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Circadian desynchronization as a cause of depression:

The involvement and mechanisms of melatonin

Bachelor Thesis
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Abstract

Depression is a big problem worldwide and the pathophysiology and causes of the disease are not understood yet. It has been proposed that desynchornization of the circadian clock might lead to depression. This is supported by the existing circadian related symptoms and the effectiveness of light therapy and sleep deprivation. As an possible internal synchronizer, melatonin's actions in depression have been widely studied. It was found that melatonin rhythms, both phase and amplitude, are altered in depressed patients. However, found results are very contradicting and it still remains unclear how the rhythm is exactly affected in depression. Exogenous melatonin has an antidepressant-like effect in different animal models of depression. Antidepressant-like effects are probably evoked by direct inhibition or long term desensitization of the melatonin receptors. Hardly any human studies on the antidepressant effects of melatonin have been done, but agomelatine (MT1/MT2 agonist, 5-HT_{2c} antagonist) was proven to be an effective antidepressant. The mechanisms by which melatonin mediates its effects are unclear. Its actions seem to be partly mediated via its own receptors, where MT1 is implicated to mediate antidepressant effects, while MT2 mediates depressant effects. But also involvement of the serotonergic, dopaminergic, GABAergic and glutamatergic systems and their receptors are found. Besides, melatonin can enhance BDNF levels and have suppressive effects on the HPA-axis. To gain better understanding, further research should be done on the effects of desynchornization in depression and the interactions between the different systems involved in melatonin's mechanisms. This could lead to new and more effective therapies for depression.

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Introduction

Depression is a big worldwide problem, more than 350 million people suffer from the disease and it is globally the leading cause of disability (WHO, 2012). The pathophysiology of the disease is very complex, with many different systems involved, and not completely understood yet. However, there are several hypotheses for the mechanisms behind the disease. The monoamine theory of depression is a widely accepted hypothesis, which states that depression is caused by too little activity of the monoaminergic nerve cells, mainly the noradrenergic and serotonergic neurons (Reviewed by: Elhwuegi, 2004). This theory is also supported by the fact that commonly used antidepressants like monoamideoxidase (MAO) inhibitors and selective serotonin reuptake inhibitors (SSRIs) increase the availability of monoamines at the synapse. However, the therapeutic effect of these drugs arise only after several weeks, which suggests that the mechanism is more complex (Reviewed by: Nestler, 2002). Another hypothesis states that depression is caused by a hyperactive hypothalamic-pituitary-adrenal axis (HPA-axis). Long-lasting hyperactivity of the HPA-axis is often seen in depressed patients and environmental and genetic risk factors for depression seem to correlate with increased HPA-axis activity in a lot of cases. High levels of glucocorticosteroids also lead to hippocampal neurodegeneration, which is a state often seen in and associated with depression (Reviewed by: Swaab, 2005). Based on this hippocampal neurodegeneration a third hypothesis was formed, stating that depression is caused by a lack of neurotrophic factors in the brain (Reviewed by: Nestler, 2002). The GABAergic hypothesis of depression links to the neurodegeneration in the hippocampus as well. A genetically induced partial GABA deficit in mice reduced hippocampal neurogenesis and cell proliferation. Besides, it also caused other behavioral, cognitive, cellular and neuroendocrine changes specific for depression and it led to reaction to antidepressant drugs (Reviewed by: Möhler, 2012). A more recent hypothesis for the mechanism of depression focuses on the biological clock and says that depression might be due to a desynchronization of circadian rhythms, in which internal rhythms are out of phase with each other and the external environment (Reviewed by: Srinivasan, 2012). The idea of possible involvement of the biological clock was based on the observation that some symptoms of depression are associated with the circadian system, like problems with the sleep/wake cycle and changes in body temperature rhythms. Besides many depressed patients experience diurnal and annual variations in mood (Reviewed by: Edgar, 2013). In several, both genetic and environmental, animals models of depression circadian rhythm disturbances are found (reviewed in: Lanfumey, 2013). The hypothesis is further supported by the effectiveness of sleep deprivation and light therapy, which are both proven to be able to reduce depressive symptoms, possibly by resetting the biological clock (Reviewed by: Golden, 2005; Reviewed by: Bunney, 2013). On the other hand, there are also studies that do not support this hypothesis for depression. In a forced desynchrony experiment in 2003, no differences were found between patients with seasonal affective disorder and healthy controls regarding the influences of both the circadian pace maker and the sleep-wake cycle on mood (Koorengevel, 2003). A study done in non-seasonal depressed patients found no abnormalities in the circadian phase of the biological clock (Gordijn, 1998). In 2013 a big genetic project was performed studying cyclic genes in patients with major depressive disorder (MDD) and healthy controls. Post mortem brain tissues were analyzed for six brain regions involved in depression. The top 16 cyclic genes found in the healthy controls were tested in the MDD patients. In the patients, only 11 of the 16 genes were

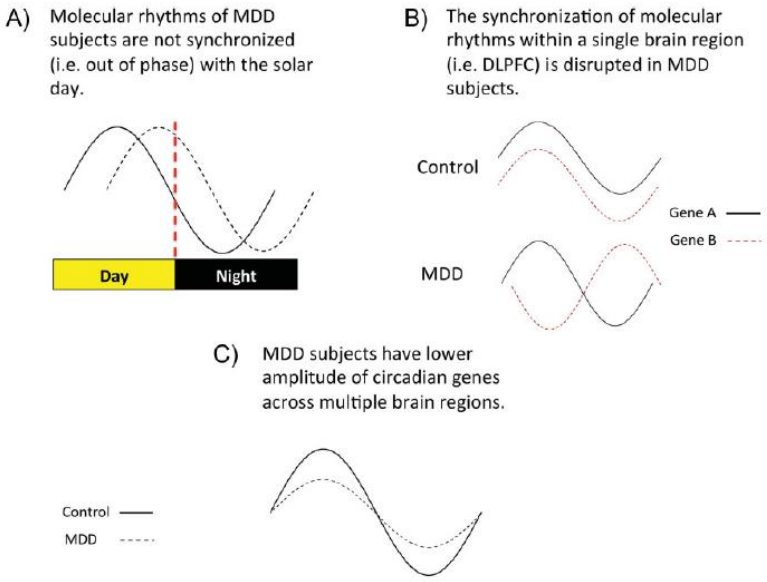


Figure 1. Desynchronization of molecular rhythms in depressed patients. Li *et al.* showed that expression rhythms of certain cyclic genes are out of phase with the external cycle (A), out of phase with each other (B) and have a lower amplitude (C) in depressed patients. (Li, 2013)

rhythmic in at least one of the six brain regions. Just two of those 11 genes showed rhythmicity in more than one of the six brain regions. Besides, the rhythms of those 11 rhythmic genes were out of phase with both the external cycle and each other and amplitude was lowered in patients with MMD (figure 1). This further supports involvement of desynchronized rhythms in depression (Li, 2013). However, if the loss of rhythmicity is a cause or a result of depression cannot be concluded yet, and the reliability is low without any studies yet performed to confirm these results.

Already in 1974 it was recognized that the retinohypothalamic projection, which projects from the retina to the suprachiasmatic nucleus (SCN), the primary circadian rhythm regulator in mammals, is important for synchronization of the internal rhythms to the external cycle. This projection results in a daily variation of the activity of the pineal enzyme N-acetyltransferase, regulated via the SCN (Moore, 1974). N-acetyltransferase is the rate limiting enzyme of the melatonin production, therefore its high activity at night results in a nocturnal melatonin peak (Klein, 1997). Melatonin can then synchronize and entrain other body rhythms, among others sleep, core body temperature and cortisol cycles (reviewed in: Pandi-Perumal, 2006). Although it needs to be mentioned that these effects are mainly proven by exogenous melatonin administration and not so much with regard to the endogenous melatonin rhythms.

Given the presumable association between desynchronized rhythms and depression, the question arises whether melatonin, as internal synchronizer, could play a role in the pathophysiology of depression as well. This review will address the changes in melatonin rhythms in depression, the effects of exogenous melatonin on depression and possible mechanisms for melatonin to act upon depression.

Endogenous melatonin rhythms in depression

Melatonin levels are found to be altered in patients suffering from MDD. Already in 1979 Mendlewicz *et al.* found no rise in melatonin at night in three of their four depressive subjects. Later, other studies also found lowered melatonin levels in persons with depression and bipolar disorder (Beck-Friis, 1984; Nair, 1984; Kennedy, 1996; reviewed in: Wetterberg, 1983). Claustrat *et al.* found

decreased nocturnal melatonin levels in depressed patients (10 women, 1 men) compared to healthy young men and ovulating women. The control group in this study does not seem to match the patient group. However, they justified their results because they found no influences of age and menstrual cycle. (Claustrat, 1984). In 1985 melatonin levels were found to be lower, but only in depressed patients with an abnormal response to the dexamethasone suppression test (DST) (Beck-Friis, 1985). In the DST reaction to dexamethasone, a glucocorticoid receptor agonist that provides negative feedback on the HPA-axis via the pituitary, on cortisol production is tested to examine HPA-axis function. Between 1980 and 1990 non-suppression of cortisol in the DST was used to diagnose depression (reviewed in: Srinivasan, 2012). Brown *et al.* found lower melatonin levels in melancholic depressed patients, compared to non-melancholic depressed patients and healthy controls. There were significantly more non-suppressors of cortisol in the DST among the melancholic depressed patients then there were among the non-melancholic patients, which is in line with the findings of Beck-Friis *et al.* (Brown, 1985).

On the other hand, also contradicting results are found. Crasson *et al.* found no significant difference between the melatonin levels of depressed patients and healthy controls (Crasson, 2004). Thompson *et al.* also reported no significant difference between depressed patients and healthy controls, but a trend to higher melatonin values in the depressed patients (Thompson, 1988). Rubin *et al.* found no significant difference over all subjects, but a trend to higher nocturnal melatonin levels in depressed premenopausal females compared to matched controls was found. (Rubin, 1992). There is a lot of variation in the results of studies done on this subject. This variation might be explained by a study done in children and young adolescents. They found children with non-psychotic depression to have higher levels of melatonin than healthy controls, while psychotic depressed children had lower melatonin secretions than healthy controls (Shafii, 1996). Almost none of the other studies mentions if patients were psychotic or not, so differences in outcomes might be due to differences in the ratio psychotic and non-psychotic patients. Although it probably adds to the clarification of the diversity in results, it is not likely to be main cause. The prevalence of psychotic depression is just 5,3% (Gaudiano, 2009), while the majority of studies finds lower melatonin secretion. The inverse would be expected, when aberrant results can be explained by differences between psychotic and non-psychotic patients. Overall a decrease in melatonin levels in depression is more supported by literature, however no explanations for aberrant results are found yet.

Beside differences in the melatonin rhythm amplitude, also phase shifts of the rhythm in depressed patients have been found, although most studies do not look into the phase shifts. Nair *et al.* found a delayed onset of the melatonin rise, but the nocturnal peak occurred earlier in depressed patients compared to healthy controls (Nair, 1984). Beck-Friis *et al.* found a trend towards a phase advance of the melatonin peak in depressed patients with an abnormal reaction to the DST, this did not reach significance. Contrastingly Crasson *et al.* found a delayed melatonin peak, depressed patients reached their maximum values 77 minutes later then the control subjects (Crasson, 2004). Corresponding, Tuunainen *et al.* found a phase delay for onset of 6-sulfatoxymelatonin excretion in the urine of depressed postmenopausal women. The number of depressed patients was however very small, only 26 out of the 382 participants had currently depressive sympthoms, 40% of the participants had a history of depressive illnesses (Tuunainen, 2002). Claustrat *et al.* did not find any phase shifts in subjects with depression (Claustrat, 1984). Results about phase shifts of the melatonin rhythm in depression are too little and too contradicting to draw any conclusions. For both melatonin levels and phase shifts results are not coupled to chronotypes and possible disturbances in the sleep-wake cycle of depressed patients. These could of course have a large influences on the melatonin

rhythms and might explain part of the large variation found. Further, all the controversy in studies on melatonin rhythms might be due to the big individual variation. In a group of 170 subjects the mean value of the melatonin peak was 17,64 ± 12,48 pg/ml, values ranged from 2,40 to 83,60 pg/ml. Also in dim light melatonin onset, a measure for the timing of the rhythm, a big range from 18:13 to 00:26 was found (Burgess, 2008). Therefore big sample sizes are needed to draw relevant conclusions. But even when consensus is found, it will still be unclear what de causal relation between melatonin rhythms and depression is and whether changes in melatonin rhythms cause depression or are a results of depression.

Effects of exogenous melatonin on depression

From the previous chapter it is clear that melatonin rhythms are changed in depressed patients, which suggests involvement of melatonin in depression. This is further supported by the effects that are found of administering exogenous melatonin in animal models of depression. In 1998 the effect of both acute and chronic treatment with a melatonin agonist and antagonist were tested, only chronic treatment with an agonist evoked significant effects. Administration of 10 mg/kg agonist reduced the immobility time in the forced swimming test, indicating a decrease in depression (Overstreet, 1998). The forced swimming test is a widely used and proven model for depression, in which the time a rat is immobile is used as a measure for depression. The longer the immobility time, the more 'depressed' a rat is. (Porsolt, 1978). Other studies also found antidepressant-like effects of chronic melatonin treatment in this animal model. Both 14 days of 2 mg melatonin in the drinking water and 20 days of 10 mg/kg subcutaneously injected melatonin led to significant decreases in immobility time (Brotto, 2000; Hill, 2003). Melatonin reversed the increase in immobility after repeated forced swimming (Raghavendra, 2000). Antidepressant-like effects were found in other models as well. 21 days pretreatment with 10 mg/kg melatonin was shown to prevent the negative alterations of dexamethasone, a glucocorticoïd receptor agonist, in the hippocampus. Sole treatment with 60 mg/kg dexamethasone led to longer immobility times in forced swimming, decreases in the number of proliferating cells and reduced the number of glucocorticoïd receptors in the hippocampus, all associated with depression and is therefore used as an animal model for depression. When dexamethasone was administered together with melatonin, those changes were not seen (Ruksee, 2014). All above mentioned studies found effects of melatonin after more than 10 consecutive days of treatment. Micale *et al.* found decreases in immobility in forced swimming after a shorter treatment period. They repeatedly administered 0.5 or 1 mg/kg melatonin at 24, 5 and 1 hour before testing (Micale, 2006). However, none of the studies found acute effects (within several hours) of melatonin treatment. This is in line with the study of Ergun *et at.* (2008), who only tested the acute effect of melatonin and found no significant changes. Two other studies did find acute effects, but

Figure 2. Rat showing typical immobility posture in forced swimming test. (Porsolt, 1978)

then after treatment with a melatonin antagonist. 30 mg/kg Luzindole (melatonin receptor 1 (MT1)/ melatonin receptor 2 (MT2) agonist) via an intraperitoneal injection showed acute antidepressant-like activity in the forced swimming test (Sumaya, 2005). 5 µg of a selective MT2 antagonist injected directly into the striatum caused decreases in immobility time as well, while 10 µg selective MT2 agonist did not (Noseda, 2014). Overstreet *et al.* (1998) did not find an acute effect of a systemically administered melatonin antagonist, this might be due to the lower dosage of antagonist used (10 mg/kg).The antidepressant-like effects of both agonists and antagonists seem contradictory, but might be explained by the timing difference. Agonists have antidepressant-like activity when they are administered for a longer period, on the other hand antagonist exert their effects directly after administration. If chronic stimulation and acute suppression both have the same effect, the antidepressant-like response is possibly due to acute blockade or long term desensitization of melatonin receptors, which in both cases leads to less signal transduction (Sumaya, 2005). However, no studies to confirm this hypothesis have been performed yet.

Hardly any studies have been done to investigate the effects of melatonin on depression in humans. One study already performed in 1976 found an increase of depressive and angry feelings in 7 patients with major depressive illness (Carman, 1976). But the period of administration and the dosages varied a lot per patient and dosages were extremely high. 5 out of 7 patients received a peak dosage of 1100-1600 mg/day. Only two double-blind, placebo-controlled trials on the effect of melatonin are performed so far. In 1998, 5 mg slow-release melatonin was administered beside 20 mg fluoxetine antidepressant medication for four weeks. No effect of melatonin on depression was found, but sleep quality and sleep length improved significantly in the melatonin group (Dolberg, 1998). In 2010, 6 mg slow-release melatonin was administered beside treatment-as-usual. After four weeks no differences in sleep, neither mood, were found between the melatonin and the placebo group (Serfaty, 2010).

Although little research is done on the effects of melatonin itself on depression in humans, agomelatine, a MT1/MT2 agonist and 5-HT2C antagonist, is excessively researched as an antidepressant drug (San, 2008). It is marketed in de EU since 2009 under the name Valdoxan (European Medicines Agency, 2014). In the US agomelatine was never approved to be marketed.[1] Antidepressant effectiveness of agomelatine was proven in preclinical studies in four different animal models of depression. Repeated administration of agomelatine (dosages varying from 4- 50 mg/kg) showed antidepressant-like activity in the chronic mild stress model, the forced swimming test, the learned helplessness test and a transgenic mouse model. All studies found antidepressant-like effects of melatonin as well, but to a lower degree (Papp, 2003; Bourin, 2004; Barden, 2006; Bertaina-Anglade, 2006). Also multiple double-blind, placebo-controlled clinical trials have been performed. All finding an antidepressant effect of 25 and 50 mg agomelatine per day in 6-8 week studies (Loo, 2002; Kennedy, 2005; Olie, 2007, reviewed in: Srinivasan, 2012;). All, or at least the vast majority of clinical trials that examined the effectiveness of agomelatine administered the drug in the evening. Nothing is known about timing differences in effectiveness. These are however presumable to exist, because of the natural fluctuations in mematonin. In addition to general antidepressant activity, agomelatine also improves subjective reports of sleep quality, sleep latency and the stability of non-REM sleep (reviewed in: San, 2008). However, it still remains unclear if the effects of agomelatine are mediated via the MT1 and MT2 receptors, or if the antidepressant effect is due to its 5-HT2c antagonism.

[1] No reference, because official documents of the US Food and Drug Administration (FDA) are not accessible.

Possible mechanisms

It is clear that melatonin and other MT1/MT2 ligands can exert different effects on depression. However there is not much clarity about the mechanisms through which these effects are mediated. Melatonin might simply use the signal transduction mediated via its own receptors, but innervation in the other systems related to the pathophysiology of depression could also be involved.

Melatonin receptors

Melatonin can act upon several receptors. The main receptors through which melatonin exerts its actions are the MT1 and MT2 receptors, both G-protein coupled receptors. Besides, melatonin can also bind to a third receptor, MT3, a quinine reductase (Reviewed in: Witt-Enderby, 2003). The signal transduction of the MT1 and MT2 receptor differs per tissue and cell type. MT1, in general, is coupled to a Gi protein, inhibiting adenyl cyclase activity and inhibiting cAMP signaling, but it can also increase Ca^{2+} levels via a Gq protein. MT2 activation leads to the same activation of signal transduction pathways, but can also decrease cGMP accumulation (figure 3) (Reviewed in: Witt-Enderby, 2003). The MT2 receptor is only expressed in the SCN, the SON and the PVN (Wu, 2013). The MT1 receptor is also expressed in other hypothalamic nuclei and the pituitary, where high expression was found in the pars tuberalis and weak expression in the anterior and posterior pituitary (Wu, 2006). The effects of the two melatonin receptors can be both complementary or contrary in different tissues. For example, MT1 activation leads to vasoconstriction, while MT2 activation leads to vasodilatation (Reviewed in: Comai, 2014). In the SCN both receptors have distinct functions as well. Activation of MT1 receptors leads to inhibition of SCN neuron firing rate (Liu, 1997). On the other hand, activation of MT2 receptors can mediate phase shifts (Pfeffer, 2012).

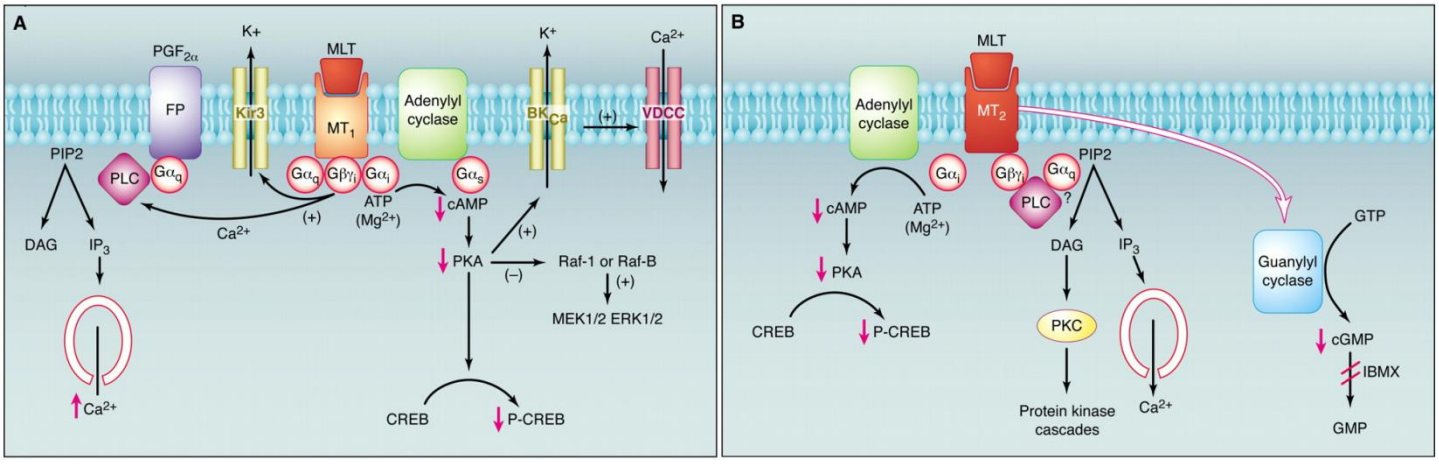


Figure 3. Signal transduction of melatonin receptor MT1 and MT2 A: MT1 receptor can cause a decrease in cAMP levels via Gi-protein activation and an increase in Ca^{2+} levels via a Gq-protein activation. B: MT2 receptor can cause a decrease in cAMP levels via Gi-protein activation and an increase in Ca^{2+} levels via a Gq-protein activation, additionally it can inhibit guanylyl cyclase and thereby reduce cGMP accumulation. http://www.lookfordiagnosis.com/mesh_info.php?term=Receptors%2C+Melatonin&lang=1 (accessed at: 13-04-2015).

The role of the two different receptors in antidepressant-like activity of melatonin is not completely understood yet. Some studies show a role for the MT1 receptor, others for the MT2 receptor. Wu *et al.* showed, by immunocytochemical staining of postmortem hypothalamic samples, that expression of MT1 receptors in the SCN was higher in depressed patients compared to healthy controls. No

difference in expression of the MT2 receptor was found, implicating a more pronounced role for the MT1 receptor in depression (Wu, 2013). Earlier, another study already found that MT1 knockout mice show more depressive-like behavior in both the forced swimming test and the open field test (Weil, 2006). Involvement of the MT2 receptor in depression was found as well. Genetic variation in the gene that encodes for the MT2 receptor influences the risk for developing recurrent depressive disorder (rDD). The C allele and T allele of the rs4753426 single nucleotide polymorphism can respectively increase and decrease the risk for rDD (Galecka, 2011). Besides, luzindole, a MT1/MT2 antagonist, showed anti-depressant like effects in the forced swimming test both in wild type mice and MT1 knockout mice. However, immobility time was not changed in MT2 knockout mice compared to vehicle treated group, suggesting the antidepressant-like effect to be mediated via the MT2 receptor (Sumaya, 2005). This was further supported in 2014 when Noseda *et al.* showed that blockade of the striatal MT2 receptor elicited antidepressant-like effects in the forced swimming test. Looking at all the studies, the effects of the two receptors may be opposite. Where MT1 might mediate antidepressive-like effects, MT2 seems to mediate depressant effects. Little research is done on the distinct functions of these two receptors, but now that more selective agonists and antagonists and genetic models are getting available, their mechanisms can be clarified.

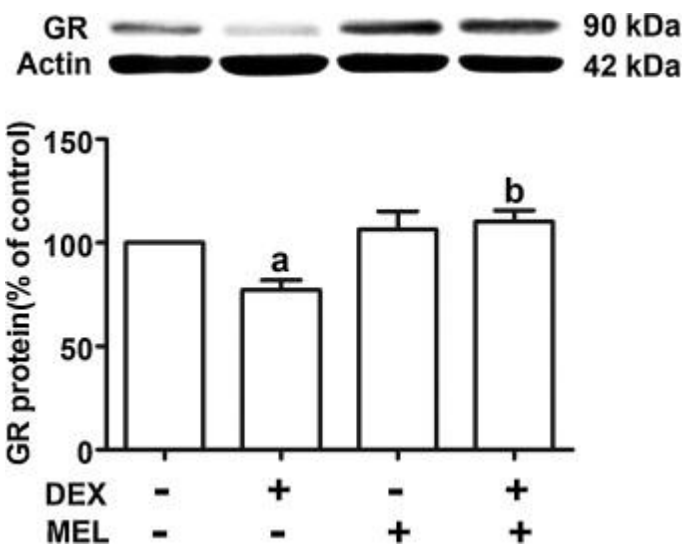
The monoaminergic system

Monoamines are extensively proven to be involved in depression. Melatonin might exert its antidepressant-like effects via the monoaminergic systems. Quite some research has been done on the relation between serotonin (5-HT) and melatonin, especially after the discovery of the effectiveness of agomelatine, a melatonin agonist and 5-HT antagonist, as an antidepressant. Although melatonin cannot bind to serotonergic receptors, it has been shown to decrease 5-HT_{2A} neurotransmission (Eison, 1995). Suppression of 5-HT_2 receptors is a possible mechanism for melatonin, because 5-HT_2 receptor density is increased in depression (D'haenen, 1992). It is further supported by the fact that corticosterone, which up regulates serotonergic receptors, can inhibit the antidepressant-like effects of melatonin (Hill, 2003). In addition, different serotonergic agonists and antagonist can enhance the effect of agomelatine and melatonin on the immobility time in the forced swimming test. Pretreatment with an agonist for postsynaptic 5-HT_{1A} receptors, as well as with an antagonist for 5-HT_{1A} autoreceptors led to a larger decrease in immobility in both agomelatine and melatonin treatment. Involvement of these serotonergic autoreceptors is however unlikely, because both agomelatine and melatonin do not have affinity for these receptors. Pretreatment with a 5-$HT_{2A/C}$ antagonist also enhanced the effect of agomelatine (Bourin, 2004). Administration of 5-HT or DOI(a 5-$HT_{2A/C}$ agonist) was shown to totally suppress the antidepressant-like effect of melatonin in the forced swimming test (Micale, 2006), which is in line with the enhancing effect of a 5-$HT_{2A/C}$ antagonist. The ability of these serotonergic ligands to exert an effect on agomelatine's and melatonin's activity supports a possible involvement of the serotonergic pathways in melatonin's mechanism in depression. On the other hand when imipramine, a tricyclic antidepressant, was co-administered with melatonin, only an additive and no synergistic effect was found. Combination of the effective dosages of both melatonin en imipramine did lead to an extra decrease in immobility time, while combination of one effective, with one below-effective dose, was not able to cause this effect. This would implicate that there is no interference between the signaling pathways of melatonin and imipramine (Ergun, 2008). Recently an interesting study was performed on the formation of MT2/5-HT_{2C} heterodimeric receptors. It was shown that these dimers can indeed be formed, and that melatonin leads to the same changes in cAMP levels in cells only expressing the

MT2 receptor as in cells expressing both receptors. Beside the cAMP changes, melatonin also activated the Gq/PLC pathway in cells with the receptor dimer. Because this activation was not seen in the MT2 monomer, the Gq/PLC activation has to be due to trans-activation of the 5-HT_{2c} receptor in the receptor dimer. The trans-activation of the dimeric receptors is unidirectional, because 5-HT stimulation does not evoke activation of either of these pathways (Kamal, 2015 *in press*). MT2 receptor activation has before been shown transduce its signals via the Gq/PLC pathway. However, because the signal transduction differs slightly per tissue and cell type, it is possible that in this experiment Gq/PLC activation was not seen in cells only expressing the MT2 receptor.
Research on the involvement of other monoamines has hardly been done. One study on the role of the dopaminergic system was performed. Both a nonselective dopmanine antagonist, a dopamine receptor 1 (D1) antagonist and a dopamine receptor 2 (D2) antagonist reversed the antidepressant-like effect of melatonin in the tail suppression test. In addition, when non-effective doses of melatonin were administered together with D1 or D2 agonist, decreases in immobility were seen (Binfare, 2010). These results implicate that melatonin might exert its antidepressant-like effects via activation of D1 and D1 receptors.

The hypothalamic-pituitary-adrenal axis
Hyperactivity of the HPA-axis is another possible explanation for depression and also this has been linked to melatonin. The start of the quiescent period of cortisol rhythms was shown to be phase locked to the dim light melatonin onset (DLMO), which indicates tight interaction between the two rhythms. This was shown in night shift workers, who have a large variation in DLMO, the beginning of the quiescent cortisol period was 1 h 28 min before the DLMO, just like in the day-active controls. (Weibel, 2002). Also suppressive actions of the HPA-axis on melatonin and vice versa have been shown. In 1997 Kellner *et al.* found an inhibitory effect of corticotrophin-releasing hormone (CRH) on melatonin, however adrenocorticotropic hormone (ACTH) did not cause the same effect. Melatonin and the melatonin agonist ramelteon on the other hand, turned out to inhibit the CRH-induced ACTH production in AtT20 cells (ACTH producing cells expressing the MT1, but not MT2 receptor) (Tsukamoto, 2013). Further melatonin also suppressed the ACTH-induced cortisol production in capuchin monkey, rat and human adrenal tissue (Torres-Farfan, 2003; Richter, 2008; Campino, 2011). Juszczak *et al.* did not find an inhibiting effect of melatonin on ACTH production, however the animals in this study were already sacrificed 10 minutes after melatonin administration, which might be a too short time span for melatonin to exert its effects and could explain the contradictory results (Juszczak, 2014). In the same study they did find that physiological melatonin concentrations led to suppression, while pharmalogical concentrations of melatonin led to a stimulation of AVP release in cells from a hypothalamo-neurohypophysial explant. Which is interesting, because AVP is stored is the same vesicles as CRH in these neurons (Sawchenko, 1984). In addition, the MT1 receptor is lolocalized with CRH neurons in the PVN, which would enable melatonin to directly affect the HPA-axis (Wu, 2006). All these results correspond with the hyperactivity of the HPA-axis and the low melatonin levels seen in depression. However the causal relation remains unknown. A hyperactive HPA-axis could lead to low melatonin levels, but low melatonin levels could also cause hyperactivity of the HPA-axis. The fact that low melatonin levels were mostly found in patients with an abnormal dexamethasone suppression test response again supports the existence of a causal relation between these two factors.
The HPA-axis is also found to be related to the antidepressant-like activity of melatonin. Three weeks of both 1 and 10 mg/kg melatonin administration led to normalization of the elevated

corticosterone levels in the chronic mild stress model. These decreases were similar to those of imipramine (Detanico, 2009). Chronic melatonin treatment was further shown to improve depressive-like behavior and hippocampal cell proliferation in chronic corticosterone-treated mice (Crupi, 2010). Which is in line with the findings that melatonin administration can prevent the effects of dexamethasone. Dexamethasone is a glucocorticoïd receptor antagonist that can induce depressive-like behavior in mice when injected acutely in a dose of 64 mg/kg or for 7 days in a dose of 16 mg/kg, besides it inhibits the effects of several known antidepressants (Wróbel, 2014). In mice that were pretreated with melatonin, the decreases in immobility time in forced swimming, cell proliferation, neurite elongation and glucocorticoid receptor (GR) numbers caused by dexamethasone, were not seen (Ruksee, 2014). In addition, corsticosterone showed depressant effects in male rats in the forced swimming test, where melatonin showed antidepressant effects in both sexes. When corticosterone was administered together with melatonin, melatonin's antidepressant activity was inhibited in both sexes (Hill, 2003). Ruksee *et al.* proposed that the increase in number of GRs seen after melatonin pretreatment (figure 4) might be an important factor in the antidepressant-like effects of melatonin. This increase in receptor expression could enable a restore of the diminished negative feedback of glucocorticoids on the HPA-axis. Which in turn could suppress the HPA-axis hyperactivity and the thereby caused depressant effects.



Fugure 4. A western blot analysis of the glucocorticoid receptor in hippocampi of melatoninin DEX-treated mice. The mice were chronically treated with DEX 60 mg/kg administered by i.p. injection for 21 days and with melatonin 10 mg/kg administered by i.p. injection 30 min prior to DEX injection. The data are expressed as a percentage of the GR value (normalized to actin) of the control animals injected with saline ($p < 0.05$). Values are means ± SEM ($n = 6$); [a]$p < 0.05$, compared to the control group and [b]$p < 0.01$, compared to the DEX group. (Ruksee, 2014).

The GABAergic system

A deficit of GABAergic neurotransmission is said to play a role in the development of depression and melatonin is able to enhance this neurotransmission, thereby creating another possible mechanism for depression. Melatonin is shown to increase the binding of GABA to its receptors in vitro in the rat brain, however diazepam, a know stimulator of GABAergic neurotransmission, was 14 times as potent in increasing the binding (Coloma, 1988). Melatonin was further shown to increase the GABA concentration in the hypothalamus, the cerebellum, the cerebral cortex and the pineal gland, but only when GABA transaminase was blocked (Rosenstein, 1986). Besides, melatonin increased the receptor density of central benzodiazepine receptors (Gomar, 1994). In 2000 an experiment was set up to test if this interaction with the GABAergic system was responsible for the antidepressant-like effects of melatonin. The antidepressant-like effects of melatonin were similar to those of both direct and indirect (via benzodiazepine) GABA ligands. Melatonin's effects were shown to be reversed by both a GABA-A receptor antagonist and a peripheral benzodiazepine receptor antagonist, a central benzodiazepine receptor antagonist on the other hand did not influence the

effects of melatonin (Raghavendra, 2000). This suggests involvement of the peripheral and not central benzodiazepine receptors in melatonin's antidepressant action.

Brain derived neurotrophic factor

Yet another hypothesis for depression states the disease to be caused by a shortage of neurotophic factors. One of these factors is brain derived neurotrophic factor (BDNF), which levels are shown to be increased by melatonin. In the cerebellar granule cells of rats, activation of both the MT1 and MT2 receptor by ramelteon, a MT1/MT2 agonist, led to higher BDNF levels (Imbesi, 2008). Agomelatine led to increases of BDNF levels in the prefrontal cortex. This effect was due to a synergism between its affinity for the melatonin receptors and the 5-HT_{2C} regeceptors, because neither melatonin nor a 5-HT_{2C} antagonist caused the same effects (Molteni, 2010). In addition, the effect of melatonin was tested after chronic dexamethasone treatment, which decreases hippocampal BDNF. When rats were treated with melatonin (10 mg/kg) beside the dexamethasone, the BDNF levels were increased compared to group treated with only dexamethasone in both the hippocampus and the prefrontal cortex (Tongjaroenbuangam, 2013). Although not much is known about the relation between BDNF and melatonin yet, these studies suggest another possible mechanism for melatonin to positively affect depression. However, no causal relation is proven yet and also no human studies have been performed so far.

The glutamatergic system

Both in preclinical and in clinical studies N-methyl-D-aspartic acid (NMDA) receptor antagonists have been found to exert antidepressant effects (reviewed in: Skolnick, 1999). Besides, melatonin can inhibit NMDA receptor responses, creating another way melatonin might mediate its antidepressant-like effects. Co-administration of melatonin made glutamate and NMDA ineffective in activating the NMDA receptor in rat striatal neurons in vivo. This action of melatonin was not mediated via one of its own receptors (Escames, 2004). Further, acute stress in rats led to an increase in extracellular glutamate in the amygdala and the hippocampus. This increase was completely prevented by administration of agomelatine (Reagan, 2012). Although acute stress is not directly linked to depression, chronic stress can cause depressive-like behavior, so increases in extracellular glutamate might play a role in depression as well. Mantovani *et al.* showed that pretreatment with an inactive dose of melatonin, combined with an inactive dose of several NMDA receptor antagonists, led to antidepressant-like effects in the tail suspension test. Showing synergistic antidepressant-like effects for these substances. Further they found that L-arginine (a nitric oxide precursor) and SNAP (a nitric oxide donor) inhibited melatonin's antidepressant-like effects. This suggests that melatonin might exert its inhibiting effect on NMDA responses by suppressing the nitric oxide (NO) production that is normally activated upon NMDA activation (Mantovani, 2003). Possibly melatonin does not directly suppress NMDA receptors, but indirectly by blocking its signal transduction. In line with these results Escames *et al.* found that melatonin blocked the potentiation of the NMDA-induced activity caused by L-arginine and SNAP (a nitric oxide donor) (Escames, 2004). On the other hand, Tongjaroenbuangam *et al.* found that NR2A/B (a NMDA receptor subunit) was decreased in the hippocampus after chronic dexamethasone treatment, which is an animal model for depression. Pretreatment with melatonin partially prevented this decrease in NR2A/B expression. These results suggest that melatonin positively influences NMDA neurotransmission. However, this study focused on the effects of chronic dexamethasone treatment and melatonin on learning and memory and not on depression. It is therefore questionable how applicable these results are. As for the involvement of BDNF in melatonin's mechanisms, also about the involvement of NMDA receptors and the L-

arginine- NO pathway too little is known. Human studies and studies on the causal relationship would help further understanding this involvement.

Conclusion

Desynchronization of the biological clock has been proposed as a possible cause for depression. There is no doubt that the biological clock and melatonin are involved in the pathophysiology of the disease. However the precise role of these two factors remains unclear and it is unknown if depression is a cause or a consequence of the disturbances in the biological clock. First of all there is no consensus in literature whether melatonin levels are elevated or decreased in patients suffering from any depressive disorder and whether the melatonin rhythm is phase advanced or delayed. Already since 1979 many studies have been performed looking into changes in melatonin secretion, but a lot of variation is found in these results. The large individual differences in both the timing and the amplitude of the melatonin rhythm make it difficult to look for differences in a specific group. Recently there is not much attention for the changes in melatonin rhythms in depression anymore and hardly any studies are performed to explain the differences found in former studies. New projects should be set up to bring clarification about natural changes in melatonin rhythms associated with depression. This would be of great use to reveal the role of melatonin in depression, because many other studies make use of artificial circumstances with exogenous melatonin. However, those studies with exogenous melatonin, which are performed in several animal models of depression, did contribute to determining melatonin's role in depression. Acute blockade or long term desensitization of melatonin receptors has been shown to have antidepressant-like effects many times. These effects were induced by administering pharmalogical dosages of melatonin (around 10 mg/kg), while no effects were found with physiological dosages. Also, in humans there is a lack of research on the effects of exogenous melatonin and the studies that are performed do not find antidepressant activity. It is therefore dubious how relevant the findings in animal models of depression are. Agomelatine, on the other hand, has been widely studied and has proven to be an effective antidepressant, in both animal models and humans. Although it is not marketed in the US due to a risk for liver toxicity.[2] If the antidepressant properties of agomelatine are due to its MT1/MT2 agonism or its 5-HT_{2c} antagonism remains unclear. Most probably both signal pathways contribute, because both MT1/MT2 agonism and 5-HT_{2C} antagonism are shown to be able to induce antidepressant effects. The mechanisms by which melatonin mediates its effects are not clarified yet. This is a topic of great interest nowadays and there is a lot of ongoing research. Because there are so many factors that are implicated in the pathophysiology of depression, possible mechanisms for melatonin have been sought in various systems. In table 1 the different ways of melatonin to exert antidepressant-like effects are summarized. Melatonin is found to be able to act on many receptors. Apart from its own MT1/MT2 receptors, effects are also mediated via serotonergic, dopaminergic, GABAergic and glutamatergic receptors. It is not yet understood how this activity is exactly mediated because in most cases melatonin does not have direct affinity for the receptors. For the effect of NMDA receptors it has been suggested that melatonin does not inhibit the receptor

[2] No reference, because official documents of the US Food and Drug Administration (FDA) are not accessible.

Mechanisms for the antidepressant-like effect of exogenous melatonin

Melatonin receptors	MT1 agonism
	MT2 antagonism
Serotonergic system	5-$HT_{2A/C}$ antagonism
	5-HT_{1A} agonism
	MT2/5-HT_{2C} receptor dimer
Dopaminergic system	D1/D2 agonism
HPA-axis	Inhibit CRH-induced ACTH
	Inhibit ACTH-induced cortisol
	Increase GR expression
GABAergic system	Central GABA-A receptor agonism
	Peripheral benzodiazepine agonism
BDNF	Increase BDNF levels (via MT1/MT2 activation)
Glutamatergic system	NMDA receptor antagonism
	Inhibition of NO production

Table 1. Mechanisms for the antidepressant-like effect of exogenous melatonin. Effects of melatonin that have been shown to contribute to its antidepressant-like activity per system. MT1/2: melatonin receptor 1/2, 5-$HT_{2A/2C/1A}$: serotonin receptor 2A/2C/1A, D1/2: dopmanine receptor ½, CRH: corticotropin-releasing hormone, ACTH: adrenocorticotropic hormone, GR: glucocorticoïd receptor, GABA: gamma-aminobutyric acid, BDNF: brain derived neurotrophic factor, NMDA: N-methyl-D-aspartic acid, NO: nitric oxide.

directly, but suppresses the NO formation caused by this receptor. This way of indirect inhibiting the signal transduction of receptors could play a role in the other receptor types as well. Involvement of melatonin in the dysregulated HPA-axis in depressed patients is presumable, because bidirectional suppressing activity was shown several times. However, the causal relationship between the two is not defined yet. Hyperactivity of the HPA-axis could cause changes in melatonin secretion or a lack of the suppressive effects of melatonin could cause the HPA-axis dysregulation. The fact that melatonin enhances GR expression and thus not only directly affects ACTH and corstisol levels, but also changes the HPA-axis regulation, suggests that melatonin might influences the HPA-axis instead of the other way around. Last of all, the effects of melatonin on BDNF were shown. There was no contradiction about the enhancing effects of melatonin, which is in line with the decreased BDNF expression in depression. In spite of the many things that have yet been discovered, no overall conclusions about the mechanisms of melatonin can be drawn yet. It is mainly important to gain knowledge about the relations of all different systems involved. Every study focuses on a certain system, but no studies have been performed looking into the relative contributions and interactions of different systems on melatonin's antidepressant activity. Furthermore, more research to the involvement of melatonin's circadian properties should be done. All the above mentioned functions of melatonin are not related to its role in the biological clock. Because circadian desynchronization is hypothesized to be the cause of depression, a bigger role for melatonins circadian properties would be expected. Studies on the effects of melatonin and agomelatine in SCN-lesioned rats might shed light on the question how important the biological clock is in depression. Also, it would be interesting to see if melatonin and agomelatine can lead to resynchronization. Agomelatine is shown to improve circadian symptoms like sleep problems (reviewed in: San, 2008), but no research on the effect on the desynchronized internal rhythms has been performed. The same is true for the more conventional antidepressants. There are indications that these do affect circadian rhythms by phase shifts, resetting and stabilizing the clock (Reviewed by: Srinivasan, 2012), but effects on internal synchronization have not been

examined yet. If circadian desynchronization is indeed a more fundamental cause of depression, the information gained from these kind of experiments could be extremely valuable. Understanding of the involvement of the biological clock could lead to development of new therapies or improvement of currently available treatments like sleep deprivation and light therapy.

A big limitation of this research field is the use of animal models. Although the used models all have been tested and currently available antidepressants are effective in these models, it remains questionable to translate a complex cognitive dysfunction like depression to animals. Research on the cause of depression is difficult in these models, because the depressive-like state is artificially induced and therefore the neurological background of the symptoms can be very different. Of course possibilities are a lot smaller in humans, but whenever possible human studies should always be preferred.

Concluding, a lot of knowledge about the effects and mechanisms of melatonin in depression is gained. However, the effects of the circadian properties of melatonin should be more emphasized. Human studies on desynchronization of internal rhythms in depressed patients and the effects of melatonin, agomelatine and other antidepressants on this internal desynchornization could be of great importance in the development of new therapies and treatments for depression.

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