BRAIN RESEARCH 1624 (2015) 479–488

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Environmental enrichment does not reverse the effects of maternal deprivation on NMDAR and Balb/c mice behaviors

Kubra Akillioglu[a,*], *M. Bertan Yilmaz*[b], *Ayper Boga*[c], *Secil Binokay*[a], *Sabriye Kocaturk-Sel*[b]

[a]*Division of Neurophysiology, Department of Physiology, Medical Faculty, University of Çukurova, Balcali, 01330 Adana, Turkey*

[b]*Department of Medical Biology and Genetics, Medical Faculty, University of Çukurova, Balcali, 01330 Adana, Turkey*

[c]*Department of Physiology, Medical Faculty, University of Çukurova, Balcali, 01330 Adana, Turkey*

ARTICLE INFO

Article history:
Accepted 13 August 2015
Available online 20 August 2015

Keywords:
Maternal deprivation
Environmental enrichment
NMDA receptor
Hippocampus

ABSTRACT

Early adverse life experiences have been associated with anxiety-like behavior and memory impairment. N-methyl-D-aspartate receptors (NMDARs) play an important role in brain development. Enriched environments are known to positively influence emotional and cognitive functions in the brain. We examined the effects of maternal deprivation (MD) on NMDAR subunits in the hippocampus, locomotor activity, anxiety behaviors, and learning-memory performance of Balb/c mice. We also examined whether these effects could be reversed by raising the offspring in an enriched environment. The mice were separated from their mothers for a single 24 h episode on postnatal day (PND) 9. The mice were weaned on day 21 and were housed under either standard (SE) or enriched (EE) environmental conditions. Emotional behaviors and cognitive processes of mice were evaluated using an open field (OF) test, an elevated plus maze (EPM) test, and a Morris water-maze (MWM). NMDAR subunits (GluN1, GluN2A, and GluN2B) mRNA expression levels in the hippocampus were examined by real-time PCR. In OF, MD had no effect on horizontal locomotor activity. MD increased anxiety-like behaviors in the EPM and decreased spatial learning performance in MWM; however, these effects were not reversed by EE. MD (in SE and EE conditions) increased GluN1, GluN2A, and GluN2B mRNA expressions in the hippocampus. In conclusion, MD led to the deterioration of the emotional and cognitive processes during adulthood. Moreover, environmental enrichment did not reverse the deleterious effects of the MD on emotional and cognitive functions and increased the NMDAR levels.

*Corresponding author.
E-mail address: kakillioglu@cu.edu.tr (K. Akillioglu).

http://dx.doi.org/10.1016/j.brainres.2015.08.009
0006-8993/

1. Introduction

Adverse life experiences during the neonatal period can have effects on behavior and on the neurobiology of brain development (Lehmann et al., 2002). Numerous animal experiments have clearly demonstrated that manipulations of neonatal rodents affect their behavioral responses later in life (Cirulli, 2001; Levine, 1957; Ellenbroek et al., 2004). In particular, the interruption of routine mother–infant interactions may lead to permanent changes in neurobiology, physiology, and emotional behavior in adulthood (Ellenbroek et al., 2004; Pryce et al., 2005). Maternal deprivation (MD) was used to examine the consequences of adverse early life experiences. In rodents, the separation of pups from their mother is typically conducted between postnatal days (PND) 2 and 14. Among these models, a 24 h time period of MD at PND 9 has been used as an animal model of early-life stress that is highly appropriate for the investigation of developmental psychiatric disorders (Navailles et al., 2008; Akillioglu et al., 2012). Many studies have demonstrated that, in rats and mice, a single or repeated separation of the pups from the mother leads to acute and long-term effects on the endocrine system and behavior (Ellenbroek et al., 2004; Lehmann et al., 1999; Daniels et al., 2004). MD in rodents, particularly at a single period of 24 h at PND 9, has been proposed as a potential animal model for psychiatric disorders (Llorente-Berzal et al., 2013; Macri and Laviola, 2004; Holmes et al., 2005; Ellenbroek et al., 2005).

N-methyl-D-aspartate receptor (NMDAR) mediated glutamate transmission is important at multiple levels of brain development and has been implicated in cognitive functions and emotional responses in the brain (Akillioglu et al., 2012; Sircar, 2000). NMDARs are hetero-oligomers consisting of GluN1 and GluN2 (GluN2A, GluN2B, GluN2C, and GluN2D) subunits (Schorge and Colquhoun, 2003; Luo et al., 1997). The GluN2 subunit, present in a functional NMDAR, determines the physiological characteristics of NMDAR (Luo et al., 1997; Boyce-Rustay and Holmes, 2006; Cull-Candy et al., 2001). In the adult hippocampus, GluN2A and GluN2B are the predominantly expressed NR2 subunits (Schorge and Colquhoun, 2003).

Enriched environments positively influence cognitive functions and produce neuroanatomical changes in the mammalian brain (Akillioglu et al., 2012; Pryce et al., 2002; Tang et al., 2001). Environmental enrichment combines many beneficial components such as novel stimuli, physical and exploratory activity, and social interaction (Tang et al., 2001). Animal studies have shown that an enriched environment is protective against neurodegenerative illnesses that affect future learning and memory performance (Nilsson et al., 1999; Monteiro et al., 2014).

Anand and Scalzo (2000) postulated that a critical window of neonatal brain development occurs around the time of birth in human neonates and at days 6–9 in rat pups. This period also coincides with the peak rates of brain growth and new synapse creation (Rakic, 1998), and with a peak density of NMDARs (Rao et al., 1997). Therefore, NMDARs may mediate the effects of MD during PND 9. Experimental studies have demonstrated behavioral or neuroendocrine changes related to hypothalamo–pituitary–adrenal (HPA) axis activity following MD (Lehmann et al., 1999; De la Fuente et al., 2009; Own et al., 2013). There is limited information on the impact of MD (24 h at PND 9) on NMDAR levels (Pickering et al., 2006; Wilber et al., 2009; Roceri et al., 2002). To the best of our knowledge, no study has simultaneously investigated the effect of MD and physical environmental enrichment on both NMDAR levels and long-term behavior. We examined the effects of MD (24 h at PND 9) on NMDAR subunits (GluN1, GluN2A, and GluN2B) in the hippocampus, locomotor activity [open field (OF)], anxiety behaviors [elevated plus maze (EPM)], and learning-memory performance [Morris water maze (MWM)] of Balb/c mice. We simultaneously examined whether these effects could be reversed by raising the offspring in an enriched environment. We selected the Balb/c mouse strain for this study because of some emotional behaviors, which may be specific to the Balb/c strain (Akillioglu et al., 2012). For example, Balb/c mice display more anxiety-like behavior (Akillioglu et al., 2012; Mehta and Schmauss 2011) and reduce maternal behavior as compared to other strains (e.g., C57BL/6) (Mehta and Schmauss, 2011). Therefore, Balb/c mice may be more susceptible to MD than other strains. In addition, Millstein and Holmes (2007) suggested that MD (180 min/day, at PND 0–13) does not alter adult emotional behavior in six inbred mouse strains.

2. Results

2.1. *Body weight*

The weights of the MD-SE and MD-EE animals were measured between PNDs 10–56. A repeated measures two-way ANOVA found a significant MD effect [$F(1,37)=26.2$ $p<0.001$], environmental effect [$F(1,37)=37.1$ $p<0.001$], and MD × environmental interaction [$F(1,37)=63.9$ $p<0.001$] on body weight measurements.

MD-SE caused a decrease in the body weight from PND 21 to PND 56 (PND 21 $p<0.01$, PND 28, 49, 56 $p<0.05$, PND 35, 42 $p<0.001$) compared to the ND-SE. MD-EE caused a decrease at PND 21 ($p<0.05$) in the body weight compared to the ND-EE. MD-EE led to a significant increase between PNDs 28–56 in the body weight compared to the MD-SE (PND 28, $p<0.01$; PND 35–56, $p<0.001$) (Fig. 1).

2.2. *OF*

There were significant differences in the distance traveled [$H(3, N=45)=11.7$, $p<0.01$]. MD-SE showed no difference in the distance traveled compared to the ND-SE ($p>0.05$). MD-EE caused an increase in the distance traveled compared to the ND-EE ($p<0.05$). The ND group in the EE led to a decrease in the distance traveled compared to the ND group in the SE ($p<0.05$) (Fig. 2).

In the OF test, the time spent in the center of the apparatus was not significantly affected by MD [$F(1,41)=1.6$ $p>0.05$], environmental effect [$F(1,36)=0.08$ $p>0.05$], or an MD × environment interaction [$F(1,41)=0.6$ $p>0.05$]. There were no significant differences in the frequency of center-crossing in the OF [$H(3, N=45)=4.8$ $p>0.05$]. A significant