Physiol. Res. 66 (Suppl. 4): S469-S479, 2017

Olfactory Bulbectomy in Methamphetamine-Treated Rat Mothers Induces Impairment in Somatic and Functional Development of Their Offspring

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Received April 10, 2017
Accepted October 25, 2017

Summary

Olfactory bulbectomy in rodents is considered a putative model of depression. Depression is often associated with drug addiction. Our previous studies demonstrated that methamphetamine (MA) administration to rat mothers affects both, mothers and their pups. The aim of the present study was to examine the effect of bulbectomy, as a model of depression, and MA administration on behavior of rat mothers and postnatal development of their pups. Adult female Wistar rats were randomly divided into two groups: bulbectomized (OBX) and sham-operated (SH). A period of 20 days was allowed for the development of the depressive-like phenotype. Animals were tested in the motor activity test and 2 % sucrose preference for anhedonia and hyperactive locomotor response to a novel environment, respectively. After then females were impregnated. Pregnant females were exposed to daily subcutaneous (s.c.) injection of MA (5 mg/kg) or saline (SA) during the entire gestation period. Postnatally, maternal behavior and pup development was examined. The effect of a challenge dose of MA (1 mg/kg, s.c.) on behavior was further examined in adult male offspring. Our results showed no differences in the maternal behavior as a matter of bulbectomy, only OBX rats slept more than all the SH controls. Pups from OBX mothers were born with lower birthweight and gained less weight during the postnatal development than pups from SH controls. Both, bulbectomy and MA administration, delayed the eyes opening. As a matter of functional development of the pups, maternal OBX procedure impaired the performance in the Bar-holding test, but only in saline group. OBX/SA group was the worst in the Bar-holding test relative to all the other groups. In addition, pups from OBX mothers dropped more boluses during the Bar-holding test, suggesting that they were more stressed. In adult male offspring, bulbectomy increased immobility only in the SA/SA group. Prenatal MA exposure increased locomotion, while decreasing immobility. In addition, challenge dose of MA in adulthood increased distance traveled, locomotion, rearing, and average and maximal velocity, while decreasing immobility and grooming. In conclusion, our results suggest that depressive-like phenotype of rat mothers induces impairment in somatic and functional development of their male offspring.

Key words

Methamphetamine • Olfactory bulbectomy • Depression • Maternal behavior • Development

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Introduction

Clinical studies demonstrated that depression may often result in drug or alcohol addiction and the opposite way around. The comorbidity between addiction and depression varies by the type of abused drugs, the rates of depression reaching 54 % in opioid-dependent, 38 % in alcohol-dependent, and 32 % in psychostimulant-dependent patients compared with only 7 % in the whole population (Filip *et al.* 2013, Kosten *et al.* 1998). Co-occurrence of substance or alcohol abuse and depression results in even greater disease burdens than

the separate disorders (Riper *et al.* 2014). Some of the burdens experienced by people with this comorbidity result in higher mortality levels, functional impairment and increased suicide risk (Riper *et al.* 2014).

As far as the neurobiology of such comorbidity is concerned, different mechanisms may be involved. Depression resulting in drug abuse may reflect an attempt to self-medicate a depression state, while drug abuse followed by depression may result from an early exposure to chronic drugs of abuse, which leads to neurobiological changes increasing the risk of depression (Filip *et al.* 2013, Volkow 2004). The Self-medication hypothesis (Hall and Queener 2007, Khantzian 2013) indicates that monoaminergic deficit present in depression may be relieved by the drug of abuse, thus individuals with depression have aberrant brain reward systems and may turn to drugs that create euphoric feelings to compensate for their anhedonia and motivational inadequacy (Baicy *et al.* 2005, Markou *et al.* 1998). In addition, experimental studies demonstrated that the rewarding activities of psychostimulant drugs are modified in experimental models of depression and that depression-like effects are observed in animals withdrawn from chronic treatment with psychostimulants such as methamphetamine (MA) and other drugs (Filip *et al.* 2013, McKernan *et al.* 2015, McKetin *et al.* 2011).

Results of preclinical studies examining drug addiction behaviors in animal models are generally conformable with the clinical experience. Bilateral olfactory bulbectomy (OBX) has become the most commonly used model of depression for study of the dual disorder (Kelly *et al.* 1997, Morales-Medina 2017, Song and Leonard 2005). In this model bulbectomized animals showed a significantly higher vulnerability in intravenous drug self-administration of MA (Kučerová *et al.* 2012) and also an increased tendency to reinstate the MA-seeking behavior after abstinence (Babinská *et al.* 2016). Furthermore, other drugs, specifically cannabinoid receptor-1 synthetic agonist (Amchová *et al.* 2014) and ketamine (Babinská and Rudá-Kučerová 2017) showed similar results. Therefore, it seems that this rat model has a high translational validity and is relevant for the study of neurochemical mechanisms underlying the depression and addiction comorbidity.

Maternal depression in humans is associated with impaired neurodevelopment in newborns (Smith *et al.* 2012). Rodent studies are in line with this evidence as OBX was previously shown to impair several components of maternal behavior in rats (Schwartz and Rowe 1976) and mice (Neckers *et al.* 1975). The rodent mothers spend less time nursing the pups and there were also more pups killed by the mother in mice. In rats these effects were reversed by apomorphine treatment suggesting at least an important role of hypodopaminergic state in the OBX model (Sato *et al.* 2010). The basal hypodopaminergic tone was confirmed in the OBX male rats (Rudá-Kučerová *et al.* 2015). Therefore, OBX procedure in rat mothers should induce early life stress in the offspring due to maternal behavior deficits which may lead to behavioral changes in adulthood. Dopaminergic system is affected not only by OBX, but also by psychostimulant drugs, such as MA (Rothman and Baumann 2006).

Psychostimulants act *via* dopaminergic, noradrenergic and serotonergic systems, though, the degree of effect of each drug differs between specific neurotransmitters (Rothman and Baumann 2006). Also MA is consistently reported to have detrimental developmental effects in human (Wouldes *et al.* 2014) as well as in laboratory animals (Malinová-Ševčíková *et al.* 2014, McDonnell-Dowling and Kelly 2015, Šlamberová 2012). Our previous studies demonstrated that MA exposure during gestation impairs maternal behavior of rat mothers (Šlamberová *et al.* 2005) and delays sensorimotor development of their pups (Šlamberová *et al.* 2006). A recent clinical study evaluated the neurodevelopmental effects of prenatal MA exposure and maternal depression and identified deficits in infants in either group. However, the combination of drug exposure and maternal depression did not lead to increased risks (Smith *et al.* 2012). To the best of our knowledge, there is no experimental study attempting a downwards translation of this situation to an animal model which could provide more information on the underlying mechanisms.

Therefore, the aim of this study was to extent our knowledge on the animals modelling of comorbid depression and addiction by evaluating developmental effects of combined MA exposure and depressive-like phenotype of the rat mothers induced by OBX. We hypothesized a differential behavioral profile and altered reactivity to MA in the offspring of the OBX mothers.

Methods

Adult female and male albino Wistar rats (375 – 400 g) provided by Charles River Laboratories

International, Inc. were delivered by VELAZ (Prague, Czech Republic). Animals were housed four per cage by sex and left undisturbed for a week in a temperature-controlled (22-24 °C) colony room with free access to food and water on a 12 h (light):12 h (dark) cycle with lights on at 06:00 h.

Olfactory bulbectomy

At the beginning of the behavioral and neurochemical experiments, rats were randomly divided into two groups: bulbectomized (OBX, n=13) and sham-operated (SH, n=9) rats and the surgery was performed as described earlier (Kučerová *et al.* 2012). Animals were anaesthetized with i.p. injections of 50 mg/kg ketamine plus 8 mg/kg xylazine. The top of the skull was shaved and swabbed with an antiseptic solution. Then, midline frontal incision was made on the skull and the skin was retracted bilaterally. Two burr holes, 1.5 mm in diameter, were drilled in the frontal bone 7.5 mm anterior and 2 mm lateral to bregma suture. Both olfactory bulbs were removed by aspiration paying particular attention to not damage the frontal cortex. Prevention of blood loss from the ablation cavity was achieved by filling the dead space with a hemostatic sponge. The skin above the lesion was closed with suture. Finally, bacitracin plus neomycin dusting powder was applied to prevent bacterial infection. During the surgery, two animals were killed to check whether the bulbs were removed entirely. All OBX rats were dissected at the end of the study in order to verify the OBX surgery.

Sham-operated rats underwent identical anesthetic and drilling procedures but their bulbs were left intact. A period of at least 20 days was allowed for the recovery from the surgical procedure and the development of the depressive-like phenotype. During this period, animals were handled daily for few minutes to eliminate aggressiveness (Kelly *et al.* 1997). Animals were then tested in the LABORAS apparatus for potential hyperactive locomotor response to a novel environment and in the 2 % sucrose preference test to assess potential anhedonia.

Spontaneous activity

After the 20-day post-surgical brake the spontaneous activity was tested in a LABORAS apparatus (Metris B.V., Netherlands) situated in a dark room. The LABORAS (Laboratory Animal Behavior Observation Registration and Analysis System) is a fully automated system for continuous behavior recognition and tracking in small rodents as described in our study (Schutová *et al.* 2013). Animals were tested in the unknown environment for 10 min. The 10-minute period was divided to ten minute measures (*Intervals*) to see the progress of the activity. The following parameters were automatically evaluated in the LABORAS: (1) time spent in locomotion [s]; (2) time spent rearing (exploratory behavior) [s]; (3) distance (trajectory length) [m]; and (4) average speed [mm/min].

Sucrose preference test

After recovery from the OBX surgery and testing in the LABORAS apparatus, female rats were transferred into single housing with free access to food and water. A two-bottle choice procedure was used to determine their sucrose intake. During the 24-h training phase, each rat was provided in their home cage with two water bottles to adapt rats drinking from two bottles. After training, one bottle was randomly (left or right) switched to contain 2 % sucrose solution. The side of sucrose presentation in the home cage was counterbalanced across rats. At 4 h and 24 h time intervals both bottles were removed and the amount of liquid remaining in each bottle was measured. After 4 h, the relative position of the bottles was inverted, i.e. they were switched from one side of the cage to the other to avoid perseveration effects. The sucrose preference score was calculated as the percentage of sucrose solution ingested relative to the total amount of liquid consumed as determined before and after each test, i.e. sucrose preference = sucrose intake / total liquid (sucrose + water) intake x 100 (Amchova *et al.* 2014).

Drug administration

After the recovery period and the behavioral tests females were impregnated with sexually mature males as described in our previous study (Šlamberová *et al.* 2005). Pregnant females (OBX or SH) were exposed to daily subcutaneous (s.c.) injection of MA (5 mg/kg) or saline (SA) during the entire gestation period (gestational day (GD) 1-22). Females were kept in group cages (4-5/cage) until GD 21. Then they were moved to maternity cages (1 female/cage). The day of the delivery was indexed as postnatal day (PD) 0. On PD 1, pups were weighed and the number of males and females were recorded. Maternal behavior was tested in two types of tests: Observation test and Retrieval test. At this time of the study, some OBX rats were lost due to surgery and some did not deliver, therefore, the final numbers of

mothers in each groups were the following: SH-SA n=4, SH-MA n=5, OBX-SA n=4 and OBX-MA n=4.

Maternal behavior - Observation test

Maternal behavior was observed daily for 50 min in the home cage of each mother and her litter between PD 1 and PD 22. The time of observation was during the light phase of the light/dark cycle between 08:00 – 09:00 h. Similar methods were used as in our previous studies (Ševčíková *et al.* 2017, Šlamberová *et al.* 2005). During each 50-minute session, each mother and her litter were observed 10 times for 5 s at 5 min intervals. Eleven types of maternal (mother in or out of the nest; mother in contact with any of her pups; mother licking or grooming any of her pups; mother carrying pups; mother manipulating nest shavings) and non-maternal (mother resting with eyes closed; mother eating; mother drinking; mother self-grooming; mother rearing; mother sniffing with head raised) activities exhibited by the mothers and three nursing positions (arched-nursing; blanket-nursing; passive-nursing) were recorded during each session. Thus, each mother and litter was observed 220 times (22 days x 10 observations / session). During each observation "1" was given if a behavior occurred and a "0" if it did not.

The occurrence of each activity (maximum 10 in each session) was counted in each of 22 sessions.

Maternal behavior – Retrieval test

The same mothers and pups were tested for Retrieval test always after the Observation test ended. The retrieval test was conducted daily between PD 1 and PD 12 between 09:00 – 10:00 h, so each mother and litter was tested 12 times. The same method was used as in our previous studies (Ševčíková *et al.* 2017, Šlamberová *et al.* 2005).

All pups were removed from their mothers and placed in a separate cage for 5 min. After this brief separation, the entire litter was returned to their mothers into the maternity cage and the pups were spread all around the cage. The mother was then observed for 10 min, and the following measures were recorded; 1) the latency to carry the first pup, 2) the latency to return the first pup into the nest, 3) the latency to return all the pups into the nest. During each observation "1" was given if a behavior occurred and a "0" if it did not. It was also noted, whether the mother carried first one of her own pup or one of the adopted pup into the nest.

Pup development

To examine pup's somatic and functional development, the same methods were used as in our previous studies (Malinová-Ševčíková *et al.* 2014, Ševčíková *et al.* 2017). The final groups of tested pups were as follows: SH-SA males (n=24), SH-SA females (n=23), SH-MA males (n=24), SH-MA females (n=23), OBX-SA males (n=14), OBX-SA females (n=15), OBX-MA males (n=15), OBX-MA females (n=15).

Litter characteristics and maturation of the pups

The same method was used as in our previous study (Ševčíková *et al.* 2017). Number of pups in the litter, number of dead pups, and percentage of males and females in each litter was recorded and compared between groups. Pups were weighted daily between PD 1 and 23 and the day of eye opening was recorded as a number of pups with eyes opened on PD 14 and PD 15.

Negative Geotaxis

Negative geotaxis was tested on PD 9 (Altman and Sudarshan 1975). Each pup was placed facing downward on a screen inclined at 30° angle. Each animal was given three trials and the best latency of turning the face upward (180° rotation) was recorded. If the pup was slid off the board, it was replaced in the downward position.

Righting reflex on surface

Righting reflex on surface was tested on PD 12 (Altman and Sudarshan 1975). Each pup was turned on its back, and the time it took for the pup to right with all four paws contacting the surface of the testing table was recorded. If righting did not occur within 60 s period, the test was terminated and a time of 60 s was given for that trial.

Righting reflex in mid-air

The righting reflex in mid-air (Altman and Sudarshan 1975) was tested on PD 17. Each pup was held on its back 40 cm above the soft pad and then released and watched in which position it reaches the soft pad. A score of "1" was given when a pup reached the ground at once with all 4 paws and a "0" when it did not.

Bar-holding test

The beam balance test on PD 23 was used to examine vestibular function and sensorimotor coordination engaged in maintenance of the balance on

the narrow bar (Malinová-Ševčíková *et al.* 2014, Murphy *et al.* 1995). A wooden bar 40 cm long with a diameter of 1 cm was suspended 80 cm above padded soft surface. The pup was placed on the bar being held by the nape of its neck and its forepaws were allowed to touch the bar. Time of fore- and hindlimb grasping reflex was recorded with a limit of 120 s. Rats were subjected to three consecutive trials.

Rotarod

Rotarod performance was examined on PD 23 to test the sensorimotor coordination and dynamic postural reactions necessary for active moving to maintain the balance on the rotating cylinder (Gerald and Gupta 1977). Pups were positioned on a rugged cylinder (11.5 cm in diameter, rotating at constant speed of 6 rpm) in the opposite direction of cylinder rotation, so they were able to walk forward. The duration of balance on the rotarod was determined for 120 s. Rats were subjected to maximum 6 trials until successfully accomplished the task. Number of falls was recorded.

Behavior of adult male offspring

For this part of the study only male offspring were used. The number of rats per group was n=8. The effect of challenge dose of MA (1 mg/kg, s.c.) on behavior in the LABORAS apparatus was further examined in adult offspring males prenatally exposed to drug and bulbectomy. Unlike the mothers (see above) male offspring were tested in the LABORAS apparatus for 60 min to see their habituation. The animals were not habituated to the apparatus before. Each rat was injected with MA (1 mg/kg) or saline (1 ml/kg) and immediately placed in the center of the LABORAS cage. The behavior was recorded for 1 h (Schutová *et al.* 2013). The 60-minute period was divided to ten 10-minute measures (*Intervals*) to see the progress of the activity.

The following parameters were automatically evaluated: (1) distance travelled (trajectory length) [m]; (2) time spent in locomotion (horizontal movement in cage) [s]; (3) velocity (average speed) [cm/s]; and (4) time spent rearing (vertical movement in cage; exploratory behavior) [s].

Statistical analyses

In the mothers, spontaneous activity, sucrose preference, observation test (22 days) data were analyzed by a one-way ANOVA (surgery) with Repeated Measure (10 min of testing). Latencies in the retrieval test were analyzed by one-way ANOVA (drug treatment) with Repeated Measure (12 days of testing). The incidence whether the mother carried one of her own or an adopted pup first to the nest was analyzed by χ^2 test. Differences were considered significant if $p<0.05$.

Litter characteristics (number of pups in each litter, the number of dead pups and the percentage of males and females in each litter) data were analyzed by a two-way ANOVA (surgery x drug exposure). A three-way ANOVA (surgery x drug exposure x sex) was used to analyze differences in birth weight and weight gain during PD 1-22. A χ^2 test was used to test eye opening in specific postnatal days. For the analysis of neonatal reflexes a three-way ANOVA (surgery x drug exposure x sex) was used. A χ^2 test was used for statistical analysis of the righting reflex in mid-air in specific days.

Behavioral test performed at adulthood were analyzed by a three-way ANOVA (surgery x prenatal drug x challenge drug) with Repeated Measure (10 min of testing).

For *post hoc* comparison a Tukey HSD test was used in all the Analysis of Variance measures. Differences were considered significant if $p<0.05$ in all analyses.

Results

Spontaneous activity of OBX females

Bulbectomy increased locomotion [$F_{(1,15)}=4.51$; $p<0.05$] and decreased immobility [$F_{(1,15)}=10.95$; $p<0.01$] of adult female rats (Fig. 1). Other measures such as distance traveled, velocity and rearing was not influenced by bulbectomy.

Sucrose preference test

There were no differences in the test of sucrose preference. All groups reached high preference of the sucrose solution.

Maternal behavior

In the observation test, there were no differences in the type of nursing or other maternal activities. The only difference induced by bulbectomy was increased sleeping [$F_{(1,13)}=5.94$; $p<0.05$], which seems to correspond with depressive-like behavior. There were no significant differences between groups in the Retrieval test either.

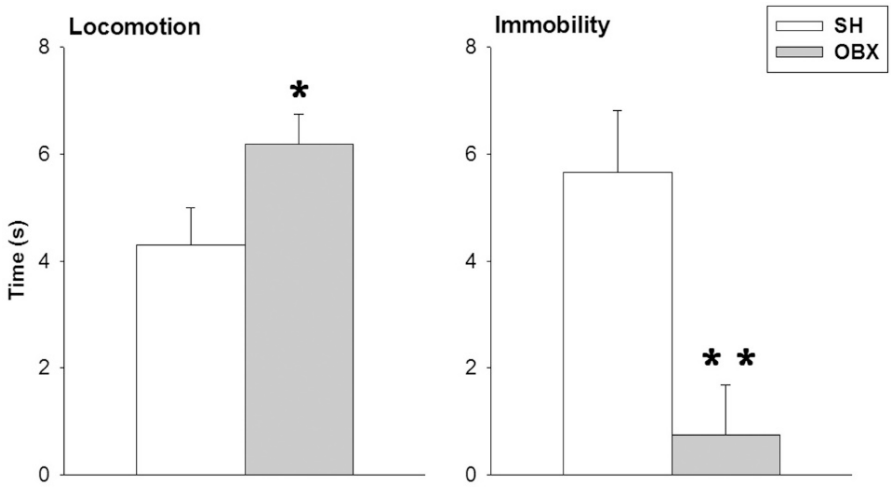

Fig. 1. The effect of bulbectomy on locomotion and immobility of adult female rats tested in the LABORAS apparatus. SH = sham, OBX = bulbectomy. Values are mean ± SEM (n=8-9); * p<0.05, ** p<0.01 vs. sham-operated controls.

Pup development

Somatic development

Pups from OBX mothers were born with lower birthweight [$F_{(1,145)}$=5.50; p<0.05] and gained less weight during the postnatal development [$F_{(1,145)}$=13.72; p<0.001] that pups from sham controls regardless of prenatal drug exposure. There were sex differences in the birth weight as expected: females had lower birthweight [$F_{(1,145)}$=13.91; p<0.001] than males.

Pups from sham-operated and saline-treated mothers (SH/SA) regardless of their sex opened eyes earlier than all the other groups (on PD 14 [χ^2=63.48; p<0.0001], on PD 15 [χ^2=53.80; p<0.0001]). Table 1 shows the percentage of animals with eyes opened on PD 14 and PD 15. These results suggest that both, the bulbectomy and MA administration, prolonged the eyes opening.

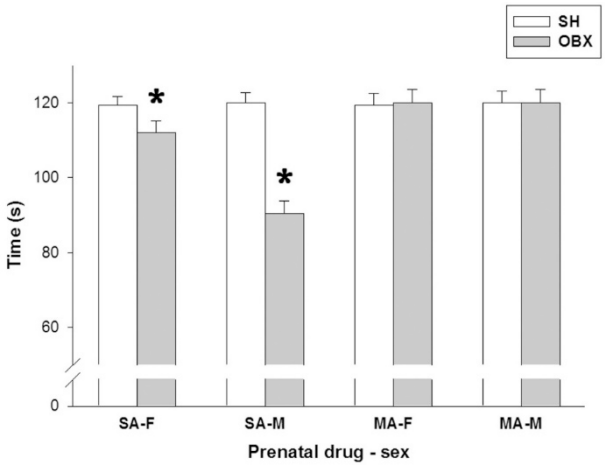

Fig. 2. The effect of maternal bulbectomy on performance of pups in Bar-holding test on PD 23. SH = sham, OBX = bulbectomy. SA = prenatal saline, MA = prenatal methamphetamine, F = female, M = male. Values are mean ± SEM (n=14-24); * p<0.05 sham-operated controls.

Functional development

Bulbectomy impaired the performance in the Bar-holding test (PD 23), but only in saline group. Pups from OBX mothers treated with saline during gestation (OBX/SA) was the worst in the Bar-holding test relative to all the other groups [$F_{(1,145)}$=17.15; p<0.0001] (Fig. 2). In addition, pups from OBX mothers dropped more boluses during the Bar-holding test [$F_{(1,145)}$=4.82; p<0.05], suggesting that they were more stressed.

In Negative geotaxis (PD 9), Righting reflex on surface (PD 12), Righting reflex in mid-air (PD 17) and Rotarod test (PD 23), there were no differences induced by bulbectomy. Only MA decreased performance in the Rotarod test [$F_{(1,145)}$=4.17; p<0.05] regardless of bulbectomy or sex.

Behavior of adult male offspring

Bulbectomy increased immobility in prenatally saline-exposed adult males rats injected prior to the test with single saline injection (SA/SA) [$F_{(1,56)}$=4.07; p<0.05] (Fig. 3B). Other measures or groups were not affected by bulbectomy. Prenatal MA exposure increased locomotion [$F_{(1,56)}$=4.03; p<0.05], while decreasing immobility [$F_{(1,56)}$=5.40: p<0.05].

Acute adult MA treatment increased locomotion [$F_{(1,56)}$=30.53; p<0.0001] (Fig. 3A), rearing [$F_{(1,56)}$=38.36; p<0.0001], distance traveled [$F_{(1,56)}$=21.29; p<0.0001] (Fig. 3C), average [$F_{(1,56)}$=21.29; p<0.0001] (Fig. 3D) and maximal velocity [$F_{(1,56)}$=16.52; p<0.001], while decreasing immobility [$F_{(1,56)}$=35.62; p<0.0001] (Fig. 3B) and grooming [$F_{(1,56)}$=40.06; p<0.0001].

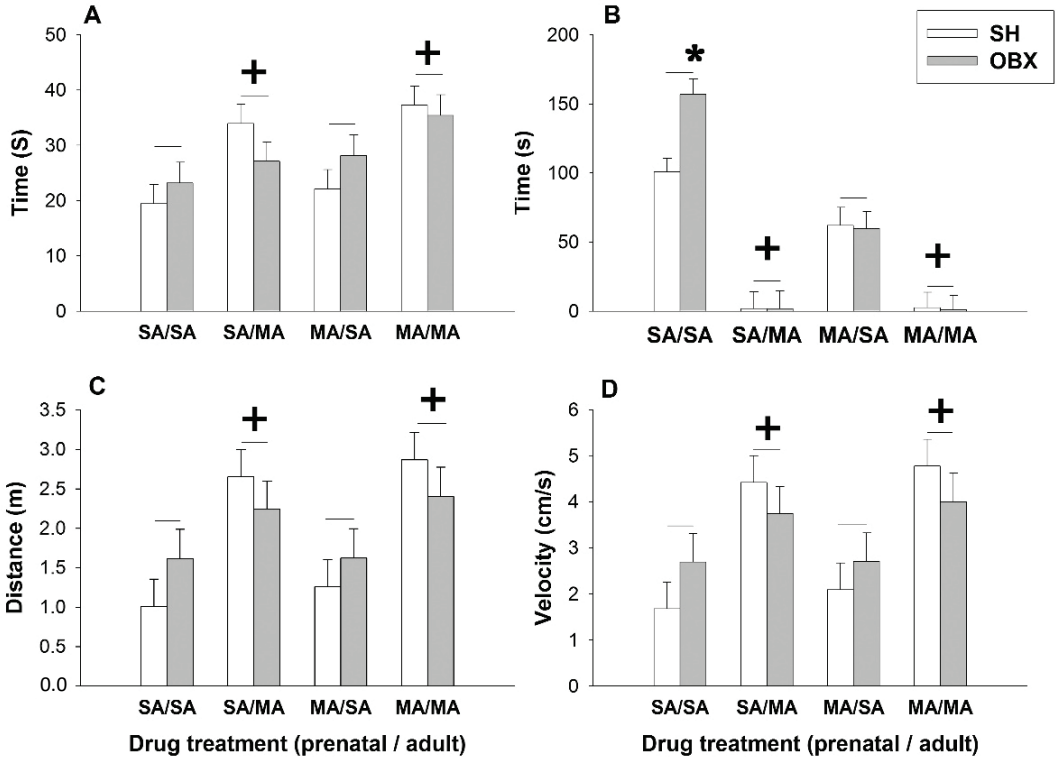

Fig. 3. The effect of maternal bulbectomy and drug exposure on locomotion (**A**), immobility (**B**), distance traveled (**C**) and average velocity (**D**) of adult offspring in LABORAS apparatus. SH = sham, OBX = bulbectomy. SA = saline, MA = methamphetamine. Values are mean ± SEM (n=8); * $p<0.05$ sham-operated controls (OBX>SH in SA/SA group, i.e. animals exposed both prenatally and postnatally to saline); $^{+}$ $p<0.05$ main effect: acute MA vs. acute saline.

Discussion

The aim of the present study was to extent our knowledge on the animals modelling of comorbid depression and addiction by evaluating developmental effects of combined MA exposure and depressive-like phenotype of the rat mothers induced by olfactory bulbectomy. As expected, OBX increased locomotion and decreased immobility in LABORAS apparatus, which is test used for rat behavior in novel environment. Enhanced locomotor activity reflects hyper-arousal of OBX animals as a response to a novel environment (Amchová *et al.* 2014, Flores *et al.* 2014, Holubová *et al.* 2016). Increased exploration in open field following OBX is one of the first reported behavioral changes evident as soon as 1 week after surgery (Hendriksen *et al.* 2015). Hyperactivity, as a consequence of novelty exposure, is probably caused by increased release of glutamate in the striatum (Ho *et al.* 2000). Increased level of glutamate was observed also in the *nucleus accumbens shell*, a reward-related brain area in the OBX rats (Rudá-Kučerová *et al.* 2015). It seems that glutamatergic system is largely distorted in the OBX model.

The present results demonstrated no differences in the maternal behavior as a matter of bulbectomy, with exception of prolonged sleeping time of OBX mothers relative to SH controls. In contrast to our results studies of others found that helplessness, as well as olfactory bulbectomy, as models of depression, decreases maternal behaviors, such as licking, grooming and nursing with arched-back position (Kurata *et al.* 2009, Sato *et al.* 2010). It should be however noted that most of these studies used mice to model the depressive-like phenotype (Neckers *et al.* 1975, Sato *et al.* 2010). In addition, there are studies showing that strain (Braw *et al.* 2009) as well as repeated pregnancy (Schwartz and Rowe 1976) may influence the impairing effect of depressive behavior on maternal behavior. In the present study primiparous albino Wistar rats provided by Charles River Laboratories International, Inc. were used. Using different species or strain of rodents might be the reason for no effect of OBX on maternal behavior observed in this experiment in the present study.

Even though this study did not show differences of bulbectomy on maternal behavior, there were some somatic as well as functional differences induced by bulbectomy of rat mothers in their offspring. Despite the fact that maternal weight did not differ, pups from OBX

mothers were born with lower birthweight and gained less weight during the postnatal development than pups from SH controls. Further, both, OBX and prenatal MA administration, delayed the eyes opening. These OBX-induced effects are in line with a previous report showing similar somatic development in offspring of rat mothers subjected to chronic mild stress before fertilization (Cankara *et al.* 2012) which is a recognized model of depression (Micale *et al.* 2013). Similarly, offspring of rats who underwent the chronic mild stress during pregnancy exhibit attenuated prepulse inhibition of the startle reaction in adulthood which indicated higher anxiety (Holubová *et al.* 2016, Hougaard *et al.* 2011, Kjaer *et al.* 2010). Therefore, it seems that maternal stress *per se* may be able to induce long-lasting behavioral alterations on the offspring. The OBX animals have higher corticosterone levels as reported previously (Yang *et al.* 2014) which could be one of the factors contributing to the offspring development.

However, no additive effect of OBX and MA exposure was observed. As a matter of functional development of the pups, maternal OBX procedure impaired the performance in the Bar-holding test, but only in saline group. OBX/SA group was the worst in the Bar-holding test relative to all the other groups. The finding that maternal OBX impaired the performance of pups in Bar-holding test only in pups of mothers treated in gestation with saline but not in mothers treated with MA, suggests that MA somehow masked or apparently diminished the effect of OBX. In addition, pups from OBX mothers dropped more boluses during the Bar-holding test, suggesting that they were more vulnerable to stress. This is in line with the increased anxiety in the offspring of chronically stressed rats (Hougaard *et al.* 2011, Kjaer *et al.* 2010). Our present data also confirm the clinical findings that maternal depression is associated with impaired neurodevelopment in newborns (Smith *et al.* 2012). To the best of our knowledge there are no experimental studies showing the effect on maternal bulbectomy on pup's development. Furthermore, there are studies showing that maternal separation impairs postnatal development of rat pups (Secoli and Teixeira 1998, Zimmerberg *et al.* 2003). Because maternal separation, as a social isolation stress, produces depressive-like behavior of rat mothers (Boccia *et al.* 2007), these studies may also confirm our results. However, our data indicate that depressive-like phenotype is able to impair pup development even in the presence of standard maternal behaviors.

In adult male offspring, the assessment of locomotor activity showed mainly the stimulating effect of MA. Challenge dose of MA in adulthood increased distance traveled, locomotion, rearing, and average and maximal velocity, while decreasing immobility and grooming. This result confirms findings of others as well as our previous results showing increased locomotion after acute MA treatment that is associated with increased dopamine levels in rats (Bubeníková-Valešová *et al.* 2009, Jones *et al.* 2007, Širová *et al.* 2016). In addition, the present results show novel results demonstrating that adult male rats from OBX mothers with no MA exposure displayed increased immobility. Interestingly, this effect of bulbectomy was not apparent in adult rats exposed to MA. The possible explanation of the absence of this phenomenon after the MA-challenge may lie in the relative non-specificity of the locomotor test together with pronounced behavioral response to MA. MA may have masked any subtle behavioral change induced by the OBX in the mother. As shown in the model of the maternal chronic mild stress (Hougaard *et al.* 2011, Kjaer *et al.* 2010) the offspring may exhibit mainly increased anxiety, which is difficult to be assessed by locomotion. Importantly, our data indicate a higher anxiety level in the Bar-holding test in the offspring of the OBX mothers. We suspect that a more complex evaluation of behavioral profile in the male offspring of the OBX mothers using paradigms sensitive for anxiety level assessment (e.g. Elevated plus maze, Ultrasonic vocalizations, etc.) may provide a better evidence of the developmental effects of maternal OBX procedure. Our previous studies (Macúchová *et al.* 2016, Pometlová *et al.* 2016, Šlamberová *et al.* 2015) described the specificity of the commonly used anxiety tests.

Conclusions

This study showed that depressive-like phenotype of rat mothers induced by olfactory bulbectomy led to impairment in somatic and functional development of their offspring in the absence of impaired maternal care. Similarly as in a clinical trial combining maternal depression and MA taking during gestation, no evidence of aggravation of the prenatal MA-induced deficits was observed in the offspring of mothers with olfactory bulbectomy.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This study was supported by projects GAUK 850317, Progres Q35 and 260388/SVV/2017 provided by Charles University and the project MUNI/A/1063/2016 with the support of the Specific University Research Grant provided by the Ministry of Education, Youth and Sports of the Czech Republic in the year 2017. The procedures for animal experimentation utilized in this report was reviewed and approved by the Institutional Animal Care and Use Committee and is in agreement with the Czech Government Requirements under the Policy of Humans Care of Laboratory Animals (No. 246/1992) and with the regulations of the Ministry of Agriculture of the Czech Republic (No. 311/1997).

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