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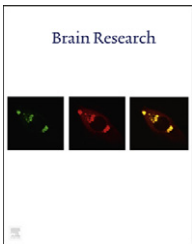

Research Report

Behavioral changes after maternal separation are reversed by chronic constant light treatment

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ABSTRACT

Background: Rats subjected to maternal separation display behavioral alterations (e.g. increased immobility in the forced swim test) and molecular changes (e.g. in growth factors and related signal transduction proteins). Light treatment has previously been shown to have antidepressant effects in rat models of depression, but has not been studied in a rodent model of maternal separation. *Methods*: This study focused on maternally separated rat pups. The aim of this study was to compare the effects of chronic constant light exposure during adolescence with the selective serotonin reuptake inhibitor (SSRI), escitalopram. Behavioral changes (exploratory activity in the open field and elevated plus maze, 22 kHz ultrasonic vocalizations, immobility in the forced swim test) and molecular changes (brain-derived neurotrophic factor (BDNF), mitogen-activated protein kinase phosphatase-1 (MKP-1) in the ventral hippocampus, and mu-opioid receptors in the nucleus accumbens) were measured. *Results*: Animals that had been subjected to maternal separation displayed an increased number and duration of 22 kHz vocalizations, increased immobility in the forced swim test, increased hippocampal BDNF, and decreased mu-opioid receptor levels in the nucleus accumbens in adulthood compared to controls. MKP-1 levels in the ventral hippocampus were not affected. After chronic light treatment, there was normalization of ultrasonic vocalizations, immobility on the forced swim test, and mu-opioid receptor levels in the nucleus accumbens. Chronic saline treatment reduced anxiety-like behavior and immobility in the forced swim test. Escitalopram did not have any significant effect in this rat model of depression. *Conclusion*: Chronic constant light treatment reversed a number of the behavioral and molecular effects of maternal separation. Light-induced up-regulation of mu-opioid receptors in the nucleus accumbens may play a key role in mediating such effects.

1. Introduction

Exposure to stressors during the early stages of development has been shown to have a negative impact on psychopathology in later life, and has been associated with a range of mood and anxiety disorders (Heim et al., 1997; Ladd et al., 2000). Rat pups subjected to repeated separation from the dam (for 3hours per day during the first 2 weeks of life) develop depression-like

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behavior (increased immobility in the forced swim test) in adulthood (Aisa et al., 2007; Marais et al., 2008). This provides a useful model to study the molecular basis of behavioral alterations induced by early adversity.

There has, for example, been significant interest in the role of the limbic serotonergic system in mediating behavioral changes induced by early exposure to stressors. Treatment with selective serotonin reuptake inhibitor (SSRI)s has been shown to normalize behavioral and molecular alterations seen after early rodent and non-human primate separation (Marais et al., 2006; Uys et al., 2006). In particular, it was found that glucocorticoid receptor down-regulation in response to trauma was corrected by escitalopram treatment (Uys et al., 2006). Escitalopram treatment also partly compensated for protein changes induced by maternal separation stress which included up-regulation of proteins involved in energy metabolism and neurotransmitter release (Marais et al., 2008). Such work may be useful in thinking through the role of the serotonin system in human depression (Binder et al., 2011; Hui et al., 2010; Williams et al., 2000). The role of many other psychobiological systems in early adversity nevertheless remains poorly explored. There is growing interest in the neurobiology of circadian rhythms, and it is notable that light exposure during the rat's dark phase may have antidepressant effects, reducing time spent immobile in the forced swim test (Iyilikci et al., 2009; Schultz et al. (2008). The effect of constant light exposure has not however been investigated in the maternal separation model of depression.

It may be hypothesized that if chronic light therapy has positive effects in the maternal separation model, that the relevant molecular systems are those that have been shown to be important in previous work on stress and depression. Signal transduction pathways active during stress and depression include mitogen-activated protein kinase (MAPK) pathways, regulated by mitogen-activated protein kinase phosphatase-1 (MKP-1) which has a dual effect on MAPK activity. MKP-1 gene expression is induced by MAPK activating stimuli and MKP-1 also deactivates MAPK by dephosphorylating ERK1/2 MAPKs and hence decreases CREB-dependent transcription of BDNF (Akbarian and Davis 2010; Jeffrey et al., 2007; Sun et al., 1993). Duric et al. (2010) found that MKP-1 is increased in post-mortem hippocampal tissue of depressed individuals, and that chronic unpredictable stress in rats, resulted in increased MKP-1mRNA and MKP-1 protein levels in the hippocampus and depression-like behavior, which were partially normalized by treatment with an SSRI. Thus MKP-1 may well play a role in mediating the effects of maternal separation.

Nocturnal induction of MKP-1 levels in the rat pineal gland has been found to be blocked by constant light treatment (Price et al., 2004), and may possibly play a role in controlling melatonin production. Melatonin secretion is controlled by the suprachiasmatic nucleus (SCN) in the ventral hypothalamus which is stimulated by light activated melanopsinergic ganglion cells in the retina (Challet, 2007; Ebling, 1996). Also, the SCN receives serotonergic inputs from the midbrain raphe nuclei in response to behavioral arousal with serotonin aiding in resetting of the SCN circadian clock (Jacobs et al. 1990; Meyer-Bernstein and Morin 1996).

Another system that appears to be particularly relevant to maternal separation, and that may therefore play a key role in mediating its behavioral effects, is the opioid system. Maternal separation stress can significantly alter the rewarding effects of opioid agonists (Michaels and Holtzman 2008). Morphine decreased separation-induced distress vocalizations, while opioid receptor antagonists potentiated separation-induced distress (Herman and Panksepp 1978; Kehoe and Blass 1986; Panksepp et al., 1978). Most rodents communicate via ultrasonic vocalizations (Portfors, 2007) of which the 22 kHz vocalization occurs in response to aversive stimuli and reflects a negative affective state (Litvin et al., 2007). It has been found that melatonin, which is regulated by light via the melanopsinergic ganglion cells in the retina influencing the stimulation of the SCN, has analgesic effects via mu-opioid receptors in the pineal gland (Wilhelmsen et al., 2011). We hypothesized that treatment of rats exposed to early maternal separation with light would result in normalization of ultrasonic vocalizations and of opioid receptors.

This study aimed (1) to compare the effects of chronic constant light stress during the adolescent period and escitalopram treatment on behavior in adulthood and (2) to investigate molecular changes (in growth factors and related signal transduction molecules, as well as in the mu-opioid system) effected by maternal separation and by such treatments.

2. Results

2.1. *Locomotor activity in the open field*

There was no difference in locomotor activity between control and maternally separated animals on P28 ($t_{(51)}=-1.508$, $p=0.137$, Table 1). The result ensured that immobility recorded in the forced swim test was not due to a locomotor deficit and hence is reflective of a depressive phenotype.

2.2. *Anxiety-like behavior assessed in the elevated plus maze*

One-way ANOVA revealed no significant differences in anxiety-like behavior between maternally separated and control rats during adolescence (P40) in terms of time spent in the open or closed arms of the elevated plus maze. However, in adulthood, at P99, a main effect of maternal separation was found in time spent in the open arms of the elevated plus maze ($F_{(1,49)}=4.510$, $p=0.039$). Maternally separated rats spent more time in the open arms than non-separated rats ($p<0.05$, Table 1). Repeated measures ANOVA of the time spent in the open arms at P40 and P99 i.e. before and after saline treatment, revealed a significant difference between groups. There was a significant main effect of stress (control, maternal separation, maternal separation followed by constant light exposure, $F_{(2,23)}=5.840$, $p=0.008$) and time (before vs after treatment with saline $F_{(1,23)}=9.606$, $p=0.005$). Duncan's post-hoc test revealed that maternally separated animals spent more time in the open arms of the elevated plus maze after saline treatment than before treatment ($p=0.003$). Repeated measures ANOVA showed that all animals spent significantly more time in the open arms after