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Biochemical and cognitive impairments observed in animal models of schizophrenia induced by prenatal stress paradigm or methylazoxymethanol acetate administration

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The aim of the study was to find whether spatial memory impairment and disruption in locomotor activity were found in prenatally stressed rats (PSG) or prenatally methylazoxymethanol acetate-treated rats (MAMG). In addition to this, we examined basal plasma corticosterone level as well as brain-derived neurothropic factor (BDNF) in the PSG and MAMG rats. The effect of prenatal stress (stress paradigm between 14 and 21 day of gestation) and methylazoxymethanol acetate (MAM) administration (17 day of gestation) to the female Wistar rats were studied on the male offspring in the Morris Water Maze (spatial memory) and locomotor activity test. Through Morris Water Maze rats were injected with saline 4 times (on 1, 7, 14 and 21 day of testing) while in locomotor activity test saline was injected only once. Corticosterone level was measured using ELISA Kit while BDNF levels were assessed using ELISA Chemikine TM BDNF kit. Results indicate that both PSG and MAMG rats deteriorate spatial memory as well as increase locomotor activity compared to the control group. Biochemical studies indicate that basal plasma corticosterone level increased in both PSG and MAMG rats compared to the control group. Analyses of the BDNF level, on the other hand, have shown decrease of the neurothropin level in both hippocampus and prefrontal cortex (PFC) in both PSG and MAMG groups of rats. As shown by the obtained results, both the prenatal stress model and prenatal MAM administration model generate a number of behavioral (e.g. spatial memory disorders, increased locomotor activity) and biochemical (e.g. increased corticosterone and decreased BDNF levels) changes in the examined offspring, Thus, these models can be successfully used in the efficacy analysis of the pharmacotherapy applied.

Key words: prenatal stress, methylazoxymethanol acetate, corticosterone, brain-derived neurothropic factor, hippocampus, prefrontal cortex

INTRODUCTION

Schizophrenia is one of the most common mental diseases with a biological background and diverse clinical course (Pers and Rajewski 2003). Schizophrenia causes a number of structural and functional brain changes, and leads, among others, to the loss of cognitive functions including operating memory disorders, which are primarily related to structural anomalies in the dorsolateral part of prefrontal cortex (PFC) (Hintze et al. 2007). Moreover, disturbed PFC and hippocampus (HIP) development plays a fundamental role in the development of schizophrenia, leading to synaptic plasticity changes and cognitive functions disorders. That confirms neurodevelopmental hypothesis of schizophrenia and at the same time provide necessary background for the animal model of disease (Ratajczak et al. 2013a).

It seems to be interesting, therefore, to define the influence of disturbances in brain structures development (HIP and PFC) due to MAM and prenatal stress exposure on spatial memory tested in the Morris Water Maze (Morris et al. 1984). Both methods, administration of methylazoxymethanol acetate (MAM) during

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gestation (Featherstone et al. 2009, Maćkowiak et al. 2014) and prenatal stress paradigm (Kinnunen et al. 2003, Ratajczak et al. 2013a, Ratajczak et al. 2013b, Koenig JI et al. 2005, O'Donnell K et al. 2009) were used in animal models of schizophrenia as factors that induce disorders in the mature offspring in primary brain structures development. MAM is a mycotoxin, which, when used in rats on day 17 of pregnancy leads to structural deficits in the growing offspring, and finally causes both neuropathological and behavioral changes, same as in clinical schizophrenia presentation (Moore et al. 2006). The use of MAM disturbs the process of neuronal cells mitotic division (Hradetzky et al. 2012), especially in the late developing paralimbic region and in the frontal and temporal cortex (Heckers 2001). Methylazoxymethanol acetate can reduce the size of the HIP and PFC in the offspring, decrease density of neurons and disturb levels of DA values determined in the PFC (Moore et al. 2006). Moreover, MAM affects behavioral flexibility, recognition memory (Flagstad et al. 2005), internal memory (Gourevitch et al. 2004) and attentional set-shifting disorders (Featherstone et al. 2007). On the other hand, exposure of pregnant mothers to stress (prenatal stress model), especially in the 3rd trimester, can cause a number of central nervous system (CNS) function disturbances in the offspring. In conjunction with genetic factors, these disorders can lead to mental disorders such as schizophrenia and depression (Szubert et al. 2008).

Stress is known to cause a release of stress mediators – glucocorticosteroids (cortisol in humans and corticosterone in animals) (Koenig et al. 2005), which induce intracellular adaptive changes. It is believed that excessive release of cortisol (corticosterone) may affect neurodegenerative processes and impair neurodevelopmental processes within the HIP, which in turn may cause, as in the case of using MAM during gestation, weight decrease in that brain structure (Ratajczak et al. 2013a). Excessive stress exposure, especially in developmental period, may lead to disorders in the mature offspring [e.g. plastic changes in the amygdaloid body, HIP and in mesolimbic part of the dopaminergic system (Charmandari et al. 2003)]. In the conducted experiments, the value of corticosterone was measured in animal blood plasma. The following deficits resulting from MAM and prenatal stress can be observed: disrupted PPI, learning deficits in the Morris water maze test (MWM), increased locomotor activity, decreased social interactions and decreased hippocampal weight (Ratajczak et al. 2013a).

Post-mortem and *in vivo* examinations of patients suffering from schizophrenia showed significant deficits in cytoarchitecture of the PFC and HIP in the affected people (Bertolino et al. 1996, Harrison 1999, Tsai et al. 1995) which is directly associated with decrease of the brain derived neurothropic factor (BDNF) level. BDNF regulates growth and development of the nervous system, synaptic plasticity and is responsible for functional activity of adult brain and behavioral reactions (Chao 2000, Connor and Dragunow 1998, Ebendal 1989). Studies of Murer others (2001) show that HIP is the brain area with the highest BDNF expression – a brain region of high synaptic plasticity responsible mainly for memory processes. As it is mainly known, schizophrenia causes a number of structural changes especially in the two examined by the authors brain areas – HIP and PFC (Hintze et al. 2007), so it was interesting to evaluate the proposed animal models of schizophrenia (prenatal stress paradigm and MAM injection) in the course of BDNF level changes observed in that regions.

METHODS

Animals

Timed pregnant Wistar female rats (44) were purchased from Poznan University of Medical Sciences, Poznan, Poland (licensed by the Ministry of Agriculture in Warsaw, Poland) and arrived at our animal facility on day 2 of gestation. The pregnant animals were housed individually in cages (size 42×26 cm) in a light- (lights on 7:00 AM–7:00 PM), temperature – and humidity-controlled animal facility. The dams had free access to rat chow (Labofeed B) and water.

For behavioral tests, male rats born to mothers either exposed to prenatal stress or prenatal MAM administration during pregnancy were used. Male rats were housed in cages (size 54×32 cm).

The total number of animals in the study was 134 (44 females, 90 males). The male rats (90 animals) were divided into three groups – CONTROL group – (30 rats – offspring of the 14 female rats (7 non-prenatally stressed and 7 non-MAM-treated females saline treated) – this group was combined because in the course of the experiments there was no statistically

significant difference between non-prenatally stressed and non-MAM-treated offspring rats), PSG group (30 rats – offspring of the 15 prenatally stressed females) and MAMG group (30 rats – offspring of the 15 MAM-treated females). Animals were selected from different litters (different mothers) and were subsequently distributed randomly into experimental groups: Morris test (30 rats), locomotor activity (30), corticosterone and BDNF analysis (30 rats).

All procedures related to the use of rats in these experiments were conducted with due respect to ethical principles regarding experiments on animals (Directive 2010/63/EU). The study protocol was approved by the Local Ethics Committee for Research on Animals in Poznan.

Drugs

Female rats were given saline (7 rats) or MAM (15 rats) at the dose of 22 mg/kg in the volume of 5 mg/ml (ip) on day 17 of gestation. MAM was obtained from BCS BUJNO Chemicals Warsaw, Poland.

Male rats (90 – offspring of the 15 MAM-treated and 15 prenatally stressed pregnant females as well as offspring of 7 non-MAM-treated females and 7 non-prenatally stressed females) were given saline intraperitoneally (*ip*) for 21 days (the 21 days of saline administration is related to the procedures of animal chronic drug treatment – drug and saline are administered to the animal from day 1 until 21 day of experiment). At the day of experiment (1, 7, 14, and 21 day) in the course of Morris water maze or locomotor activity test the animals received saline 30 min before the test.

Saline solution used in the experiments contained sodium chloride NaCl dissolved in 1% carboxymethylcellulose vehicle in the 1:1 ratio.

Prenatal stress procedure (Kinnunen et al. 2003) (Fig. 1)

Beginning on day 14 of gestation, the pregnant dams (15) were exposed to a repeated variable stress paradigm until delivery of their pups on gestational day 22 or 23 as previously described. The stresses used in this paradigm consisted in: (1) restraint in metal tube for 1 h, (2) exposure to a cold environment (4°C) for 6 h, (3) overnight food deprivation, (4) 15 min of swim stress in water of ambient temperature, (5) lights on for 24 h, and (6) social stress induced by overcrowded housing conditions (Lee PR et al. 2007) during the dark phase of the cycle. Pregnant control dams (7) remained in the animal room from gestational days 14–21 and were only exposed to normal animal room husbandry procedures. All delivered their pups vaginally (fertilization found in 5 out of 7 control dams and 11 out of 15 prenatally stressed dams). At weaning, male offspring from each litter were placed with either same sex litter mates per cage with free access to rat chow and water.

MAM (Featherstone et al. 2009) (Fig. 1)

On embryonic day 17 (ED 17 – day 0 defined as the day of conception) female rats were treated by saline (7) or MAM methylazoxymethanol acetate (15) in dose 22 mg/kg in volume of 5 mg/ml; (BCS BUJNO

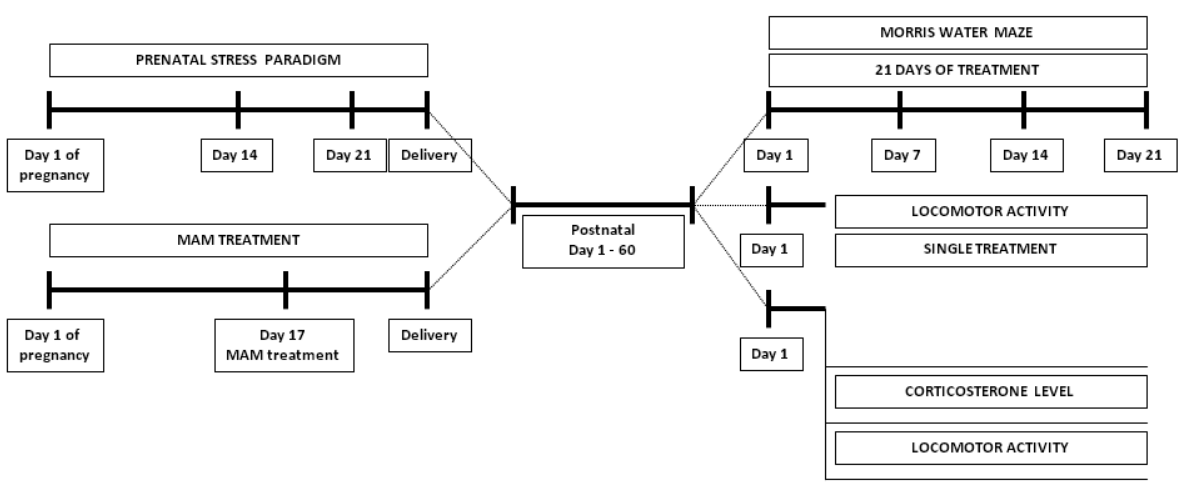

Fig 1. Schedule of the experiments.

Chemicals, Warsaw) administered via intraperitoneal (*ip*) injection. All dams delivered their pups vaginally (fertilization found in 6 out of 7 control dams and 12 out of 15 prenatally stressed dams). At weaning, male offspring from each litter were placed with either same sex litter mates per cage with free access to rat chow and water.

Basal corticosterone analyses (Koenig et al. 2005) (Fig. 1)

Corticosterone level was measured with corticosterone ELISA Kit ADI-900-097 (Biomibo – Mieczysław Bogus, Warszawa, Poland).

Male rats from the CONTROL (10), PSG (10) and MAMG (10) groups were moved to the laboratory 5−7 days prior to experimental procedures. This period allows the animals to become domesticated to the experimental environment and to alleviate the stress induced by moving the animals from the animal room to the experimental laboratory. On the morning of the experiment, beginning at approximately 9.00 h, a CONTROL (10), PSG (10) and MAMG (10) groups of animals were sacrificed by decapitation and trunk blood was collected (rapidly) to establish basal corticosterone level in each group of animals (without acute restraint).

Blood was collected immediately to plastic tubes containing 10% ethylenediaminetetraacetate (EDTA). The blood was spun at 3,000 rpm and the plasma placed in a fresh tube and frozen. Plasma samples were stored at −80°C until use. Plasma levels of corticosterone were determined using Corticosterone ELISA Kit purchased from Biomibo – Mieczysław Bogus, Warszawa, Poland. Assays were performed according to the protocols provided by the manufacturer.

BDNF assay (Fig. 1)

BDNF levels were assessed by an ELISA test. Frozen tissues from CONTROL (10), PSG (10) and MAMG (10) groups which were earlier sacrificed by decapitation for the corticosterone level evaluation were homogenized in 1.5–3.0 ml of an ice-cold buffer consisting of 100 mM Tris/HCl (pH 7.0), containing 2% bovine serum albumin, 1 M NaCl, 4 mM Na2EDTA, 2% Triton X-100, 0.1% sodium azide, and the protease inhibitors 5 µg/ml aprotinin, 5 µg/ml leupeptin, 0.1 µg/ml pepstatin A and 17 µg/ml phenylmethylsulfonyl fluoride (PMSF), using a Potter-Elvehjem homogenizer (Wheaton Industries, NJ, USA). Supernatants were collected by centrifugation at 14.000×g for 30 min at 4°C, stored at −70°C and were used for ELISA. Total protein content in tissue extracts was determined by Lowry's et al. method (Lowry et al. 1951). Endogenous BDNF was quantified by a sandwich enzyme immunoassay method using ChemiKineTM BDNF kits (Chemicon, Millipore Group, MA, USA). Data are represented as pg/mg protein, and all assays were performed in triplicate. The sensitivity of the ELISA was 7.8 pg/ml and no significant cross-reactivity was observed with other neurotrophins, such as NGF, NT4/5 or NT3.

Morris water maze test (Morris 1984) (Fig. 1)

The water maze apparatus was a circular basin (diameter=180 cm, height=50 cm) filled with water (approximately 22–24°C) to a depth of 24 cm, and pieces of Styrofoam were hiding an escape platform (diameter=8 cm) that was placed 1 cm below the water surface (learning place, invisible condition). Many extra-maze visual cues surrounding the maze were available, and the observer remained in the same location for each trial. The rats from the CONTROL (10), PSG (10) and MAMG (10) groups were placed in the water facing the midpoint section of the wall at one of 4 equally spaced locations: North (N), East (E), South (S), and West (W). The pool was divided into 4 quadrants: NW, NE, SE, and SW. The rats were allowed to swim freely until they found and climbed onto the

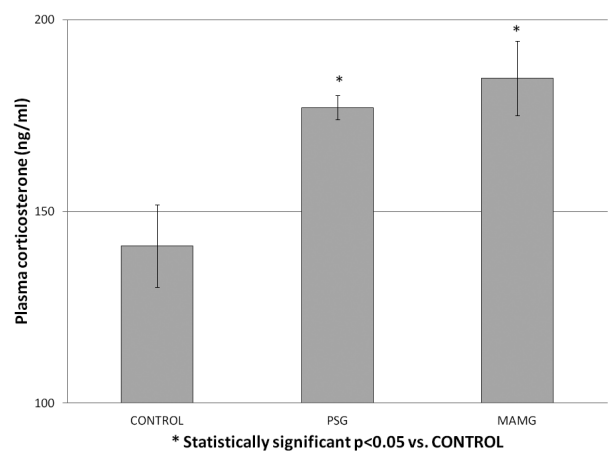

Fig 2. Concentration of basal plasma corticosterone in prenatally stressed and MAM treated rats.

Table I

Effect of prenatal stress and prenatal administration of MAM on spatial memory tested in the Morris water maze test (number of escape latencies)

Group	Escape latencies				Friedman H[3.37]
	Single administration (x ± SEM)	Chronic treatment			
		7 days (x ± SEM)	14 days (x ± SEM)	21 days (x ± SEM)	
Saline (0.5 ml/rat) CONTROL	19.37 ± 2.45	14.18 ± 0.91	11.62 ± 0.78	11.70 ± 1.35	2.5
Saline (0.5 ml/rat) PSG	**27.90 ± 3.20*** *p=0.0485*	**23.00 ± 1.50*** *p<0.0001*	12.90 ± 1.70	11.30 ± 1.90	5.7
Saline (0.5 ml/rat) MAMG	**28.25 ± 1.75*** *p=0.0086*	**17.75 ± 1.46*** *p=0.0526*	**17.00 ± 1.01*** [x] *p=0.0005 vs. CON* *p=0.0528 vs. PSG*	**17.70 ± 1.98*** [x] *p=0.0221 vs. CON* *p=0.0315 vs. PSG*	6.3
Kruskal-Wallis H [2.28]	8.4	9.2	7.3	7.9	

Number of housed animals=10.
* Statistically significant difference p<0.05 *vs.* CONTROL
[x] Statistically significant difference p<0.05 *vs.* PSG

platform. If a rat failed to locate the platform within 60 s, it was placed on the platform for 5 s. Each rat was submitted to 6 trials per day, and the starting position was changed at each trial (starting on the N side, followed by E, S, W sides, in that order). The interval was 5 min between trials 1–3 and 4–6 and 10 min between trials 3 and 4. For the first 3 days of maze testing, the submerged platform was placed in the NW quadrant. The platform was subsequently placed in the SE quadrant for the following 3 days. On day 7, the platform was lifted above the water level and placed in the SW quadrant, and rats were injected saline 30 min before the test (1, 7, 14 and 21 day of the experiment). Each rat was subjected to a one probe trial consisting of 6 individual trials. The total number of times each rat crossed the probe target area and the time of the probe trial swim were recorded by the observer. The time of each of the 6 trials was noted, and a mean value for each rat was calculated (number of escape latencies). Moreover total number of times each rat crossed the area of quadrant – NW, NE, SE, and SW – (crossed quadrants) were recorded by the observer and a mean value for each rat was calculated (crossed quadrants). The same procedures were followed until 21 day of experiment.

Measurement of locomotor activity (Fig. 1)

Locomotor activity was measured in rats using eight 20.5×28×21 cm wire grid cages, each with two horizontal infrared photocell beams along the long axis, 3 cm above the floor. Photocell interruptions were recorded by electromechanical counters in an adjacent room. Before the test, all groups of animals were habituated to a novel cage within 30 min period. Subjects were tested for single administration of saline 30 min (*ip*) before the test in experimental groups: CONTROL (10), PSG (10) and MAMG (10). Then, photocell activity would be recorded at 10-min intervals for 1 h. This test provided an index of basal locomotor activity of animals in a familiar environment, necessary to indicate the presence of a central stimulant or sedating effects of the drug used in the test.

Table II

Effect of prenatal stress and prenatal administration of MAM on spatial memory tested in the Morris water maze test (crossed quadrants)

Group	Crossed quadrants				
	Single administration (x ± SEM)	Chronic treatment			Friedman H[3.37]
		7 days (x ± SEM)	14 days (x ± SEM)	21 days (x ± SEM)	
Saline (0.5 ml/rat) CONTROL	5.31 ± 0.80	4.37 ± 0.43	3.00 ± 0.46	2.68 ± 0.43	2.2
Saline (0.5 ml/rat) PSG	5.90 ± 1.00	4.80 ± 0.80	**4.20 ± 0.35*** *p=0.0525*	**3.80 ± 0.32*** *p=0.0511*	3.0
Saline (0.5 ml/rat) MAMG	**8.00 ± 1.02*** *p=0.0526*	**6.00 ± 0.61*** *p=0.0424*	**4.75 ± 0.47*** *p=0.0159*	**4.25 ± 0.52*** *p=0.0319*	4.3
Kruskal-Wallis H [2.28]	5.7	5.9	4.2	5.2	

Number of housed animals=10.
* Statistically significant difference $p<0.05$ *vs.* CONTROL

Statistical analysis

The data are shown as the mean values ±SEM. The data distribution pattern was not normal (unlike Gaussian function). Statistical analyses for the memory test, locomotor activity and BDNF level were carried out using the nonparametric Kruskal-Wallis H test for unpaired data and ANOVA Friedman two-way analysis of variance test for paired data. Statistical significance was tested using Dunn's *post-hoc* test. In neuroendocrine analyses, significant differences between group means were determined by analysis of variance with post-hoc testing using Tukey's test.

RESULTS

1) Effect of prenatal stress and prenatal administration of MAM on spatial memory tested in the Morris water maze test (number of escape latencies).

PSG group has shown statistically significant increase in the number of escape latencies which was observed in the **1** and **7** day of the experiment compared to the control group ($p<0.05$ *vs.* CONTROL). The statistically significant increase in the number of escape latencies has been also shown in the MAMG group in the **1**, **7**, **14** and **21** day of the experiment compared to the control group ($p<0.05$ *vs.* CONTROL). Results proving spatial memory impairment in both prenatally stressed and MAM treated groups compared to the control group (Table I). Moreover the increase in the number of escape latencies has been shown in the MAMG compared to the PSG group of rats in the **14** and **21** day of experiment.

2) Effect of prenatal stress and prenatal administration of MAM on spatial memory tested in the Morris water maze test (crossed quadrants).

PSG group has shown statistically significant increase in the number of crossed quadrants which was observed in the **14** and **21** day of the experiment compared to the control group ($p<0.05$ *vs.* CONTROL). The statistically significant increase in the number of crossed quadrants has been also shown in the MAMG group in the **1**, **7**, **14** and **21** day of the experiment compared to the control group ($p<0.05$ *vs.* CONTROL). Results proving spatial memory impairment in both prenatally stressed and MAM treated groups compared to the control group (Table II).

3) Effect of prenatal stress and prenatal administration of MAM on locomotor activity in rats.

Both PSG and MAMG groups of animals has shown statistically significant increase in the locomotor activity compared to the control group ($p<0.05$ *vs.* CONTROL) proving that a correct animal models of schizophrenia were used (Table III). Moreover it was noted that the increase in the locomotor acivity of the PSG rats was statistically significant higher then increase observed in the MAMG rats.

4) Concentration of basal plasma corticosterone in prenatally stressed and MAM treated rats.

In PSG rats, a statistically significant increase of plasma basal corticosterone level (177.17±3.07 ng/ml) was found compared to the control group (CONTROL 141.07±10.78 ng/ml). Statistically significant increase of plasma basal corticosterone level was also found in the MAMG rats (184.78±9.67 ng/ml) compared to the control group (CONTROL 141.07±10.78 ng/ml) (Fig. 2).

5) BDNF values in the prefrontal cortex of prenatally stressed and MAM treated rats.

Both in PSG (288.16±9.14) and MAMG (314.05±15.34) groups of animal a statistically significant decrease in the BDNF level in the rat PFC versus CONTROL group (346.89±8.71) was observed (Fig. 3).

6) BDNF values in the hippocampus of prenatally stressed and MAM treated rats.

Analysis of the BDNF level in the rats HIP shown that in both PSG (402.39±18.90) and MAMG (456.78±20.81) groups a statistically significant decrease in the BDNF level in the rat hippocampus versus CONTROL (593.51±16.35) was observed (Fig. 3).

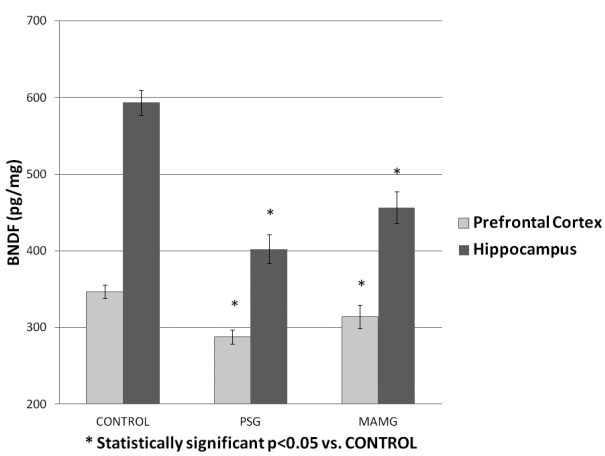

Fig 3. BDNF values in the hippocampus and prefrontal cortex of prenatally stressed and MAM treated rats.

DISCUSSISON

Considering high incidence of schizophrenia in the global population (approx. 1%) (Nagai et al. 2010), as well as continuous development of studies aimed at explaining the background of the disease, animal models of mental disorders seem to be an important tool in understanding key theories related to pathophysiology of the schizophrenia. Animal model is only an experimental method that allows to achieve results in the context of specific parameters depending on the model used (Ratajczak et al. 2013a). High scientific value of these models is associated with the neurodevelopmental theory which stipulates that at an early stage of body development, a number of interactions between genetic and environmental factors may present themselves and, consequently, adversely affect the development of neurons (their stratification and spatial arrangement) which may, in turn, cause disorders of brain cytoarchitecture development (Gabryel 2008). Developmental abnormalities originating in the foetal period may become permanent in the perinatal period, and are usually fully expressed in the early adolescence period (Arnold and Trojanowski 1996). The analyzed study focuses mainly on neurodevelopmental models using prenatal stress exposure or methylazoxymethanol acetate (MAM) administration, and on comparing the generated spatial memory disorders and locomotor activity changes. The study also focused on the plasma corticosterone level changes as well as BDNF level changes in the HIP and PFC observed in the prenatally stressed rats, MAM-treated rats and control groups (naive rats).

It is believed that the background of cognitive function disorders in patients with diagnosed schizophrenia is the attentional disruption, disturbed basic social functions, working and verbal memory disorders as well as spatial memory deterioration (Addington and Addington 2000). Results of spatial memory examinations using the Morris water maze show that both prenatal stress and MAM administration cause a statistically significant deterioration of spatial memory compared to the control group. These results corroborate with studies by many other authors (Ratajczak et al. 2013b, Lucas et al. 2011, Cattabeni and Di Luca 1997, Karnam et al. 2009a, Karnam et al. 2009b).

Schizophrenia features a general deterioration of cognitive functions which is mainly related to disturbances in development of the HIP – the key brain structure responsible for neurocognitive processes, in particular memory, as well as for synaptic plasticity (Manahan-Vaughan et al. 2008, Sheline et al. 1996). Conclusions of the published study results indicate that stress exposure related with the increase of glucocorticosteroid levels during foetal development result in disturbed development of brain structures and thus may cause a number of behavioral changes related with reduction of social interactions, enhanced body response to psychostimulants such as amphetamine and apomorphine (Ratajczak et al. 2013a) and may generate manifestation of depression symptoms, deterioration of spatial memory or enhanced locomotor activity. Experimental studies on animals show that the HIP in both juvenile and adult rats exposed to prenatal stress has less neurons (which entails lower BDNF concentration) and these animals have more difficulty in performing simple tasks related, for instance, with recognition (tested in the Morris test) (Lemaire et al. 2000). Studies of Cattabeni and others (1997), in turn, show that administration of MAM to pregnant females can cause not only structural changes in the offspring's HIP but also generates structural disturbances in the brain cortex (including PFC) and subcortical regions (brain weight diminishes as well). A detailed results analysis based on MAM model showed that disturbances in HIP and PFC development are identical to disturbances observed in humans (Chevassus-Au-Louis et al. 1998). Research conducted by Lucas and others (2011) show that MAM induces spatial memory disturbances in the offspring irrespective of the period when the substance had been administered to pregnant mothers – drug administration in day 15 or 17 of pregnancy caused a similar memory deterioration in animals. Deficits in these and similar paradigms are consistently observed in human schizophrenia patients (Lodge and Grace 2009, Lipska and Weinberger 2000). A detailed description of disturbances occurring in the analysed schizophrenia animal models was published in our other papers (Ratajczak et al. 2013a).

Results of motor activity evaluation in rats indicate increased locomotor mobility both in rats that have been exposed to prenatal stress and in MAM-treated animals. Moreover, prenatally stressed rats showing greater locomotor activity increase compared to the MAM-treated rats. The results are consistent with previously published papers (Ratajczak et al. 2013b, Kinnunen et al. 2003, Koenig et al. 2005, Balduini et al. 1991, Cannon-Spoor and Freed 1984, Ferguson et al. 1993, Hanada et al. 1982) and confirm that increased animal mobility is one of the factors that indicate correctness of animal schizophrenia model implementation. Increased locomotor activity in rats in the prenatal stress and MAM models is probably related to the increased DA release in the striatum and the nucleus accumbens (Gulley et al. 1999, Sharp et al. 1987).

Table III

Effect of prenatal stress or prenatal administration of MAM on locomotor activity in rats

	Activity counts / mean
Group	Single treatment (x ± SEM)
Saline (0.5 ml/rat) CONTROL	69.33 ± 5.83
Saline (0.5 ml/rat) PSG	**141.30 ± 9.10*** *p<0.0001*
Saline (0.5 ml/rat) MAMG	**113.50 ± 4.66*** [x] *p<0.0001 vs. CON; p=0.0138 vs. PSG*
Kruskal-Wallis H [2.28]	9.6

Number of housed animals=10.

* Statistically significant difference p<0.05 *vs.* CONTROL.

[x] Statistically significant difference p<0.05 *vs.* PSG

Analysis of rat corticosterone plasma levels showed that in both PSG and MAMG groups the levels of glucocorticoid hormone were increased compared to the CONTROL group. These results corroborate with our previous research (Ratajczak et al. 2013b) as well as with other authors' reports (Kinnunen et al. 2003, Sahu SS et al. 2012, Ohta et al. 2000). Prenatal or post-natal stress increases the risk of schizophrenia development (Corcoran et al. 2001) in the offspring. Stress reaction activates brain monoaminergic transmission and increases cortisol/corticosterone secretion. In rats, chronic glucocorticosteroids administration leads to neurotoxic structural changes within the HIP and to

degenerative changes in the PFC (Conrad et al. 2007). It has been also proven that chronic hypercortisolaemia due to permanently high corticosterone levels leads to a decrease in 5HT1 receptors density and sensitivity and to changes in apical neurons' dendrite sizes in the rat HIP, regardless of the chosen model (Conrad et al. 1999). The obtained results clearly show that the basal corticosterone level was significantly higher in rats stressed prenatally as well as in MAM-treated rats. Research conducted in 2000 by Ohta and others (2000) using MAM in HAA (high-avoidance animals) and LAA (low-avoidance animals) rats indicate that in the conducted shuttle-box avoidance task (Ohta et al. 1995), increased basal corticosterone levels appeared only in HAA rats – animals with high avoidance of the task, while in LAA animals no statistical significance was shown compared to the control group. McEwen et al. (McEwen 2008) also pointed out that structural disorders in the HIP affect stimulation/inhibition of receptors responsible for glucocorticosteroids release, leading to neurogenesis inhibition (which is directly related to the BDNF level) and the resulting cognitive disorders in the discussed animal models of schizophrenia.

BDNF level analysis in PSG and MAMG rats showed that in both regions (PFC and HIP) significantly lower levels of the neurotrophin were observed compared to the control group. It has been also observed that the BDNF protein level in the PFC was lower than in the HIP. These results corroborate with studies by other authors (Madhyastha et al. 2013, Fiore et al. 2002). BDNF is a neurotrophine responsible primarily for behavior regulation and for emergence of neurodevelopmental disorders (Alleva et al. 1993, Bersani et all. 1999, Nawa et al. 2000) which could lead to depression and schizophrenia development.

In the case of discussed animal models of schizophrenia the use of MAM leads to neuroblastic cells apoptosis caused by mitotic division inhibition (Fiore et al. 1999, Talamini et al. 1999) and thus eventually reduces weight of each brain structure as well as the whole brain. Fiore et al. (Fiore et al. 2002) indicate that due to MAM administration in rats BDNF level decreases both in the PFC and HIP, and suggests that this is associated with MAM's effect on the hippocampal-entorhinal cortex axis. On the other hand, research conducted by Di Fausto and others (2007) shows that prenatal MAM injection may increase BDNF level in the HIP regardless of its weight reduction by 21%. It is probably caused by an increased expression of TrkA and TrkB receptors [responsible for proliferation of nervous cells (Pencea et al. 2001)]. Conducted studies with animal model of schizophrenia using prenatal stress, in turn, also confirm reduced BDNF expression in the rats' HIP (Madhyastha et al. 2013) and PFC (Luoni et al. 2013). Prenatal stress exposure disturbs proliferation and neuronal differentiation as well as induces memory disorders (Karten et al. 2005). As indicated by Madhyastha and others (Madhyastha et al. 2013) prenatal stress causes neurogenesis reduction in the dentate gyrus. Also using of Maternal Deprivation – one of the developmental animal model of schizophrenia – leads to decrease of the hippocampal volume as well as decrease in the size of pyramidal and granular cell layers (Aksić et al. 2013). Author observed also the decrease in the thickness of the prefrontal, retrosplenial and motor cortex (Aksić et al. 2013). Calabrese and others (2013) indicated that the reduced BDNF level may result from conversion between the animals' life periods. It is also remarkable that the BDNF level may be regulated by mRNA transcription and dopaminergic (mainly D2) and serotonin (mainly 5-HT2A) receptors expression (Fiore et al. 2002).

CONCLUSION

It can be assumed that experimental trials using animal models of schizophrenia are the basic tool for a better understanding of mechanisms related to the incidence and treatment of this disease, especially when the etiology of disease has not been entirely discovered. As shown by the obtained results, both the prenatal stress model and prenatal MAM administration model generate a number of behavioral (e.g. spatial memory disorders, increased locomotor activity) and biochemical (e.g. increased corticosterone and decreased BDNF levels in HIP and PFC) changes in the examined offspring and thus these models can be successfully used in the efficacy analysis of the pharmacotherapy applied (Ratajczak et al. 2013a, Ratajczak et al. 2013b).

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