Chapter 1

Clock Genes in Depression

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Additional information is available at the end of the chapter

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Abstract

Data demonstrate that abnormal regulation of the circadian system can result in cardiovascular disease, metabolic syndrome, obesity, immune dysfunction, increased risk for cancer, reproductive complications, etc. It is highly individual among depressed patients and may be expressed as a phase advance or phase delay of rhythms and/or increase or decrease in the amplitude. The stress-induced anhedonic-like state characterizes by hypothermia, hypercortisolemia, and hypermelatoninemia associated with disturbances in the circadian system. Mainly Per2 and Bmal1 demonstrate altered expression in the brain and liver: expression of Per2 is sensitive to stress and changes in Bmal1 mostly associated with depressive behavior. The Per1 expression is sustainable in maintaining the circadian rhythm. A normalization of the expression patterns is likely to be essential for the recovery from the pathological state. Depression is a high prevalent disorder. The number of incidents is rising due to changes in lifestyle. The symptomatology is inconsistent and it is difficult to agree on one hypothesis. The disturbances of the 24 h circadian rhythm may be a factor in the development of major depressive disorder. The molecular biology underlying a causal relationship between circadian rhythm and mood disorders is slowly being unraveled. However, many questions still need to be answered.

Keywords: depression, anhedonia, diurnal rhythms, clock genes, phase markers, chronic mild stress

1. Hypothesis of disturbed circadian rhythms in depression: evidence in support of a dysfunction of the endogenous clock machinery in depression

For more than 40 decades, several lines of evidence have linked depression to disturbances of the circadian system. Abnormalities in the sleep pattern, such as early awakening in the morning hours, are found in up to 80% of the depressed patients [1]. Treatment with antide-

pressants can restore the chronobiological changes [2]. Work shift or jetlag (manipulations of the circadian rhythm) increases the risk of developing a depression [3]. Individuals born with a shifted or arrhythmic biological clock have a higher risk of becoming depressed [4]. Circadian manipulations, such as bright light therapy and total sleep deprivation, are capable to reverse depressive symptoms within hours [2, 5]. The severity of the depressive symptoms follows a 24 h rhythm most dominant in the morning [6]. Blunted or abnormal circadian rhythm of temperature and hormone secretion is a prominent feature in depressed people. Also, depressed individuals elicit altered brain and locomotor activity [7]. Decreased hippocampal neurogenesis is found in depressed patients [8], and neurogenesis is under the control of the so-called clock genes (clock genes are making up the biological clock of the body) [9]. Clock gene polymorphism has been found to be associated with mood disorders [10]. Involvement of the circadian system in depression is emphasized by the seasonal affective disorder (SAD), a subtype of depression also called winter depression. SAD is defined as recurrent episodes of depression in the autumn and winter [11]. It is shown that SAD is more common in areas of the world receiving less sunlight [12]. The late chronotype/eveningness is associated with increased risk of developing a depression compared to the early chronotype/morningness [13]. Treatment with a third-generation antidepressant, agomelatine, is known to act through the recovery of the disturbed circadian rhythm [14].

Besides the involvement of the circadian system in depression, disturbances of the 24 h rhythm also possess a major risk to health in general [15]. Abnormal regulation of the circadian system can result in cardiovascular disease, metabolic syndrome, obesity, immune dysfunction, increased risk for cancer, and reproductive complications [16].

In the context of a disturbed circadian rhythm, it is also relevant to comment on the possible types of rhythmic abnormalities, which are highly individual among depressed patients. The circadian rhythm abnormalities may be expressed as a phase advance or a phase delay of rhythms and/or increase or decrease in the amplitude [17].

2. Introduction to circadian rhythms

So, what is a circadian rhythm exactly?

The word circadian is derived from Latin and means *about a day*. The most prominent circadian rhythm is the sleep/wake cycle, but most physiological and behavioral processes of the body follow a 24 h rhythm, such as activity, core body temperature, hormone levels, cognition, attention, and even mood [18].

One of the most essential time givers or zeitgebers (ZTs) is the light since it has the ability to entrain the organism to the 24 h circadian day [19]. Entraining information reaches the master clock of the body, the suprachiasmatic nucleus (SCN), via the retinohypothalamic tract [20] (**Figure 1A**). The SCN neurons project to multiple areas in the brain (for a review, see [21]). However, the paraventricular nucleus (PVN) of the hypothalamus and the pineal gland are the major SCN output [22].

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Before presenting the mechanism of the molecular clock, two core hormones, melatonin and cortisol (corticosterone in rodents), of the circadian system deserve attention.

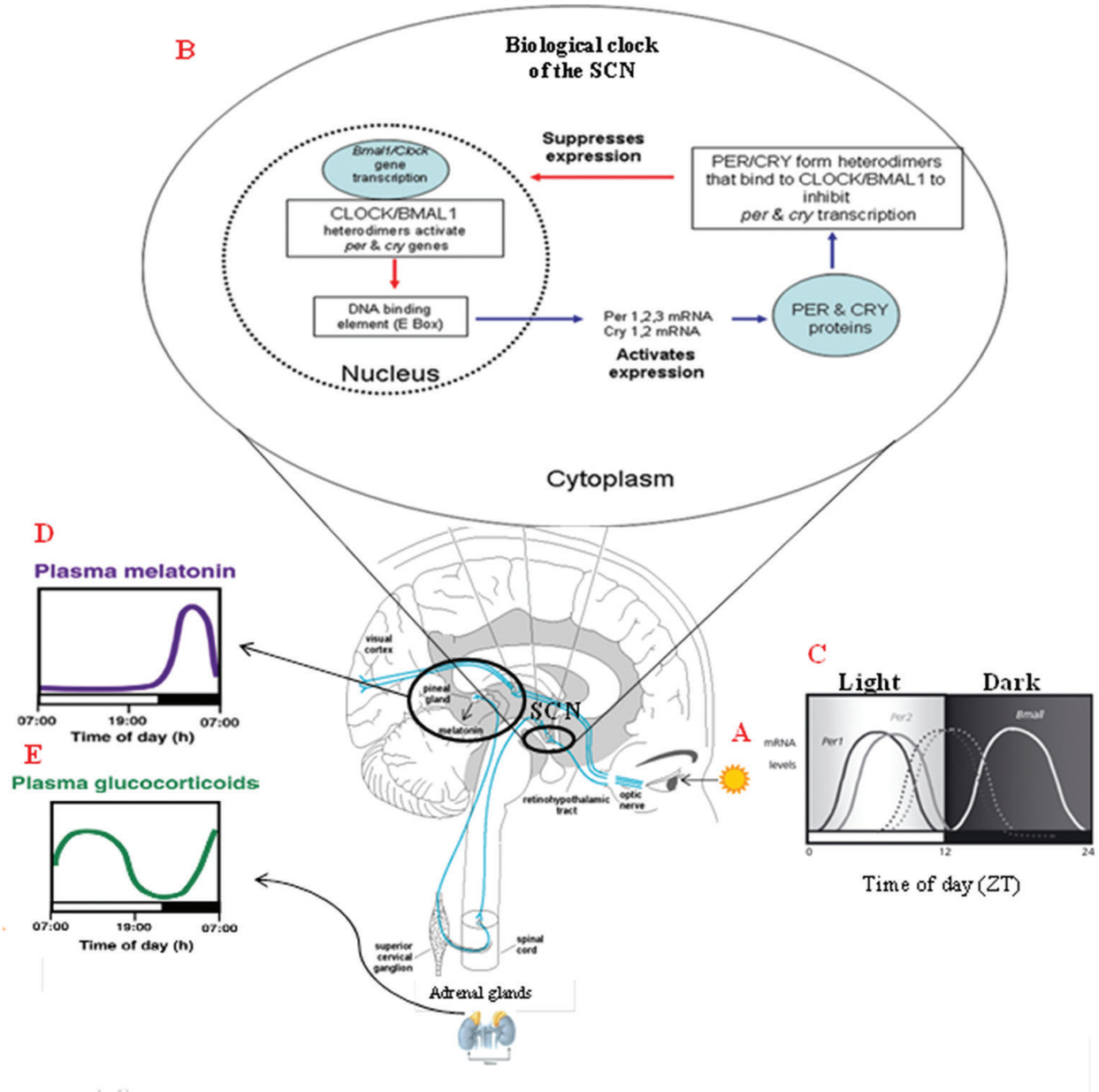

Figure 1. Essential components of the human circadian system. (A) Light and dark cues are the strongest zeitgebers of the circadian rhythm. The light sends photic information to the retina, which is the inner and light-sensitive layer of the eye. Through the optic nerve, the signal reaches the brain. A projection innervates the master clock, the SCN, via the retinohypothalamic tract that is anatomically and functionally different from the other neural pathways that reach the visual cortex. (B) Positive and negative feedback loop of the clock genes organizes the biological clock of the SCN. (C) Through rhythmic expression of the clock genes (light-sensitive genes have been selected as an example), the circadian rhythm is resynchronized every day. (D) From the SCN, a multisynaptic pathway leads to the pineal gland, where melatonin is secreted in the rhythmic manner as a signal of darkness. Entraining cues also affect the adrenal gland, which secretes corticosterone. (E) The maximum of corticosterone secretion occurs in the morning and associated with awakening. Modified from Refs. [120–122].

2.1. The role of melatonin in the development of depression with focus on the circadian rhythm

Melatonin is a hormone under direct control of the SCN and is one of the most important players in resetting the circadian rhythm every day [23]. It is primarily secreted from the pineal gland and mainly synthesized at night in all species [24] (**Figure 1D**). Due to minor sensitivity to the environment, melatonin is a stable marker of the circadian phase [25]. In humans, the circadian phase is determined by measuring the onset of melatonin secretion by dim light in the evening, the so-called dim light melatonin onset (DLMO). Thus, from 18:00 until prior to bedtime, the concentration of plasma melatonin is measured every 30 min [26]. The DLMO was first used in the 1980s [27], and today it is acknowledged as one of the best markers of the phase [28].

Since the 1980s, melatonin has been linked to depression, and low melatonin levels have been observed in depressed patients [29, 30]. Since serotonin is the precursor of melatonin, the low levels of melatonin can partly be explained by the low serotonin level found in some depressed individuals [31]. In contrast, other studies have reported elevated levels of melatonin during depression [32, 33]. Finally, a phase shift in the secretion of melatonin has been linked to depression [34].

2.2. The role of cortisol in depression with focus on the circadian rhythm

Cortisol is an important element for maintaining the daily circadian rhythm. The secretion of cortisol is associated with awakening and increases shortly after awakening: the cortisol awakening response (CAR) [35] (**Figure 1E**). A rise in the early morning level of cortisol is stated to be a reliable marker of the adrenocortical activity if measured repeatedly at the time of awakening. The lowest concentration is found in the beginning of the evening [36]. Compared to melatonin cortisol is a less robust phase marker since its secretion is affected by environmental factors, most importantly stress.

An abnormal circadian rhythm of cortisol is well described in a subgroup of depressed patients. Also, a blunted circadian rhythm [37] and an elevated level of cortisol are specific features of depressed individuals [38].

3. The mechanism of the molecular clock

The internal biological clock or the master clock is believed to hierarchically control all circadian rhythms in the body. It is located deep inside the brain in the anterior part of the hypothalamus and named by its location, the SCN [39]. The SCN is built up from the positive and negative feedback loops of so-called clock genes. Some of the most essential clock genes are the period genes (*Per*) 1–3, bone and muscle ARNT-like 1 (*Bmal1*), circadian locomotor output cycle caput (*Clock*), and the cryptochrome (*Cry*) 1–2. However, the clock genes are not exclusive components of the clockwork. Other well-known signaling proteins and cytosolic factors have been revealed as important players of the circadian machinery. Briefly, the

main regulatory unit of the clock genes, a heterodimeric complex of CLOCK and BMAL1, is formed in the cytoplasm and translocated to the cell nucleus. In the nucleus, the dimer binds to an e-box motif and drives the transcription of the *Per* and *Cry* genes. These genes are translated into the corresponding protein products, and like the BMAL1 and CLOCK, they dimerize, enter the nucleus, and interfere with the BMAL1/CLOCK complex, thus inhibiting its transcription. For a review, see [40] (**Figure 1B**). This so-called cycle of gene activation and inhibition is self-sustained and takes about 24 h [41]. The expression of *Per1* and *Per2* genes is the light-sensitive elements of the cycle. Hence expression starts by light activation and reaches peak level at noon. The protein product reaches peak level approximately 6 h later (**Figure 1C**).

It is a well-known fact that the clock genes are not only found in the SCN machinery, but in most central regions [42] and peripheral tissues, including the heart and liver [43–46]. A functional molecular clock is even observed in cell cultures [47]. Food is the strongest cue able to entrain peripheral clocks without affecting the SCN rhythm [48], but social activity and locomotor activity are also known to synchronize the phase [49].

4. The clock genes in major depression

Implications of the circadian system in depression have gained much attention in recent years. However, the biology underlying the association or causal relationship between circadian rhythm and mood disorders is still mostly unknown, and no clock genes specific for the disease have been convincingly identified yet.

In particular, in the late 1990s, the clock genes gained increased awareness due to important breakthroughs in the understanding of the molecular clock [18]. The following quote is from Science (December, 1998):

"Nineteenth-century philosophers proposed that God was a clockmaker who created the world and let it run. Modern biologists might in part agree, for it's clear that evolution has carefully crafted clocks that allow almost all organisms to follow the rhythm of the sun. In 1998, a volley of rapid-fire discoveries revealed the stunning universality of the clock workings. Across the tree of life, from bacteria to humans, clocks use oscillating levels of proteins in feedback loops to keep time. Perhaps more amazing, fruit flies and mice—separated by nearly 700 million years of evolution—share the very same timekeeping proteins. Now that they better understand the cellular clock, scientists can begin to manipulate it, with applications from curing jet lag to brightening winter depression."[1]

Two studies, published in 2012 and 2013, demonstrate the implication of dysfunction of clock genes in human depression [50, 51]. The later work is most convincing. Li and coworkers used transcriptome-wide analysis on high-quality postmortem brain tissue and showed that several hundred transcripts in six selected structures of the human brain had 24 h rhythmic-

[1]http://science.sciencemag.org/content/282/5397/news-summaries

ity. Most interestingly, they measured a much weaker 24 h rhythm in the brains of depressed patients and postulated that it could be a consequence of a shift in peak and a dislocated phase relationships between different clock genes. Sequeira and coworkers report a reduced *Per1* expression at one time point in postmortem brain tissue from depressed suicide individuals compared to non-suicide depressed individuals, indicating for the first time the association between the clock genes and depression [50].

Few other studies also report abnormal clock gene expression in the human brain, but not in relation to depression [52, 53].

In general, postmortem studies are challenged by difficulties related to the differences in the precise time of death, which is of great importance in the studies of the clock genes. Furthermore, the length of the postmortem interval is a potential confound in all studies [54].

The involvement of the clock genes in depression is also evident from several genetic studies. Polymorphisms of clock genes have been reported in depressed patients [55–59]. Despite the number of studies investigating the polymorphism in clock genes, the validity of the studies might be discussed due to small sample size and low reproducibility [60].

5. The clock genes in animal models of depression

Most studies on clock genes have been conducted in animal models of depression, and manipulations of the clock genes in these models have been reported to induce depression-like behavior. Strong evidence for a likely role of the clock genes in depression is found in a recent study showing that SCN-specific *Bmal1* knockdown mice exhibit depressive-like behavior [61]. However, it should be noted that SCN-specific *Bmal1* knockdown mice do not have a reduced intake in the sucrose consumption test indicative for hedonic status.

A disruption of the clock genes has a considerable effect on memory and thinking. Bearing in mind that depressed patients often suffer from cognitive deficits, Snider and colleagues demonstrated that selectively deleted *Bmal1* from excitatory forebrain neurons results in deficits in cognitive tests [62].

A differential expression of clock genes in the amygdala in the dark phase of a standard 12:12 light/dark cycle (LD) was measured in *Cry2*$^{-/-}$ mice compared to wild-type animals. Most importantly, *Cry2*$^{-/-}$ mice also exhibit anhedonic-like behavior in the sucrose preference test. In mice, a mutation in the *Per2* clock gene increased the depression- and anxiety-like behavior showed by using despair-based tests [63]. Furthermore, knockdown of the *Clock* in CA1 caused depressive-like behavior [64].

As aforementioned, the SCN is not exclusive timekeeper of the body, but rather coordinator of activity between a wide range of brain regions and peripheral sub-oscillators. Thus, the fact that depression-like behavior can be induced by manipulations with core clock genes outside the SCN raises the question about the top position of the SCN and interaction between the SCN and sub-oscillators [62].

Another approach used to investigate a role of the clock genes in the development of depression is an examination of the consequences of stress exposure on the expression of the clock genes [65–70]. All studies reported that stress significantly alters the expression pattern of the clock genes independently on applied stressors, animals strain, and time of the termination of the experiment. For instance, altered expression of the *Per2* gene in the SCN and nucleus accumbens is observed in mice exposed to chronic unpredictable mild stress [71]. In the chronic mild stress (CMS) model, *Bmal1* and *Clock* showed significantly reduced expression in the prefrontal cortex in the anhedonic-like rats [72]. Likewise, the disturbance in the circulation of corticosterone caused altered rhythm of the *Per2* gene expression in the rat brain [73, 74].

Chronological study on clock genes in rat CMS model of depression [75] demonstrated robust expression of *Per1* gene in all analyzed brain areas. The anhedonic-like behavior was associated with delayed peak in *Bmal1* expression in the SCN and completely abolished rhythmicity of the *Bmal1* expression in the nucleus accumbens. Furthermore, the expression of *Per2* was affected by CMS in all three regions of the hippocampus (DG, CA1, and CA3). In the lever, the anhedonic-like effect of CMS was pronounced in the decrease of *Per2* expression and increase in the expression of *Bmal1*. However, the rhythmicity in the expression of three clock genes was not affected by stress.

6. Stress and depression go hand in hand

6.1. When stress is assumed as a key factor in the etiology of depression

Charles B. Nemeroff said in 1996: "One way to conceptualize depression is a pathological stress response gone awry." In our days, stress is defined as any situation able to disturb physiological or psychological homeostasis [76].

However, the word stress is often incorrectly used to describe the matters of hassles in daily life. Correctly used, stress describes life experiences resulting in a specific behavior involving a serious threat to health; burnout, including anxiety and depressed mood; disturbance of sleep; difficulties handling obstacles of daily life; and abuse of stimulants and/or medicine [77]. It is important to distinguish between acute and chronic stress and between controlled and uncontrolled stress. Chronic and uncontrolled stress highly increases the risk of developing a depression.

The first response of the body to either acute or chronic stress is activation of the HPA axis [78]. A prominent feature of the HPA axis is the negative feedback mechanism upon multiple targets including the hypothalamus, the anterior pituitary, and the limbic system [79]. A substantial subgroup of depressed individuals show an increased cortisol level [80]. It has been hypothesized that dysfunction of the glucocorticoid receptors could explain the elevated cortisol level.

Glucocorticoids, the end result of stress activation of the HPA axis, are well known to affect metabolism in the liver and entrainment of the circadian rhythm in peripheral organs,

including the liver, kidneys, and heart [43]. It is broadly accepted that stress activates the HPA axis and that depression is likely to be induced by stress. However, a big conundrum in the modern stress research is why some people are able to cope with a certain intensity of stress exposure and others are not.

6.2. How to successfully cope with stress

How to handle exposure to stress? The keyword is adaptation [76], and the key player is the brain determining whether a situation is threatening to the body [81]. Or as Hans Selye ("the father" of the term stress) opined: "It's not stress that kills us, it is our reaction to it."

The ability to successfully adapt to stress very much depends on early life experience. Abuse and neglect in childhood is the most prominent risk factor for ineffective stress coping [82]. A comprehensive study was done to investigate the stress-coping abilities of littermates according to the postnatal maternal care. While analyzing maternal care, the score system was used, and a score was defined by maternal behavior, where five types of maternal behaviors were distinguished: licking and/or grooming, arched-back nursing (dam shows an obvious arch in her back while nursing), blanket nursing (dam engages in nursing postures with no obvious arch in her back), passive nursing (dam is lying on her side or back while nursing her pups), and no maternal contact. Each dam received a score for a combination of leaking/grooming behavior and either one of the three nursing postures or just the nursing position alone with no leaking/grooming. The sum of 7 days of leaking/grooming scores was used as the parameter for dividing pups into groups. Dams were divided in group of low leaking/grooming mothers and in group of high leaking/grooming mothers. When pups reached age of 6 weeks, they were exposed to standard CMS procedure including initial adaptation to consume the palatable sucrose solution. It was shown that even in stress-free control conditions, offspring from damps with low maternal care activity demonstrated increased level of anxiety and rats from damps with low maternal care activity demonstrated increase in fecal concentration of corticosterone metabolites after initiation of CMS procedure. Also the susceptibility to stress was higher in animals exposed to low level of postnatal maternal care [83].

In terms of circadian rhythm and successful adaptation to the seasonal variations (mostly the related variation in daylight hours), we may assume that the coping mechanism becomes more challenged at the northern latitude of the northern hemisphere. As aforementioned, certain subtypes of depression are more pronounced at the northern latitudes, which could be the result of the challenges in the clock genes' adaptation to the seasonal variations. The sensitivity of the circadian system is also affected by daylight saving time (DST). DST is extracting one hour in spring and returns it in autumn. The major propose of this change is providing more efficient industrial usage of the sunlight. Depending on age, gender, and chronotype, the adaptation to the change in time takes from 2 to 14 days [84]. It is tempting to speculate that inaccurate correction of DST might in some rare cases result in development of depression. A study conducted in the diurnal Siberian hamster showed that shortening the length of the day induced depressive-like behavior [85].

The etiology of depression is still largely unknown although the disease has been known for centuries [86]. In recent years, evidence points to involvement of the circadian system in major depression [54].

Investigating the circadian system is of major importance in order to find new molecular targets, hence aiming for new and better treatment strategies. This does not necessarily imply novel drugs, but it could be an intervention targeting the circadian system by manipulating environmental conditions.

7. Altered 24 h rhythm in phase markers is associated with anhedonic-like behavior

Three classical phase markers (body core temperature, blood levels of melatonin, and corticosterone) exhibit a 24 h diurnal rhythm in both anhedonic-like and control rats with altered levels at specific time points of the day in the anhedonic-like rats: corticosterone levels showed an additional peak during the light (resting in nocturnal animals) phase, whereas melatonin levels were elevated during the last period of the dark phase. The core body temperature was significantly decreased during the last period of the dark phase [87].

It is reasonable to believe that the circadian machinery is involved in the depressive-like state in the CMS model, since the anhedonic-like behavior correlates well with disturbances of the circadian system, which are also observed in the clinical depression.

The most common disturbance of the circadian rhythm observed in depressed individuals is altered sleep architecture [88]. Some patients also experience a dysfunction of the HPA axis [89–91], altered 24 h rhythm of body temperature and melatonin [92], and reduced psychomotor activity [93]. These disturbances have also been reported in the CMS model of depression: sleep disturbances [94], dysfunction of the HPA axis [95], altered 24 h rhythm of core body temperature, and reduced circadian rhythm of locomotor activity [96].

However, measurements of the 24 h rhythm of phase markers are more indicative of circadian rhythm disturbances. It is important to measure the phase markers simultaneously due to their interplay and role in stress response, especially corticosterone. Furthermore, inconsistencies among the findings complicate the modeling of the chronopathology in depression [97].

The corticosterone level in animals exposed to chronic mild stress (CMS) protocol is associated with development of anhedonic-like behavior [66, 95, 98]. The additional peak in corticosterone level during the light phase has also been reported in a clinical study performed on patients with depression [99]. Landgraf and coworkers demonstrated that SCN-*Bmal1* knockdown mice after 25 h in total darkness exhibit depression-like behavior in several behavioral tests. They also have a second peak in corticosterone secretion compared to the control mice [61].

These data could provide clues to focus on another important time point for measuring the level of corticosterone/cortisol. The daily occurrence of a physiological increase in the cortisol

level, associated with awakening (CAR), is normally the target point for measuring the plasma concentrations of cortisol in depressed individuals [100]. Taking into consideration the results obtained on animals, the evaluation of cortisol level at the time point, when its level is not expected to be high, might be relevant for the ongoing diagnosis of depression.

The 24 h secretion pattern of melatonin in relation to depression is mostly studied in humans, where there is a report on an elevated melatonin level in depressed individuals [31], a report on the delay in the nocturnal melatonin peak secretion in depressed patients [33], and one report on recovery of the phase shift in patient treated with melatonin [101].

Zurawek and coworkers did not find differences in levels of melatonin measured during the light phase of the light/dark cycle between resilient and anhedonic-like animals compare to the controls after 2 and 7 weeks of CMS [102]. According to the result of Christiansen et al. [87], the level of melatonin is only affected by CMS during the dark phase.

Melatonin, corticosterone/cortisol, and core body temperature are all important factors for regulating the sleep pattern. Therefore, the altered 24 h pattern in anhedonic-like rats could explain the disturbances of the sleep pattern previously demonstrated in CMS rats [94].

8. Altered expression pattern of the core clock genes might partially explain changes in the 24 h pattern of phase markers

In line with disturbances of the circadian rhythm in clinical depression, disturbances of the circadian rhythm have also been observed in animal models of depression, but only in recent years, the clock genes have been linked to the disturbances. In study of Christiansen et al. [87], expression patterns of the clock genes were significantly altered in three out of the nine brain areas investigated in the anhedonic-like rats: the hippocampus, the lateral habenula, and the nucleus accumbens. In addition, changes in clock gene expression were also observed in the liver of CMS-susceptible rats.

The diurnal pattern of *Per1* expression was significantly altered in CA1 of the hippocampus, whereas the diurnal pattern of *Per2* expression was significantly altered in all subregions of the hippocampus, in the lateral habenula, and in the liver. *Bmal1* expression was altered in the nucleus accumbens and liver [87].

At first glance, the effect of the stress paradigm on the 24 h expression pattern of the clock genes might be evaluated as minor. However, minor alterations may have a major impact. Jiang and coworkers demonstrated that specific knockdown of the clock gene called *clock* in the CA1 region of the hippocampus led to development of the depressive-like behavior. The presence of depressive-like phenotype was demonstrated by using the sucrose consumption test and the forced swim test [64]. Knockdown of *Bmal1* in the SCN also induced depressive-like behavior, such as despair and helplessness [61]. Interestingly, these SCN-specific *Bmal1*-knockdown mice did not exhibit anhedonic-like behavior tested by sucrose consumption test. Knockdown of the gene in sub-oscillators might have given rise to a different result, indicating that SCN may not be directly involved in stress-induced mood disorders, presumably due

to lack of the glucocorticoid receptors in the SCN [43, 103]. This might be a reason why it is widely accepted that this area of the brain is naturally protected from stress.

Remarkably, the areas of the rat brain that demonstrate most changes in the CMS paradigm are the structures, which are known to be affected in major depression.

The hippocampus is one of the most studied brain structures in depression; since the hippocampal formation is involved in learning and memory, structural and functional deficits in this area are most often accompanying clinical depression [104]. In study of Christiansen et al. [87], *Per2* was found to be most affected clock gene in the hippocampal formation. This is intriguing since Borgs and coworkers found a link between *Per2* expression and neurogenesis [105]. Using the CMS model, it has been demonstrated that anhedonic-like rats have a decreased neurogenesis [106]. The involvement of hippocampal *Per2* in the development of the depression-like state was emphasized by the results from the resilient rats showing no effect of CMS on *Per2* expression in any subregions of the hippocampus [107].

Anhedonia is a core symptom of depression, and the nucleus accumbens is a key structure in the reward circuit of the brain [108]. The observed changes in *Bmal1* expression in the nucleus accumbens could therefore be implicated in the development of the anhedonic-like behavior in the CMS model which was also underlined by the results of clock gene expression in the resilient rats where no differences were demonstrated.

The lateral habenula has been suggested to be an important structure involved in the development of the depressive phenotype [109, 110] and as a brain structure, which must be taken into consideration when studying the circadian rhythm [111]. In the CMS-exposed rats, the expression level of *Per2* in this region was altered only in anhedonic-like rats, but not in stress resilient [107]. This is indicative for the inducible character of *Per2* expression in lateral habenula and its role in the development of the depression-like phenotype.

In human postmortem brain tissue, *Bmal1* has been ranked as the gene showing the most robust circadian rhythm in control individuals. *Per2* and *Per1* were ranked on a second and a ninth place, respectively [51]. In the Li study, six regions of the human brain were investigated for diurnal expression of genes, and they reported a lack of rhythmicity in *Per1*, *Per2*, and *Bmal1* (among several other genes) in depressed individuals, indicating a disturbed circadian rhythm [51]. Although the pattern of alteration differs between studies, a link between altered clock gene expression and depression is clearly illustrated.

Thus, the inducible control for the expression of *Per2* in the hippocampus and lateral habenula as well as *Bmal1* in the nucleus accumbens and liver might be proposed to be specific for the depression-like state.

Takahashi and coworkers [112] showed that expression of *Per1* gene in the liver was highly affected by CMS after 1 week of stress exposure as demonstrated by a shifted phase in the diurnal expression, while Christiansen et al. [75] demonstrated that *Per1* was not affected by the CMS after 3.5 weeks. Together these observations indicate a highly adaptive nature of *Per1* expression, hinting that the *Per1* expression, sensitive to CMS paradigm in the beginning of the stress exposure, adapts faster than the other tested genes. Indeed, altered expression

pattern of *Per2* clock gene was associated with both anhedonic-like and stress-resilient phenotypes, while changes in expression of *Bmal1* were associated with anhedonic-like phenotype only, indicating the prominent role of *Bmal1* in the development of the depressive reaction.

9. Stress resilience might be explained by the absence in disturbances in core phase markers and stress-resilient profile in the expression of clock genes

Some individuals find it challenging to live up to the conflicting roles that exist in the modern society lifestyle of today, such as performing well at home, at work, and socially. Presumably as a consequence, the number of individuals feeling burnout and depressed is increasing. However, most individuals can cope to even severe stress without getting symptoms of depression. It was shown using the CMS model of depression that part of animals exposed to chronic stress will not develop anhedonic-like behavior [113–117]. These stress-resilient animals identified by the absence of decrease in consumption of palatable sucrose solution [118] do not exhibit either loss in weight gain or cognitive deficit [119]. Neither corticosterone nor melatonin concentrations in the blood were increased as an effect of chronic (3.5 weeks) exposure to mild stressors in the stress-resilient animals, but expression of *Per2* clock gene was lower in three areas (CA1, CA3, and dentate gyrus) of the hippocampus, of the lateral habenula, and in the liver in resilient animals than unstressed controls at the late onset of the dark phase of light/dark circle [107]. Anhedonic-like animals demonstrated increased expression of *Per2* in all aforementioned brain areas, but expression level of *Per2* in the liver was also decreased [75]. It was shown that regulation of *Bmal1* expression is involved in the development of depression-like phenotype [75], but in stress-resilient rats, its expression was affected by CMS only in the nucleus accumbens [107]. Altogether, by the analysis of CMS-induced effects between stress-resilient and stress-susceptible individuals, it is possible to differentiate between general effects of stress per se and effects precipitating an anhedonic-like reaction measured on molecular, cellular, and behavioral levels. Most likely the coping mechanisms associated with stress resilience based on fast and adequate response to increased corticosterone upon the stress in turn prevent disturbances in clock gene machinery, associated with development of the depressive behavior.

10. Conclusions

Thus, the depression-like phenotype is associated with changed in 24 h rhythm of key phase markers: corticosterone, melatonin, and core body temperature. Expression of the clock genes in the master clock, the SCN, is not sensitive to stress and does not associate with the development of the depressive-like phenotype. The analysis of clock gene expression in specific brain regions and in the liver allows distinguishing between stress-resilience and stress-induced depression. The *Per1* demonstrated constitutive expression profile vigorously protected from stress effect both centrally and periphery. The analysis of *Per2* expression might be used to

identify the overall effect of stress on clock gene machinery while changes in expression of *Bmal1* associated with depression-like behavior. Thereby, manipulations with circadian system might be considered as an important factor to compensate effects of chronic stress and in treatment of stress-induced pathology.

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