ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research paper Acta Neurobiol Exp 2013, 73: 394–403

Long-term effects of the maternal deprivation on the volume and number of neurons in the rat neocortex and hippocampus

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Maternal deprivation (MD) leads to a variety of behavioral changes in rats which closely resemble the symptoms of schizophrenia in humans. With the aim to investigate the morphological changes which underlie the behavioral insults in this experimental paradigm we exposed 9-day-old Wistar rats to a 24 h MD. At the period of young adulthood rats were sacrificed for morphometric analysis and their brains were compared to the control group. Rats exposed to MD had a decrease in hippocampal volume (71% of the control value) as well as a decrease in the size of pyramidal (62% of the control) and granular (60% of the control) cell layers. Also, there was a decrease in the thickness of the prefrontal, retrosplenial and motor cortex compared to the control group. Analysis of the density of NeuN-immunolabeled neurons revealed a reduction in retrosplenial and prefrontal cortex (70% and 81% of the control, respectively), while there was no difference in the motor cortex. Western blot analysis confirmed a decrease in NeuN expression in the MD group compared to the control rat brain homogenates. The results of this study show that early stress in life has a long-term effect on the morphology of cognitive brain regions, most probably due to the loss of neurons during postnatal development and further contributes to our understanding of the effects of maternal separation on brain development.

Key words: cortical thickness, hippocampal volume, maternal deprivation, schizophrenia

INTRODUCTION

Available evidence suggests that schizophrenia is associated with subtle but widespread changes in brain morphology (Shelton and McNamara 2001). Gross morphometric studies of brain structures in schizophrenic patients generally confirm *in vivo* neuroimaging findings of enlarged ventricles, a decreased size of ventromedial temporal lobe structures and parahippocampal cortical thickness and an increase in the gyrification index (DeLisi et al. 1988, Buschbaum 1990, Bogets et al. 1991, Degreef et al. 1992, Harvey et al. 1993, Kleinschmidt et al. 1994, Arnold and Trojanowski 1996, Honer et al. 1996, Frith 1997). Morphometric microscopy studies revealed alterations in neuron density, size and shape in limbic, temporal (Scheibel and Kovelman 1981, Kovelman and Scheibel 1984, Falkai and Bogets 1986, Arnold and Trojanowski 1996, Zaidel et al. 1997), and frontal cortical regions (Akbarian et al. 1993a,b, Benes and Bird 1987, Benes et al. 1987, 1991, Goldman and Seleman 1997). Vita and coauthors (2006) showed that morphological changes are already present in the first-episode psychosis patients. Furthermore, studies of unaffected adult first-degree relatives of schizophrenic patients demonstrated reversed hemispheric asymmetry (Goghari et al. 2007a) and reduced cortical surface area (Goghari et al. 2007b) in the cingulate and the superior temporal cortex (Schultz et al. 2009). However, several longitudinal magnetic resonance imaging (MRI) studies in the first-episode schizophrenic patients have demonstrated progressive brain changes in the years following illness onset (Gur et al. 1998, Ho et al. 2003, Kasai et al. 2003, Nakamura et al. 2007, Sun et al. 2009). These data suggest that brain morphologic abnormalities in psychotic disorders might reflect

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Received 14 August 2012, accepted 29 May 2013

a combination of pre-existing vulnerability and changes associated with the first expression of psychotic symptoms (Pantelis et al. 2005, 2007).

Since the etiology and neuropathology of schizophrenia still largely elude us, development of the animal model for this severe psychiatric disease has remained a difficult and somewhat controversial undertaking. Consequently, there is no 'gold standard' for animal model of schizophrenia. Nonetheless, the models which focus on the neurodevelopmental hypothesis of schizophrenia have so far been the most successful. One of these is the maternal deprivation (MD) model in which rat pups are separated from their mothers for a single period of time (Ellenbroek and Cools 1998, 2000b, Husum and Mathe 2002). A wealth of information shows that exposure of mammals to early-life stress, such as MD or social isolation, adversely affect brain development and adult behavior (Harlow et al. 1965, Heim et al. 2004, Rapoport et al. 2005). It has been shown that MD leads to a large variety of behavioral changes in rats, including the disruption of the prepulse inhibition, an effect which could be temporarily reversed by antipsychotic treatment (Ellenbroek et al. 1998, Ellenbroek and Cools 2000b, Husum et al. 2002), and which closely resembles the symptoms of schizophrenia in humans. Moreover, MD leads to neurochemical changes, predominantly in the hippocampal formation, a neural structure often implicated in the neuropathology of schizophrenia, suggesting reduced synaptic plasticity (Harrison 1999, Weinberger 1999). Although the molecular mechanisms involved in producing these developmental adaptations are unclear, similar environmental disruptions during early life in humans may contribute to the development of common psychiatric disorders, such as bipolar disorder and schizophrenia, in genetically predisposed individuals.

The aim of this study was to elucidate long-term effects of MD on the rat brain morphology. We have studied the key cortical areas involved in the processing of information, i.e. the hippocampus and prefrontal and retrosplenial cortex, and the motor cortex as a control area using a morphometric approach. We show that in maternally deprived young adult rats, the volume of the hippocampus and cortical thickness are significantly reduced compared to the controls. Additionally, we demonstrate reduced density of neurons in the retrosplenial (RSCX) and prefrontal (PFCX), but not in the motor cortical area, confirmed as well by immunoblot. These findings further contribute to the bulk of evidence that early life stress can substantially alter brain morphology and thus its function.

METHODS

Animals and procedures

Male and four nulliparous female Wistar rats 3-months-old were put together in standard plexiglass cages with sawdust (26 × 42 × 15 cm), in a temperature controlled room (23 ± 1°C). The rats were on a standard 12h light/dark cycle with lights on from 07:00 AM to 07:00 PM, with water and food available *ad libitum*. Two weeks later the males were removed and the dams were checked twice daily for delivery. The day of delivery was denoted as postnatal day zero (P0). On P9, two litters were subjected to the MD procedure according to the previously published protocol (Ellenbroek et al. 1998, Roceri et al. 2002). Briefly, dams were removed from the litter at 10:00 AM, after which the pups were weighed and returned to their home cage. They remained in their home cage at room temperature for 24 h. On P10, the pups were weighed again and dams were returned to their cages. The dams of the control litters (2 groups) were briefly (3 min) removed from their home cages and the pups were weighed on both P9 and P10. All litters were later left undisturbed except for the routine cleaning of the cages, until P21 when litters were weaned and classified according to sex. For morphological and biochemical studies only male rats were used in order to avoid sexual dimorphism (Wooley and McEwen 1992) and many of the previous studies were performed on males (Own and Patel 2013, Vivinetto et al. 2013). Animals were sacrificed at the period of young adulthood (P60). All efforts were made to minimize animal suffering and reduce the number of animals used in the study. All experiments were carried out according to the NIH Guide for Care and Use of Laboratory Animals and were approved by the Local Bioethics Committee.

Tissue processing

For morphological analysis, 5 male animals from the control and experimental group were anaesthetized with chloral hydrate (3 mg/kg, i.p.) and transcardially

perfused with the fixative (4% formaldehyde in 0.1 M phosphate buffer). The brains were post-fixed for 24 h at +4°C and cryoprotected by infiltration with sucrose for 2 days at 4°C (20% sucrose in 0.1 M phosphate buffer). The brains were frozen by immersion in 2-methylbutane (Sigma-Aldrich, St. Louis, MO) precooled to −80°C and stored at −80°C until cutting. Serial transverse sections (25-µm-thick) were cut on a cryostat (Leica Instruments, Nußloch, Germany). Sections were collected on SuperFrost Plus glass slides (Menzel, Braunschweig, Germany) in a standard sequence so that four sections 250 µm apart were present on each slide.

Estimations of the hippocampal volume and cortical thickness

Whole hippocampus and its subfields were delineated (Fig. 1A) and the area was measured from the micrographs using Image Tool 3.0 software. The volumes of each subfield (CA1, CA2-3, in further text abbreviated as CA3, and the dentate gyrus, DG) and the whole dorsal hippocampus were calculated according to Cavalieri's principle. The same principle was applied to the measuring area and calculating the volume of the pyramidal cell layer of the whole Ammon's horn (CA1-3) and granular cell layer of the DG (see Fig. 1B) and soma area of the pyramidal and granular cells of the hippocampus. All measurements were performed bilaterally.

The area of the cortical field (excluding layer I) was delineated and its surface measured from the micrographs. To estimate the average cortical thickness, the delineated cortical area was divided by the length of its boundary facing the meningeal surface. All measurements were performed bilaterally.

Immunohistochemistry

Immunohistochemical staining was performed after water-bath antigen retrieval in 0.01 M sodium

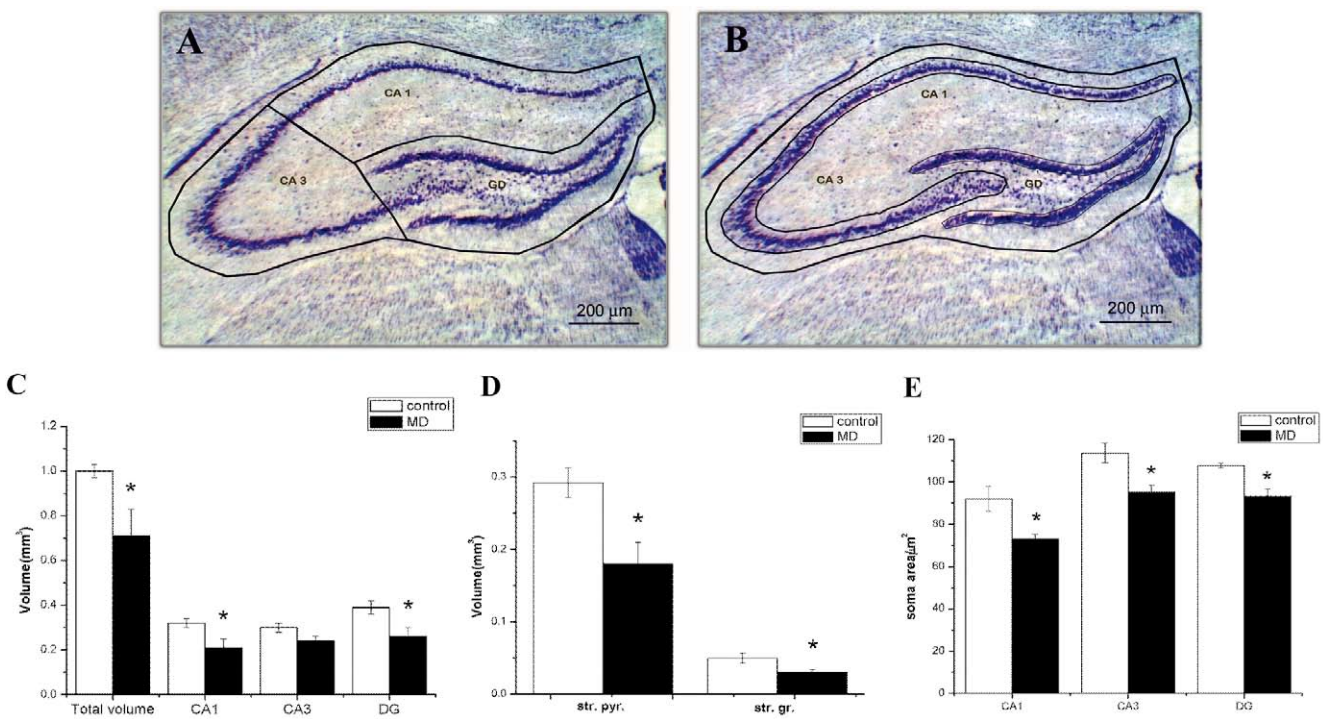

Fig. 1. Total volume of the hippocampus and its subfields and volume of the pyramidal and granular cells layer and its soma areas. (A, B) Representative micrograph of the Nissl stained section of the control hippocampus with traced areas for volume analysis. (C) Volume of the whole dorsal hippocampus and its subfields CA1, CA3 and DG in maternally deprived (MD) and control rats. (D) Volume of the pyramidal cell layer and granular cell layer in the dorsal hippocampus in MD and control rats. (E) Cell soma areas of the pyramidal (CA1, CA3) and granular (DG) cells in the hippocampus in MD and control rats. Results are presented as the mean values + SEM. Asterisk indicates significant differences between group mean values (Student's *t* test for two independent groups, $P<0.05$).

citrate solution, pH 9.0, for 30 min at 80°C. Non-specific binding was blocked using 5% normal goat serum, dissolved in phosphate buffered saline (PBS), pH 7.3 and supplemented with 0.2% Triton X-100, 0.02% sodium azide for 1 h at RT. Incubation with the primary antibody (mouse monoclonal NeuN antibody, Millipore, Schwalbach, Germany), diluted in PBS containing 0.5% lambda-carrageenan (Sigma-Aldrich) and 0.02% sodium azide, was carried out for 2 days at 4°C. After washing in PBS (3 × 15 min at RT), the endogenous peroxidase activity was blocked by submerging sections in 3% H_2O_2 solution for 10 min. The sections were then incubated for 30 min at RT with EnVision®+ Dual Link System-HRP (Dako, Carpinteria, CA). After a subsequent wash in PBS, the sections were incubated with diaminobenzidine with chromogen (Dako, Carpinteria, CA) for approximately 20 min, until the immune reaction was visible. Finally, the sections were counterstained in Mayer's hematoxylin (Fisher Scientific, Leicestershire, UK) for 30 s, rinsed with PBS, dehydrated, and mounted with DPX (Sigma Aldrich).

Estimations of pyramidal and granule cells soma area

Estimations of the pyramidal and granular cell soma areas were performed at the level of the largest cell body cross-sectional area. Coronal brain sections stained for NeuN were selected for analyses. Four sections 250 μm apart were analyzed per animal. Pyramidal and granular neurons were identified by their position in the pyramidal and granular layers in the hippocampus. The sample size was between 20 and 30 neurons per animal. Areas were measured using the ImageTool 2.0 (University of Texas, San Antonio, TX).

Image acquisition and quantitative analysis of immunolabeled neurons

Pictures were taken on an optical microscope (DM4000 Leica) with a 40× objective and analyzed in Photoshop 7.0 software (Adobe, San Jose, CA), using a 1-cm grid. NeuN-immunoreactive cells were counted in stereological sections of the rat brains on the same distance from bregma (2.52 mm for prefrontal cortex and −2.76 mm for retrosplenial and motor cortex). The counted number of NeuN-immunoreactive cells was expressed per unit area (mm^2), and we will further refer to it as profile density. At least 200 random microscope fields (area 400 μm^2) were counted in the retrosplenial, motor cortex and prefrontal cortex of each section.

Western blot analysis

The dorsolateral frontal cortex and hippocampi were homogenized in lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail) on ice for 30 min, centrifuged at 18 000× g for 15 min at 4°C, and the supernatant was collected. An equal amount of protein from each sample was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 12% gel and transferred to a nitrocellulose membrane (Amersham, Buckinghamshire, UK). Mouse monoclonal anti-NeuN primary antibody was used. All membranes were stripped and re-probed with anti-actin antibody (Sigma-Aldrich) to ensure equal loading. Western blots were scanned and densitometric analysis was performed using ImageQuant 5.2 (GE Healthcare, Buckinghamshire, UK).

Statistical analysis

All numerical data are presented as group mean values with standard errors of the mean (SEM). Morphological analysis was performed bilaterally, and if no difference was observed data were pooled together for presentation of results. All comparisons were performed by the Student's *t* test, and the threshold value for acceptance of the difference was 5%.

RESULTS

Maternal deprivation reduces volume of the hippocampus

First, on P9 we measured body weight of both control and MD pups and showed that groups were matched (16.05 ± 2 g and 15.37 ± 1 g, respectively; P=0.8). Also, we compared body weights of maternally deprived pups before (15.37 ± 1 g) and after (14.72 ± 0.84 g) separation and there was no reduction in the body weight (P=0.6). On P60, we examined if

exposure to MD alters gross morphology of the key congitive brain structures. We started by measuring volumes of the hippocampus and its subfields in MD treated rats and controls (Fig. 1A). The volume of the whole dorsal hippocampus in the control group was 1 ± 0.03 mm^3, while the volumes of its subfields were: CA1 0.32 ± 0.02 mm^3, CA3 0.30 ± 0.02 mm^3 and DG 0.39 ± 0.03 mm^3. In the MD group, the volume of the whole hippocampus was 0.71 ± 0.12, in CA1 0.21 ± 0.04 mm^3, in CA3 0.24 ± 0.02 mm^3 and in DG 0.26 ± 0.04 mm^3 (Fig. 1C). Analysis of the obtained results by *t* test showed statistically significant decrease in the volume of whole hippocampus (P=0.03) and volumes of CA1 (P=0.04) and DG (P=0.04) but not in the CA3 (P=0.06).

Reduced volumes of str. pyramidale and str. granulare in maternally deprived animals

Because the reduction in volume of a brain structure could result from the loss of neurons or the reduction of neuropil, i.e. loss of dendritic processes and synapses, we measured the volume of the pyramidal cell layer in the Ammon's horn and the volume of the granulare cell layer in the gyrus dentatus (Fig. 1B). The volume of the pyramidal cell layer in the control group was 0.29 ± 0.02 mm^3 while in MD group was 0.18 ± 0.03 mm^3 (Fig. 1D) and this difference was statistically significant (P=0.03). Reduction was also observed in the volume of the granule cell layer: in the control group volume was 0.05 ± 0.007 mm^3 while in MD group 0.03 ± 0.004 mm^3 (P=0.03) (Fig. 1D).

Maternal deprivation reduces cell soma areas of hippocampal pyramidal and granule cells

In order to respond to the question if the decrease in the volume of pyramidal and granule cell layer is due to a decrease in the number of cells or a decrease in their size, we measured the cell soma area of the pyramidal and granule cells. The cell soma area of the pyramidal cells in the CA3 region of the hippocampus in the control group was 114 ± 5 μm^2, while in the CA1 and DG region was 92 ± 6 μm^2 and 108 ± 1 μm^2, respectively. In the MD group, the cell soma area of pyramidal cells in CA3 was 95 ± 3 μm^2, in CA1 73± 2 μm^2 and in DG 93 ± 3 μm^2. Analysis of the obtained results by *t* test showed that this difference is statistically significant in all investigated subfields [CA1 (P=0.02), CA3 (P=0.01), DG (P=0.03)].

Maternal deprivation reduces thickness of prefrontal, retrosplenial and motor cortex

Next we analyzed the gross anatomy of the neocortex. Cortical thickness of the prefrontal cortex (PFCX) was measured bilaterally in stereological sections at distance of 2.52 mm from bregma while the thickness of retrosplenial (RSCX) and motor cortex (MCX) was measured at the distance of −2.76 mm from bregma (Paxinos and Watson 2005) (Fig. 2A, B). The thickness of the PFCX in the control group of rats was 1 771 ± 40 μm while in the maternally deprived group cortical thickness was 1 300 ± 123 μm; this difference was statistically significant (P=0.02) (Fig. 2C). The thickness of the RSCX in the control group of rats was 1 641 ± 45 μm and in MD group 1 414 ± 58 μm (Fig. 2C). Analysis of the obtained results by *t* test showed that this difference is statistically significant (P=0.02). The thickness of the MCX was as well reduced in MD group compared to controls (1 411 ± 57 μm and 1 650 ± 39 μm, respectively; P=0.01).

Effects of maternal deprivation on NeuN immunoreactivity in the cerebral cortex

To demonstrate if the thinning of the neocortical structures in maternally deprived rats is due to neuronal loss we counted profile densities of NeuN-immunolabeled neurons in stereological sections of the rat brains at the same distance from bregma, as described previously in the PFCX, RSCX, and MCX (Fig. 2A, B). The profile density of NeuN-immunoreactive neurons in the PFCX in the control group was 3 415 ± 60 cells/mm^2, whereas in the MD group density was 2 779 ± 118 cells/mm^2 and this difference was statistically significant (P=0.003) (Fig. 2D). Profile densities of NeuN-immunoreactive neurons in the RSCX of the control and MD group were 3 170 ± 218 cells/mm^2 and 2 197 ± 205 cells/mm^2 respectively (P=0.01) (Fig. 2D). The profile density of the NeuN-immunoreactive neurons in MCX in the control group was 1 427 ± 69 cells/mm^2 while in MD group was 1 342 ± 59 cells/mm^2 (Fig. 2D) and there was no statistical siginificance (P=0.37).

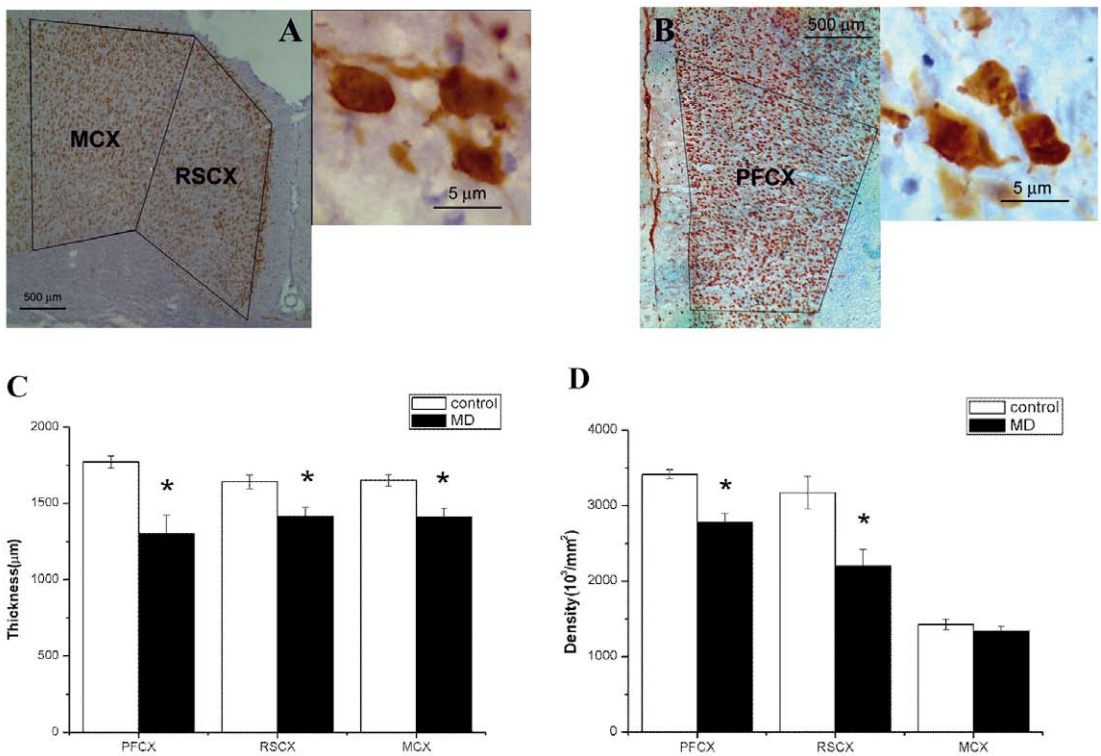

Fig. 2. Cortical thickness and density of NeuN-immunoreactive neurons. (A, B) Representative micrographs, in high and low magnification, of the NeuN-immunolabeled sections of the control retrosplenial cortex (RSCX), motor cortex (MCX) and prefrontal cortex (PFCX). (C) Cortical thickness of the retrosplenial cortex (RSCX), motor cortex (MCX) and prefrontal cortex (PFCX) in maternally deprived (MD) and control rats. (D) Profile densities of NeuN-immunolabeled neurons in the retrosplenial cortex (RSCX), motor cortex (MCX) and prefrontal cortex (PFCX) of MD and control rats. Results are presented as the mean values + SEM. Asterisk indicates significant differences between group mean values (Student's *t* test for two independent groups, $P<0.05$).

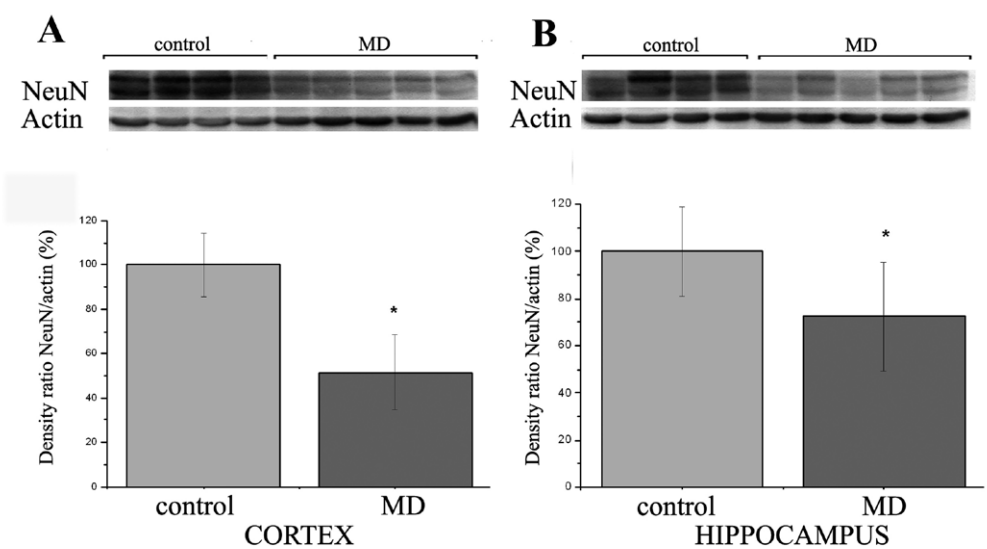

Fig. 3. Expression of NeuN in cortex (A) and whole hippocampus (B) homogenates. Figures are accompanied by representative immunoblots from the same gel. Results are presented as the mean values + SEM. Asterisk indicates significant differences between group mean values (Student's *t* test for two independent groups, $P<0.05$).

Maternal deprivation reduces expression of NeuN protein in the hippocampus and neocortex

To confirm the results of the quantification of NeuN-immunolabeled cells, we performed immunoblot experiments from the tissue homogenates of the whole hippocampus and neocortex from control and maternally deprived rats at P60 (Fig. 3A). Quantitative analysis of the immunoblot data shows that in the hippocampus NeuN expression was reduced by approximately 30% in the MD group compared to the control. In the tissue homogenates from the neocortex the reduction in NeuN expression reached almost 50% (Fig. 3B). Both these differences were statistically significant. We can conclude that in both hippocampus and neocortex substantial loss of neurons occurs in animals stressed by MD and/or the levels of NeuN expression in neurons decrease.

DISCUSSION

In this study we demonstrate that MD at P9 has a long-term effect on volume and number of neurons in the rat neocortex and hippocampus. Reduction in the hippocampal volume was at least in part due to a reduction of the volume of pyramidal and granular cell layers as well as a decrease in pyramidal and granular cell soma size. Furthermore, we show that MD leads to a reduction of the cortical thickness in the PFCX, RSCX and MCX. These results were further corroborated with reduced NeuN expression in hippocampus and neocortex by Western blot analysis. Decrease in the number of NeuN-immunolabeled neurons in the hippocampus and neocortex was also present, suggesting that the reduction in volume was due to neuronal loss during postnatal development. Decreased thickness of the motor cortex with no changes in density of NeuN-positive cells implies that there is a reduction in dendritic branching and synapse formation in the motor cortex upon MD. Also, we hypothesize that even though in motor cortex there was no change in neuron density, reduced NeuN expression in neocortex reflects overall result, presumably due to changes in prefrontal and retrosplenial cortex. Further studies are necessary to test this hypothesis.

Thinning of the anterior, subcallosal and rostral cingulate along with paracingulate regions predicted development of psychosis among the "ultra-high risk" subjects (Fornito et al. 2008). This suggests predictive ability of region-specific surface parameter changes in this population. Changes in the position and density of neuronal clusters within the entorhinal cortex (Falkai and Borgets 1986, Jakob and Beckman 1994, Arnold et al. 1995) and alterations in the positioning of neurons in the frontal cortex imply distorted neuronal migration (Akbarian et al. 1993a,b).

Maternally deprived rats (deprived from their mothers for a single period of 24 h, shortly after birth, typically at P9) have a diminished prepulse inhibition (Ellenbroek and Cools 1998, 2000b, Ellenbroek et al. 1998) and latent inhibition (Ellenbroek and Cools 1995b), as well as an increased sensitivity to dopamine agonist, apomorphine (Ellenbroek and Cools 1995a). The latter is accompanied by an increase in tyrosine hydroxylase mRNA in the substantia nigra (Rots et al. 1996a,b). It is important to realize that MD not only deprives rat pups from their mother, but also mother from her pups. This 24 h isolation of the mother may very well lead to alterations in maternal behavior and possibly milk production, which might influence the derivational effect (Ellenbroek and Cools 2002). The authors subsequently showed that these maternally deprived animals also share other features with schizophrenic patients, such as an enhanced sensitivity to dopaminergic drugs and stress.

All these data together have led to the hypothesis that the maternally deprived rat might represent an interesting animal model for specific aspects of schizophrenia. Both maternally deprived rats (Zimmerberg and Shartrand 1992, Ellenbroek and Cools 1995a, Rots et al. 1996a) and schizophrenic patients (Leiberman et al. 1987, Muller-Spahn et al. 1998), show a hypersensitivity to dopamimetic agents such as amphetamine or apomorphine. Maternally deprived rats (Rots et al. 1996b, Penke et al. 2001) and schizophrenic patients (Lammers et al. 1995, Altamura et al. 1999) have increased levels of adrenocorticotropic hormone (ACTH) and corticosterone, as well as an increased response to stress (Ellenbroek et al. 2004).

Schizophrenia is a severe psychiatric disorder with multifactorial ethiopathogenesis. It is considered that several genetic predisposing factors interact with environmental events, such as sexual, emotional, or physical abuse. There is ample evidence that these non-ge-

netic factors are important early in life (pre- and perinatally) as well as later in life (around the age of puberty) (Gutman and Nemerolf 2003).

CONCLUSION

In conclusion, this is the first study to provide evidence that early stress caused by MD in rats leads to alteration in morphological and biochemical parameters, including the size of the neocortex and hippocampus, number of neurons in the prefrontal and retrosplenial cortex and NeuN expression in the hippocampus and neocortex. Considering the role that these structures play in cognitive functions, it is tempting to speculate that similar mechanisms might be involved in the pathogenesis of schizophrenia in humans.

ACKNOWLEDGEMENTS

This study was supported by grants III 41020 and 175058 from the Ministry for Science and Enviromental Protection of the Republic of Serbia. We are grateful to Dr. Igor Jakovcevski (University Medical Center Hamburg-Eppendorf) for help in editing manuscript.

REFERENCES

Akbarian S, Bunney WE Jr, Potkin SG, Wigal SB, Hagman JO, Sandman CA, Jones EG (1993a) Altered distribution of nicotinamide-adenine dinucleotide phosphate-diaphorasencells in frontal lobe of schizophrenics implies disturbences of cortical development. Arch Gen Psychiatry 50: 169–177.

Akbarian S, Vinuela A, Kim JJ, Potkin SG, Bunney WE Jr, Jones EG (1993b) Distored distribution of nicotinamide-adenine dinucleotide phosphate-diaphorase neurons in temporal lobe of schizophrenics implies anomalous corticaldevelopment. Arch Gen Psychiatry 50: 178–187.

Altamura AC, Boin F, Maes M (1999) HPA axis and cytokines dysregulation in schizophrenia: Potential implications for the antipsychotic treatment. Eur Neuropsychopharmacol 10: 1–4.

Arnold SE, Trojanowski JQ (1996) Recent advances in defining the neurophatology of schizophrenia. Acta Neuropathol (Berl) 92: 217–231.

Arnold SE, Franz BR, Gur RC, Gur RE, Shapiro RM, Moberg PJ, Trojanowski JQ (1995) Smaller neuron size in schizophrenia in hippocampal subfields that mediate cortical-hippocampal interactions. Am J Psychiatry 152: 738–748.

Benes FM, Bird ED (1987) An analysis of the arrangement of neurons in the cingulate cortex of schiziphrenic patients. Arch Gen. Psychiatry 44: 608–616.

Benes FM, Majocha R, Bird ED, Marotta CA (1987) Increased vertical axon number in cingulate cortex of shizophrenics. Arch Gen Psychiatry 44: 1017–1021.

Benes FM, McSparren J, Bird ED, San Giovannni JP, Vincent SL (1991) Deficits in small interneurons in prefrontal cortex and anterior cingulate cortices of schizophrenic and schizoaffective patients. Arch Gen Psychiatry 48: 996–1001.

Bogets B, Falkai P, Greve B (1991) Evidence of reduced temporolimbic structure volume in schizophrenia. Arch Gen Psychiatry 48: 956–958.

Buchsbaum MS (1990) Frontal lobes, basal ganglia, temporal lobe-three sites for schizophrenia? Schizophr Bull 16: 377–378.

Degreef G, Borgets B, Falkai P, Greve B, Lantos G, Ashtari M, Leiberman J (1992) Increased prevalence of the cavum septum pellucidum in magnetic resonance scans and post-mortem brains of schizophrenic patients. Psychiatry Res 45: 1–13.

DeLisi LE, Dauphinais ID, Gershon ES (1988) Perinatal complications and reduced size of brain limbic structures in familial schizophrenia. Schizophr Bull 14: 185–191.

Ellenbroek BA, Cools AR (1995a) The behavior of APOSUS rats in animal models with construct validity for schizophrenia. J Neurosci 15: 7604–7611.

Ellenbroek BA, Cools AR (1995b) Maternal separation reduces latent inhibition in the conditioned taste aversion paradigm. Neurosci Res Commun 17: 27–33.

Ellenbroek BA, Cools AR (1998) The neurodevelopmental hypothesis of schizophrenia: clinical evidence and animal models. Neurosci Res Commun 22: 127–136.

Ellenbroek BA, Cool AR (2000) The long-term effects of maternal deprivation depend on the genetic background. Neuropsychopharmacology 23: 99–106.

Ellenbroek BA, Cools AR (2002) Early maternal deprivation and prepulse inhibition – The role of the postdeprivation environment. Pharmacol Biochem Behav 73: 177–184.

Ellenbroek BA, van den Kroonenberg PTJM, Cools AR (1998) The effects of an early stressful life event on sensorimotor gating in adult rats. Schizophr Res 30: 251–260.

Ellenbroak BA, de Bruin NMWJ, van Den Kroonenburg PTJM, van Luijtelaar ELJM, Cools AR (2004) The effects of early maternal deprivation on auditory information processing in adult Wistar rats. Biol Pshychiatry 55: 701–707.

Ellenbroek BA, Zhang XX, Jin GZ (2006) Effects of (-) stepholidine in animal models of schizophrenia. Acta Pharmacol Sin 27: 1111–1118.

Falkai P, Bogets B (1986) Cell loss in the hippocampus of schizophrenics. Eur Arch Psychiatry Neurol Sci 236: 154–161.

Fornito A, Yucel M, Wood SJ, Adamson C, Velakoulis D, Saling MM (2008) Surface-based morphometry of the anterior cingulate cortex in the first episode schizophrenia. Hum Brain Mapp 29: 478–489.

Frith CD (1997) Functional brain imaging and neurophatology of schizophrenia. Schizophr Bull 23: 525–527.

Goghari VM, Rehm K, Carter CS, MacDonald AW (2007a) Sulcal thickness as a vulnerability indicator for schishophrenia. Br J Psychiatry 191: 229–233.

Goghari VM , Rehm K, Carter CS, MacDonald AW (2007b) Regionally specific cortical thining and gray matter abnormalities in the healthy relatives of schisophrenia patients. Cereb Cortex 17: 415–424.

Goldman RP, Seleman LD (1997) Functional and anatomical aspects of prefrontal pathology in schizophrenia. Schizophr Bull 23: 437–458.

Gur RE, Cowell P, Turetsky BI, Gallacher F, Cannon T, Bilker W, Gur RC (1998) A follow-up magnetic resonance imaging study of schizophrenia. Relationship of neuroanatomical changes to clinical and neurobehavioral measures. Arch. Gen. Psychiatry 55: 145–152.

Gutman DA, Nemerolf CB (2003) Persistent central nervous system effects of an adverse early enviroment: clinical and preclinical studies. Physiol Behav 79: 471–478.

Harlow HF, Dodsworth RO, Harlow MK (1965) Total social isolation in monkeys. Proc Natl Acad Sci U S A 54: 90–97.

Harrison PJ (1999) The neurophatology of schiziophrenia: A critical review of the date and their interpretation. Brain 122: 593–624.

Harvey I, Ron MA, Du BG, Wicks D, Lewis SW, Murray RM (1993) Reduction of cortical volume in schizophrenia on magnetic resonance imaging. Psychol Med 23: 591–604.

Heim S, Kissler J, Elbert T, Rockstrah B (2004) Cerebral lateralization in schizophrenia and dyslexia: neuromagnetic responses to auditory stimuli. Neuropshychologia 42: 692–697.

Ho BC, Alticata D, Ward J, Moser DJ, O'Leary DS, Arndt S, Andreasen NS (2003) Untreated initial psychosis: relation to cognitive deficits and brain morphology in first-episode schizophrenia. Am J Psychiatry 160: 142–148.

Honer WG, Bassett AS, Falkai P, Beach TG, Lapointe JS (1996) A case study of temporal lobe development in familial schizophrenia. Psychol Med 26: 191–195.

Husum H, Mathe AA (2002) Early life stress affects concentrations of Neuropeptide Y and Corticotropin-releasing hormone in adult rat brain. Lithium alleviates these changes. Neuropsychopharmacology 27: 756–764.

Husum H, Termeer E, Mathe AA, Bolwing TG, Ellenbroek BA (2002) Early maternal deprivation alters hippocampal levels of neuropeptide Y and calcitonon-gene related peptide in adult rats. Neuropharmacology 42: 798–806.

Jakob H, Beckman H (1994) Circumscribed malformation and nerve cell alterations in the entorhinal cortex of shizophrenics. Pathogenetic and clinical aspects. J Neural Transm Gen Sect 98: 83–106.

Kasai K, Shenton ME, Salisbury Df, Onitsuka T, Toner SK, Yurgelun-Tood D, Kikinis R, Jolesz FA, McCarley RW (2003) Differences and similarities an insular and temporal pole MRI gray matter volume abnormalities in first episode schizophrenia and affective psychosis. Arch Gen Psychiatry 60: 1069–1077.

Kleinschmidt A, Falkai P, Huang Y, Schneider T, Furst G, Steinmetz H (1994) In vivo morphometry of planum temporale asymetry in first-episode schizophrenia. Schizophr Res 12: 9–18.

Kovelman JA, Scheibel AB (1984) A neurohistological correlate of schizophrenia. Biol Psychiatry 19: 1601–1621.

Lammers CH, Garcia-Borreguero D, Schmider J, Gotthardt U, Dettling M, Holsboer F (1995) Combined dexamethasone/corticotropin-releasing hormone test in patients with schizophrenia and in normal controls II. Biol Psychiatry 38: 803–807.

Leiberman JA, Kane JM, Alvir J (1987) Provocative tests with psychostimulant drugs in schizophrenia. Psychopharmacology (Berl) 91: 415–533.

Muller- Spahn F, Modell S, Ackenheil M, Brachner A, Kurtz G (1998) Elevated response of growth hormone to graded doses of aphomorphine in schizophrenic patients. J Psychiatr Res 32: 265–271.

Nakamura M, Salisbury DF, Hairayasu Y, BouixnS, Pohl KM, Yoshida T, Koo MS, Shenton ME, Mc Carley RW (2007) Neocortical gray matter volume in first-episode schizophrenia and first-episode affective psychosis: a cross-sectional and longitudinal MRI study. Biol Psychiatry 62: 773–783.

Own LS, Patel PD (2013) Maternal behavior and offspring resiliency to maternal separation in C57B/6 mice. Horm Behav 63: 411–417.

Pantelis C, Yucel M, Woood SJ (2005) Structural brain imaging evidence for multiple pathological processes at different stages of brain development in schizophrenia. Schizophr Bull 31: 672–696.

Pantelis C, Velakoulis D, Wood SJ, Yucel M, Yung AR, Phillips J, Sun DQ, McGorry PD (2007) Neuroimiging and emerging psychotic disorders: The Melbourne ultra-high risk studies. Int Rev Psychiatry 19: 371–381.

Paxinos G, Watson C (2005) The Rat Brain in Stereotaxic Coordinates (5th Edition). Elsevier Academic Press, Amsterdam, NL.

Penke Z, Felszeghy K, Fernette B, Sage D, Nyakas C, Burlet A (2001) Postnatal maternal deprivation produces long-lasting modifications of the stress response, feeding and stress-related behaviour in the rat. Eur J Neurosci 14: 747–755.

Rapoport JL, Addington A, Frangou S (2005) The neurodevelopmental model of schizophrenia: what can very early onset cases tell us? Curr Psychiatry Rep 7: 81–82.

Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA (2002) Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus: implications for cellular plasticity. Mol Psychiatry 7: 609–616.

Rots NY, Cools AR, Berad A, Voorn P, Rostene W, de Kloet ER (1996a) Rats bred for enchanced apomorhine susceptibility have elevated tyrosine hydroxylase mRNA and dopamine D2 receptor binding sites in nigrostiatal and tuberoinfundibular dopamine systems. Brain Res 710: 189–196.

Rots NY, de JJ, Workel JO, Levine S, Cools AR, de KE (1996b) Neonatal maternally deprivated rats have as adults elevated basal pituitary-adrenal activity and enhanced susceptibility to apomorphine. J Neuroendocrinol 8: 501–506.

Scheibel AB, Kovelman JA (1981) Disorientation of the hippocampal pyramidal cell and its processes in the schizophrenic patients. Biol Psychiatry 16: 101–102.

Schultz CC, Koch K, Wagner G, Roebel M, Schachtzabel C, Gaser C, Nenadic I, Reichenbach JR, Sauer H, Schlosser RGM (2009) Reduced cortical thickness in first episode schizophrenia. Schizophr . Res 116: 204–209.

Shelton AL, McNamara TP (2001) Systems of spatial reference in human memory. Cogn Psychol 43: 274–310.

Sun D, van Erp TG, Thompson PM, Bearden CE, Daley M, Kushan L, Hardt ME, Nuechterlein KH, Toga AW, Cannon TD (2009) Elucidating a magnetic resonance imaging-based neuroanatomic biomarker for psychosis: classification analysis using probabilistic brain atlas and machine learning algorithms. Biol Psychiatry 66: 1055–1060.

Vita A, De Peri L, Silenzi C, Dieci M (2006) Brain morphology in first-episode schizophrenia: A meta-analysis of quantitative magnetic resonance imaging studies. Schizophr Res 82: 75–88.

Vivinetto AL, Suarez MM, Rivarola MA (2013) Neurogiological effects of neonatal maternal separation and post-weaning enviromental enrichment. Behav Brain Res 240: 110–118.

Weinberger DR (1999) Cell biology of the hippocampal formation in schizophrenia. Biol Pschiatry 45: 395–402.

Woolley CS, McEwen BS (1992) Estradiol mediates fluctuations in hippocampal synapses density during the estrous cycle in the adult rat. J Neurosci 12: 2549–2554.

Zaidel DW, Esirii MM, Harrison PJ (1997) The hippocampus in schizophrenia: Lateralized increase in neuronal density and altered cytoarchitectural asymmetry. Psychological Medicine 27: 703–713.

Zimmerbergand B, Shartrand AM (1992) Temperature-dependent effects of maternal separation on growth, activity, and amphetamine sensitivity in the rats. Dev Psychobiol 25: 213–226.