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Chronic mild stress-induced alterations of clock gene expression in rat prefrontal cortex: modulatory effects of prolonged lurasidone treatment

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ABSTRACT

Disruptions of biological rhythms are known to be associated with depressive disorders, suggesting that abnormalities in the molecular clock may contribute to the development of these disorders. These mechanisms have been extensively characterized in the suprachiasmatic nucleus, but little is know about the role exerted by individual clock genes in brain structures that are important for depressive disorders. Using the chronic mild stress model we found a significant reduction of BMAL1 and CLOCK protein levels in the nuclear compartment of the prefrontal cortex of CMS rats, which was paralleled by a down-regulation of the expression of several target genes, including *Per*s and *Cry*s but also *Reverbβ* and *Pparα*.

Interestingly, chronic treatment with the multi receptor modulator lurasidone (3 mg/kg for 5 weeks) was able to normalize the molecular changes induced by CMS exposure in prefrontal cortex, but it was also able to regulate some of these genes within the hippocampus.

We believe that changes in clock genes expression after CMS exposure may contribute to the disturbances associated with depressive disorders and that the ability of chronic lurasidone to normalize such alterations may be relevant for its therapeutic properties in ameliorating functions that are deteriorated in patients with major depression and other stress-related disorders.

1. Introduction

Depressive disorders are characterized by complex and heterogeneous symptoms, including alterations of biological rhythms that can manifest, for example, with changes in sleep-wake cycle [1]. At the bases of these modifications there is a disruption of the circadian clock since it has been demonstrated that the intensity of major depressive symptoms in human is correlated with the misalignment of circadian rhythms [2]. Furthermore mutations in circadian genes are found in depressed subjects and may contribute to specific symptoms [3]. Moreover, considering the importance of cell birth and proliferation in the hippocampus in mood and in the antidepressant activity, the alterations in neurogenesis observed following chronic circadian disruption [4] confirm that this process may contribute to the development or exacerbation of depressive disorders.

While the pacemaker cells controlling most of the circadian rhythms are located in the suprachiasmatic nucleus (SCN), it has been recently demonstrated that patients with major depressive disorder show profound alterations of hundreds of genes that have a rhythmic transcriptional activity in regions outside the SCN [5].

At molecular level, the circadian clock involves periodic changes in gene expression achieved by transcription-translation feedback loops whereby the protein product of transcribed genes auto-regulate their own transcription. In mammals, the circadian clock is composed of an auto-regulatory transcriptional network with an interlocked feedback loop. Specifically, the core transcriptional circuit comprises the transcription factors BMAL1 and CLOCK that heterodimerize and activate the transcription of *Period* (*Per*) and *Cryptochrome* (*Cry*) genes. PER/CRY proteins then repress their own transcription by inhibiting the activity of CLOCK:BMAL1 until they are degraded to allow a new cycle of transcription to begin [6]. In addition, the interlocking feedback loop regulated rhythmic expression of *Bmal1* through opposing action of the ROR and

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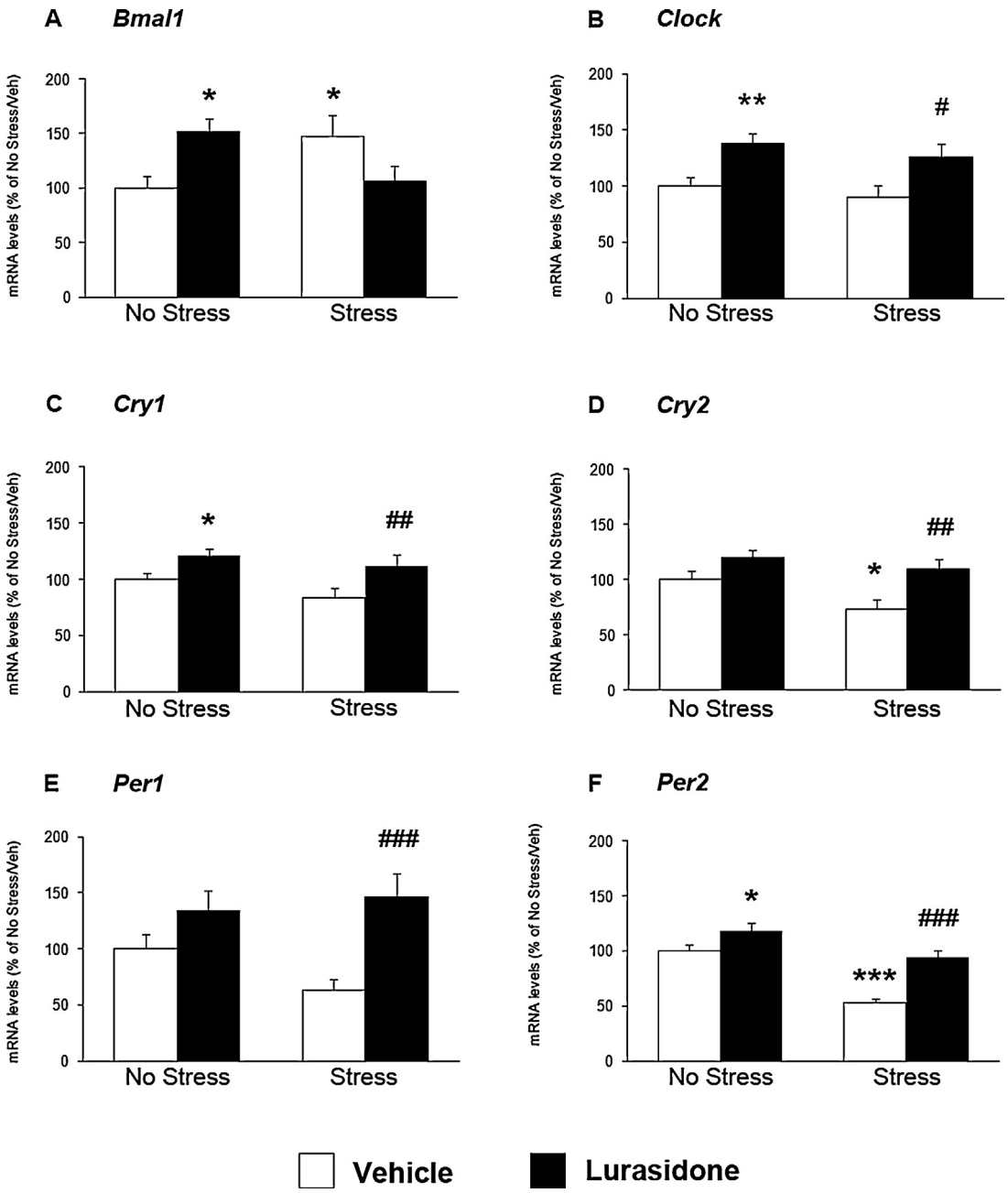

Fig. 1. Analysis of clock genes expression in the prefrontal cortex of rats exposed to CMS: effect of lurasidone treatment. The mRNA levels of *Bmal1* (A), *Clock* (B), *Cry1* (C), *Cry2* (D), *Per1* (E) and *Per2* (F) were measured in the prefrontal cortex of non-stressed or chronically stressed rats, treated for 5 weeks with vehicle or lurasidone and killed 24 h after the last administration. The data, expressed as a percentage of No Stress/Vehicle (set at 100%), are the mean ± SEM of at least 9 independent determinations. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* No Stress/Vehicle; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs* Stress/Vehicle (two-way ANOVA with PLSD).

REV-ERB families that activate and repress *Bmal1* transcription respectively and whose expression is controlled by the core loop [6].

Since depression can be the consequence of altered and often maladaptive response to stress, models in which animals are exposed to different paradigms of stress during adult life are supposed to disrupt normal homeostasis, leading to pathologic alterations. Among the wide array of experimental paradigms employed in rodents to investigate the mechanisms that may contribute to stress-related disease susceptibility, the chronic mild stress (CMS) model is a well-validated paradigm to induce depressive-like behavior, which is also associated with significant alterations of circadian rhythms [7].

On these bases, in the present study we have used the CMS paradigm to establish possible changes in the expression of the clock genes in brain regions different from the SCN, such as prefrontal cortex and hippocampus, which play a key role in core symptoms of major depressive disorders. Furthermore, we have investigated the ability of the multi-receptor modulator lurasidone to normalize stress-induced changes of clock genes expression. Even if lurasidone was initially introduced as an antipsychotic drug, recent evidence indicate that it also has antidepressant [8,9] and procognitive activity both in rats and primates [10,11].

Moreover, we have recently shown that prolonged treatment with lurasidone normalizes the behavioral as well as the molecular changes at synaptic levels due the exposure to CMS [12].