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Research report

Behavioral and molecular effects of prenatal continuous light exposure in the adult rat

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ABSTRACT

Disruption of the maternal environment during pregnancy leads to behavioral changes and diseases in the adult offspring. To explore the influence of prenatal continuous light exposure (PCLE) on the adult offspring, we exposed pregnant Wistar rats to constant light during late gestation. Adult PCLE offspring showed an anxiety-like behavior and impairment of short-term memory in different tests. Measurements in the whole brain homogenates from newborn and adult offspring indicated decreased melatonin and serotonin levels and increased reactive oxygen species level in PCLE offspring. Further, we determined melatonin-, serotonin-, oxidative stress-, apoptosis-, and circadian system-related genes expression in different brain areas of adult offspring. The serotonin reuptaker *Slc6a4* displayed a decreased expression in the prefrontal cortex of PCLE group. The circadian rhythm-related gene *Rora* was upregulated in the amygdala of PCLE offspring. Our results point to adverse behavioral effects of PCLE on adult offspring, involving serotonin and melatonin signaling dysregulation, increased chronic oxidative stress, and altered gene expression.

1. Introduction

Continuous light exposure is a strong stressful stimulus, leading to the disruption of circadian rhythms (Fonken and Nelson, 2014) and a subsequent altered melatonin secretion (Wideman and Murphy, 2009). Circadian disruption has been associated with different psychiatric disorders in adult humans like bipolar disorder, depression, schizophrenia, and obsessive compulsive disorder (Pacchierotti et al., 2001; Salgado-Delgado et al., 2011). Maternal circadian rhythm is involved in the programming of fetal and newborn circadian clocks (Irmak et al., 2005; Reiter et al., 2014) and can influence the pineal-defining transcriptome, shown to be established prior to the neonatal period (Hartley et al., 2015). Disturbances of the fetal circadian system have been linked to long-term metabolic and behavioral consequences in the adult offspring (Cisternas et al., 2010; Ferreira et al., 2012; Voiculescu et al., 2015).

Circadian melatonin secretion from the pineal gland exhibits a nocturnal maximum value. Its role during fetal development, when the pineal gland is immature, is supported by its progressively increased concentration in the maternal blood and amniotic fluid during late gestation peaking at term and during delivery (Kivelä, 1991; Nakamura et al., 2001; Okatani et al., 1998; Tamura et al., 2008). Continuous light exposure, known as functional pinealectomy (Briaud et al., 2004; Delibas et al., 2002), is a potent circadian rhythm disruptor suppressing the endogenous circulating melatonin levels (Lewy et al., 1980; Revell and Skene, 2007). This leads to pathophysiological changes, including altered metabolism, endocrine system malfunction, free radical-induced molecular damage, and abnormal behavior in adults (de Matos Cavalcante et al., 2012; Erren and Reiter, 2009; Hardeland et al., 2012; Milczarek et al., 2010; Reiter et al., 2009; Tamura et al., 2013).

Up to date, the impact of prenatal continuous light exposure

Abbreviations: EPM, elevated plus maze; NOR, novel object recognition; OF, open field; PCLE, prenatal continuous light exposure; ROS, reactive oxygen species

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(PCLE) on adult offspring behavior is still poorly understood. Fetal circadian clocks begin to form in the second half of the pregnancy (Sladek et al., 2004), thus in the present study we explored the long-term effects of PCLE during this period of gestation. We assessed the behavior of adult offspring male rats and inquired the related molecular changes. We assessed brain melatonin, serotonin, and reactive oxygen species (ROS) levels and explored expression of genes related to melatonin/serotonin and their respective receptors, oxidative stress balance, apoptosis, and circadian rhythm.

2. Results

2.1. Behavioral changes

2.1.1. Open field test

In the open field (OF) test, PCLE rats showed significantly decreased mobility and exploratory activity, as indicated by an 11.7-fold reduction of the time spent in the central zone (Fig. 1A) and absence of central area crossings (Fig. 1B). The significantly higher number of defecations (3.2-fold) in the PCLE group compared to controls was also suggestive for increased anxiety (Fig. 1C).

2.1.2. Elevated plus maze test

In the elevated plus maze (EPM) test, PCLE rats moved inside the maze over a mean distance of 798 cm, while controls were significantly more mobile ($p=0.007$), with an average distance of 1,288 cm (Fig. 2A). Time spent in the central area was decreased by 5.9-fold (Fig. 2B) and time spent in the open arms of the maze was decreased by 6.5-fold (Fig. 2C) in PCLE group vs. controls. A similar reduction was observed in the number of crossings (2.9-fold) through the central area of the maze (Fig. 2D). Conversely, time spent in the closed arms of the maze was significantly higher (1.5-fold) in the PCLE group compared to controls (Fig. 2E).

2.1.3. Novel object recognition test

In the novel object recognition (NOR) test, during the short-term memory trial, with a retention period of 5 min, PCLE rats took significantly less time (1.5-fold) to explore the novel object (Fig. 3A). However, the time spent with the familiar object did not differ significantly between the two groups. The preference for novel vs. familiar objects, assessed through the discrimination index, was mainly for the novel object in the case of the control group (6 out of 8 rats), while the PCLE rats showed a strong preference ($p=0.027$) for the familiar object (7 out of 8 rats) (Fig. 3B).

The long-term memory trial, with a retention period of 24 h, showed no significant differences between PCLE group and controls (data not shown).

2.2. Molecular evidence for neural alterations

The PCLE model is the equivalent of a functional pinealectomy (Briaud et al., 2004; Delibas et al., 2002). To test the efficacy of the PCLE used, we measured melatonin levels. Since melatonin does not have a specific target in the brain, as it can act for example on the hypothalamus, where it inhibits neuronal firing, or in the midbrain and the result is forebrain dopamine regulation (Smith, 1985), we considered that whole-brain melatonin levels are more informative than the regional ones. Serotonin acetylation is involved in melatonin synthesis (Ganguly et al., 2002) and both melatonin (Cisternas et al., 2010; Ferreira et al., 2012; Voiculescu et al., 2015) and serotonin levels (Gurtman et al., 2002) can influence behavior. We thus measured melatonin and serotonin levels and expression levels of genes related to their metabolism. Moreover, since melatonin influences oxidative stress balance (Reiter et al., 2000) we also inquired the effect of PCLE on ROS and oxidative stress enzyme expression levels. Lastly, we investigated the effect of PCLE on different circadian-related genes.

2.2.1. Melatonin and serotonin levels in the brain

Melatonin levels in offspring brains at the time of birth and in adulthood showed a significant decrease both immediately after birth ($p=0.022$; Fig. 4A) and in adult offspring ($p=0.009$; Fig. 4B). Serotonin levels in offspring brains were decreased ($p=0.01$) in PCLE adults (Fig. 4C), but not in newborn rats.

2.2.2. ROS levels in the brain

Our study showed a significant increase of ROS measured in whole brain homogenates from PCLE offspring immediately after birth ($p=0.006$; Fig. 5A) and from adult PCLE offspring ($p=0.002$; Fig. 5B) compared to controls.

2.2.3. qPCR results

2.2.3.1. Melatonin-related genes. Gene expression levels of the rate-limiting melatonin synthesizing enzyme *Aanat* (arylalkylamine N-acetyltransferase), and melatonin receptors (*Mtr1a, Mtr1b)* were not significantly different between controls and the PCLE group in none of the regions (data not shown).

2.2.3.2. Serotonin related genes. Tryptophan hydroxylase *Tph1* and *Tph2* mRNA levels did not reveal any significant difference between the control and PCLE groups (data not shown). There were no significant differences in the serotonin receptor *Htr1a* gene expression levels between the two groups in either of the investigated brain areas (data not shown). For the serotonin

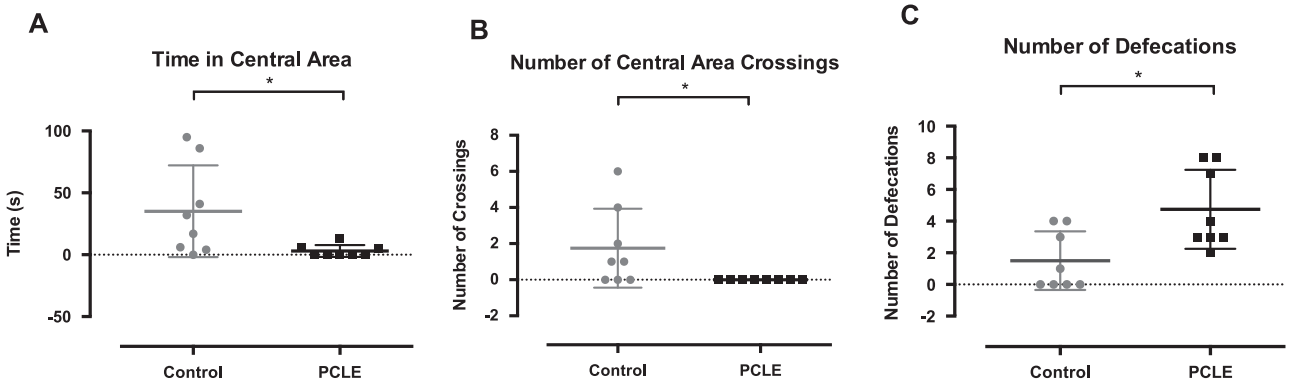

Fig. 1. Anxiety assessment in the OF test. PCLE rats spent shorter time in the central area of the open arena (A) and did not cross the central area (B). The higher number of defecations in the PCLE group is suggestive for increased anxiety (C). Data are expressed as mean ± SD, $n=8$ rats per group, $^{*}p < 0.05$ calculated with a non-parametric Mann-Whitney-Test.