

## MATERNAL DEPRIVATION OF RAT PUPS REDUCES BODY WEIGHT AND ALTERS BEHAVIOR IN ADULTHOOD IN A GENDER-SPECIFIC MANNER

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**Abstract** - The early postnatal environment is critical for its capacity to influence adult behavior, and is associated with traits of altered physiological and neurobiological function and long-term predisposition to depression. Here we describe the delayed effects of maternal deprivation (MD) in male and female Wistar pups on their physical development and behavior in adulthood in tasks designed to explore depressive-like (forced swimming test, FST), and anxiety-like behaviors (elevated plus maze, EPM). We observed that MD led to reduced body weight in adulthood, anxiety-like traits in the EPM test and increased activity in the phases of the FST. Particularly, a consistent sexual dimorphism was observed in the responses to MD. A lower increase in body weight during maturation of MD rats was more pronounced in males than in females. MD anxiogenic effects were more pronounced in females, while in FST only MD males showed a marked increase in swimming activity followed by decreased immobility.

**Key words:** Maternal deprivation; behavior; forced swimming test; elevated plus maze

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### INTRODUCTION

There is accumulating evidence that early life stress exposure produces a diverse range of developmental outcomes, and predisposes to later vulnerability for physical and mental health problems (Agid et al., 1999; Heim and Nemeroff, 2001; Takase et al., 2012). This is because early experiences occur during a time of extraordinary brain plasticity, when the brain is maximally capable of being programmed in an enduring way (Knudsen, 2004). Animals subjected to early stress show a hyperactive HPA axis, alterations in neurotransmitter systems, increased responses to novelty and greater vulnerability for learned helplessness and drug self-admin-

istration. In rodent models, MD protocols represent models of disrupted parenting. They are based on the disruption of the mother-infant bond and they differ according to the duration of the separation period, the number of separation sessions and the timeframe in which the stressful conditions are applied (Veenema, 2009). Pups of most rodent species are poorly developed at birth and require intensive care by the dam to ensure homeostatic control and normal sensory and motor development. Later on, additional interactions with conspecifics are required for the development of appropriate social behaviors. Manipulations of dam-pup interactions as well as of early interactions with conspecifics can serve as useful tools to investigate the importance

of the early social environment for the development of social, emotional, and cognitive systems. The pups are not only deprived of maternal care during the separation, but maternal behavior typically remains aberrant even after reunion (Huot et al., 2004). Maternally separated female offspring is also more likely to display aberrant maternal care with their own litters (Gonzalez et al., 2001; Lovic et al., 2001), although not all studies have demonstrated this finding (Pryce et al., 2001). Despite the well-known higher prevalence in women, the majority of studies seeking to link the model of MD and depressive-like behavior have been conducted almost exclusively on male rats.

In the present study, we were particularly interested in gender-specific effects of diminished maternal care on adult behavior, since it is important to recognize sexual dimorphisms in the susceptibility to psychiatric disorders (Simic et al., 2013). MD has been reported to provoke behavioral abnormalities that resemble psychotic-like symptoms such a disruption in the pre-pulse inhibition response (Ellenbroek et al., 2005; Ellenbroek and Riva, 2003), neuroendocrine alterations related to stress reactivity (Lehmann et al., 2002; Levine et al., 1991; Rentesi et al., 2010), as well as cognitive impairments in adult animals (Llorente et al., 2011). Our primary aim was to characterize animal behavior that was triggered by MD, particularly those aspects that are similar to symptoms of depressive disorder, such as anxiety, despair and helplessness symptoms. Elevated plus maze (EPM) was used to assess the anxiety-like behavior (Walf and Frye, 2007), where anxious rodents show natural tendency to move in the closed space and to avoid the open zone of the maze. In addition to EPM, we also employed the forced swimming test (FST), which we used to measure behavioral despair, where immobility is a behavioral correlate of negative mood, representing helplessness (Porsolt et al., 1977). We demonstrated that the isolation of pups from the dam and littermates for 3 h per day on postnatal days (PND) 1-14 produced long-term behavioral impairments that are gender specific and reduced body weight in adulthood.

## MATERIALS AND METHODS

### *Maternal deprivation*

Adult pregnant Wistar rats were housed individually in Plexiglas cages and checked daily for delivery. Light was kept on, between 07:00 am and 07:00 pm; room temperature was kept at  $22\pm 2^\circ\text{C}$  and humidity at 50-55%. Rats were offered food (commercial rat pellets) and water *ad libitum*. The day of delivery was designated as postnatal day (PND) 0. Maternal deprivation was performed daily for three consecutive hours from 9:00 to 12:00 am, between PND 1 and PND 14, during which time the pups were removed from their home cage and kept in separate cages at  $26\pm 2^\circ\text{C}$ , where the bedding was changed every day. During MD, pups were individually isolated. Control pups were left undisturbed with their dam. From days 15 to 22, all control and MD pups were maintained with their dam. Rats of the same gender were weaned at PND 22 and housed in groups of four until adulthood (14 weeks). The body weight of each animal was measured once per week, starting PND22. All animal procedures were approved by the Ethical Committee for the Use of Laboratory Animals of the VINCA Institute of Nuclear Sciences, according to the guidelines of the EU registered Serbian Laboratory Animal Science Association (SLASA).

### *Elevated plus maze*

The standard elevated plus maze is commonly used to assess anxiety-like behavior in laboratory animals (Walf and Frye, 2007). The maze contains two open arms, 50 x 10 cm (length x width) and two closed arms, 50 x 10 x 50 cm (length x width x height). The maze is elevated to a height of 50 cm. The test relies upon the animal's natural tendency to stay in enclosed spaces and their unconditioned fear for open spaces and heights – anxious animals will spend more time in the closed arms than less anxious animals. The rats were placed individually in the center of the maze facing a closed arm and allowed 5 min of free exploration. The behavior of each animal in the maze was analyzed and scored by two observers unaware of the treatments applied, taking into account

the standard measures recorded in each section of the maze, comprising total arm entries (arm entry defined as all four paws into an arm), open arm entries, and time spent in the open arms of the maze. After each testing, the maze was cleaned. Male and female offspring were tested on the EPM at adulthood (PND 99).

#### *Forced swimming test*

FST is considered a standard test for the evaluation of depressive-like behavior in laboratory rodents (Porsolt et al., 1977; Mitic et al., 2013). FST was conducted 24 h after elevated plus maze testing. Briefly, rats were placed in a Plexiglas cylinder (50 cm tall, 20 cm in diameter) filled to 30 cm with water ( $24\pm 0.5^\circ\text{C}$ ). They were allowed to swim in the cylinder under conditions in which escape was not possible. After each test, the cylinder was cleaned. Each test session lasting 5 min was videotaped in a room dimly illuminated and later scored by two observers unaware of the treatments applied. A time-sampling technique (Page et al, 1999) was employed to score, every 5 s, one of the following behaviors: a) immobility, defined as the minimum movements done by animals to keep their nostril above the water (i.e. floating); b) swimming, active motions made by animals that result in movements within the pool (i.e. moving around the jar); c) climbing (and diving), defined as strong movements executed with forepaws in and out of the water usually against the walls. Thus, in a 5-min test a total of 60 counts, including immobility, swimming or climbing were obtained.

#### *Statistical analysis of data*

Data are presented as mean  $\pm$  SD for 8 animals per experimental group. For analyzing the body weight during maturation a General Linear Model for repeated measures (Mixed ANOVA) was used with time as within-subject factor and MD and gender as between-subject factors. The data from the behavioral test were analyzed using two-way ANOVA with MD treatment and gender as independent factors, followed by post hoc Tukey test. Values were considered statistically significant if the p value was less than 0.05.

## RESULTS

### *The effect of maternal deprivation on body weight*

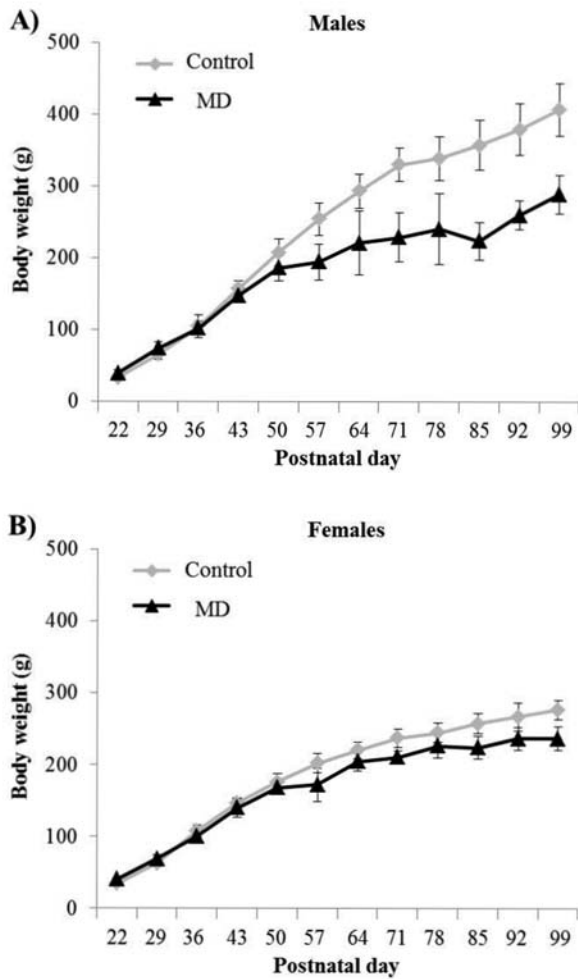
Body weight was measured in MD animals, as well as in the corresponding age-matched controls, once per week starting PND22. As can be seen in Fig. 1, the increase in body weight during maturation of both male and female MD rats was significantly lower than that of the corresponding controls (time x MD interaction:  $F=49.621$ ,  $p<0.001$ ). Additionally, the effect of MD on body weight was more pronounced in males than in females (time x MD x gender interaction:  $F=17.423$ ,  $p<0.001$ ).

### *The effects of maternal deprivation on animal behavior in the elevated plus maze*

Elevated plus maze (EPM) is a test used for assessing anxiety-like behavior. Our results showed a significant effect of MD on the percent of open arm entries ( $F=10.427$ ,  $p=0.004$ ) and closed arm entries ( $F=10.427$ ,  $p=0.004$ ), as well as on the time spent in the open arms ( $F=8.459$ ,  $p=0.007$ ) and closed arms ( $F=5.804$ ,  $p=0.023$ ). As shown in Fig. 2, only MD females had a significant decrease in the percent of open arm entries and in the time spent in the open arms ( $*p<0.05$ ) compared to female controls, which was followed by an increase in the percent of closed arm entries and the time spent in them ( $*p<0.05$ ).

### *The effects of maternal deprivation on animal behavior in the forced swimming test*

In the forced swimming test, MD had a significant effect on swimming ( $F=47.881$ ,  $p=0.000$ ) and immobility ( $F=45.704$ ,  $p=0.000$ ), while there was no significant effect on climbing activity ( $F=1.006$ ,  $p=0.326$ ). There was also a significant effect of the interaction of gender and MD on swimming ( $F=13.728$ ,  $p=0.001$ ) and immobility ( $F=11.578$ ,  $p=0.002$ ). Females demonstrated higher climbing activity than males (gender effect,  $F=15.298$ ,  $p=0.001$ ). The results presented in Fig. 3 show a significant increase in swimming ( $*p<0.05$ ), followed by a decrease in immobility time in male MD rats compared to controls ( $*p<0.05$ ), while climbing remained unaffected. The



**Fig. 1.** Body weight of control and maternally deprived (MD) male A) and female B) rats measured once per week starting postnatal day (PND) 22. Data and presented as means  $\pm$  SD.

behavior of the MD females showed a similar trend, but there was no statistical significance.

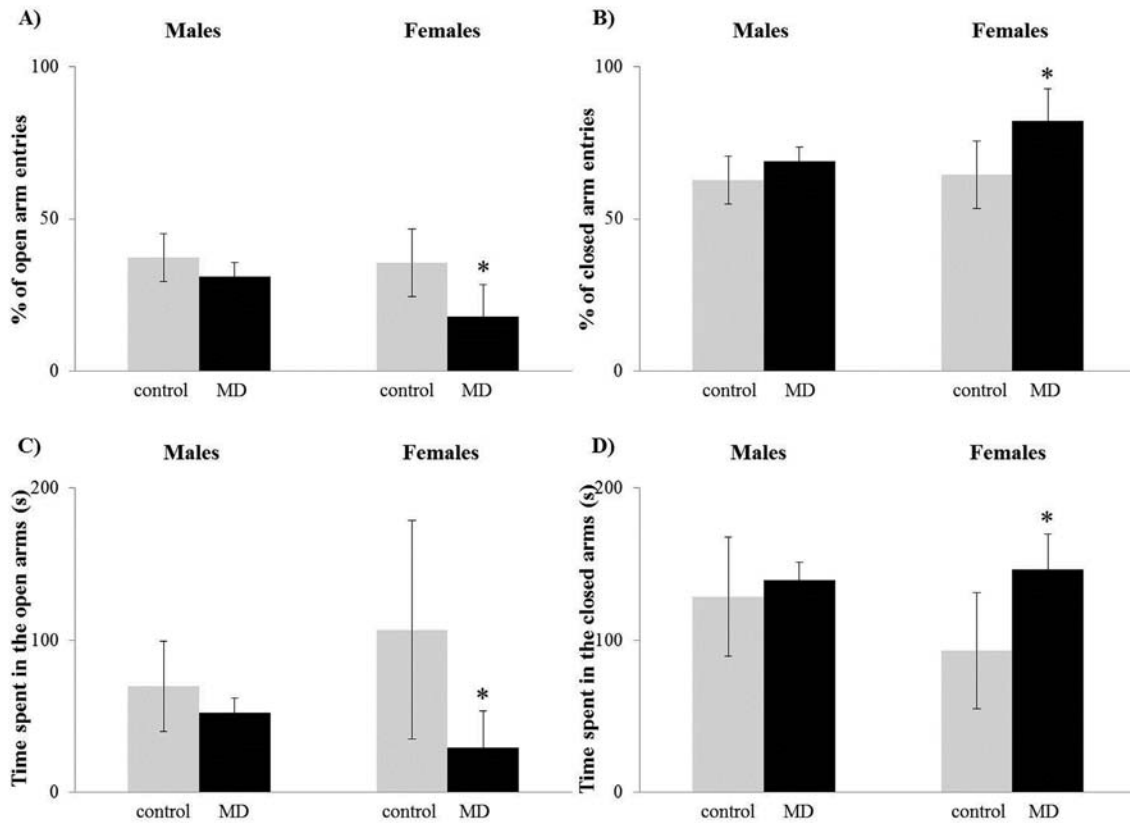
## DISCUSSION

Over the last few years, several lines of investigation have suggested that adverse life events during infancy are able to affect the immature neural circuitry and increase susceptibility to depression (Newport et al., 2002). The relation between the quality of the early environment and health in adulthood appears to be mediated by parental influences on the devel-

opment of neural systems that underlie the expression of behavioral and endocrine responses to stress later in life. The overall aim of this study was to determine whether early MD could influence behavior in adult Wistar rats, particularly those aspects that are similar to the symptoms of depressive disorders, such as anxiety, despair and helplessness symptoms. Our findings demonstrated that early MD stress produced long-term behavioral impairments and reduced body weight in adulthood in a gender-specific manner.

Growth assessment is an essential component of pediatric health surveillance because almost any problem within the physiologic, interpersonal, and social domains can adversely affect growth (Ali, 2013). It is reported that cardiac response, secretion of growth hormone (GH), sleep/wake rhythms and metabolism are all disturbed after maternal separation. The reduced body weight in MD animals that we detected was in accordance with other studies. Namely, decreased body weight was observed not only in MD pups, but also during their adolescence and adulthood (Husum et al., 2002; Ellenbroek et al., 2005; Llorente et al., 2007). The persistent nature of this effect suggests that it might not be solely due to the lack of milk ingestion during deprivation period. Viveros et al. (2009) propose that the decreased leptin levels and increased corticosterone they found in neonatal MD rats (PND 13) might affect the metabolic developmental programming of the hypothalamic circuitry involved in the regulation of energy balance. Hupa et al. (2014) showed that MD pups had a retarded postnatal lung development compared with untreated controls. Some authors suggest that MD is associated with a specific suppression of tissue response to growth-promoting peptide hormones such as GH (Kuhn et al., 1979), since circulating GH is responsible for the generation of the somatomedins, which are among the major regulators of muscle and possibly organ growth.

Beside physical development, MD has been reported to provoke behavioral abnormalities. The elevated plus maze is one of the most widely used tests in animal models in contemporary preclinical research



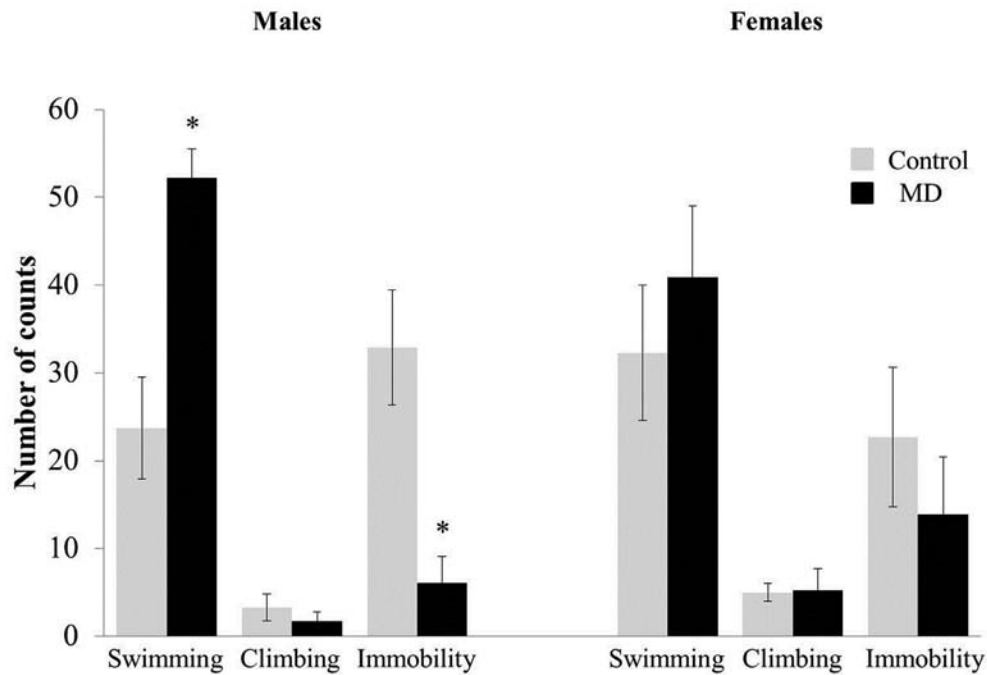
**Fig. 2:** Percent of open arm entries A), closed arm entries B), time spent in the open arms C) and time spent in the closed arms D) of the elevated plus maze in control and maternally deprived (MD) male and female rats. Data and presented as means  $\pm$  SD, \* $p < 0.05$  MD vs. control.

on anxiety (Handley and Mc Blane, 1993; Hog, 1996). Our results revealed that MD significantly reduced the percent of open arm entries and the time spent in them, which reflects the conflict between fear and exploratory activity and serves as a common indicator of anxiety-like behavior. Although the absolute number of closed arm entries was not altered, MD animals spent significantly more time in these arms of the maze than controls. Interestingly, our results also showed that anxiogenic effects of MD were statistically significant only in females, while in males there was only a trend. Anxiety-related behaviors in MD animals were also reported in the studies of Jia et al. (2009) and Rentesi et al. (2010). Sex-dependent alterations in the behavior of adolescent rats upon MD have already been reported in the studies of Viveros and coworkers (Viveros et al., 2009; Marco et

al., 2013), and they linked these alterations with the decreased concentration of the stress hormone, corticosterone, which was found only in MD females.

The results of the FST were somewhat surprising since the test phases of the FST showed pronounced swimming activity only in MD males, with almost no immobility segments. Results from other laboratories are rather contradictory. While some authors reported increased immobility in MD rats (Hui et al., 2011), others demonstrated increased locomotor activities (Brake et al., 2004; Hall et al., 1999). Contradictory results can be attributed to different procedural approaches or rat strains used in individual experiments. Mueller and Bale (2008) showed that male offspring exposed to prenatal stress had altered stress-coping responses and depressive-like behav-





**Fig. 3.** Changes in swimming, climbing and immobility in the forced swimming test (FST) in control and maternally deprived (MD) male and female rats. Data and presented as means  $\pm$  SD, \* $p < 0.05$  MD vs. control.

iors, whereas females were not affected. Although very unlikely, MD of rodent pups can be a stimulus for maternal grooming (Newport et al., 2002) so that behavioral changes induced by MD may be mediated indirectly through changes in maternal care. Increased maternal care may be one mechanism by which MD reduces immobility, but it may not be the only or even a necessary mechanism for the FST-reported MD effect. The decrease in immobility was due to increased swimming activity, while no alterations were detected in the climbing activity. It is important to make a distinction between climbing and swimming activities since they appear to be two separate responses, linked sequentially in series that facilitate escape during the FST. Literature data show that the integrity of the serotonin (5-HT) pathway is indispensable for the selective serotonin reuptake inhibitor reduction of immobility time and increase of swimming, but not necessary for actions of selective noradrenalin reuptake inhibitor (Page et al., 1999). Since swimming is the behavioral mani-

festation of selective treatment with 5-HT reuptake inhibitors (Djordjevic et al., 2012), our results indicate that a possible candidate in the association between early life MD and adulthood psychiatric disorders in males could be the central serotonergic system, which is also abnormal in psychiatric illnesses. Maternal deprivation has been associated with decreased 5-HT concentrations in the dorsal hippocampus and prefrontal cortex of offspring, but this finding is in contrast to other results demonstrating no alterations in the density of 5-HT fibers in the prelimbic, anterior cingulate, and precentral medial cortices of MD rats (Newport et al., 2002). Our study also showed that regardless of the MD treatment, there was a gender-specific profile of the behavior in the FST, where females displayed more climbing activity than males. According to the literature, an enhancement of climbing behavior appeared to be a product of noradrenergic reuptake inhibition, so gender differences in susceptibility to stress could lie in the noradrenergic system.

Overall, our study showed that early MD stress produces long-term behavioral impairments that are gender specific. These divergent responses in male and female rats emphasize the necessity of considering the potential influence of gender in animal and human studies related to neurodevelopmental psychiatric disorders. Schmidt (2011) proposes that sexual dimorphisms could be due to differences in adaptive plasticity and the ability to respond properly to the environmental conditions, while Schwarz and McCarthy (2008) believe that these differences may be attributable to the organizational effects of gonadal steroids in the brain. It is also worth mentioning that sensitive periods vary between males and females. Namely, studies showed increased depressive-like behavior in female but not male rats following chronic adolescent stress, while early developmental models showed that males are more affected by stress (Pignatelli et al., 2006).

Animal studies based on early life adversity can potentially yield environmental models of the developmental behavioral neurobiology of depression. Further investigation is needed to understand the neurobiological mechanisms underlying the sexual dimorphisms associated to MD effects, and thereby better understand the specific sex-dependent vulnerabilities to early life stress.

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