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Early maternal deprivation retards neurodevelopment in Wistar rats

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Abstract

A single 24 h period of maternal deprivation (MD) in rats has been shown to induce, in adulthood, a number of abnormalities in brain and behaviour that also occur in patients with schizophrenia. However, the short-term behavioural effects of MD have not been studied in detail. Since patients with schizophrenia are characterized by a retardation of normal development, we aimed in the present study to investigate the development of control rats and rats that were exposed to MD on postnatal day 9. Compared to control animals, MD rats showed (1) a reduction in body weight, (2) an increased in reversal latency in negative geotaxis, (3) a delayed eye opening, (4) a delayed emergence of walking and rearing; and (5) a delayed emergence of the behavioural response to amphetamine (amph). On the other hand, MD and control rats responded similarly to the non-competitive NMDA antagonist MK801. These data clearly show that early MD delays development, especially of the dopaminergic system and confirm our hypothesis that MD may represent an interesting animal model for the neurodevelopmental hypothesis of schizophrenia.

Keywords: *Amphetamine, animal model, dopamine, locomotor activity, MK801, negative geotaxis*

Introduction

The neurodevelopmental hypothesis of schizophrenia states that this disease results from a disturbance in the normal development of the central nervous system, resulting from a complex gene–environment interaction (Weinberger 1987; Pilowsky et al. 1993). Although there is a large body of evidence to support the neurodevelopmental hypothesis of schizophrenia (Ellenbroek and Cools 1998), probably the most convincing data come from large scale prospective epidemiological studies, such as the two birth-cohort studies performed in the UK in 1946 the National Survey of Health and Development (NSHD) and 1958 National Child Development Study (NCDS). The NSHD was a study of all children born in the UK during the week 3–9 March 1946 and the NCDS comprised about 98% of all the children born in the same week in 1958. These children were followed up

throughout life and data have clearly shown that the children that developed schizophrenia were delayed in many developmental milestones, including sitting, standing, walking alone and talking (Jones et al. 1994; Jones 1997). Since then, these developmental disturbances, especially in early motor behaviour have been replicated in several independent studies, including a large birth cohort study in Finland (Isohanni et al. 2000) and New Zealand (Cannon et al. 2002). In the latter study, the authors did not find a similar retardation in children that later developed anxiety/depression or bipolar disorder, which is in good agreement with studies by Walker et al. (1994). It thus seems that retardation in development and early motor behaviour is one of the first symptoms of schizophrenia, occurring long before the onset of psychotic symptoms.

The neurodevelopmental hypothesis also led to a plethora of new models to investigate aspects of

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schizophrenia in animals. Until the middle of the 1980s virtually all animal models were based on the acute or (sub)chronic administration of psychotomimetic drugs such as amphetamine (amph) or phencyclidine (Ellenbroek et al. 2000; Ellenbroek and Cools 2002a). However, in more recent years, numerous animal models have focussed on the long-term consequences of early lesions or other environmental manipulations. The most prominent of these are early postnatal lesions of the ventral hippocampus (Lipska et al. 1993), the amygdalae (Daenen et al. 2003) or the prefrontal cortex (Schwabe et al. 2004). In addition the long-term changes after environmental challenges such as prenatal stress (Koenig et al. 2005), perinatal hypoxia (Boksa 2004b) or early postnatal maternal deprivation (MD) (Ellenbroek and Riva 2003) have been studied in relation to schizophrenia. Although, all these models show a number of similarities with schizophrenia, most studies have focussed on the long-term (post-pubertal) consequences of such early manipulations.

Several years ago we started to investigate the long-term consequences of a single 24 h period of MD. Since it was already well established that MD led to an enhanced sensitivity of the hypothalamus–pituitary–adrenal axis to stressors (Levine et al. 1991) as well as an increased behavioural response to amph (Zimmerberg and Shartrand 1992), i.e. features that also occur in patients with schizophrenia (Lieberman et al. 1987; Lammers et al. 1995), we set out to investigate whether MD also led to other abnormalities observed in schizophrenia. Indeed, we found that maternally deprived rats showed, in adulthood, disturbances in prepulse inhibition (Ellenbroek et al. 1998), latent inhibition (Ellenbroek and Cools 1995), auditory sensory gating and startle habituation (Ellenbroek et al. 2004), all abnormalities also observed in patients with schizophrenia (Adler et al. 1982; Braff et al. 1978, 1992). In addition to this, other similarities (behavioural, pharmacological and neurochemical, see Discussion) between MD and schizophrenia were also observed, as was recently reviewed (Ellenbroek and Riva 2003). Moreover, there is epidemiological evidence that early stressors, including maternal separation (Mednick 1970) and early parental loss (Agid et al. 1999) increase the risk for schizophrenia. All these data together have led us to propose that MD might be an interesting animal model to study specific aspects of schizophrenia. Using different strains of rats, we could show that these long-term consequences were due to a gene–environment interaction, as F344 and Lewis rats responded differently to an identical MD (Ellenbroek and Cools 2000). However, so far we focussed predominantly on the long-term consequences, and have only shown that the deficits in prepulse inhibition and in BDNF were not detectable before

puberty at postnatal day 35 (Ellenbroek et al. 1998) or 21 (Roceri et al. 2002), respectively.

Given the similarities between the effects of MD and schizophrenia, we aimed to test the hypothesis that early MD would retard normal (motor) development. To that extent behaviour and aspects of general development were assessed in young maternally deprived and control pups. In addition, the sensitivity of these pups to the dopamine releasing drug amph and the NMDA receptor antagonist MK801 (both of which stimulate motor activity) was also investigated.

Material and methods

Rats and housing

Male and female (nulliparous) Wistar rats were obtained from the Central Animal Laboratory of the Radboud University of Nijmegen. The rats were housed in pairs of one male and one female in a standard Macrolon[®] cage (26 × 42 × 15 cm) in a temperature controlled room (22 ± 2°C) on a standard 12 h light/dark (light on at 07.00 a.m.) cycle. Food and water were available *ad libitum*. Two weeks later the males were removed and the females were checked twice daily (08.00 and 17.00 h) for delivery. The day of delivery was designated postnatal day 0. Within 24 h after birth, the litters were culled to eight animals (six males and two females) and animals were weighed. MD took place at postnatal day 9. The mothers were removed from the cage around 10.00 h and placed in a single cage, housed in the same room as the pups. The pups were weighed and left in the home-cage at room temperature. In control (Ctrl) litters, mothers were removed at postnatal day 9, pups were weighed and mothers returned immediately (within 3 min). At postnatal 10 (24 h later) the deprived pups were weighed again and the mothers placed back. In the control litter the mothers were again removed, pups weighed and mothers placed back. On different days after the MD several experiments were performed as described below. Since experiments in the past have shown that prior manipulations of young rats can influence their speed of development (Degen et al. 2005), each rat was used for only one behavioural paradigm. All experiments were performed in accordance with (inter)national laws and institutional guidelines.

Negative geotaxis

Rats were weighed and placed in the centre of a 25° inclined wooden platform (27 × 23 cm), head facing downward. The platform was covered with fine sandpaper. Reversal time was defined as the time the animal needed to complete a 135° turn of the spine

from the original position, thus head facing upwards. For statistical comparison, reversal time was analysed with a two-way analysis of variance (ANOVA) with factors early manipulation (MD vs. Ctrl) and age.

Eye opening

Eyes of both males and females were examined on postnatal days 9–11, 13, 15–18. Any degree of eyelid separation was scored as a positive eye opening. For statistical comparison, the percentages of MD and Ctrl animals with eyes opened were compared with a χ^2 test over all days and for each day separately.

Open field behaviour

Open field behaviour was measured in a black arena of 50 × 50 cm with black walls of 60 cm height. Rats from postnatal days 12, 14, 16, 18 and 21 were injected with either saline, amph (1 mg/kg) or MK-801 (0.1 mg/kg, both from Sigma/RBI, Zwijndrecht, the Netherlands) subcutaneously in the neck 5 min prior to being placed in the arena and behaviour was recorded for 1 h and videos were analysed by an observer blind to the treatment using a well defined ethogram (Table I). Since the same control group was used for both the amph and the MK-801 treated rats, we first analysed the duration of the different behaviours for only the saline treated animals with a two-way ANOVA (with factors early manipulation and age) to investigate the effects of MD. In this respect, it is important to note that all the experiments were done in the same time period and rats for the saline, amph and MK-801 treatments were obtained from the same litters (ensuring that animals within each treatment group all came from different litters). After this analysis, the effects of amph and MK801 were separately analysed using a three way ANOVA with factors early manipulation (MD vs. Ctrl), drug treatment

(saline vs. amph, or saline vs. MK801) and age. Where appropriate, a subsequent two-way and/or one-way ANOVA was performed to identify the source of the significance (especially focussing on the possible differential response of MD and Ctrl rats to amph or MK-801).

Results

Body weight

Body weights of all the rats from postnatal day 1–24 are shown in Figure 1. The data also include rats that were not used for the present experiments (especially at postnatal days 1, 9, 10 and 24). A two-way ANOVA showed a significant effect of early treatment ($F_{(1,1366)} = 264.9$, $p < 0.001$) and of days ($F_{(7,1366)} = 2166$, $p < 0.001$) as well as a significant interaction between early treatment and days ($F_{(7,1366)} = 264.9$, $p < 0.001$). Post hoc *t*-tests and inspection of Figure 1 showed that MD and Ctrl rats had similar body weights on postnatal days 1 and 9, but were significantly reduced in weight at all subsequent time points. The data, therefore, indicate that MD led to a significant reduction in body weight from pnd10 until the end of the measurement at pnd24.

Negative geotaxis

Reversal time in the negative geotaxis test is represented in Figure 2. The two-way ANOVA showed a significant effect of early treatment ($F_{(1,505)} = 110.3$, $p < 0.001$) and of days ($F_{(3,505)} = 78.0$, $p < 0.001$), as well as a significant interaction between early treatment and days ($F_{(3,505)} = 94.7$, $p < 0.001$). Post-hoc *t*-test showed that MD and Ctrl rats did not differ on postnatal day 9, but MD rats had significantly

Table I.

Behaviour	Description
Walking	Displacement of the rat in a horizontal plane for at least 5 cm
Sitting	The rat is not moving his body in a horizontal or vertical plane
Rearing free	The rat raises its forelimbs and torso without support of a wall
Rearing wall	The rat raises its forelimbs and torso without support of a wall
Grooming	The rat cleans its fur and snout with his tongue and/or forelimbs
Scratching	The rat scratches its back and side with its hindlimb
Sniffing	The rat sniffs on an object or wall or freely in the air

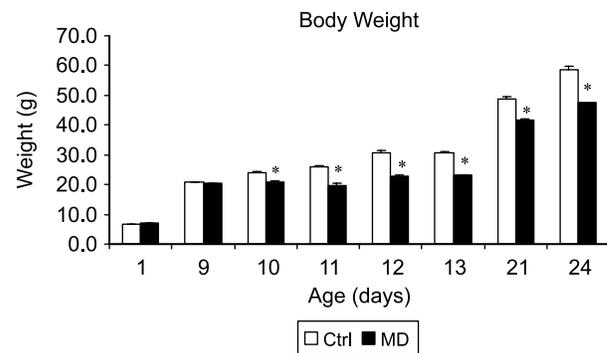


Figure 1. Body weight of MD and Ctrl rats from postnatal one to postnatal day 24. The data are a summary of a large series of animals. The total number are: pnd1: Ctrl $N = 159$, MD $N = 151$; pnd9: Ctrl $N = 196$, MD $N = 183$; pnd10: Ctrl $N = 188$, MD $N = 166$; pnd11: Ctrl $N = 16$, MD $N = 16$; pnd12: Ctrl $N = 16$, MD $N = 16$; pnd13: Ctrl $N = 16$, MD $N = 16$; pnd21: Ctrl $N = 175$, MD $N = 155$; pnd24: Ctrl $N = 16$, MD $N = 13$. Values are mean \pm SEM. * represents a significant difference between MD and Ctrl groups.

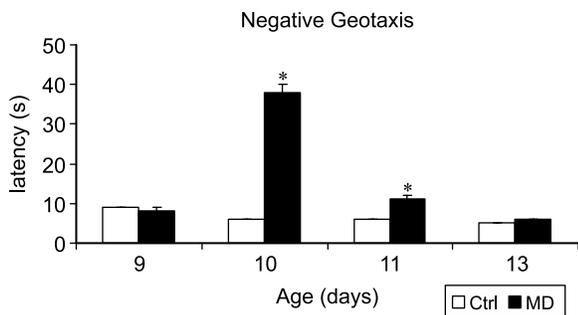


Figure 2. Reversal latency in the negative geotaxis experiment in MD and Ctrl rats. pnd9: Ctrl $N = 92$, MD $N = 63$; pnd10: Ctrl $N = 92$, MD $N = 63$; pnd11: Ctrl $N = 54$, MD $N = 54$; pnd13: Ctrl $N = 48$, MD $N = 47$. Values are mean \pm SEM. * represents a significant difference between MD and Ctrl groups.

longer latencies on postnatal day 10 and 11. On postnatal day 13, MD and Ctrl rats were not significantly different anymore. In other words, MD led to an increase in the negative geotaxis latency on postnatal days 10 and 11, but not 13.

Eye opening

On postnatal days 9–13 none of the Ctrl and MD rats had opened their eyes (data not shown). The cumulative percentage of animals that opened their eyes between postnatal day 15 and 18 is shown in Figure 3. It is clear that Ctrl rats were somewhat faster in opening their eyes than rats that were exposed to MD. A χ^2 analysis confirmed that there was a significantly different distribution between the MD and the Ctrl rats ($\chi^2 = 17.1$, $p < 0.001$). When analysed per day, a significant difference was observed between MD and Ctrl rats on postnatal day 15 ($\chi^2 = 6.8$, $p < 0.01$) and 16 ($\chi^2 = 17.7$, $p < 0.001$), with a tendency to be different on postnatal day 17 ($\chi^2 = 3.1$, $p = 0.08$). Thus, MD led to a delay in eye opening.

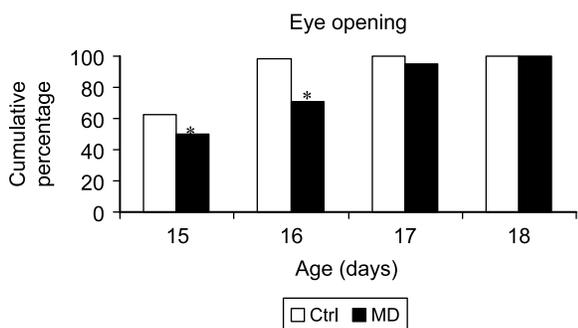


Figure 3. Eye opening in MD and Ctrl rats. On the y-axis the cumulative percentage of rats that had their eyes open is represented. The Ctrl groups consisted of 61 animals, the MD groups of 62 animals. * represents a significant difference between MD and Ctrl groups.

Open field behaviour—effects of MD

Before presenting the effects of amph and MK801, the effects of MD *per se* will first be described (Figures 4 and 5). Since several of the behaviours listed in Table I occurred only rarely, we will limit our discussion to walking, sitting, rearing (wall) and grooming. When the results of only the saline-treated rats were investigated with a two-way ANOVA (with factors early manipulation and age), there was a significant effect of early manipulation for rearing ($F_{(1,49)} = 7.3$; $p < 0.01$) and grooming ($F_{(1,49)} = 6.1$; $p < 0.02$). In addition, there was a significant effect of age for walking ($F_{(4,49)} = 6.9$; $p < 0.001$), rearing ($F_{(4,49)} = 4.4$; $p < 0.004$) and grooming ($F_{(4,49)} = 5.4$; $p < 0.001$). Finally, there was a significant interaction between early manipulation and age with respect to grooming ($F_{(4,49)} = 3.2$; $p < 0.02$). In order to identify the source of the statistical significance for early manipulation, the data were separately analysed per day. The one-way ANOVA revealed that MD rats showed significantly less rearing on postnatal days 12, 14 and 18, whereas grooming was significantly reduced in the MD rats on postnatal days 12 and 16.

Open field behaviour—effects of amphetamine

For walking (Figure 4, upper left corner), the three-way ANOVA showed a significant effect of early manipulation ($F_{(1,100)} = 7.1$, $p < 0.01$), of drug treatment ($F_{(1,100)} = 65.7$, $p < 0.001$) and of age ($F_{(4,100)} = 17.6$, $p < 0.001$). In addition a significant two-way interaction was observed for early manipulation—drug treatment ($F_{(1,100)} = 4.9$, $p < 0.05$), for early manipulation—age ($F_{(4,100)} = 4.1$, $p < 0.005$) and for drug treatment—age ($F_{(4,100)} = 6.3$, $p < 0.001$), as well as a significant three-way interaction ($F_{(4,100)} = 3.7$, $p < 0.01$).

Given the early manipulation—drug treatment interaction, we investigated the effects of amph in the Ctrl and the MD rats separately. In the Ctrl animals, a two-way ANOVA showed a significant effect of drug treatment ($F_{(1,52)} = 39.9$, $p < 0.001$) and of age ($F_{(4,52)} = 8.7$, $p < 0.001$) as well as a significant interaction ($F_{(4,52)} = 5.1$, $p < 0.002$). When analysed separately for each age, we found that amph significantly increased the duration of walking on all postnatal days. In the MD animals, a two-way ANOVA showed a significant effect of drug treatment ($F_{(1,48)} = 29.4$, $p < 0.001$) and of age ($F_{(4,48)} = 19.3$, $p < 0.001$) as well as a significant interaction ($F_{(4,48)} = 6.7$, $p < 0.001$). Separated for age, a one-way ANOVA showed that amph only significantly increased the duration of walking on postnatal days 18 and 21.

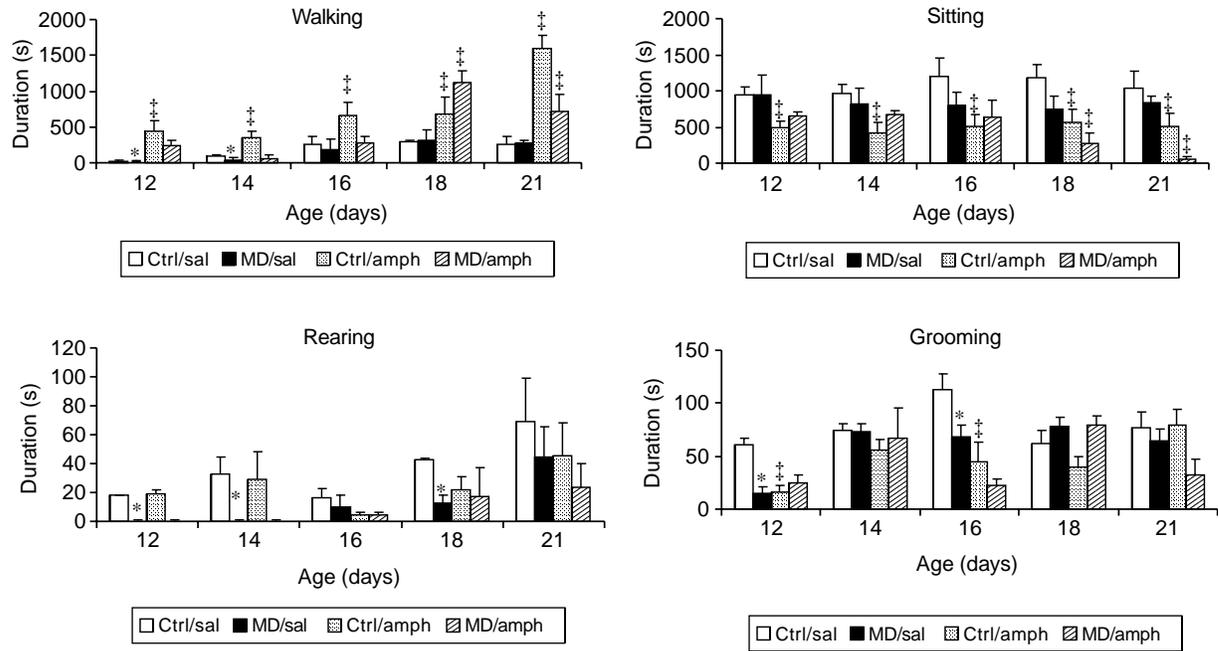


Figure 4. The effects of amphetamine (amph) on the duration of various components of motor behaviour in the open field of MD and Ctrl rats. Each group consisted of 5–7 animals and each day new animals were used. Values are mean ± SEM. * represents a significant difference between MD and Ctrl groups. ‡ represents a significant difference between amphetamine and saline treatment.

For sitting (Figure 4, upper right corner), the three-way ANOVA showed a tendency for early manipulation ($F_{(1,100)} = 2.7, p = 0.09$), a significant effect of drug treatment ($F_{(1,100)} = 65.7, p < 0.001$) but no

significant effect of age ($F_{(4,100)} < 1.0$). In addition a significant two-way interaction was observed for early manipulation—drug treatment ($F_{(1,100)} = 4.8, p < 0.05$), but not for early manipulation—age

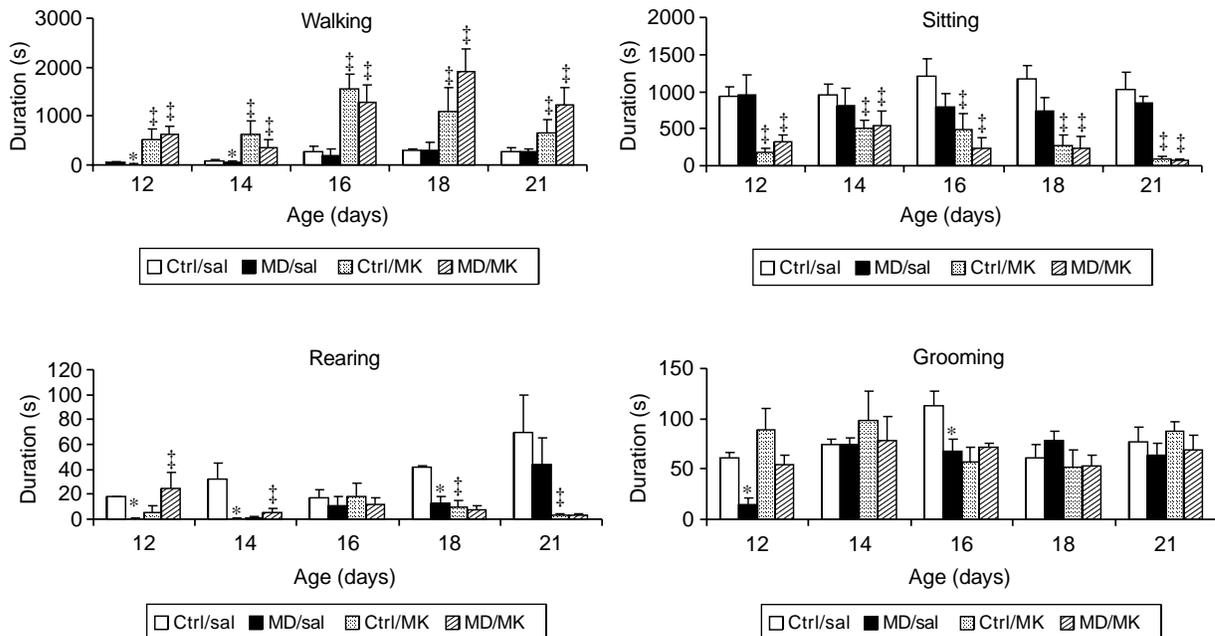


Figure 5. The effects of MK801 (MK) on the duration of various components of motor behaviour in the open field of MD and Ctrl rats. Each group consisted of 5–7 animals and each day new animals were used. Values are mean ± SEM. * represents a significant difference between MD and Ctrl groups. ‡ represents a significant difference between MK801 and saline treatment.

($F_{(4,100)} = 1.1, p > 0.3$) nor for drug treatment—age ($F_{(4,100)} < 1.0$). The three-way interaction was also not significant ($F_{(4,100)} = 1.2, p > 0.3$).

In view of the significant early manipulation—drug treatment interaction, we investigated the effects of amph in the Ctrl and the MD rats separately. In the Ctrl animals, a two-way ANOVA showed a significant effect of drug treatment ($F_{(1,52)} = 19.6, p < 0.001$) but not of age ($F_{(4,52)} < 1.0$) nor a significant interaction ($F_{(4,52)} < 1.0$). Separated for age, a one-way ANOVA showed that amph significantly reduced the duration of sitting at all postnatal days in Ctrl rats. In the MD animals, a two-way ANOVA showed a significant effect of drug treatment ($F_{(1,48)} = 18.0, p < 0.001$) but not of age ($F_{(4,48)} = 1.4, p > 0.2$) nor a significant interaction ($F_{(4,48)} = 1.1, p > 0.3$). Separated for age, a one-way ANOVA showed that amph significantly reduced the duration of sitting on postnatal days 18 and 21 in MD rats.

For rearing, the three-way ANOVA showed a significant effect of early manipulation ($F_{(1,100)} = 11.5, p < 0.001$), and of age ($F_{(4,100)} = 5.9, p < 0.001$) but not of drug treatment ($F_{(4,100)} = 2.2, p > 0.1$). There was no significant two-way interaction observed for early manipulation—drug treatment, of early manipulation—age ($F_{(4,100)} < 1.0$), nor of drug treatment—age (all $F_{(4,100)} < 1.0$). The three-way interaction was also not significant ($F_{(4,100)} < 1.0$). Since there were no significant drug effects, nor interactions, we did not analyse the effects of amph in the Ctrl and MD rats separately.

For grooming, the three-way ANOVA showed no significant effect of early manipulation ($F_{(1,100)} = 2.5, p = 0.12$), a significant effect of drug treatment ($F_{(1,100)} = 13.3, p < 0.001$) and a significant effect of age ($F_{(4,100)} = 5.1, p < 0.001$). In addition, no significant two-way interaction was observed for early manipulation—drug treatment ($F_{(1,100)} = 1.6, p > 0.2$). The early manipulation—age interaction was almost significant ($F_{(4,100)} = 2.1, p = 0.08$) but the drug treatment—age was not ($F_{(4,100)} = 1.3, p > 0.2$). The three-way interaction was also not significant ($F_{(4,100)} = 1.2, p > 0.3$).

Given the significant drug effect, we investigated the effects of amph in the Ctrl and the MD rats separately. In the Ctrl animals, a two-way ANOVA showed a significant effect of drug treatment ($F_{(1,52)} = 13.5, p < 0.001$) and of age ($F_{(4,52)} = 3.7, p < 0.01$). The interaction approached significance ($F_{(4,52)} = 2.3, p = 0.08$). Separated for age, a one-way ANOVA showed that amph significantly reduced the duration of rearing at postnatal days 12 and 16. In the MD animals, a two-way ANOVA showed no significant effect of drug treatment ($F_{(1,48)} = 2.6, p > 0.1$) but a significant effect of age ($F_{(4,48)} = 5.1, p < 0.005$). The interaction was not significant ($F_{(4,48)} = 1.31, p > 0.28$).

In summary, amph increased walking in Ctrl rats on all postnatal days, whereas in MD rats this drug only increased walking on postnatal days 18 and 21. This was accompanied by a similar reduction in sitting behaviour. Amph did not increase rearing or affect grooming in either Ctrl or MD rats.

Open field behaviour—effects of MK801

The effects of saline and MK801 (0.1 mg/kg s.c.) on the behaviour in the open field test are shown in Figure 5. As with amph only the duration of walking, sitting, rearing and grooming will be discussed.

For walking, the three-way ANOVA showed no significant effect of early manipulation ($F_{(1,101)} < 1.0$), but a significant effect of drug treatment ($F_{(1,101)} = 55.9, p < 0.001$) and of age ($F_{(4,101)} = 5.6, p < 0.001$). None of the two or three way interactions were significant.

Given the significant effect of drug treatment, we also investigated the effects of MK801 in the Ctrl and the MD rats separately. In the Ctrl animals, a two-way ANOVA showed a significant effect of drug treatment ($F_{(1,51)} = 21.6, p < 0.001$) and a trend towards a significant effect of age ($F_{(4,51)} = 2.3, p = 0.07$) but no significant interaction ($F_{(4,51)} = 1.1, p > 0.35$). Separated for age, a one-way ANOVA showed that MK801 significantly increased the duration of walking on all postnatal days. In the MD animals, a two-way ANOVA showed a significant effect of drug treatment ($F_{(1,50)} = 34.8, p < 0.001$) and of days ($F_{(4,50)} = 4.5, p < 0.005$) but no significant interaction ($F_{(4,50)} = 2.0, p > 0.1$). Separated for age, a one-way ANOVA showed that MK801 significantly increased the duration of walking on all postnatal days.

For sitting, the three-way ANOVA showed no significant effect of early manipulation ($F_{(1,101)} < 1.0$), but a significant effect of drug treatment ($F_{(1,101)} = 76.3, p < 0.001$) and of age ($F_{(4,101)} = 2.7, p < 0.05$). None of the two or three way interactions were significant.

Given the significant drug effect, we also investigated the effects of MK801 in the Ctrl and the MD rats separately. In the Ctrl animals, a two-way ANOVA showed a significant effect of drug treatment ($F_{(1,51)} = 60.0, p < 0.001$) but no significant effect of age ($F_{(4,51)} = 1.3, p > 0.27$) and no significant interaction ($F_{(4,51)} < 1.0$). Separated for age, a one-way ANOVA showed that MK801 significantly reduced the duration of sitting on all postnatal days in Ctrl animals. In the MD animals, a two-way ANOVA showed a significant effect of drug treatment ($F_{(1,50)} = 25.6, p < 0.001$) but not of age ($F_{(4,50)} = 1.4, p > 0.25$) and no significant interaction ($F_{(4,50)} < 1.0$). Separated for age, a one-way ANOVA showed that MK801 significantly decreased the duration of sitting on postnatal days 12, 18 and 21, with a strong tendency ($p < 0.07$) at postnatal day 14.

For rearing, the three-way ANOVA showed a significant effect of early manipulation ($F_{(1,101)} = 4.7$, $p < 0.05$), of drug treatment ($F_{(1,101)} = 12.4$, $p < 0.001$) and of age ($F_{(4,101)} = 2.7$, $p < 0.05$). In addition, there was a significant early manipulation—drug treatment interaction ($F_{(1,101)} = 8.1$, $p < 0.005$) and a significant drug treatment—age interaction ($F_{(4,101)} = 5.5$, $p < 0.001$). The early manipulation—age interaction ($F_{(4,101)} < 1.0$) as well as the three-way interaction ($F_{(1,101)} < 1.0$) was not significant.

Given the significant effect of drug treatment and the significant interaction between early manipulation and drug treatment, we also investigated the effects of MK801 in the Ctrl and the MD rats separately. In the Ctrl animals, a two-way ANOVA showed a significant effect of drug treatment ($F_{(1,51)} = 17.0$, $p < 0.001$) but no significant effect of age ($F_{(4,51)} = 1.7$, $p > 0.17$). The interaction, on the other hand, was significant ($F_{(4,51)} = 2.7$, $p < 0.05$). Separated for age, a one-way ANOVA showed that MK801 significantly reduced the duration of rearing on all postnatal days except postnatal day 16 in Ctrl animals. In the MD animals, a two-way ANOVA showed no significant effect of drug treatment ($F_{(1,50)} < 1.0$) nor/ of age ($F_{(4,50)} = 1.5$, $p > 0.2$). However, the interaction was significant ($F_{(4,50)} = 3.4$, $p < 0.02$). Separated for age, a one-way ANOVA showed that MK801 significantly increased the duration of rearing on postnatal days 12 and 14 in MD rats.

For grooming, the three-way ANOVA showed a significant effect of early manipulation ($F_{(1,101)} = 5.0$, $p < 0.05$), but not of drug treatment ($F_{(1,101)} < 1.0$). The effect of age was almost significant ($F_{(4,101)} = 2.47$, $p = 0.06$). In addition, there was a significant drug treatment—day interaction ($F_{(4,101)} = 2.8$, $p < 0.05$). The other two and three way interactions were not significant. Since there was no significant drug effect, we did not analyse the effects of MK801 for Ctrl and MD rats separately.

In summary MK801 increased walking and reduced sitting in both Ctrl and MD rats at all postnatal days tested. This drug also reduced rearing in both Ctrl and MD rats, but did not significantly affect grooming.

Discussion

Our main hypothesis was that early MD would retard normal (motor) development. The data obtained indeed seem to confirm this hypothesis. In short, we found that, compared to Ctrl rats, MD rats showed [1] a reduction in body weight from postnatal day 10 until at least day 24 (the last day of measurement); [2] a prolonged reversal time in the negative geotaxis on postnatal days 10 and 11; [3] a retardation of eye opening at postnatal days 15 and 16; [4] a reduction in walking on postnatal days 12–14, and rearing on

postnatal days 12–18; and [5] a reduced response to amph on postnatal days 12–16.

The reduction in body weight after MD is in line with our own previous papers (Husum et al. 2002; Ellenbroek et al. 2004), as well as with those of others (Workel et al. 1997; Penke et al. 2001). In a previous paper, we pointed out that the relative reduction in body weight was larger at weaning (postnatal 21) than immediately after the deprivation on postnatal day 10 (Ellenbroek et al. 2004). In the present paper, we extended this by also looking at intermediate days. Interestingly, the largest relative decrease in body weight was seen on postnatal days 11 (24%) and 12 (26%), whereas it was only 14% at postnatal day 10. Moreover, MD rats at postnatal day 11 weighed less than those at postnatal day 10 (Figure 1). This suggests that changes in maternal behaviour or milk production have occurred, which last beyond the 24 h period of deprivation. In agreement with this, we recently showed that the long-term consequences of MD are reduced when the maternally deprived rats are being fostered by non-deprived mothers (Ellenbroek and Cools 2002b).

Negative geotaxis is an early righting reflex that appears to be particularly sensitive to early manipulations. Thus prenatal treatment with drugs such as alcohol (Hannigan 1995), 2,4-dichlorophenoxyacetic acid (Bortolozzi et al. 1999) and 1,1,1-trichloroethane (Coleman et al. 1999) selectively retard the development of negative geotaxis. On the other hand, prenatal treatment with drugs like 5-bromo-2'-deoxyuridine (Kuwaigata and Nagao 1998), phenytoin (McCartney et al. 1999) or maternal exposure to picrotoxin during early lactation (Baso et al. 2003) improves negative geotaxis. In addition, prenatal stress (Patin et al. 2004), neonatal hypoxia (Lubics et al. 2005) and neonatal dexamethasone (Gramsbergen and Mulder 1998; Ferguson et al. 2001) also retard the development of the negative geotaxis reflex. Prenatal stress (Koenig et al. 2005) neonatal hypoxia (Boksa 2004a, c), and MD (Ellenbroek and Riva 2003) have all been proposed to model specific aspects of schizophrenia. In agreement with this, we found a significant increase in the reversal latency on postnatal days 10 and 11 in MD rats as well. However, from the data, it is not clear whether this increase really represents a delay in maturation. In fact, the rats at postnatal day 9 already had a fast geotaxis reflex. In this respect, it is important to realise that during the MD, the rats were kept at room temperature, implying that at the time of the geotaxis experiment on postnatal day 10, they were hypothermic, in relation to the non-deprived rats, which might explain the large increase. Although the MD rats also showed a significantly longer latency at postnatal day 11, this increase was much smaller than at postnatal day 10 (Figure 2). The data on negative geotaxis, therefore, do not really allow a conclusion with respect to the retardation of

development. Although differences in the protocol may be of importance too (Kreider and Blumberg 1999), a more important factor is presumably the time of measurement. As shown by others, negative geotaxis starts to occur on postnatal day 4 and is almost complete by postnatal day 9 (Patin et al. 2004), and may, therefore, not be an ideal parameter to assess after the MD (which starts at postnatal day 9).

The effects of MD on eye opening provide more convincing evidence for a delay in development. Thus, whereas virtually all control rats had opened their eyes on postnatal 16 (99%), 29% of all MD rats still had their eyes closed. Although, the delay may seem to be small, 24 h during this developmental period may be of crucial importance. Indeed most of the pre- and early postnatal manipulations that affect negative geotaxis do not delay eye opening (Gramsbergen and Mulder 1998; Baso et al. 2003), with the exception of neonatal hypoxia (Lubics et al. 2005) and prenatal stress (Patin et al. 2004), both of which also delay eye opening by about 24 h.

As with eye opening, MD rats showed signs of a retardation of motor development in the open field test. Thus maternally deprived rats showed significantly less walking and rearing on postnatal days 12 and 14. Especially the differences in rearing (reduced in MD rats on postnatal day 12, 14 and 18) is interesting, in view of the data presented by Eilam and Golani (1988), showing that normal development of motor behaviour in rats follows a series of very strict rules, starting with sideward (scanning) movements, followed by horizontal forward (walking) movements and the development is completed by vertical (rearing) movements (Eilam and Golani 1988). Given the fact that MD rats showed no rearing at all at postnatal days 12 and 14, and a reduction in postnatal days 16 and 18, this is a clear indication that MD retards normal (motor) development.

Finally, we found that both amphetamine (amph) and MK801 increased locomotor activity, and decreased sitting in MD and Ctrl rats. However, whereas MK801 was equally effective in both groups of rats, there was a significant drug treatment—early manipulation interaction in the case of amph. Close inspection of Figure 4 showed that whereas amph increased the duration of walking (and concomitantly decreased the duration of sitting) at all days (from postnatal day 12 to 21) in Ctrl rats, it was only effective on postnatal days 18 and 21 in MD rats.

Amphetamine is known to increase extracellular dopamine concentrations in the brain, by blocking the dopamine transporter and by enhancing dopamine release through a process known as reverse transport (Sulzer et al. 2005). The increased locomotor activity seen after amph administration is generally considered to be due to an increase in extracellular dopamine, especially in the nucleus accumbens (Broekkamp et al. 1975; Kuczenski and Segal 1990). The differential

response of amph in MD vs. Ctrl rats thus suggests a difference in the development of the mesolimbic dopaminergic system. The ontogeny of many aspects of the dopaminergic system in rats has been described, including the number of dopamine transporters (Coulter et al. 1996; Galineau et al. 2004), the dopamine D1 and D2 receptor densities (Jung and Bennett 1996) and the methamphetamine-induced dopamine release (Tsuchida et al. 1998). All these data clearly show that during postnatal 9 and 10 (when MD takes place) the dopaminergic system is still developing, with levels being much less than in adulthood. Given the fact that MD acutely leads to high levels of corticosterone (Suchecki et al. 1993), and that high levels of corticosterone are detrimental to cell development (Gould and Tanapat 1999), it is tempting to speculate that MD stops or retards the normal development of the dopaminergic system through its actions on the HPA axis. In agreement with this, MD has been shown to reduce neurogenesis (Park et al. 2002) and to increase cell death (Zhang et al. 2002). Although it has been shown that early environmental manipulations (such as repeated maternal separation) can reduce the number of dopamine transporters (Meaney et al. 2002), so far this has only been observed in adulthood. In fact it is unlikely that our MD procedure will also lead to a reduction in dopamine transporters in adulthood, as we did not find any differences in amph response in rats at postnatal day 18 and 21 (and 30, unpublished data), and it has been found that maternally deprived rats, in adulthood, are actually more sensitive to apomorphine (Ellenbroek and Cools 1995; Rots et al. 1996) and amph (Zimmerberg and Shartrand 1992).

Interestingly, MD rats did not differ from Ctrl rats in their behavioural response to MK801. This implies first of all that even at pnd12, MD rats can show walking and rearing, when stimulated with a drug, and secondly that the differential sensitivity of MD and Ctrl rats to drugs, depends on the type of pharmacological challenge. In contrast to amph, MK801 selectively targets the glutamatergic system, by non-competitively blocking the NMDA receptor. It has been shown that this receptor is already functionally active at a very young age. Indeed it has been shown that a single injection of MK801 at postnatal day 7 can lead to long lasting changes in neurochemistry and behaviour, such as a reduction in prepulse inhibition (Harris et al. 2003). In addition, even prenatal injections of MK801 induce long lasting changes (Ikonomidou et al. 1999). Thus, one might hypothesize that the development of the NMDA receptors is already in such an advanced state at postnatal day 9, when MD commences, that this stressor cannot affect its development. However, additional experiments are needed to test this hypothesis.

In summary, the present set of data show that a single 24 h period of MD on postnatal day 9 leads to a long lasting reduction in body weight, a delay in

eye opening, in walking and in rearing and in a delayed behavioural response to amph. As discussed in the introduction, early MD leads to a large number of abnormalities in adulthood, that show similarity with known signs and symptoms of schizophrenia, including reductions in acoustic sensory gating, startle habituation, prepulse inhibition and an increased stress response (Ellenbroek and Riva 2003). In addition, both MD rats and patients with schizophrenia have reduced hippocampal levels of brain derived neurotrophic factor (Roceri et al. 2002; Weinberger 1999), neuropeptide Y (Frederiksen et al. 1991; Husum et al. 2002), polysialylated-neuronal cell adhesion molecule (Barbeau et al. 1995; Foley et al. 2000) and mRNA levels for the NR₁ subunit of the NMDA receptor (Roceri et al. 2002; Harrison et al. 2003; Meador-Woodruff et al. 2005). Although none of the items, by themselves, are specific or pathognomic for schizophrenia, together they substantiate the claim that MD might represent an interesting animal model for several aspects of schizophrenia.

The present data, which show that MD also leads to a retardation of normal development, seem to further substantiate the claim that early MD might represent an interesting animal model for schizophrenia. Although rats show a different development after birth than humans, for example, with respect to eye opening, the retardation in walking and rearing in rats is reminiscent of the retarded development of motor behaviour in children that develop schizophrenia (Jones et al. 1994; Jones 1997).

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