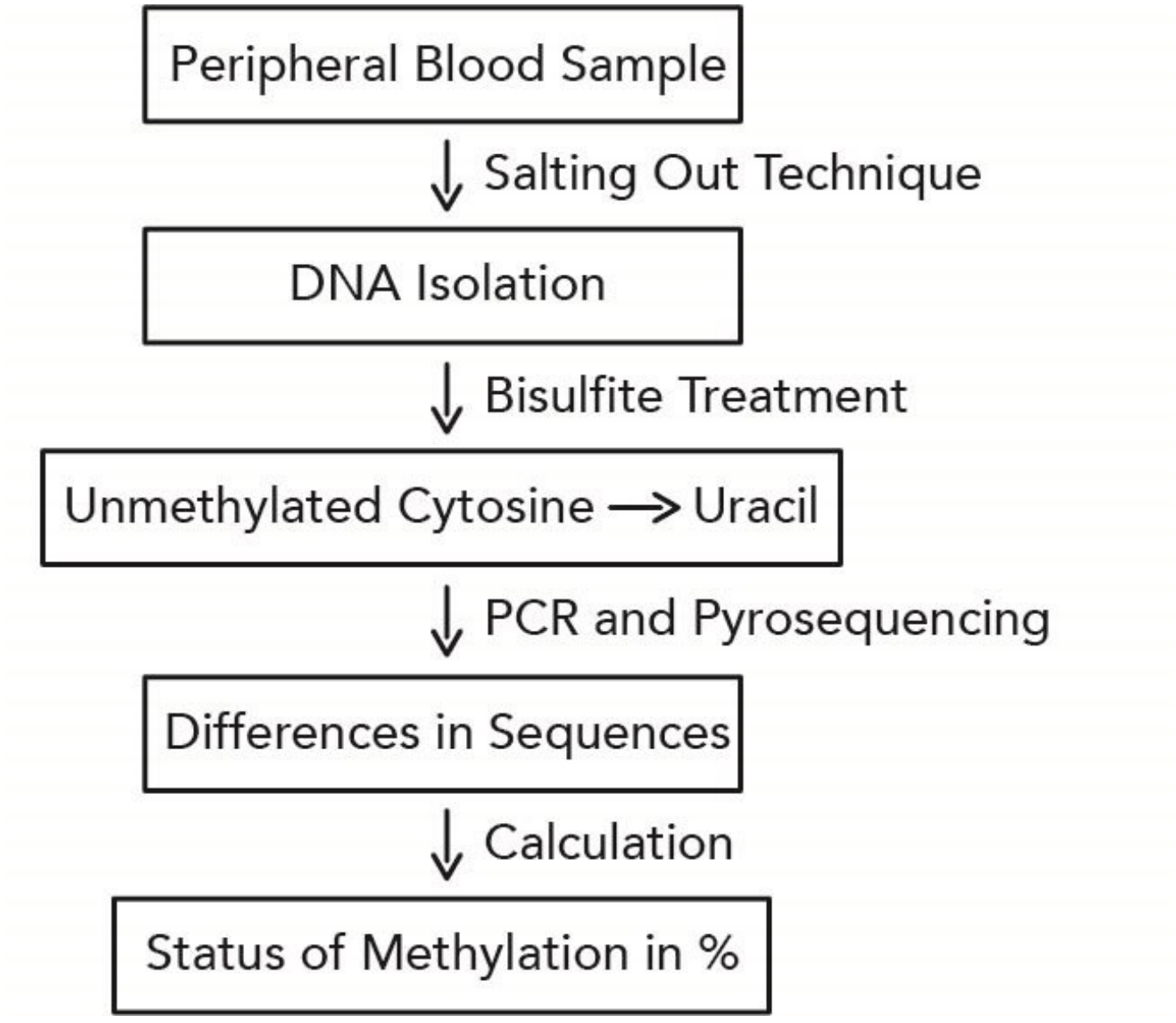


Figure 5 Workflow of the analysis of methylated ARNTL

2.4. Analysis of Dopamine and Norepinephrine levels in peripheral blood

The analysis of the catecholamines DA and NE of peripheral blood was performed by a *high performance liquid chromatography* (HPLC) with a kit called "*ClinRep HPLC Komplettkit Katecholamine im Plasma*" of the company "*Recipe*". The underlying principle in the measure is an electrochemical detection of the turnover of substances. This turnover leads to a donation or acceptance of electrons at the working electrode, depending on oxidation or reduction. Thereby electric current, which is directly proportional to the concentration of the substances, is generated and afterwards measured by a detector. Finally, through the signals of the HLPC's detector, the concentration of the measured samples can be assessed by a comparison with known standards and is shown in a chromatogram.

An example for a chromatogram is given in figure 6. The letters *A, B, C* stand for fictive substances. The retention times (measured in minutes) of the substances are the known standards and with this knowledge you can identify, which peak (measured in millivolt) belongs to which substance. In a simplified conclusion you can say, the bigger the area under the peak, the higher is the concentration of the substance.

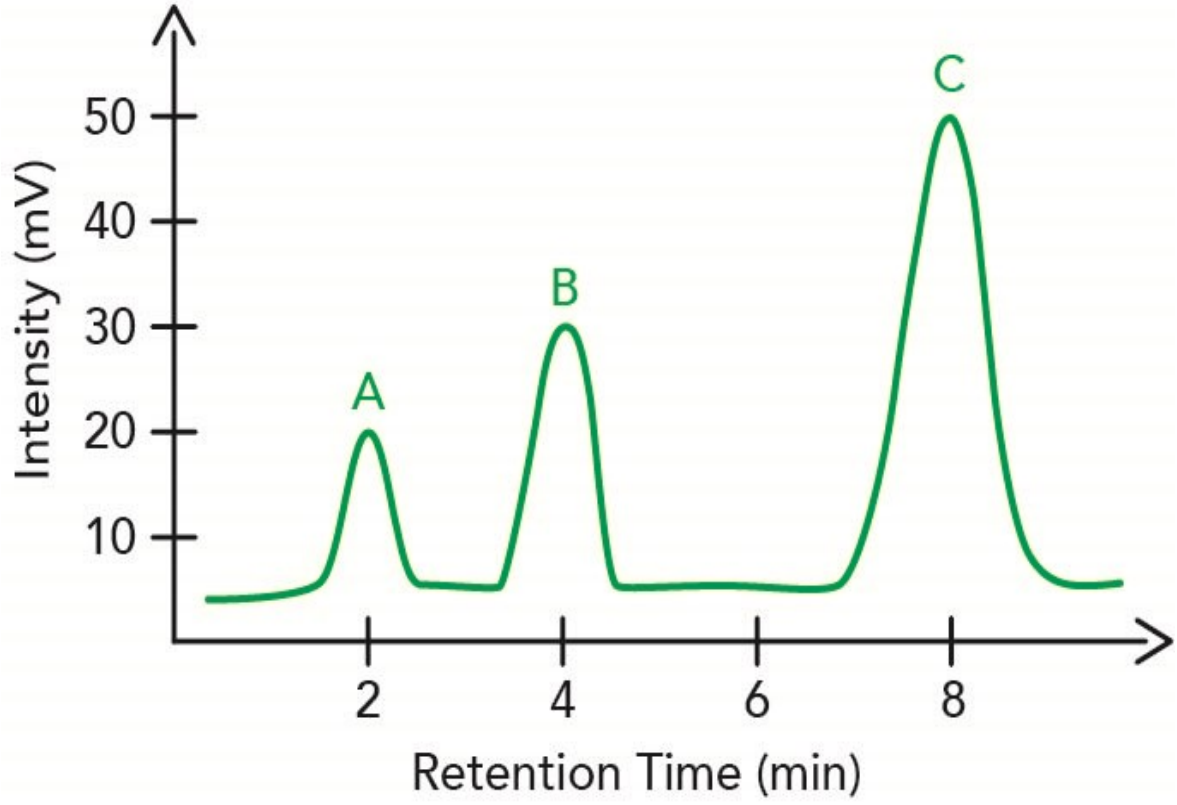


Figure 6 Model of a chromatogram with fictive substances A, B and C. The higher the area under the peak, the higher is the concentration of the substance; mV... millivolt, min… minutes

Before analysis with HPLC, a chemical treatment of the sample is essential. Therefore, following procedure was performed:

1. Application of 1.0ml plasma and 0.05ml Internal Standard in a clean-up column
2. Centrifugation and removal of the supernatant
3. Consecutive washing (3x) of the sample with 1.0ml washing solution
4. Application of 0.12ml elution reagent
5. Injection of 0.04ml eluate into the HPLC

As described above, the chromatogram reveals the concentrations of the free catecholamines. Since there are also bound catecholamines in blood, a second sample was incubated in sulfatase and β-glucuronidase before the treatment with the kit. The HPLC-result of this sample provides data about the total amount of catecholamines. The concentration of the bound catecholamines results in a difference between total and free catecholamines.

2.5. Statistical analysis

The statistic tool IBM SPSS version 23 was used for the analysis in this thesis and a significance level of $p < 0.05$ was set. Prior to testing, I excluded 12 dopamine and 6 norepinephrine values because of extreme spikes in the boxplot. Normality distribution was tested with the Kolmogorov Smirnov test. The homogeneity of the sample was additionally tested with the Levene's test. In case of a normal distribution the t-test and in case of a non-normal distribution Mann-Whitney-U test was used to investigate if there are differences in the levels of bound dopamine and norepinephrine in bipolar patients and controls.

The non-parametric Spearman's rank correlation coefficient was used to analyse the correlation of methylated *ARNTL* at the cg05733473 site and bound dopamine and bound norepinephrine.

3. Results

3.1. Description of the cohort

At the time of evaluation data of 229 participants were collected. A comparison between BD-patients and controls is shown in table 9 below.

Table 9 Description of the cohort; M… mean, N… number, SD… standard deviation, kg… kilogram, m… metre.

	BD-patients	**Controls**
Number of participants (N)	159	70
Males (N)	76	26
Females (N)	83	44
Age [years] (M +/- SD)	44.27 (+/- 13.91)	41.23 (+/- 15.6)
Body Mass Index [kg/m²] (M +/- SD)	28.07 (+/- 6.14)	25.08 (+/- 4.73)
Lithium Intake [%]	26%	-

The stage of overall 71 BD-patients was known and the result is shown in the following table.

Table 10 Clinical classification of BD-patients

Stage	**Number**
Manic	0
Hypomanic	2
Euthymic	49
Depressive	20

3.2. Analysis of bound catecholamines in BD- patients and controls

In BD–patients 78 valid values of bound dopamine and 84 of bound norepinephrine were measured and in controls 46 bound dopamine and 47 bound norepinephrine data were analysed. The descriptive statistic is shown in table 11 and 12.

Table 11 Bound catecholamines in BD–patients; Abbreviations: Min… Minimum, Max... Maximum, DA… dopamine, NE… norepinephrine; values of DA and NE in [pg/ml]

	N	Min	Max	M	SD
Bound DA	78	1494.80	7467.70	3719.72	1418.94
Bound NE	84	436.00	4132.20	1665.95	812.43

Table 12 Bound catecholamines in controls; Abbreviations: Min… Minimum, Max... Maximum, DA… dopamine, NE… norepinephrine; values of DA and NE in [pg/ml]

	N	Min	Max	M	SD
Bound DA	46	1591.40	8819.70	4342.60	2093.08
Bound NE	47	487.10	4155.40	1960.02	889.19

Furthermore, an illustration of the descriptive statistic in form of a boxplot (figure 7 and 8) is shown to compare the two groups and histograms were made for the evaluation of the distribution (figure 9-12).

Prior testing I evaluated the homogeneity with Levene's test. The values of bound dopamine showed a heterogeneity between the group of BD and control ($F(1,122)= 12.842$, $p< 0.001$). As a consequence, I used the non-parametric Mann-Whitney- U test. No significant difference was found in the levels of bound DA between individuals with BD and controls ($p= 0.255$).

According to Levene's test, the bound NE values were revealed a homogeneity ($F(1,129)= 0.498$, $p= 0.482$). Hence, I conducted the parametric t-test. The result was a $p= 0.057$, which is not a significant outcome as well.

Figure 7 Boxplot of the levels of bound DA between BD and controls; BD… bipolar disorder, DA… dopamine, pg… picogram, ml… millilitre

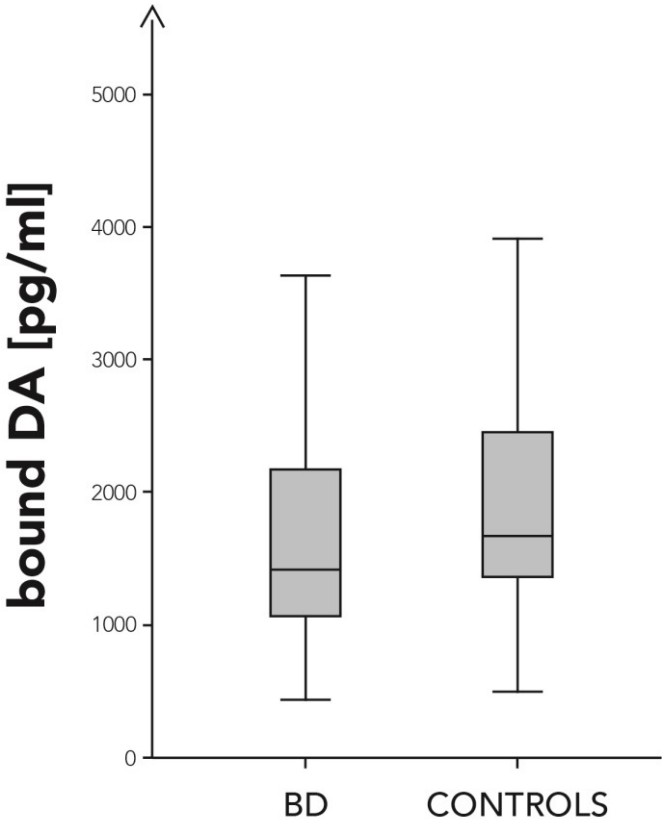


Figure 8 Boxplot of the levels of bound NE between BD and controls; BD… bipolar disorder, NE… norepinehprine, pg… picogram, ml… millilitre

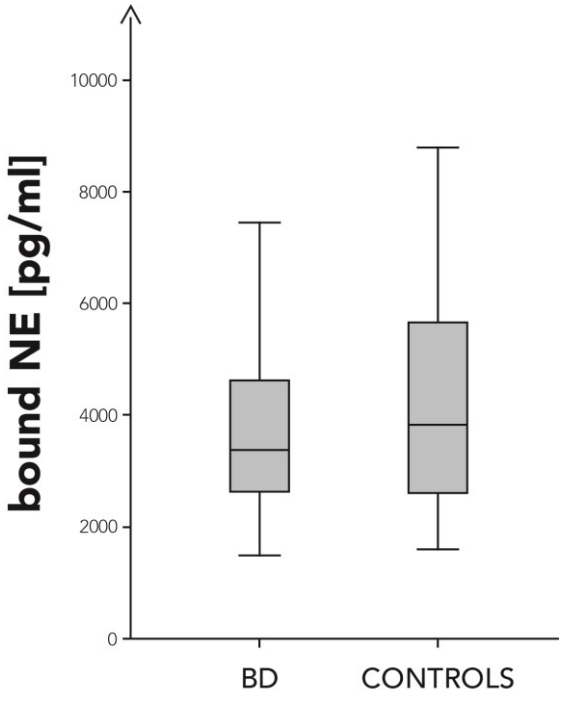


Figure 9 Histogram of the distribution of the levels of bound DA in BD; BD… bipolar disorder, DA… dopamine, pg… picogram, ml… millilitre, M… mean, SD… standard deviation, N… number

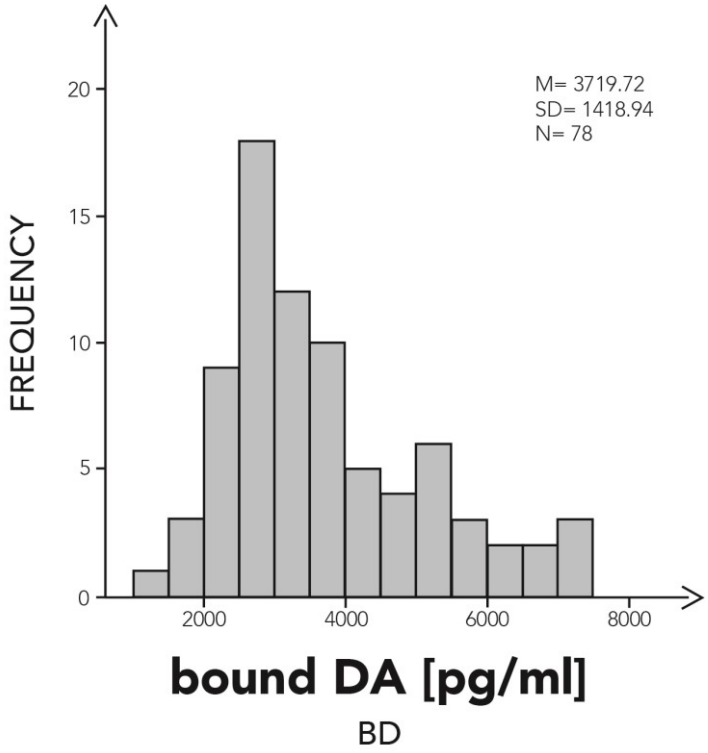


Figure 10 Histogram of the distribution of the levels of bound DA in controls; DA… dopamine, pg… picogram, ml… millilitre; M… mean, SD… standard deviation, N… number

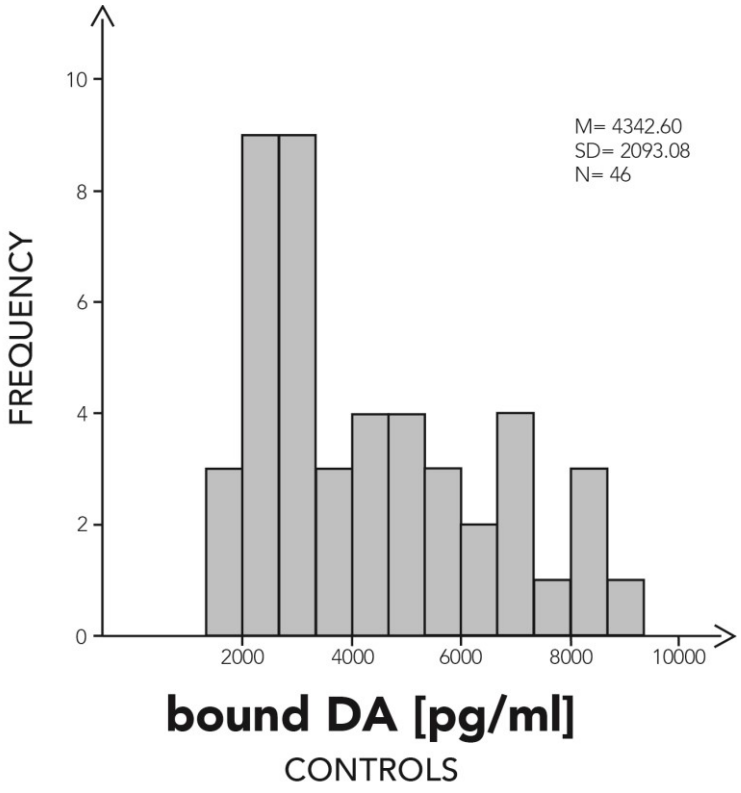


Figure 11 Histogram of the distribution of the levels of bound NE in BD; BD… bipolar disorder, NE… norepinephrine, pg… picogram, ml… millilitre, M… mean, SD… standard deviation, N… number

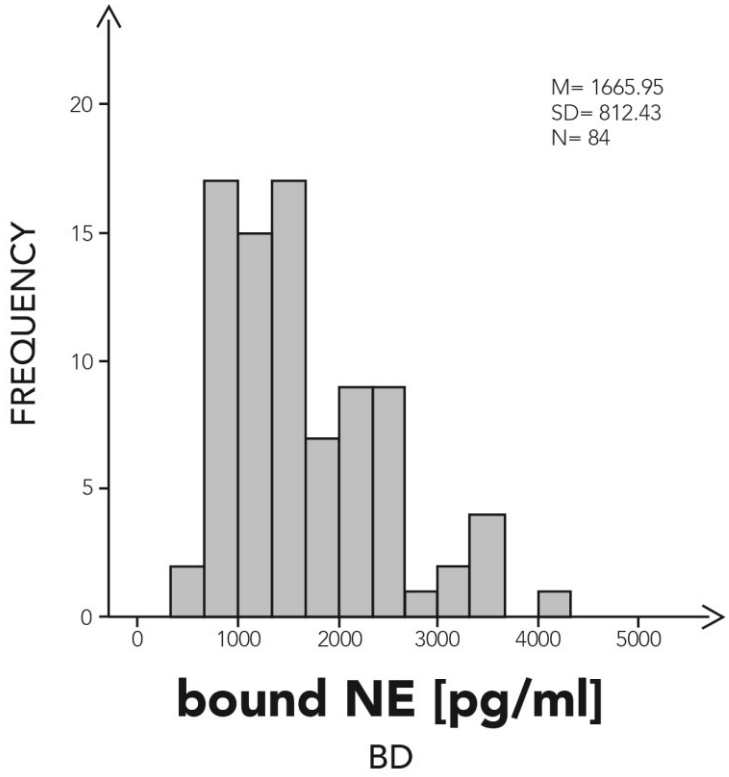
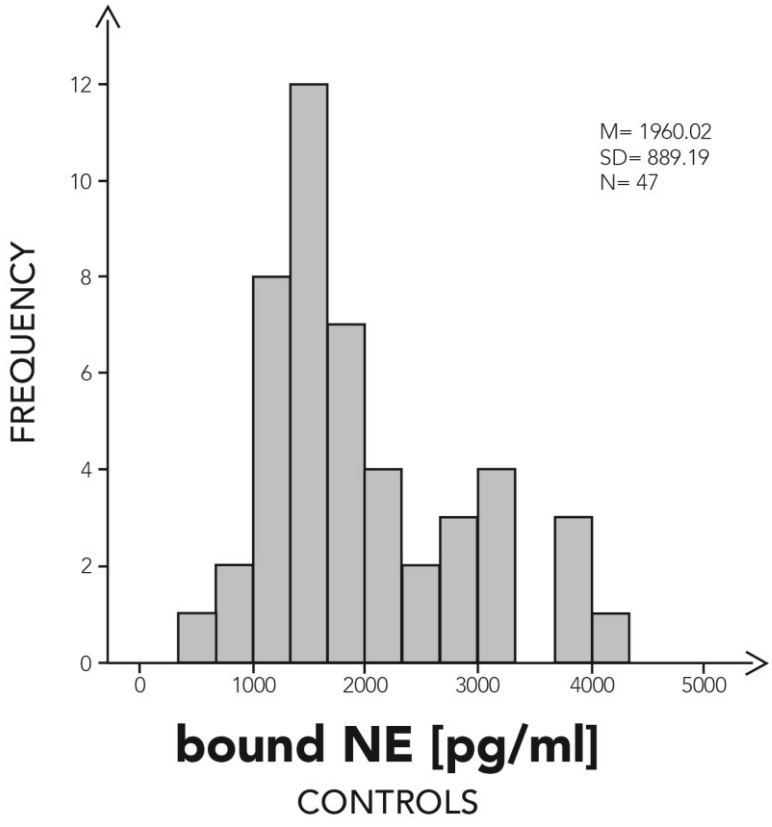


Figure 12 Histogram of the distribution of the levels of bound NE in controls; NE… norepinephrine, pg… picogram, ml… millilitre, M… mean, SD… standard deviation, N… number



3.3. Analysis of a correlation between status of methylated *ARNTL* and bound DA and NE

The analysis of correlation was conducted using the non-parametric Spearman's rank correlation. No significant result was found for both groups. The statistical outcomes are displayed in table 13 and 14.

Table 13 Spearman correlation between bound DA and NE and status of methylated ARNTL in BD- patients; BD… bipolar disorder, DA… dopamine, NE… norepinephrine

	Bound DA	Bound NE
Status of methylated *ARNTL* in BD-patients	*r*= -0.091 *p*= 0.426	*r*= -0.083 *p*= 0.454

Table 14 Spearman correlation between bound DA and NE and status of methylated ARNTL in controls; DA… dopamine, NE… norepinephrine

	Bound DA	Bound NE
Status of methylated *ARNTL* in controls	*r*= -0.059 *p*= 0.738	*r*= -0.161 *p*= 0.340

4. Discussion

The aim of this thesis was to investigate, if there is a correlation between the status of methylation of *ARNTL* and the bound forms of the catecholamines dopamine and norepinephrine in peripheral blood. My main hypothesis was based on the research result of Bengesser et al. (2016), who found a significant higher methylation of *ARNTL* at the cg05733473 site in BD than in controls and Hampp et al. (2008) as well as Hampp et Albrecht (2008), who proved the influence of the circadian rhythm in the expression of MAOA and as a further consequence in raised or decreased levels of neurotransmitters.

In a further hypothesis I investigated if there are differences in the levels of bound dopamine and norepinephrine in peripheral blood between BD and controls. We did not find significant differences between individuals with BD and controls in this study.

4.1. Discussion of the catecholamine levels

Individuals with BD are suffering from pathological mood disturbances, experiencing episodes of (hypo-) manic and depressive episodes. There are numerous studies that have proven a correlation between mood episodes and changes in neurotransmitter levels (described in chapter 1.3.7. and 1.3.8.), even more neurotransmitter hypothesis of depression is one of the oldest and has risen since decades.

My consideration, regarding the measurements of distinctive levels of catecholamines in association with current mood symptoms in this study, was that it could be a simply performable way to identify early changes in the peripheral levels of DA and NE in a venepuncture as a routine check. At best, differences in these levels could be detected before the occurrence of distinctive mood episodes and in a synopsis with the clinical presentation of the patient the attending psychiatrist could adapt the pharmacological treatment as well as influence the individual’s behaviour to avoid or at least soften episodes with clinical relevance.

For the identification of BD-biomarkers, one role model was the meta-analysis by Fernandes et al. (2015), who claimed a link between brain-derived neurotrophic factor (BDNF) in peripheral blood and BD. The findings of this study showed decreased levels of BDNF in both forms of bipolar episodes, while in contrast normal levels in euthymic state were found. Furthermore, the levels of BDNF correlated negatively with the severity of mood symptoms. In summary, BDNF was identified as a biomarker for disease activity but no as a biomarker for episodes (Fernandes et al. 2015).

A further study showed an association of 3-methoxy-4-hydroxyphenylglycol (MHPG) and BD I. MHPG, which is a metabolite of NE's breakdown in the CNS, was found in significantly lower levels in the plasma of individuals with BD in manic and remission stages (Kurita et al. 2014).

To serve as a biomarker of BD it is obvious, that this scenario requires a proven relationship between these monoamines in peripheral blood and the BD mood states which is leading to a limitation factor in my research. Hitherto literature confirmed only the link between BD and the neurotransmitters in the CNS and not in peripheral blood levels (excluded are metabolites of DA and NE).

Since it is technically difficult and in respect of ethical reasons barely possible to perform a biopsy of the CNS, I used the peripheral levels of dopamine and norepinephrine for my research. This is of important relevance, as the so called blood-brain barrier (BBB) controls the transfer of CNS and peripheral blood. The BBB is primarily formed by endothelial cells, astrocytes, pericytes, perivascular macrophages and a basal membrane. Through a specifically adjacency the cells build tight junctions with the main function of the maintenance of a homeostatic milieu in the CNS. Therefore, large hydrophilic molecules cannot pass the barrier, while small lipophilic are able to diffuse along the concentration gradients (Patel et Frey 2015). Dopamine and Norepinephrine are not able to conquer an intact BBB but their metabolites (e.g. MHPG) do. Beside the catecholamines synthetized in the brain, there is a second source of catecholamines in the body: The adrenal medulla with its chromaffin cells (Eisenhofer et al. 2004).

So why are the BBB and the adrenal medulla worth mentioning? In my second hypothesis I analysed the catecholamines levels of peripheral blood. Neither there

was a significant difference in the levels of bound dopamine (p= 0.255), nor in the bound norepinephrine levels (p= 0.057) between individuals with BD and healthy controls. However, regarding a p= 0.057, one could call it a trend, because of its closeness to the significance threshold of $p < 0.05$. In the analysis, the control group had higher levels of bound NE (1960.02 pg/ml +/-889.19) in contrast to the BD group (1665.95 pg/ml +/-812.43).

Possibly, disturbances in the HPA-axis are involved in this outcome of my research. Particularly in relation to the adrenal gland this axis is of important relevance. It is a well-known mechanism that a stimulation of the hypothalamus leads to the release of CRH, which causes the pituitary gland's expression of ACTH. Consequently, ACTH triggers the adrenal, resulting in the release of their hormones. In view of BD an over-activity of this axis has been described several times (Belvederi Murri et al. 2016). In this context it is quite remarkable that the trend of my research points in the inverse direction. Though, it has to be added, that the over-activity of the HPA-axis in BD primarily manifests itself in the levels of cortisol, which were not collected in this study, and not in the catecholamines.

Another potential reason for this not clearly significant result was the fact that a high number of the BD-group in this research was inpatient and received mood-stabilizing pharmacological therapy, as well as relaxation therapy in a clinical setting.

Nevertheless, in my opinion the peripheral levels of bound DA and bound NE are not representative for the CNS turnover due to the BBB and an extraneuronal synthesis of catecholamines (adrenal medulla) and consequently are not appropriate as specific BD biomarker.

4.2. Discussion of the status of methylated ARNTL and the correlation with the peripheral levels of DA and NE

As declared earlier, Bengesser et al. (2016) proved a higher status of methylation at the cg05733473 site of *ARNTL* in BD patients. This result gave me reason to widen the research focusing on that site. It is important to know, that the methylation of DNA belongs to epigenetic mechanisms to regulate genetic expression of genes without changing the DNA sequence itself.

Basically, during the process of methylation, a methyl group is added to cytosine at the carbon 5-site. The product is a modified cytosine, called 5-methylcytosine. Methylation of cytosine appears in so called CpG dinucleotides. Regions, with a high amount of CG repeats are called CpG islands and are primary found around the promotor of a gene. Although there are exceptions, most literature linked a high status of methylation with a silencing of a gene, resulting in a decreased gene expression (Bengesser et al. 2016, Fries et al. 2016).

My main hypothesis, that there is a positive correlation between the catecholamines DA and NE and the status of methylated *ARNTL* developed from results of hitherto literature. Several studies proved a disruption in the circadian rhythm in BD. Pivotal for me was the research of Hampp et al. (2008) and Hampp et Albrecht (2008), who claimed a reduced ARNTL and MAOA expression in mania. As pointed out in chapter 1.2. *ARNTL*, as one representative of the clock genes, plays a crucial role in the circadian rhythm and furthermore *ARNTL* is involved in the expression of MAOA. A higher status of methylation of *ARNTL* should silence then the expression of MAOA. Consequently, lower levels of MAOA should cause higher levels of catecholamines, as MAOA is responsible for their inactivation and metabolism. Finally, higher levels of catecholamines could explain the presence of mania.

Unfortunately my results could not support this hypothesis. We neither found for bound DA nor for bound NE a significant correlation between BD and controls. To interpret the outcome of the study, I have to state, that the investigated region cg05733473 is only one position at *ARNTL*. There are other regions of the gene as well, which could impact genetic expression.

A further relevant point is that my hypothesis is based on the degradation of the catecholamines by MAOA. Indeed, I have not measured MAOA itself or its gene expression in my thesis. Hence, the outcome could have only indicated indirectly to an altered expression of MAOA. In addition, it is no note that the breakdown of catecholamines is not exclusively performed by MAOA. COMT is involved in the process of catecholamine-inactivation as well and was not included in this analysis (Teroganova et al. 2016).

To my best knowledge, there is no other research, which investigated peripheral levels of catecholamines and their correlation to the status of a methylated gene concerning BD, but there are other studies, which report an effect of hypo- and hypermethylated genes in BD.

Teroganova et al. (2016) summed up the current state of knowledge about hypo- and hypermethylation in BD, including all studies, which analysed DNA methylation in peripheral tissue. Hypermethylation was found in gene loci of Serotonin (5-HT1A gene and SLC6A4) and BDNF (BDNF exon 1 promotor), while hypomethylation was observed in membrane-bound (MB-) COMT, which is in context of my research relevant, as COMT is jointly involved in the breakdown of DA and NE (Teroganova et al. 2016).

Interestingly, research by Fries et al. (2016) stated that a hypomethylation of MB-COMT in the promotor region in the frontal lobe was leading to an increased neurotransmitter breakdown resulting in a hypodopaminergic state. At least this outcome facilitates my hypothesis.

4.3. Limitations

As already mentioned in the chapters before, there are some limitations in my research. First of all, all data were measured in peripheral blood. Therefore, the issue with peripheral levels of catecholamine and the BBB and adrenal medulla was elucidated in chapter 4.1. Additionally, the status of methylation was generated from peripheral blood as well and thus is not exactly the same as in brain tissue, although peripheral levels of methylation might mirror concentrations in the brain (Bengesser et al. 2016; Fries et al. 2016).

Further limitations concern a potentially lack of measurements of MAOA to support my hypothesis. Even if I had found a significant positive correlation between the status of methylation and DA and NE, without a measurement of MAOA, I only could have speculated about the relation. Additionally it is worth mentioning, that data of catecholamines were gained in cross section measurements. This could be a bias, since many factors (e.g. physical activity or stress) could influence the transient catecholamine levels.

Finally, there is a last limitation in my thesis. The pharmacological treatment of BD intervenes in the circadian rhythm. Especially Lithium, which is the gold standard in the therapy of BD, as well as valproate interact with the molecular clock. Gsk3B and *REV-ERBa* are particular sensitive for these substances (Bengesser et al. 2016). The results of my research could therefore have been affected, as the patients had on-going treatment, resulting in possibly balanced neurotransmitter levels.

4.4. Conclusion and Implication

In my research I concerned myself with the complex subject of the circadian rhythm in BD, which is primarily affected by so called clock genes. Typically changes in behaviours (e.g. reduced need for sleep or decreased appetite, raised libido and so on) characterize manic state, while depressive episodes are often defined by lethargy, feebleness and joylessness as well as disturbances in sleep rhythms. There is a lot of literature, which proved a disturbance in the circadian rhythm in BD and driven by these results of hitherto literature I analysed peripheral blood levels of catecholamines, to see whether there is a relation to current mood status. The reason I chose peripheral catecholamines was a pragmatically one. It is easily performable to gain the data with a blood sampling. The effect of DA and NE in the CNS regarding BD is well known already. Although I could not find a significant difference in the level of catecholamines, I noticed a trend (p= 0.057), where the BD group had lower levels of NE in contrast to controls. This could either be explained by the on-going pharmacological treatment of the BD patients in a clinical setting or it could be a random finding.

Furthermore it could be of interest to investigate manic and depressive patients separated. Unfortunately, I was not able to do it in this cohort, due to a lack of (hypo-) manic patients in my cohort. Since DA and NE are not able to conquer the BBB, their peripheral levels might not be reliable measures of the respective levels in the CNS. Therefore a study with their precursors, which are able to cross BBB, could be more meaningful.

Continuing, the field of epigenetic studies will rise in future due to improving equipment and better knowledge about the mechanisms. In my thesis I tried to combine epigenetics (status of methylation) and the circadian rhythm. Regrettably, I failed to prove my main hypothesis: a higher status of methylation of *ARNTL* would lead to increased levels of peripheral catecholamines. Even though I analysed the region, where Bengesser et al. (2016) claimed a significant hypermethylation in BD, it has to be noted, that there are also other positions, which could mainly be responsible for gene expression.

To gain better understanding of BD-mechanism in the future, I suggest that a higher sample size and measurements of peripheral levels of MAOA and COMT in combination with the status of methylation of clock genes, such as *ARNTL*, could be useful. Furthermore, the gene expression of MAOA, COMT and ARNTL itself should be measured. Due to the influence of the circadian rhythm on the transient release of catecholamines, multiple testing at different time points during the day and during the course of disease would be of advantage. Therefore follow-up studies would be adequate, as one could detect alterations in their levels, depending on the individual's stage. Moreover, a comparison between manic and depressive BD-patients in their MAOA and COMT levels would be interesting and could probably serve as a state-marker for BD.

To sum up, I state that my hypothesis could not be verified, but that does not mean that it was a false hypothesis per se. It could be possible, that I have not analysed the right position yet.

References

ACOSTA-GALVAN, G., YI, C.X., VAN DER VLIET, J., JHAMANDAS, J.H., PANULA, P., ANGELES-CASTELLANOS, M., DEL CARMEN BASUALDO, M., ESCOBAR, C. and BUIJS, R.M., 2011. Interaction between hypothalamic dorsomedial nucleus and the suprachiasmatic nucleus determines intensity of food anticipatory behavior. *Proceedings of the National Academy of Sciences of the United States of America,* 108(14), pp. 5813-5818.

AKASHI, M. and TAKUMI, T., 2005. The orphan nuclear receptor RORalpha regulates circadian transcription of the mammalian core-clock Bmal1. *Nature structural & molecular biology,* 12(5), pp. 441-448.

AKISKAL, H.S., BOURGEOIS, M.L., ANGST, J., POST, R., MOLLER, H. and HIRSCHFELD, R., 2000. Re-evaluating the prevalence of and diagnostic composition within the broad clinical spectrum of bipolar disorders. *Journal of affective disorders,* 59 Suppl 1, pp. S5-S30.

ANAND, S.N., MAYWOOD, E.S., CHESHAM, J.E., JOYNSON, G., BANKS, G.T., HASTINGS, M.H. and NOLAN, P.M., 2013. Distinct and separable roles for endogenous CRY1 and CRY2 within the circadian molecular clockwork of the suprachiasmatic nucleus, as revealed by the Fbxl3(Afh) mutation. *The Journal of neuroscience : the official journal of the Society for Neuroscience,* 33(17), pp. 7145-7153.

ANGST, J., GAMMA, A., BENAZZI, F., AJDACIC, V., EICH, D. and ROSSLER, W., 2003. Toward a re-definition of subthreshold bipolarity: epidemiology and proposed criteria for bipolar-II, minor bipolar disorders and hypomania. *Journal of affective disorders,* 73(1-2), pp. 133-146.

ARNONE, D., CAVANAGH, J., GERBER, D., LAWRIE, S.M., EBMEIER, K.P. and MCINTOSH, A.M., 2009. Magnetic resonance imaging studies in bipolar disorder and schizophrenia: meta-analysis. *The British journal of psychiatry: the journal of mental science,* 195(3), pp. 194-201.

ASHOK, A.H., MARQUES, T.R., JAUHAR, S., NOUR, M.M., GOODWIN, G.M., YOUNG, A.H. and HOWES, O.D., 2017. The dopamine hypothesis of bipolar affective disorder: the state of the art and implications for treatment. *Molecular psychiatry,* 22(5), pp. 666-679.

BALDESSARINI, R.J., BOLZANI, L., CRUZ, N., JONES, P.B., LAI, M., LEPRI, B., PEREZ, J., SALVATORE, P., TOHEN, M., TONDO, L. and VIETA, E., 2010. Onset-age of bipolar disorders at six international sites. *Journal of affective disorders,* 121(1-2), pp. 143-146.

BELVEDERI MURRI, M., PRESTIA, D., MONDELLI, V., PARIANTE, C., PATTI, S., OLIVIERI, B., ARZANI, C., MASOTTI, M., RESPINO, M., ANTONIOLI, M., VASSALLO, L., SERAFINI, G., PERNA, G., POMPILI, M. and AMORE, M., 2016. The HPA axis in bipolar disorder: Systematic review and meta-analysis. *Psychoneuroendocrinology,* 63, pp. 327-342.

BENEDETTI, F., SERRETTI, A., COLOMBO, C., BARBINI, B., LORENZI, C., CAMPORI, E. and SMERALDI, E., 2003. Influence of CLOCK gene polymorphism on circadian mood fluctuation and illness recurrence in bipolar depression. *American journal of medical genetics.Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics,* 123B(1), pp. 23-26.

BENGESSER S, R.E., ed, 2013. *Genetics of Bipolar Disorder, Peter-Lang.*

BENGESSER, S.A., REININGHAUS, E.Z., LACKNER, N., BIRNER, A., FELLENDORF, F.T., PLATZER, M., KAINZBAUER, N., TROPPER, B., HORMANSEDER, C., QUEISSNER, R., KAPFHAMMER, H.P., WALLNER-LIEBMANN, S.J., FUCHS, R., PETEK, E., WINDPASSINGER, C., SCHNALZENBERGER, M., REININGHAUS, B., EVERT, B. and WAHA, A., 2016. Is the molecular clock ticking differently in bipolar disorder? Methylation analysis of the clock gene ARNTL. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry,* , pp. 1-9.

BERK, M., DODD, S., KAUER-SANT'ANNA, M., MALHI, G.S., BOURIN, M., KAPCZINSKI, F. and NORMAN, T., 2007. Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder. *Acta psychiatrica Scandinavica.Supplementum,* (434):41-9. doi(434), pp. 41-49.

BIRNER, A., SEILER, S., LACKNER, N., BENGESSER, S.A., QUEISSNER, R., FELLENDORF, F.T., PLATZER, M., ROPELE, S., ENZINGER, C., SCHWINGENSCHUH, P., MANGGE, H., PIRPAMER, L., DEUTSCHMANN, H., MCINTYRE, R.S., KAPFHAMMER, H.P., REININGHAUS, B. and REININGHAUS, E.Z., 2015. Cerebral White Matter Lesions and Affective Episodes Correlate in Male Individuals with Bipolar Disorder. *PloS one,* 10(8), pp. e0135313.

BUIJS, F.N., LEON-MERCADO, L., GUZMAN-RUIZ, M., GUERRERO-VARGAS, N.N., ROMO-NAVA, F. and BUIJS, R.M., 2016. The Circadian System: A Regulatory Feedback Network of Periphery and Brain. *Physiology (Bethesda, Md.),* 31(3), pp. 170-181.

CASSIDY, F., ZHAO, C., BADGER, J., CLAFFEY, E., DOBRIN, S., ROCHE, S. and MCKEON, P., 2007. Genome-wide scan of bipolar disorder and investigation of population stratification effects on linkage: support for susceptibility loci at 4q21, 7q36, 9p21, 12q24, 14q24, and 16p13. *American journal of medical genetics.Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics,* 144B(6), pp. 791-801.

CECIL, C.A., SMITH, R.G., WALTON, E., MILL, J., MCCRORY, E.J. and VIDING, E., 2016. Epigenetic signatures of childhood abuse and neglect: Implications for psychiatric vulnerability. *Journal of psychiatric research,* 83, pp. 184-194.

CHARRIER, A., OLLIAC, B., ROUBERTOUX, P. and TORDJMAN, S., 2017. Clock Genes and Altered Sleep-Wake Rhythms: Their Role in the Development of Psychiatric Disorders. *International journal of molecular sciences,* 18(5), pp. 10.3390/ijms18050938.

COUSINS, D.A., BUTTS, K. and YOUNG, A.H., 2009. The role of dopamine in bipolar disorder. *Bipolar disorders,* 11(8), pp. 787-806.

CZEISLER, C.A., DUFFY, J.F., SHANAHAN, T.L., BROWN, E.N., MITCHELL, J.F., RIMMER, D.W., RONDA, J.M., SILVA, E.J., ALLAN, J.S., EMENS, J.S., DIJK, D.J. and KRONAUER, R.E., 1999. Stability, precision, and near24-hour period of the human circadian pacemaker. *Science (New York, N.Y.),* 284(5423), pp. 2177-2181.

DAHLSTROEM, A. and FUXE, K., 1964. Evidence for the Existence of Monoamine-Containing Neurons in the Central Nervous System. I. Demonstration of Monoamines in the Cell Bodies of Brain Stem Neurons. *Acta physiologica Scandinavica.Supplementum,* , pp. SUPPL 232:1-55.

DALLASPEZIA, S., LORENZI, C., PIROVANO, A., COLOMBO, C., SMERALDI, E. and BENEDETTI, F., 2011. Circadian clock gene Per3 variants influence the postpartum onset of bipolar disorder. *European psychiatry : the journal of the Association of European Psychiatrists,* 26(3), pp. 138-140.

DARUY-FILHO, L., BRIETZKE, E., LAFER, B. and GRASSI-OLIVEIRA, R., 2011. Childhood maltreatment and clinical outcomes of bipolar disorder. *Acta Psychiatrica Scandinavica,* 124(6), pp. 427-434.

DIENES, K.A., HAMMEN, C., HENRY, R.M., COHEN, A.N. and DALEY, S.E., 2006. The stress sensitization hypothesis: understanding the course of bipolar disorder. *Journal of affective disorders,* 95(1-3), pp. 43-49.

EISENHOFER, G., KOPIN, I.J. and GOLDSTEIN, D.S., 2004. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacological reviews,* 56(3), pp. 331-349.

ERTEN, E., FUNDA UNEY, A., SAATCIOGLU, O., OZDEMIR, A., FISTIKCI, N. and CAKMAK, D., 2014. Effects of childhood trauma and clinical features on determining quality of life in patients with bipolar I disorder. *Journal of affective disorders,* 162, pp. 107-113.

ETAIN, B., JAMAIN, S., MILHIET, V., LAJNEF, M., BOUDEBESSE, C., DUMAINE, A., MATHIEU, F., GOMBERT, A., LEDUDAL, K., GARD, S., KAHN, J.P., HENRY, C., BOLAND, A., ZELENIKA, D., LECHNER, D., LATHROP, M., LEBOYER, M. and BELLIVIER, F., 2014. Association between circadian genes, bipolar disorders and chronotypes. *Chronobiology international,* 31(7), pp. 807-814.

ETCHEGARAY, J.P., MACHIDA, K.K., NOTON, E., CONSTANCE, C.M., DALLMANN, R., DI NAPOLI, M.N., DEBRUYNE, J.P., LAMBERT, C.M., YU, E.A., REPPERT, S.M. and WEAVER, D.R., 2009. Casein kinase 1 delta regulates the pace of the mammalian circadian clock. *Molecular and cellular biology,* 29(14), pp. 3853-3866.

EWALD, H., FLINT, T., KRUSE, T.A. and MORS, O., 2002. A genome-wide scan shows significant linkage between bipolar disorder and chromosome 12q24.3 and suggestive linkage to chromosomes 1p22-21, 4p16, 6q14-22, 10q26 and 16p13.3. *Molecular psychiatry,* 7(7), pp. 734-744.

FERNANDES, B.S., MOLENDIJK, M.L., KOHLER, C.A., SOARES, J.C., LEITE, C.M., MACHADO-VIEIRA, R., RIBEIRO, T.L., SILVA, J.C., SALES, P.M., QUEVEDO, J., OERTEL-KNOCHEL, V., VIETA, E., GONZALEZ-PINTO, A., BERK, M. and CARVALHO, A.F., 2015. Peripheral brain-derived neurotrophic factor (BDNF) as a biomarker in bipolar disorder: a meta-analysis of 52 studies. *BMC medicine,* 13, pp. 289-015-0529-7.

FERNSTROM, J.D. and FERNSTROM, M.H., 2007. Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *The Journal of nutrition,* 137(6 Suppl 1), pp. 1539S-1547S; discussion 1548S.

FERRARI, A.J., BAXTER, A.J. and WHITEFORD, H.A., 2011. A systematic review of the global distribution and availability of prevalence data for bipolar disorder. *Journal of affective disorders,* 134(1-3), pp. 1-13.

FRIES, G.R., LI, Q., MCALPIN, B., REIN, T., WALSS-BASS, C., SOARES, J.C and QUEVEDO, J., 2016. The role of DNA methylation in the pathophysiology and treatment of bipolar disorder. *Neuroscience and biobehavioral reviews,* 68, pp. 474-488.

GAWESKA, H. and FITZPATRICK, P.F., 2011. Structures and Mechanism of the Monoamine Oxidase Family. *Biomolecular concepts,* 2(5), pp. 365-377.

GEOFFROY, P.A., LAJNEF, M., BELLIVIER, F., JAMAIN, S., GARD, S., KAHN, J.P., HENRY, C., LEBOYER, M. and ETAIN, B., 2015. Genetic association study of circadian genes with seasonal pattern in bipolar disorders. *Scientific reports,* 5, pp. 10232.

GERSHON, A., JOHNSON, S.L. and MILLER, I., 2013. Chronic stressors and trauma: prospective influences on the course of bipolar disorder. *Psychological medicine,* 43(12), pp. 2583-2592.

GLASER, B., KIROV, G., GREEN, E., CRADDOCK, N. and OWEN, M.J., 2005. Linkage disequilibrium mapping of bipolar affective disorder at 12q23-q24 provides evidence for association at CUX2 and FLJ32356. *American journal of medical genetics.Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics,* 132B(1), pp. 38-45.

GOLDBERG, J.F., HARROW, M. and WHITESIDE, J.E., 2001. Risk for bipolar illness in patients initially hospitalized for unipolar depression. *The American Journal of Psychiatry,* 158(8), pp. 1265-1270.

GREEN, E., ELVIDGE, G., JACOBSEN, N., GLASER, B., JONES, I., O'DONOVAN, M.C., KIROV, G., OWEN, M.J. and CRADDOCK, N., 2005. Localization of bipolar susceptibility locus by molecular genetic analysis of the chromosome 12q23-q24 region in two pedigrees with bipolar disorder and Darier's disease. *The American Journal of Psychiatry,* 162(1), pp. 35-42.

GROSS, S., GAROFALO, D.C., BALDERES, D.A., MASTRACCI, T.L., DIAS, J.M., PERLMANN, T., ERICSON, J. and SUSSEL, L., 2016. The novel enterochromaffin marker Lmx1a regulates serotonin biosynthesis in enteroendocrine cell lineages downstream of Nkx2.2. *Development (Cambridge, England),* 143(14), pp. 2616-2628.

GUERRERO-VARGAS, N.N., SALGADO-DELGADO, R., BASUALDO MDEL, C., GARCIA, J., GUZMAN-RUIZ, M., CARRERO, J.C., ESCOBAR, C. and BUIJS, R.M., 2014. Reciprocal interaction between the suprachiasmatic nucleus and the immune system tunes down the inflammatory response to lipopolysaccharide. *Journal of neuroimmunology,* 273(1-2), pp. 22-30.

HAJEK, T., KOPECEK, M., KOZENY, J., GUNDE, E., ALDA, M. and HOSCHL, C., 2009. Amygdala volumes in mood disorders--meta-analysis of magnetic resonance volumetry studies. *Journal of affective disorders,* 115(3), pp. 395-410.

HAMPP, G. and ALBRECHT, U., 2008. The circadian clock and mood-related behavior. *Communicative & integrative biology,* 1(1), pp. 1-3.

HAMPP, G., RIPPERGER, J.A., HOUBEN, T., SCHMUTZ, I., BLEX, C., PERREAU-LENZ, S., BRUNK, I., SPANAGEL, R., AHNERT-HILGER, G., MEIJER, J.H. and ALBRECHT, U., 2008. Regulation of monoamine oxidase A by circadian-clock components implies clock influence on mood. *Current biology : CB,* 18(9), pp. 678-683.

HIBAR, D.P., WESTLYE, L.T., VAN ERP, T.G., RASMUSSEN, J., LEONARDO, C.D., FASKOWITZ, J., HAUKVIK, U.K., HARTBERG, C.B., DOAN, N.T., AGARTZ, I., DALE, A.M., GRUBER, O., KRAMER, B., TROST, S., LIBERG, B., ABE, C., EKMAN, C.J., INGVAR, M., LANDEN, M., FEARS, S.C., FREIMER, N.B., BEARDEN, C.E., COSTA RICA/COLOMBIA CONSORTIUM FOR GENETIC INVESTIGATION OF BIPOLAR ENDOPHENOTYPES, SPROOTEN, E., GLAHN, D.C., PEARLSON, G.D., EMSELL, L., KENNEY, J., SCANLON, C., MCDONALD, C., CANNON, D.M., ALMEIDA, J., VERSACE, A., CASERAS, X., LAWRENCE, N.S., PHILLIPS, M.L., DIMA, D., DELVECCHIO, G., FRANGOU, S., SATTERTHWAITE, T.D., WOLF, D., HOUENOU, J., HENRY, C., MALT, U.F., BOEN, E., ELVSASHAGEN, T., YOUNG, A.H., LLOYD, A.J., GOODWIN, G.M., MACKAY, C.E., BOURNE, C., BILDERBECK, A., ABRAMOVIC, L., BOKS, M.P., VAN HAREN, N.E., OPHOFF, R.A., KAHN, R.S., BAUER, M., PFENNIG, A., ALDA, M., HAJEK, T., MWANGI, B., SOARES, J.C., NICKSON, T., DIMITROVA, R., SUSSMANN, J.E., HAGENAARS, S., WHALLEY, H.C., MCINTOSH, A.M., THOMPSON, P.M. and ANDREASSEN, O.A., 2016. Subcortical volumetric abnormalities in bipolar disorder. *Molecular psychiatry,* 21(12), pp. 1710-1716.

HILLEGERS, M.H., BURGER, H., WALS, M., REICHART, C.G., VERHULST, F.C., NOLEN, W.A. and ORMEL, J., 2004. Impact of stressful life events, familial loading and their interaction on the onset of mood disorders: study in a high-risk cohort of adolescent offspring of parents with bipolar disorder. *The British journal of psychiatry : the journal of mental science,* 185, pp. 97-101.

HOLSBOER, F., 2000. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology,* 23(5), pp. 477-501.

HORN, F., ed, 2009. *Biochemie des Menschen.* Thieme Verlag.

JAGER, J., WANG, F., FANG, B., LIM, H.W., PEED, L.C., STEGER, D.J., WON, K.J., KHARITONENKOV, A., ADAMS, A.C. and LAZAR, M.A., 2016. The Nuclear Receptor Rev-erbalpha Regulates Adipose Tissue-specific FGF21 Signaling. *The Journal of biological chemistry,* 291(20), pp. 10867-10875.

JUDD, L.L., AKISKAL, H.S., SCHETTLER, P.J., CORYELL, W., ENDICOTT, J., MASER, J.D., SOLOMON, D.A., LEON, A.C. and KELLER, M.B., 2003. A prospective investigation of the natural history of the long-term weekly symptomatic status of bipolar II disorder. *Archives of General Psychiatry,* 60(3), pp. 261-269.

JUDD, L.L., AKISKAL, H.S., SCHETTLER, P.J., ENDICOTT, J., MASER, J., SOLOMON, D.A., LEON, A.C., RICE, J.A. and KELLER, M.B., 2002. The long-term natural history of the weekly symptomatic status of bipolar I disorder. *Archives of General Psychiatry,* 59(6), pp. 530-537.

KARTHIKEYAN, R., MARIMUTHU, G., RAMASUBRAMANIAN, C., ARUNACHAL, G., BAHAMMAM, A.S., SPENCE, D.W., CARDINALI, D.P., BROWN, G.M. and PANDI-PERUMAL, S.R., 2014. Association of Per3 length polymorphism with bipolar I disorder and schizophrenia. *Neuropsychiatric disease and treatment,* 10, pp. 2325-2330.

KEMPTON, M.J., GEDDES, J.R., ETTINGER, U., WILLIAMS, S.C. and GRASBY, P.M., 2008. Meta-analysis, database, and meta-regression of 98 structural imaging studies in bipolar disorder. *Archives of General Psychiatry,* 65(9), pp. 1017-1032.

KIM, H.I., LEE, H.J., CHO, C.H., KANG, S.G., YOON, H.K., PARK, Y.M., LEE, S.H., MOON, J.H., SONG, H.M., LEE, E. and KIM, L., 2015. Association of CLOCK, ARNTL, and NPAS2 gene polymorphisms and seasonal variations in mood and behavior. *Chronobiology international,* 32(6), pp. 785-791.

KOVANEN, L., KAUNISTO, M., DONNER, K., SAARIKOSKI, S.T. and PARTONEN, T., 2013. CRY2 genetic variants associate with dysthymia. *PloS one,* 8(8), pp. e71450.

KRIPKE, D.F., NIEVERGELT, C.M., JOO, E., SHEKHTMAN, T. and KELSOE, J.R., 2009. Circadian polymorphisms associated with affective disorders. *Journal of circadian rhythms,* 7, pp. 2.

KURITA, M., NISHINO, S., NUMATA, Y., OKUBO, Y. and SATO, T., 2014. The noradrenaline metabolite MHPG is a candidate biomarker from the manic to the remission state in bipolar disorder I: a clinical naturalistic study. *PloS one,* 9(6), pp. e100634.

LAI, Y.C., KAO, C.F., LU, M.L., CHEN, H.C., CHEN, P.Y., CHEN, C.H., SHEN, W.W., WU, J.Y., LU, R.B. and KUO, P.H., 2015. Investigation of associations between NR1D1, RORA and RORB genes and bipolar disorder. *PloS one,* 10(3), pp. e0121245.

LANDGRAF, D., WANG, L.L., DIEMER, T. and WELSH, D.K., 2016. NPAS2 Compensates for Loss of CLOCK in Peripheral Circadian Oscillators. *PLoS genetics,* 12(2), pp. e1005882.

LAVEBRATT, C., SJOHOLM, L.K., SORONEN, P., PAUNIO, T., VAWTER, M.P., BUNNEY, W.E., ADOLFSSON, R., FORSELL, Y., WU, J.C., KELSOE, J.R., PARTONEN, T. and SCHALLING, M., 2010. CRY2 is associated with depression. *PloS one,* 5(2), pp. e9407.

LE-NICULESCU, H., PATEL, S.D., BHAT, M., KUCZENSKI, R., FARAONE, S.V., TSUANG, M.T., MCMAHON, F.J., SCHORK, N.J., NURNBERGER, J.I.,JR and NICULESCU, A.B.,3RD, 2009. Convergent functional genomics of genome-wide association data for bipolar disorder: comprehensive identification of candidate genes, pathways and mechanisms. *American journal of medical genetics.Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics,* 150B(2), pp. 155-181.

LINKOWSKI, P., 2003. Neuroendocrine profiles in mood disorders. *The international journal of neuropsychopharmacology,* 6(2), pp. 191-197.

MACQUEEN, G.M., YOUNG, L.T. and JOFFE, R.T., 2001. A review of psychosocial outcome in patients with bipolar disorder. *Acta Psychiatrica Scandinavica,* 103(3), pp. 163-170.

MANJI, H.K., QUIROZ, J.A., PAYNE, J.L., SINGH, J., LOPES, B.P., VIEGAS, J.S. and ZARATE, C.A., 2003. The underlying neurobiology of bipolar disorder. *World psychiatry : official journal of the World Psychiatric Association (WPA),* 2(3), pp. 136-146.

MANSOUR, H.A., TALKOWSKI, M.E., WOOD, J., CHOWDARI, K.V., MCCLAIN, L., PRASAD, K., MONTROSE, D., FAGIOLINI, A., FRIEDMAN, E.S., ALLEN, M.H., BOWDEN, C.L., CALABRESE, J., EL-MALLAKH, R.S., ESCAMILLA, M., FARAONE, S.V., FOSSEY, M.D., GYULAI, L., LOFTIS, J.M., HAUSER, P., KETTER, T.A., MARANGELL, L.B., MIKLOWITZ, D.J., NIERENBERG, A.A., PATEL, J., SACHS, G.S., SKLAR, P., SMOLLER, J.W., LAIRD, N., KESHAVAN, M., THASE, M.E., AXELSON, D., BIRMAHER, B., LEWIS, D., MONK, T., FRANK, E., KUPFER, D.J., DEVLIN, B. and NIMGAONKAR, V.L., 2009. Association study of 21 circadian genes with bipolar I disorder, schizoaffective disorder, and schizophrenia. *Bipolar disorders,* 11(7), pp. 701-710.

MANSOUR, H.A., WOOD, J., LOGUE, T., CHOWDARI, K.V., DAYAL, M., KUPFER, D.J., MONK, T.H., DEVLIN, B. and NIMGAONKAR, V.L., 2006. Association study of eight circadian genes with bipolar I disorder, schizoaffective disorder and schizophrenia. *Genes, brain, and behavior,* 5(2), pp. 150-157.

MAZZOCCOLI, G., LAUKKANEN, M.O., VINCIGUERRA, M., COLANGELO, T. and COLANTUONI, V., 2016. A Timeless Link Between Circadian Patterns and Disease. *Trends in molecular medicine,* 22(1), pp. 68-81.

MCCARTHY, M.J., WEI, H., MARNOY, Z., DARVISH, R.M., MCPHIE, D.L., COHEN, B.M. and WELSH, D.K., 2013. Genetic and clinical factors predict lithium's effects on PER2 gene expression rhythms in cells from bipolar disorder patients. *Translational psychiatry,* 3, pp. e318.

MCGRATH, C.L., GLATT, S.J., SKLAR, P., LE-NICULESCU, H., KUCZENSKI, R., DOYLE, A.E., BIEDERMAN, J., MICK, E., FARAONE, S.V., NICULESCU, A.B. and TSUANG, M.T., 2009. Evidence for genetic association of RORB with bipolar disorder. *BMC psychiatry,* 9, pp. 70-244X-9-70.

MERIKANGAS, K.R., AKISKAL, H.S., ANGST, J., GREENBERG, P.E., HIRSCHFELD, R.M., PETUKHOVA, M. and KESSLER, R.C., 2007. Lifetime and 12-month prevalence of bipolar spectrum disorder in the National Comorbidity Survey replication. *Archives of General Psychiatry,* 64(5), pp. 543-552.

MÖLLER, H., LAUX, G. and KAPFHAMMER, H., 2007. *Psychiatrie und Psychotherapie: Band 1: Allgemeine Psychiatrie Band 2: Spezielle Psychiatrie.* Springer-Verlag.

MOORE, R.Y., 1997. Circadian rhythms: basic neurobiology and clinical applications. *Annual Review of Medicine,* 48, pp. 253-266.

MUHLEISEN, T.W., LEBER, M., SCHULZE, T.G., STROHMAIER, J., DEGENHARDT, F., TREUTLEIN, J., MATTHEISEN, M., FORSTNER, A.J., SCHUMACHER, J., BREUER, R., MEIER, S., HERMS, S., HOFFMANN, P., LACOUR, A., WITT, S.H., REIF, A., MULLER-MYHSOK, B., LUCAE, S., MAIER, W., SCHWARZ, M., VEDDER, H., KAMMERER-CIERNIOCH, J., PFENNIG, A., BAUER, M., HAUTZINGER, M., MOEBUS, S., PRIEBE, L., CZERSKI, P.M., HAUSER, J., LISSOWSKA, J., SZESZENIA-DABROWSKA, N., BRENNAN, P., MCKAY, J.D., WRIGHT, A., MITCHELL, P.B., FULLERTON, J.M., SCHOFIELD, P.R., MONTGOMERY, G.W., MEDLAND, S.E., GORDON, S.D., MARTIN, N.G., KRASNOW, V., CHUCHALIN, A., BABADJANOVA, G., PANTELEJEVA, G., ABRAMOVA, L.I., TIGANOV, A.S., POLONIKOV, A., KHUSNUTDINOVA, E., ALDA, M., GROF, P., ROULEAU, G.A., TURECKI, G., LAPRISE, C., RIVAS, F., MAYORAL, F., KOGEVINAS, M., GRIGOROIU-SERBANESCU, M., PROPPING, P., BECKER, T., RIETSCHEL, M., NOTHEN, M.M. and CICHON, S., 2014. Genome-wide association study reveals two new risk loci for bipolar disorder. *Nature communications,* 5, pp. 3339.

MULLENDERS, J., FABIUS, A.W., MADIREDJO, M., BERNARDS, R. and BEIJERSBERGEN, R.L., 2009. A large scale shRNA barcode screen identifies the circadian clock component ARNTL as putative regulator of the p53 tumor suppressor pathway. *PloS one,* 4(3), pp. e4798.

NIEVERGELT, C.M., KRIPKE, D.F., BARRETT, T.B., BURG, E., REMICK, R.A., SADOVNICK, A.D., MCELROY, S.L., KECK, P.E.,JR, SCHORK, N.J. and KELSOE, J.R., 2006. Suggestive evidence for association of the circadian genes PERIOD3 and ARNTL with bipolar disorder. *American journal of medical genetics.Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics,* 141B(3), pp. 234-241.

NIEVERGELT, C.M., KRIPKE, D.F., REMICK, R.A., SADOVNICK, A.D., MCELROY, S.L., KECK, P.E.,JR and KELSOE, J.R., 2005. Examination of the clock gene Cryptochrome 1 in bipolar disorder: mutational analysis and absence of evidence for linkage or association. *Psychiatric genetics,* 15(1), pp. 45-52.

PATEL, J.P. and FREY, B.N., 2015. Disruption in the Blood-Brain Barrier: The Missing Link between Brain and Body Inflammation in Bipolar Disorder? *Neural plasticity,* 2015, pp. 708306.

PICHOT, P., 2004. Circular insanity, 150 years on. *Bulletin de l'Academie nationale de medecine,* 188(2), pp. 275-284.

PERRIS, C., 1990. The importance of Karl Leonhard's classification of endogenous psychoses. *Psychopathology,* 23(4-6), pp. 282-290.

REININGHAUS, E.Z., ZELZER, S., REININGHAUS, B., LACKNER, N., BIRNER, A., BENGESSER, S.A., FELLENDORF, F.T., KAPFHAMMER, H.P. and MANGGE, H., 2014. Oxidative stress in bipolar affective disorder. *Der Nervenarzt,* .

REPPERT, S.M. and WEAVER, D.R., 2002. Coordination of circadian timing in mammals. *Nature,* 418(6901), pp. 935-941.

ROYBAL, K., THEOBOLD, D., GRAHAM, A., DINIERI, J.A., RUSSO, S.J., KRISHNAN, V., CHAKRAVARTY, S., PEEVEY, J., OEHRLEIN, N., BIRNBAUM, S., VITATERNA, M.H., ORSULAK, P., TAKAHASHI, J.S., NESTLER, E.J., CARLEZON, W.A.,JR and MCCLUNG, C.A., 2007. Mania-like behavior induced by disruption of CLOCK. *Proceedings of the National Academy of Sciences of the United States of America,* 104(15), pp. 6406-6411.

RYBAKOWSKI, J.K., DMITRZAK-WEGLARZ, M., DEMBINSKA-KRAJEWSKA, D., HAUSER, J., AKISKAL, K.K. and AKISKAL, H.H., 2014. Polymorphism of circadian clock genes and temperamental dimensions of the TEMPS-A in bipolar disorder. *Journal of affective disorders,* 159, pp. 80-84.

RYBAKOWSKI, J.K. and TWARDOWSKA, K., 1999. The dexamethasone/corticotropin-releasing hormone test in depression in bipolar and unipolar affective illness. *Journal of psychiatric research,* 33(5), pp. 363-370.

SCHEER, F.A., WRIGHT, K.P.,JR, KRONAUER, R.E. and CZEISLER, C.A., 2007. Plasticity of the intrinsic period of the human circadian timing system. *PloS one,* 2(8), pp. e721.

SCHULZE, T.G., 2010. Genetic research into bipolar disorder: the need for a research framework that integrates sophisticated molecular biology and clinically informed phenotype characterization. *The Psychiatric clinics of North America,* 33(1), pp. 67-82.

SERRETTI, A., BENEDETTI, F., MANDELLI, L., LORENZI, C., PIROVANO, A., COLOMBO, C. and SMERALDI, E., 2003. Genetic dissection of psychopathological symptoms: insomnia in mood disorders and CLOCK gene polymorphism. *American journal of medical genetics.Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics,* 121B(1), pp. 35-38.

SHI, J., WITTKE-THOMPSON, J.K., BADNER, J.A., HATTORI, E., POTASH, J.B., WILLOUR, V.L., MCMAHON, F.J., GERSHON, E.S. and LIU, C., 2008. Clock genes may influence bipolar disorder susceptibility and dysfunctional circadian rhythm. *American journal of medical genetics.Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics,* 147B(7), pp. 1047-1055.

SHIMBA, S., OGAWA, T., HITOSUGI, S., ICHIHASHI, Y., NAKADAIRA, Y., KOBAYASHI, M., TEZUKA, M., KOSUGE, Y., ISHIGE, K., ITO, Y., KOMIYAMA, K., OKAMATSU-OGURA, Y., KIMURA, K. and SAITO, M., 2011. Deficient of a clock gene, brain and muscle Arnt-like protein-1 (BMAL1), induces dyslipidemia and ectopic fat formation. *PloS one,* 6(9), pp. e25231.

SIDOR, M.M., SPENCER, S.M., DZIRASA, K., PAREKH, P.K., TYE, K.M., WARDEN, M.R., AREY, R.N., ENWRIGHT, J.F., JACOBSEN, J.P., KUMAR, S., REMILLARD, E.M., CARON, M.G., DEISSEROTH, K. and MCCLUNG, C.A., 2015. Daytime spikes in dopaminergic activity drive rapid mood-cycling in mice. *Molecular psychiatry,* 20(11), pp. 1479-1480.

SJOHOLM, L.K., BACKLUND, L., CHETEH, E.H., EK, I.R., FRISEN, L., SCHALLING, M., OSBY, U., LAVEBRATT, C. and NIKAMO, P., 2010. CRY2 is associated with rapid cycling in bipolar disorder patients. *PloS one,* 5(9), pp. e12632.

SMOLLER, J.W. and FINN, C.T., 2003. Family, twin, and adoption studies of bipolar disorder. *American journal of medical genetics.Part C, Seminars in medical genetics,* 123C(1), pp. 48-58.

SORIA, V., MARTINEZ-AMOROS, E., ESCARAMIS, G., VALERO, J., PEREZ-EGEA, R., GARCIA, C., GUTIERREZ-ZOTES, A., PUIGDEMONT, D., BAYES, M., CRESPO, J.M., MARTORELL, L., VILELLA, E., LABAD, A., VALLEJO, J., PEREZ, V., MENCHON, J.M., ESTIVILL, X., GRATACOS, M. and URRETAVIZCAYA, M., 2010. Differential association of circadian genes with mood disorders: CRY1 and NPAS2 are associated with unipolar major depression and CLOCK and VIP with bipolar disorder. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology,* 35(6), pp. 1279-1289.

TAHARA, Y. and SHIBATA, S., 2013. Chronobiology and nutrition. *Neuroscience,* 253, pp. 78-88.

TEROGANOVA, N., GIRSHKIN, L., SUTER, C.M. and GREEN, M.J., 2016. DNA methylation in peripheral tissue of schizophrenia and bipolar disorder: a systematic review. *BMC genetics,* 17, pp. 27-016-0332-2.

WOON, P.Y., KAISAKI, P.J., BRAGANCA, J., BIHOREAU, M.T., LEVY, J.C., FARRALL, M. and GAUGUIER, D., 2007. Aryl hydrocarbon receptor nuclear translocator-like (BMAL1) is associated with susceptibility to hypertension and type 2 diabetes. *Proceedings of the National Academy of Sciences of the United States of America,* 104(36), pp. 14412-14417.

YATHAM, L.N., KENNEDY, S.H., PARIKH, S.V., SCHAFFER, A., BEAULIEU, S., ALDA, M., O'DONOVAN, C., MACQUEEN, G., MCINTYRE, R.S., SHARMA, V., RAVINDRAN, A., YOUNG, L.T., MILEV, R., BOND, D.J., FREY, B.N., GOLDSTEIN, B.I., LAFER, B., BIRMAHER, B., HA, K., NOLEN, W.A. and BERK, M., 2013. Canadian Network for Mood and Anxiety Treatments (CANMAT) and International Society for Bipolar Disorders (ISBD) collaborative update of CANMAT guidelines for the management of patients with bipolar disorder: update 2013. *Bipolar disorders,* 15(1), pp. 1-44.

YI, C., MU, L., DE LA LONGRAIS, I.A., SOCHIRCA, O., ARISIO, R., YU, H., HOFFMAN, A.E., ZHU, Y. and KATSARO, D., 2010. The circadian gene NPAS2 is a novel prognostic biomarker for breast cancer. *Breast cancer research and treatment,* 120(3), pp. 663-669.

YIN, L., WANG, J., KLEIN, P.S. and LAZAR, M.A., 2006. Nuclear receptor Rev-erbalpha is a critical lithium-sensitive component of the circadian clock. *Science (New York, N.Y.),* 311(5763), pp. 1002-1005.

YOUNG, L.T., WARSH, J.J., KISH, S.J., SHANNAK, K. and HORNYKEIWICZ, O., 1994. Reduced brain 5-HT and elevated NE turnover and metabolites in bipolar affective disorder. *Biological psychiatry,* 35(2), pp. 121-127.

YUAN, P., LI, J., ZHOU, F., HUANG, Q., ZHANG, J., GUO, X., LYU, Z., ZHANG, H. and XING, J., 2017. NPAS2 promotes cell survival of hepatocellular carcinoma by transactivating CDC25A. *Cell death & disease,* 8(3), pp. e2704.