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Long-term consequences of early maternal deprivation in serotonergic activity and HPA function in adult rat

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ABSTRACT

Increasing body of evidence indicates that early life stressful events may induce permanent alterations in neurodevelopment, which in turn, could lead to the development of psychopathologies in adulthood. In particular, maternal deprivation (MD) for 24 h in rats has been associated with several abnormalities in brain and behaviour during adulthood, relevant to the neurobiological substrate of anxiety disorders. The aim of the present study was to clarify the long-term effects of MD, on hypothalamo–pituitary–adrenal (HPA) axis activity and serotonergic (5-HT) function, in adulthood, subjects that have not been yet thoroughly investigated. For this purpose, Wistar rat pups were deprived from their mothers for a 24-h single period at postnatal day 9 (pnd 9) and were examined when aged 69–90 days. Plasma corticosterone and ACTH levels along with the animal's behaviour in an open field were used as indices of stress. Moreover, serotonergic activity was estimated in hypothalamus and hippocampus, key structures in the coordination of neuroendocrine and behavioural responses to stress. Interestingly, in adulthood, MD rats compared to controls, displayed decreased body weight, increased serotonergic activity and "anxiety" related behaviour, as well as elevated plasma corticosterone and ACTH levels. The findings of this study showed that MD results in long-term modifications in HPA axis and serotonergic activity indicating a clear relationship between early life stressful events and the development of anxiety-like disorders later in adulthood.

Accumulating evidence suggests that early life stressful events may induce permanent alterations in neurodevelopment [29] that in turn could be associated with the development of several psychopathologies during adulthood [15,16]. Studies involving animal models have shown that environmental rearing conditions during the neonatal period play a pivotal role in the establishment of neurobiological factors controlling behaviour and stress responsiveness [25]. In particular, maternal deprivation (MD) is known to produce a variety of behavioural and neurobiological alterations that are related to the pathophysiology of anxiety/depression [3,26,27] and schizophrenia [7,10].

A number of experimental studies have focused on behavioural or neuroendocrine alterations related to hypothalamo–pituitary–adrenal (HPA) axis activity, following MD (24 h) at postnatal day (pnd) 9. However, most of these studies investigated the short-term effects of MD in anxiety models on corticosterone (CORT) and adrenocorticotropin hormone (ACTH) levels, focusing mainly on the post-weaning period until adolescence [4,36,35]. There is limited information on the impact of MD (at pnd 9) in adult animals concerning stress-related variables.

Previous studies have shown that early MD at pnd 9 may affect dopaminergic activity in the adulthood [6,11]. Nonetheless, the effect of MD on serotonergic (5-HT) activity remains unclear. Although, 5-HT system dysfunction has been associated with dysregulation of the HPA axis in the pathophysiology of anxiety-like disorders [24,30], no study has yet focused simultaneously on the effect of early MD (at pnd 9) on both, serotonergic activity and HPA function as well.

Therefore, this study aimed to investigate the long-term effects of early MD at pnd 9, on HPA axis activity and 5-HT function, in the adulthood. For this purpose, we evaluated the "anxiety" profile of MD rats using the time spent in the periphery versus the time spent in the center of an open field apparatus, along with their plasma CORT and ACTH levels, as indices of their stress status. Tissue contents of 5-HT and 5-HIAA (its metabolite) were also evaluated

Abbreviations: ACTH, adrenocorticotropin hormone; ANOVA, analysis of variance; CORT, corticosterone; HPA, hypothalamo–pituitary–adrenal axis; HPLC, high-performance liquid chromatography; MD, maternal deprivation; MD rats, maternally deprived rats; PND, postnatal day; SHRP, stress-hyporesponsive-period; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid.

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in the hippocampus and hypothalamus of MD rats and controls, since it has been previously well defined as being implicated in anxiety disorders and associated with various neuroendocrine and behavioural responses to stress.

Male and female Wistar rats were obtained from the Animal Center of the University of Ioannina and were used throughout the experiments. The animals were housed in Techniplast cages (425 mm × 266 mm × 155 mm) in sets of one male and two females in a temperature-controlled room (21 ± 1 °C) on a standard 12 h light/dark (lights on at 7:00 h) cycle. Food and water were available ad libitum. Ten days later, male rats were removed and the females were left undisturbed until they gave birth. Approximately 21 days later, animals were checked twice daily (10:00 and 18:00 h) for delivery. The delivery day was designated pnd 0. All animal experiments were reviewed and approved by the local committee and all studies have been carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Efforts were made in order to use the minimal amount of rats necessary for experiments and minimize pain or discomfort.

Rat pups were separated from their mothers for a 24 h single period at pnd 9 ($n = 42$) [11]. The mothers were removed from the cage at 10:00 a.m. and placed in a single cage in the same room as the pups in order to minimize the possible existence of any other stressors except of MD for the dam and pups respectively. The pups were weighed and then returned in their home cages. Twenty four hours later, the pups were weighed again and the mothers returned to their litters. Control pups ($n = 34$) were weighed and returned immediately in their home cages. Twenty four hours later, pups were again weighed and placed back in their cages. After this, the litters were left undisturbed until weaning (pnd 21). At weaning (pnd 21) the male animals ($n = 45$, controls = 20, MD rats = 25) were housed in groups of 3 per cage and all experiments were performed between postnatal days 69 and 90.

MD and control rats ($n = 20$ and $n = 25$, respectively) were accustomed to the experimental room for at least 40 min prior to the experiment. The rats were then introduced into the testing cage (40 cm × 40 cm × 40 cm), in a low-luminosity environment and the time spent in the center along with the time spent in the peripheral zone was recorded during a 75 min registration period, using a computerized activity monitoring system (ENV515, Activity Monitor, version 5, Med Associates).

A subset of rats was used for CORT and ACTH determinations (MD = 5, controls = 5). These rats were handled for approximately 20 days and were accustomed to the experimental room (quiet and low-luminosity environment) for 1 h prior to decapitation. They were then sacrificed by rapid decapitation upon removal from their home cages and this procedure was performed between 10.00 and 14.00 for purposes of appropriate conditions needed for hormone's determinations. Blood samples were collected from the trunk in EDTA-containing tubes, centrifuged (4000 rpm for 15 min at 4 °C) and plasma was collected and stored at −80 °C until analysis. Plasma CORT was determined by RIA using the Coat-A-Count kit (Diagnostic Products Corporation, Los Angeles, USA). The detection limit was 5.7 ng/ml and the intra-assay coefficient of variation was 4.0%. Plasma ACTH was measured using a RIA (^{125}I-labeled) rat ACTH kit (DiaSorin, Hellas) with detection limit 15 pg/ml.

Another subset of rats (MD = 10, controls = 10) was used for the estimation of serotonergic activity. After decapitation, rat brains were rapidly removed. Hypothalamus and hippocampus were dissected on ice and they were immediately placed in liquid nitrogen. Once the tissue was weighed, each sample was homogenized and deproteinized using 0.2 N perchloric acid (Merck KgaA, Darmstadt, Germany) containing 7.9 mM $Na_2S_2O_5$ (Riedel-de Haën AG, Seelze, Germany) and 1.3 mM Na_2EDTA (Riedel-de Haën AG, Seelze, Germany). Homogenates were then centrifuged

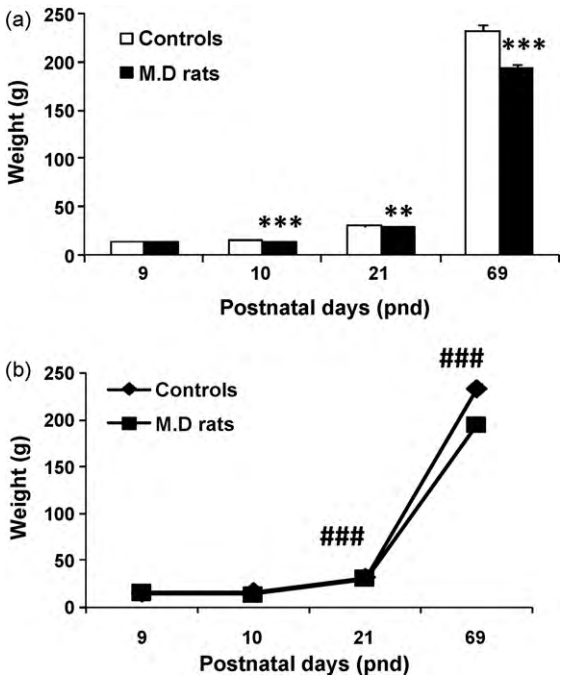

Fig. 1. The effects of MD stress on body weight (postnatal days 9, 10, 21 and 69). MD took place on postnatal day 9. The total numbers for pnd 9, pnd 10 and pnd 21 are: controls $n = 58$, MD rats $n = 65$; pnd 69: controls $n = 30$, MD rats $n = 35$. Means of body weight ± S.E.M. (a) Body weight over time in both experimental groups. $^{###}p \leq 0.001$ denotes a significant difference in body weight on pnd 21 and pnd 69 versus pnd 9. (b) Body weight of control and MD rats. $^{***}p \leq 0.001$, $^{**}p \leq 0.01$ denotes a significant difference between MD rats versus controls.

at 14000 rpm for 30 min at 4 °C. Supernatants were aspirated and stored at −80 °C until analysis. Tissue samples were analyzed for serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) using reverse phase ion-pair chromatography on a high-performance liquid chromatography system with electrochemical detection (HPLC-ED) [1]. The turnover ratio, 5-HIAA/5-HT, was then estimated and used as an index of 5-HT release, re-uptake and metabolism to 5-HIAA [1].

The statistical software SPSS 14.0 was used for all the statistical analyses. One-way analyses of variance (one-way ANOVA) with MD as factor were performed for all available data. A repeated two-way ANOVA with MD as a between factor and time as within factor, was used for body weight measurements. All figures presented here display mean values ± S.E. Student's t-test comparisons (two-tailed test) were used for comparison between time spent in the periphery and time spent in the center of the open field apparatus. Probability value of $p \leq 0.05$ was considered as significant.

A repeated two-way ANOVA with MD as between factor and time as within factor revealed a significant time effect [$F_{(3,189)} = 3691.895$, $p < 0.001$] and stress (MD) effect [$F_{(1,63)} = 35.143$, $p < 0.001$] as well as a significant interaction between MD and time [$F_{(3,189)} = 32.276$, $p < 0.001$] on body weight. Subsequent repeated ANOVAs revealed an increase in body weight over time in both experimental groups [$F_{(3,102)} = 1624.718$, $p < 0.001$ for controls, and $F_{(1,87)} = 3142.362$, $p < 0.001$ for MD rats, respectively] (Fig. 1a). Interestingly, additional one-way ANOVAs per day revealed that MD rats displayed a reduction in body weight in comparison with controls at pnd 10 [$F_{(1,122)} = 37.946$, $p < 0.001$], at pnd 21 [$F_{(1,122)} = 8.767$, $p = 0.004$] and at pnd 69 [$F_{(1,64)} = 33.506$, $p < 0.001$] (Fig. 1b).

MD rats during the 75 min registration period spent more time in the periphery and less time in the center of the open field apparatus as compared to controls [$F_{(1,44)} = 6.089$, $p = 0.01$; Fig. 2]. MD rats displayed a lower delay to enter in the center of the open field as