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Research report

Effects of neonatal lesions of the medial prefrontal cortex on adult rat behaviour

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Abstract

While prefrontal lesions in rodents serve as models for frontal lobe syndromes, neonatal lesions are considered as models for disconnection syndromes, such as schizophrenia. We investigated the effect of neonatal lesions of the rat medial prefrontal cortex (mPFC) together with pubertal dexamethasone-challenge on adult rat behaviour and on apomorphine-induced behavioural changes. Adult lesions were used as controls. Rats with neonatal (postnatal day 7) or adult excitotoxic lesions or sham-lesions of the mPFC were tested 9 weeks after surgery. At postnatal day 49 one group of neonatal operated rats were systemically injected with the glucocorticoid receptor agonist dexamethasone (20 mg/kg), in order to simulate stress-induced glucocorticoid receptor activation. Working memory and perseveration was tested in T-maze tasks (continuous delayed alternation and reversal learning). Additionally, locomotor activity and prepulse inhibition (PPI) of startle was tested with and without apomorphine-treatment. Brain tissue damage was assessed using Nissl-staining and parvalbumine-immunocytochemistry.

Pronounced thinning of the prelimbic-infralimbic subregion of the mPFC accompanied by altered cytoarchitecture and reduced number of parvalbumine-immunopositive neurones was found after neonatal lesions while adult lesions resulted in loss of neurones accompanied by gliosis. Neonatal lesions increased perseveration in the T-maze tasks and enhanced PPI, while adult lesions induced a working memory deficit. This differential behavioural outcome presumably reflects neurodevelopmentally induced alterations in neuronal circuits after neonatal lesions versus damage to mPFC alone after adult lesions. Dexamethasone-injection at day 49 did not alter behaviour in these tasks. Motor activity was not affected by neonatal or adult lesions but dexamethasone reduced apomorphine-induced hyperlocomotion.

Keywords: Neonatal lesion; Neurodevelopment; Two hit model; Dexamethasone; Apomorphine; Working memory; Prepulse inhibition; Motor activity

1. Introduction

The outcome of damage to the brain depends upon the developmental phase during which the lesion occurs. While lesions of the adult brain usually result in immediate behavioural deficits, lesions of the juvenile brain are generally compensated during ontogeny [33]. However, early brain damage may also lead to disturbances of neurodevelopmental programs and may thus induce behavioural deficits in adults that are different from those seen after adult lesions of certain brain regions.

In this context, adult animals that have sustained neonatal lesions are considered useful models to study neurodevelopmental deficits underlying neuropsychiatric disorders [36,40]. Neonatal excitotoxic hippocampal damage in rats induced postpubertal behavioural abnormalities, such as hyperlocomotion, deficits in working memory and in prepulse inhibition (PPI) of the acoustic startle response (ASR), whereas rats with adult lesions were not altered in these tasks [38,39]. Additionally, neonatal lesions of the amygdala resulted in deficient social behaviour whereas lesions later in life had no such effect [10,57].

We here investigated whether lesions of the medial prefrontal cortex (mPFC) would also lead to disturbance of neurobehavioural development. Functions of the mPFC include various aspects of higher cognitive functions, e.g. executive control and behavioural flexibility [19]. Previous studies showed that lesions of the rat mPFC sustained in the first days of life or in adulthood led to abnormal cortical morphology and severe behavioural deficits [11,31–33] whereas lesions of the rat mPFC on postnatal days (PND) 7–12 led to spontaneous regeneration of much of the lost

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tissue with relative normal cortical cytoarchitecture and sparing, or recovery of behaviour. However, it has been reported that certain aspects of adult behaviour after neonatal mPFC lesions were altered by environmental or pharmacological challenges [18]. For instance, neonatal mPFC lesions have been shown to alter striatal dopamine systems [18], which resulted in enhanced responsivity to direct postsynaptic dopamine agonists, such as apomorphine. This reduced functional capacity of the regenerated tissue might render the brain vulnerable to neurodevelopmental disturbances that interfere with normal maturation and refinement of the brain during puberty. This would then cause behavioural changes and deficits in cognitive function as proposed in the "two hit model of schizophrenia" [16,17,56].

Stress has been shown to exacerbate the symptoms of schizophrenia as well as precipitate the onset and relapse of symptoms [20,44,45]. Acute administration of the synthetic glucocorticoid-receptor agonist dexamethasone in rats induced apoptosis in striatal cells [22,42], worsen psychotic symptoms in patients [23,43] and impairs cognitive function in healthy volunteers [58]. Therefore, we here investigated the effects of neonatal excitotoxic lesions of the rat mPFC together with a single dexamethasone-treatment challenge around puberty (day 49) on behavioural tasks known to be sensitive to the integrity of the rat mPFC. Rats were tested for working memory and behavioural flexibility in T-maze tasks (spatial continuous alternation and reversal learning) as adults. Additionally, rats were tested with and without apomorphine-challenge for locomotor activity in an open field and for PPI of the ASR, a test for sensorimotor gating. For comparison, adult rats were lesioned in the mPFC and tested in the same paradigms. Finally, brain tissue damage was assessed using Nissl-staining and parvalbumine-immunocytochemistry (a marker for a subset of GABAergic neurones).

2. Methods

2.1. Animals

A total of 125 male Wistar rats (offspring of parents from Harlan–Winkelmann, Borchen, Germany) were used in the experiments. At birth the litters were culled to eight male pups (in case of less than eight male pups, females were used to fill up the litter). The day of birth was designated as PND 0. After weaning (PND 21) the rats were housed in groups of six to eight in Macrolon cages (type IV). Additionally, adult male rats ($n = 34$) were purchased from Harlan–Winkelmann weighing 180–200 g upon arrival. They were housed in groups of six to eight animals per cage. All rats were kept under controlled environmental conditions (ambient temperature 22 °C, 12 h light/dark cycle, light on at 7:00 a.m.). During the light cycle a softly playing radio was used to provide a continuous background noise and minimise the disturbing effects of sudden noise. Standard laboratory chow (Altromin 1324 standard diet; Lage, Germany) and tab water were allowed ad libitum until rats weighed approximately 200 g; thereafter, food intake was restricted to 12 g pellets per animal per 24 h. This controlled feeding schedule was continued throughout the whole testing period, keeping the animal's body weigh on approximately 85% of the free feeding weight. All behavioural testing was performed during the rats light cycle between 12 a.m. and 6 p.m. The experimenters were blind with respect to the animal's treatment. The experimental protocols used in this study were in line with national and international ethical guidelines, conducted in compliance with the German Animal Welfare Act and approved by the local authorities, including approval by an animal ethics committee.

2.2. Surgery

2.2.1. Neonatal lesions

Equal numbers of animals of each individual litter were assigned to the different treatment groups in order to avoid litter effects. On PND 7, male pups were anaesthetised using hypothermia (placed on ice for 15–20 min), divided into two groups (sham and lesion) and placed in a stereotaxic apparatus adapted for neonatal rats (TSE, Bad Homburg, Germany). An incision was made in the skin overlying the skull that was then penetrated with a microliter syringe (SGE Deutschland GmbH). Two micrograms of ibotenic acid (Sigma, Deisenhofen, Germany) in 0.3 μl PBS for lesion or 0.3 μl PBS for sham-lesion were infused bilaterally into the mPFC at a rate of 0.1 μl/min at the following co-ordinates: anterior–posterior (AP) +2.7 mm, lateral (L) ±0.3 mm, ventral (V) 3.2 mm, relative to Bregma. These co-ordinates were chosen on the basis of preliminary experiments and data from other groups [18,37]. In pilot experiments, the dosage of ibotenic acid was determined to induce selective lesions within the mPFC. The needle was withdrawn 5 min after completion of the infusion and the skin wound closed using medical adhesive. After surgery pups were placed on a warming pad and then returned to their mother. Rats with lesions or sham-lesions inflicted on PND 7 will in the following be referred to as neonatal lesioned or sham-lesioned rats.

2.2.2. Adult lesions

Adult rats (weight about 200 g) were anaesthetised with chloralhydrate (360 mg/kg intraperitoneally) and placed in a stereotaxic instrument (tooth bar set at −3.3 mm). Ibotenic acid (1.6 μg in 0.25 μl) or PBS was administered at a rate of 0.1 μl/min bilaterally into the mPFC at the co-ordinates AP +2.7, L ±0.5, V 4.5 according to the atlas of Paxinos and Watson [47]. In pilot experiments, this dosage was determined to induce selective lesions that were similar in extent compared to neonatal lesions. This dosage was lower than that used for neonates probably due to the hypothermia-induced neuroprotective effects of cryo-anaesthesia, which was used for surgery of neonates.

The cannula remained in place for 5 min after the injection, then it was withdrawn. After completion of the injection procedure the skin wound was sutured. Rats with lesions or sham-lesions inflicted in adulthood will in the following be referred to as adult lesioned or sham-lesioned rats.

2.2.3. Challenge with dexamethasone

On PND 49 neonatally lesioned and sham-lesioned rats were randomly assigned to receive dexamethasone (20 mg/kg in aqua dest; Sigma, Deisenhofen, Germany) or vehicle injection.

3. Behavioural testing

3.1. T-maze

Behavioural tests of lesioned and sham-lesioned rats commenced 9 weeks after surgery (PND 70 for neonatal lesioned rats). For behavioural tests we used three independent cohorts of neonatal operated rats, two for a spatial alternation task with ($n = 45$) and without ($n = 39$) delay and one for a reversal-learning task ($n = 41$). Moreover, we used two cohorts of adult operated rats for a spatial alternation task with ($n = 17$) and without delay ($n = 17$).

3.2. Spatial alternation

Rats were tested for spatial continuous delayed alternation in a T-maze, four groups (one with neonatal and one with adult lesions and the respective shams) with an intertrial interval (ITI) of 0 s (ITI 0) and four groups (one with neonatal and one with adult lesions and the respective shams) with an ITI of 30 s (ITI 30). The T-maze was constructed from black plastic (main alley: 70 cm × 10 cm × 30 cm and two side alleys: 50 cm × 10 cm × 30 cm). At the end of each alley was a 1 cm deep cavity (diameter 4.5 cm) that concealed the food reward (casein pellets, Bioserv, Germany). A movable guillotine door was mounted in the main alley to separate a start box (20 cm). During the test the animals had to alternate between the arms to get a reward. Rats were habituated to the maze for 4 days. First animals were placed in the maze in groups of three rats and allowed to explore the maze with the door open and pellets distributed in the entire maze. During the following 3 days, rats were placed in the start box, trained to find food in the cavity of both alleys and were accustomed to the movement of the door of the start box. After this adaptation period spatial continuous delayed alternation testing started. During the first trial of each day, food was presented in both goal arms. During the next 15 trials, the arm opposite to the one the animal had entered on the previous trial was baited, except when the animal had gone to the empty arm on the last trial. Each daily session consisted of 16 trials and tests were run on 5 days a week for two consecutive weeks for the groups with ITI 0 and for three consecutive weeks for the groups with ITI 30. During the ITI animals were kept in the start box. Errors were divided into two types. A ‘working memory error’ (type I) was made when the rat entered the arm that was the correct one in the previous trial (i.e. the first entry into an unbaited arm or incorrect choice). A ‘perseverative error’ (type II) was made when the rat continued to choose the incorrect arm (i.e. re-entries into an unbaited arm).

3.3. Reversal learning

Rats were adapted to the maze in a similar procedure as described for the alternation task. They were then trained for 5 days to collect pellets from only one arm of the T-maze. During the next 5 days pellets were placed in the arm opposite to the previously baited arm. Each daily session consisted of 10 trials with an ITI 30. Entering the arm that was baited in the training period was counted as an error.

3.4. Statistical analysis for T-maze tasks

Data for continuous alternation and reversal learning were first analysed per day using a three-way analysis of variance (ANOVA) with lesion status and dexamethasone-challenge as independent factors and day as within subject factor followed by Tukey *t*-test for post-hoc pairwise comparisons. For the continuous alternation task errors were subsequently analysed separately for types I and II errors. Since type II errors were not evenly distributed over days but appeared to form clusters at different days, we chose to pool these errors in blocks of 5 days. Type I errors were also blocked in order to be able to compare between type I and II errors. These data were then evaluated by three-way ANOVA with lesion status and dexamethasone-challenge as independent factors and week as within subject factor, followed by Tukey *t*-test. All tests were performed two-sided and $P < 0.05$ was considered significant.

3.5. Open field

For this experiment we used 15 neonatal lesioned (6 with pubertal dexamethasone-injection and 9 with pubertal vehicle-injection) and 13 neonatal sham-lesioned rats (5 with pubertal dexamethasone-injection and 8 with pubertal vehicle-injection) of the group previously used for the continuous alternation task. Additionally 13 adult lesioned and 12 sham lesioned rats were tested. Motor activity was recorded using a computerised photocell system. The testing field consisted of Plexiglas cages (42 cm × 42 cm × 42 cm) equipped with photocell monitors (AktiMot; TSE-systems, Bad Homburg, Germany). On the first day rats were placed into the box for 35 min to familiarise them to the new environment. On days 3 and 5, rats were monitored for 35 min in the box, on day 3 they were injected with vehicle (0.1% ascorbic acid in aqua dest) and on day 5 with apomorphine (0.5 mg/kg apomorphine in 0.1% ascorbic acid solution). The following parameters were recorded: (1) horizontal

activity: number of beam interruptions in the horizontal sensors; (2) vertical activity (rearings): number of beam interruptions in the vertical sensors; (3) percent time spend in the centre of the field.

3.6. Statistical analysis

The data were analysed by three-way ANOVA with lesion status and dexamethasone-challenge as independent factors and apomorphine-treatment as within subject factor, followed by Tukey *t*-test. All tests were performed two-sided and $P < 0.05$ was considered significant.

3.7. ASR and PPI

Startle measurements were made using the Startle Response System (TSE, Bad Homburg, Germany) consisting of two sound-attenuated startle chambers. The motor response of the rats was registered using a motion-sensitive measuring platform (piezoelectronic accelerometer) and transmitted to a PC after analogue to digital (AD) conversion. In each chamber, acoustic stimuli were delivered by two loud-speakers mounted on both sides of the platform at a distance of 4 cm.

For this experiment we used 11 lesioned (4 with dexamethasone-challenge and 7 with vehicle-challenge) and 12 sham-lesioned rats (6 with dexamethasone-challenge and 6 with vehicle-challenge) of the group previously used for the reversal learning experiment. Rats were placed in wire mesh cages (24.5 cm × 9 cm × 10 cm) that were fixed on the startle platform and allowed to acclimatize to the startle chamber for a period of 4 min. Thereafter, each animal was given five startle stimuli (20 ms white noise pulse, 100 dB sound pressure level (SPL)) at an ITI of 10 s, in order to habituate the animal to a stable baseline of responding. After that the test session started, which consisted of six different trial types that were presented 10 times each in a random order: (1) pulse alone ("pulse-alone trial"; 20 ms white noise pulse, 100 dB SPL, 0 ms rise/fall times), (2, 3, and 4) pulse preceded by a 64, 68 and 72 dB SPL prepulse ("prepulse-pulse trial", 20 ms pure tone 10 kHz, 0 ms rise/fall times, 100 ms before onset of the pulse), (5) 72 dB prepulse alone and (6) no stimulus. Test duration was 35 min and ITI 20–30 s. All rats were first tested directly after vehicle-injection and 2 days later directly after 2 mg/kg apomorphine-injection.

3.8. Statistical analysis

The ASR magnitudes of each different trial type were averaged. PPI was the percent decrease of the ASR in pulse-alone trials compared to an ASR in prepulse-pulse trials (100 × (pulse-alone trial – prepulse-pulse trial)/pulse-alone trial). The data were analysed by four-way ANOVA with lesion status and dexamethasone-challenge as independent factors and PPI after different prepulse-intensities and apomorphine-treatment as within subject factors, followed by Tukey *t*-test. All tests were performed two-sided and $P < 0.05$ was considered significant.

3.9. Histology

After termination of the experiments the extent of the ibotenic acid-induced lesions were determined histologically. Rats were deeply anaesthetised with chloralhydrate and transcardially perfused with 0.01 M phosphate-buffered saline (PBS), pH 7.4, followed by 4% paraformaldehyde-PBS. The brains were then placed in 30% sucrose-PBS for at least 24 h at 4 °C. Thereafter, they were cut on a freezing microtome and divided into six series of 40 μm-thick coronal sections. One series was Nissl-stained with thionine. One series of neonatal lesioned rats without dexamethasone-treatment was processed immunohistochemically for the detection of parvalbumine-immunoreactive neurones, in order to detect possible cell type-specific lesion effects. Free-floating sections were preincubated in a blocking solution containing normal rabbit serum and bovine serum albumine in Tris buffered saline (TBS, pH 7.4) for 60 min. They were then incubated for 20 h at room temperature in a monoclonal mouse anti-parvalbumine IgG1 isotype (1:2000; Sigma, Deisenhofen, Germany), 1% normal rabbit serum and 1% Triton-X in 0.1 M PBS. After washing the sections three times in TBS, sections were placed in biotin-labelled secondary antiserum (rabbit anti-mouse IgG, 1:500; Sigma) for 60 min. After washing they were incubated in TBS containing streptavidin coupled to horseradish peroxidase (1:375; DAKO, Hamburg) for 60 min. The free-floating sections were again washed and transferred to a solution containing 0.05% 3.3-diaminobenzidine (DAB) tetrahydrochloride and 0.6% ammonium nickel sulphate (both from Sigma, Deisenhofen, Germany) in TBS for 15 min. Then, 0.01% hydrogen peroxide (H_2O_2) was added which led to the production of a black DAB precipitate by peroxidase activity. Incubation in H_2O_2 was terminated after 15 min, the sections were washed in TBS, mounted on gelatine coated glass slides, air-dried, dehydrated in graded ethanols, cleared in xylene and coverslipped in entellan (Merck, Darmstadt, Germany).

The extent of mPFC-subregions (anterior cingulate cortex, prelimbic cortex and infralimbic cortex) of neonatal lesioned and sham lesioned rats was measured in three coronal Nissl-stained sections (distance: 240 μm, since every sixth section was Nissl-stained) at a standardised position according to the atlas of Paxinos and Watson [47] (see Fig. 2B) beginning at 3.2 mm anterior from Bregma using a light microscope (Zeiss, Göttingen, Germany) and the MetaMorph image analysis system (Visitron-Systems, Puchheim, Germany). The boundaries of these regions are similar to the dorsal anterior cingulate cortex and the prelimbic cortex of the mPFC described in the study of Eden and Uylings (1985) [55]. All other groups were qualitatively analysed for a similar extent of the lesions in Nissl-stained sections and absence of lesions was verified in sham-lesioned rats. Digital

photomicrographs of the mPFC of lesioned rats and sham-lesioned rats were taken.

Additionally, all parvalbumine-immunopositive neurones in the mPFC-subregions in three sections adjacent to the Nissl-stained sections used for the area-measurements were counted. We first projected the frame used for measuring the area of the mPFC-subregions onto the parvalbumine-stained sections. The number of parvalbumine-immunopositive neurones within the left and right subregions of the mPFC was then determined per section for the entire regions by counting all parvalbumine-immunopositive neurones emerging into focus through the 40 μm sections at 200 times magnification. Since the area of the different subregions was expected to be smaller after neonatal lesions, we then calculated the number of parvalbumine-immunopositive neurones per area of the different mPFC-subregions (a measure of cell density).

4. Results

4.1. Neonatal lesions

After a survival period of 2 days ibotenic acid induced a complete loss of neurones at the injection site accompanied by little gliosis (data from a pilot study assessing immediate lesion size). The lesions were restricted to the infralimbic, prelimbic and the ventral part of the anterior cingulate cortices. Adult rats that had been lesioned on PND 7 showed atrophy and retraction of tissue at the level of the infralimbic-prelimbic cortex with abnormal cytoarchitecture (Fig. 1A). These alterations were generally found at the anterior–posterior co-ordinates approximately from 3.7 to 2.2 mm from bregma, according to the atlas of Paxinos and Watson [47]. Other areas of the frontal cortex adjacent to the anterior cingulate cortex and infralimbic cortex were spared. The reconstruction of a typical neonatal lesion is depicted in Fig. $1A_1$ with the corresponding Nissl-stained section in Fig. $1A_{4,5}$. Additionally, in about 30% of the lesioned animals the lateral ventricle of one hemisphere was enlarged. Eleven animals (five rats used for the T-maze alternation task with ITI 30 and six rats used for the reversal learning task) were deleted from further analysis because of inappropriate location of the lesion or due to the lack of discernible damage. The sham-lesioned rats showed no detectable damage of the cortical tissue (Fig. $1A_{2,3}$). The area of different mPFC-subregions was determined in one neonatal lesioned ($n = 10$) and one sham-lesioned ($n = 9$) group. Compared to sham-lesioned rats the extent of all subregions of the medial PFC was significantly smaller in neonatal lesioned rats than in shams ($P < 0.05$, Student t-test; Fig. $2A_1$). Neonatal lesions of all other groups were qualitatively similar to the lesions of the group used for area measurements.

Compared to sham-lesioned rats the number of parvalbumine-immunopositive neurones in lesioned rats was reduced within all mPFC-subregions ($P < 0.05$; Fig. $2A_2$). Since the area of all mPFC-subregions was also reduced in lesioned rats, we additionally calculated the density (number of cells per area) of parvalbumine-immunopositive neurones. Density of parvalbumine-immunopositive neurones was reduced in the infralimbic cortex ($P < 0.05$) but not in the anterior cingulate and prelimbic cortices (Fig. $2A_3$). Fig. 2C shows examples of parvalbumine-immunopositive neurones in mPFC-subregions of neonatal lesioned and sham-lesioned rats.

4.2. Adult lesions

Histological analysis of lesions revealed a loss of tissue similar in extent as acute neonatal lesions. In contrast to neonatal lesioned rats, however, the remaining tissue consisted mainly of glial cells. Therefore, lesions were only qualitatively analysed and not processed for parvalbumine-immunohistochemistry. Gliosis was restricted to the infralimbic and prelimbic cortices and to the ventral parts of the anterior cingulate cortex. The reconstruction of a typical adult lesion is depicted in Fig. $1B_1$ with the corresponding Nissl-stained section in Fig. $1B_{4,5}$. Similar to neonatal sham-lesioned rats, adult sham-lesioned rats showed no damage of the cortical tissue (Fig. $1B_{2,3}$). Two animals of the group used for the T-maze alternation task with ITI 30 were excluded from the statistical analysis because of lack of discernible damage.

4.3. Behavioural tests

4.3.1. T-maze

During the autoshaping period all animals learned to collect pellets from both arms of the T-maze. Pubertal dexamethasone-treatment did not affect behavioural performance of neonatal lesioned or sham-lesioned animals compared to vehicle-treatment in any T-maze test (P-values > 0.2). Therefore, dexamethasone- and vehicle-treated rats were grouped and will be referred to as neonatal lesioned or sham-lesioned animals. Data were then evaluated using a two-way ANOVA with day or week as within subject factor and lesion as independent factor.

4.4. Continuous spatial-delayed alternation task

4.4.1. Neonatal lesions

All groups learned the task with no difference between lesioned and sham-lesioned rats. Analysis of the total number of errors (types I and II) with a two-way ANOVA during acquisition for groups with ITI 0 and ITI 30 showed a significant effect of day during training (ITI 0: $F_{9,389} = 8.815$, $P < 0.001$; ITI 30: $F_{14,599} = 7.422$, $P < 0.001$) and a significant effect of lesion (ITI 0: $F_{1,389} = 5.071$, $P = 0.03$; ITI 30: $F_{1,599} = 4.914$, $P = 0.033$). The interaction between day and lesion was not significant (ITI 0: $F_{9,389} = 1.391$, $P = 0.191$; ITI 30: $F_{14,599} = 1.318$, $P = 0.191$).

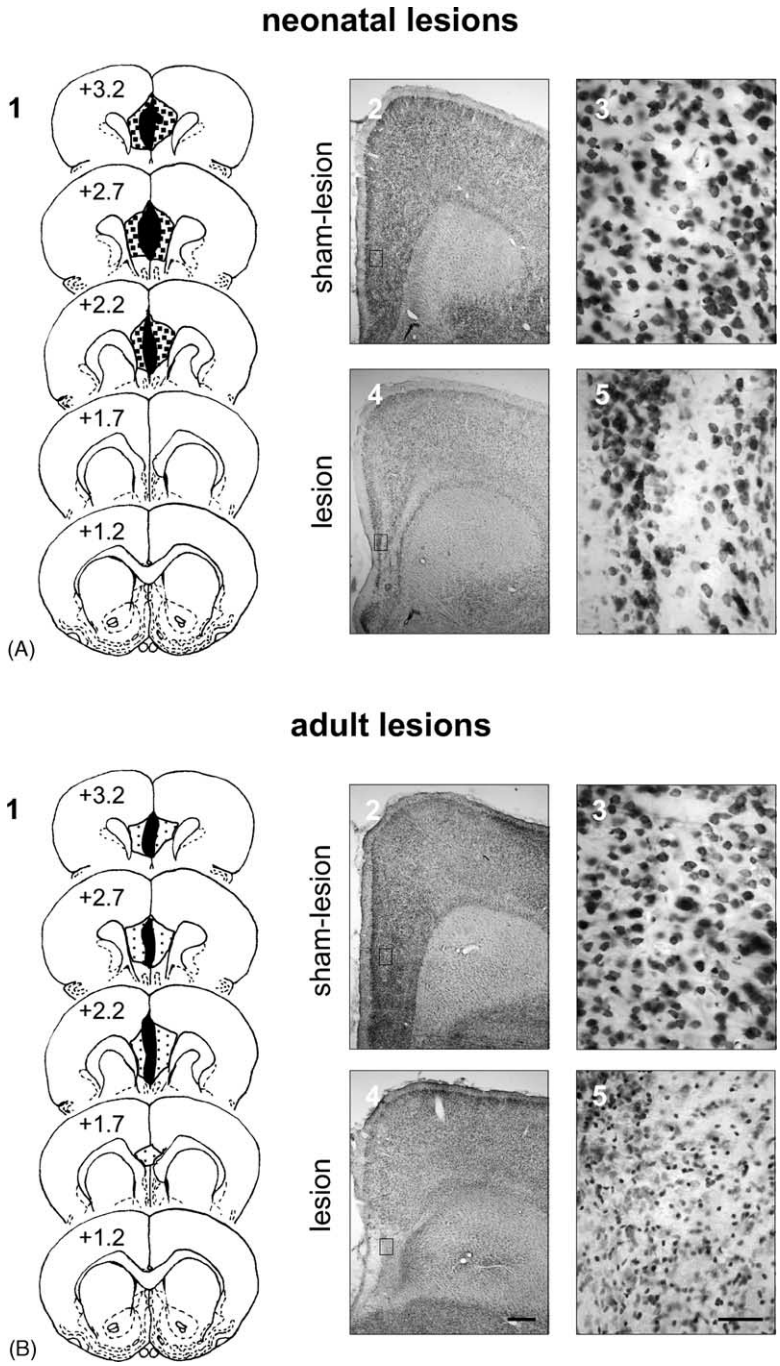

Fig. 1. The medial prefrontal cortex (mPFC) after neonatal (A) and adult (B) excitotoxic lesions. The extent of a typical neonatal (A1) and adult (B1) lesion is shown on schematic drawings of coronal brain sections according to the atlas of Paxinos and Watson (1997). Black areas correspond to the loss of tissue and dotted areas depict areas of abnormal cytoarchitecture (A) or gliosis (B). A/B2-5 are Nissl-stained coronal sections of the right mPFC of sham-lesioned (A/B2,3) and lesioned (A/B4,5) rats. A/B3,5 are greater magnifications of areas indicated in A/B2,4. Ibotenic acid-injections induced thinning of the mPFC with normal morphology in neonatal and glia reaction in adult lesions. No morphological or cytoarchitectural alterations are seen after vehicle-injection (sham-lesions). Calibration bar: A/B2,4500 μm, A/B3,550 μm.

Post-hoc analysis revealed that in the alternation task without delay lesioned rats made more errors on days 1 and 10 and in the alternation task with delay on days 4 and 9 compared to sham-lesioned rats ($P < 0.05$; Fig. 3). Since groups differed in the total number of errors during acquisition, we subsequently differentiated between types I and II errors. For this purpose, the number of errors were grouped for 1 week. Comparison for type I errors showed no differences between lesioned and sham-lesioned groups. A two-way ANOVA showed a significant effect of week (ITI 0: $F_{1,77} = 6.457$, $P = 0.015$; ITI 30: $F_{2,119} = 14.405$, $P < 0.001$), no effect of lesion status (ITI 0: $F_{1,77} = 1.662$, $P = 0.205$; ITI 30:

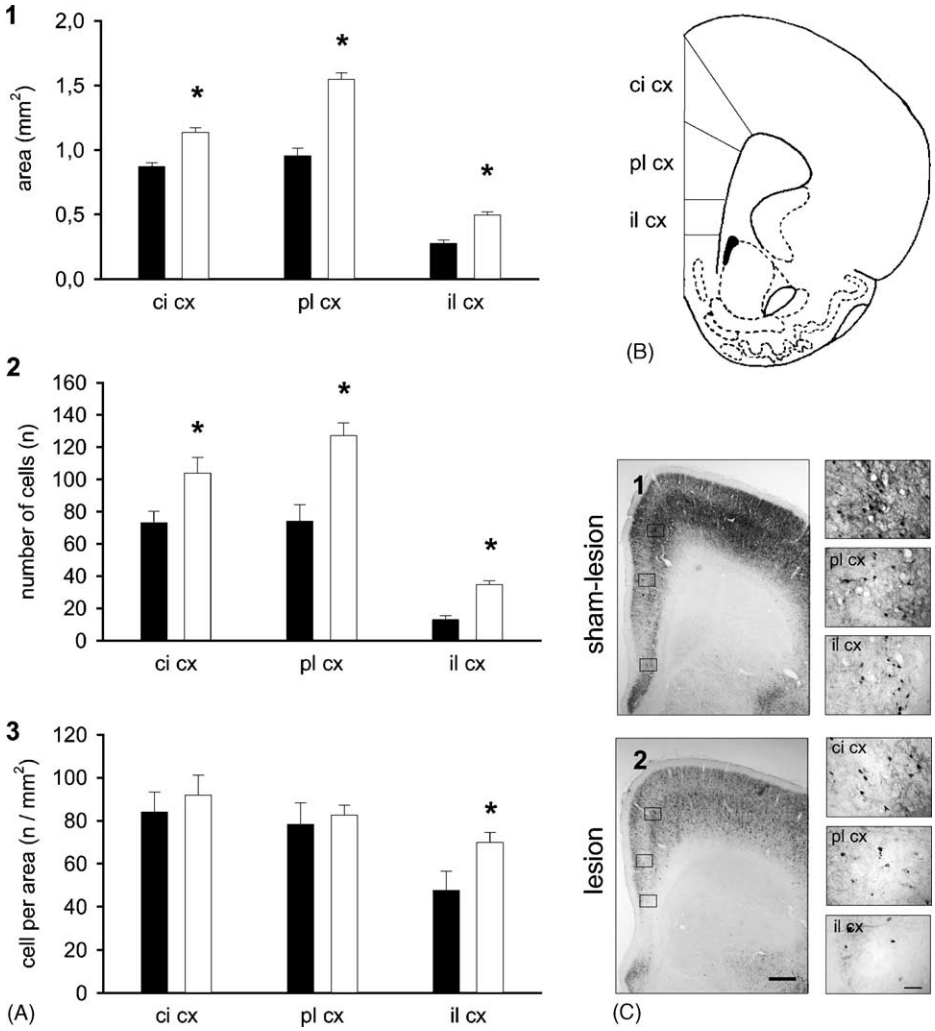

Fig. 2. Area (extent) of different subregions (mean of three sections between +3.2 and +2.2 mm, relative to Bregma; A_1) of the medial prefrontal cortex (mPFC; the anterior cingulate cortex (ci cx), the prelimbic cortex (pl cx) and infralimbic cortex (il cx)) indicated in the schematic drawing (B) with total number (A_2) and density (number of cells per area; A_3) of parvalbumine-immunopositive neurones. Data are mean + S.E. of the left and right hemisphere of rats with neonatal lesions (black bars, $n = 10$) and rats with sham-lesions (white bars, $n = 9$). Significant differences in the area, the number of neurones and in the density of neurones within the mPFC-subregions are indicated by asterisks ($^*P < 0.05$). $C_{1,2}$ show coronal brain sections with typical examples of distribution of parvalbumine-immunopositive neurones in different mPFC-subregions of neonatal sham-lesioned (C_1) and lesioned (C_2) rats. Calibration bar: C1 500 μm, ci cx, pl cx, il cx 50 μm.

$F_{1,119} = 1.828$, $P = 0.369$) and no interaction between day and lesion status (ITI 0: $F_{1,77} = 1.204$, $P = 0.280$; ITI 30: $F_{1,119} = 1.291$, $P = 0.281$; Table 1), indicating no effect of lesion on working memory represented by type I errors. However, neonatal lesioned rats made more type II errors in all training weeks. A two-way ANOVA showed a significant effect of week (ITI 0: $F_{1,77} = 27.599$, $P < 0.001$; ITI 30: $F_{2,119} = 40.898$, $P < 0.001$), a significant effect of lesion status (ITI 0: $F_{1,77} = 11.274$, $P = 0.002$; ITI 30: $F_{1,119} = 9.478$, $P = 0.004$), and a trend of interaction between these factors in groups with ITI 0 ($F_{1,77} = 2.978$, $P = 0.093$) which was significant in groups trained with an ITI 30 ($F_{2,119} = 3.212$, $P = 0.046$). Post-hoc testing revealed that all lesioned groups made significantly more type II errors in weeks 1 and 2 compared to sham-lesioned groups ($P < 0.05$), indicating that neonatal lesions caused an increase in perseverative behaviour (Fig. 4A).

4.4.2. Adult lesions

Lesions sustained in adulthood did not affect type II errors but lesioned rats made more type I errors during the first week when tested with delay (Table 1). A two-way ANOVA showed a significant effect of week in groups trained without delay (ITI 0: $F_{1,29} = 8.457$, $P = 0.012$) but not for groups trained with delay (ITI 30: $F_{2,50} = 1.232$, $P = 0.306$). There was no effect of lesion status in both groups (ITI 0: $F_{1,29} = 1.669$, $P = 0.219$; ITI 30: $F_{1,50} = 1.661$, $P = 0.217$) and no interaction between day and lesion status in groups trained without delay (ITI 0: $F_{1,29} = 0.0703$, $P = 0.795$). Groups trained with delay showed an inter-

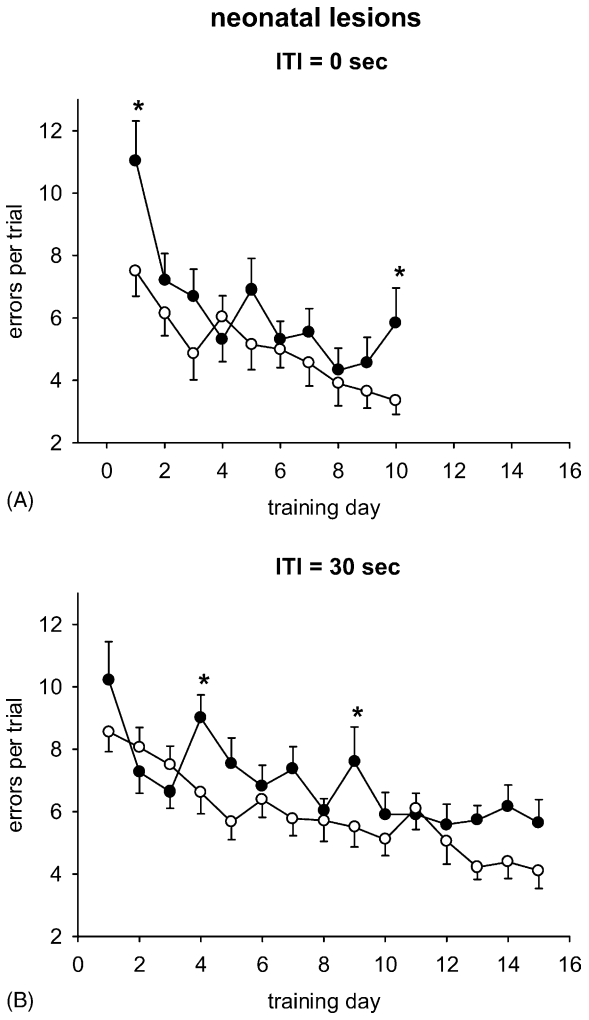

Fig. 3. Effect of neonatal lesions of the medial prefrontal cortex on acquisition of spatial continuous alternation with intertrial interval (ITI) of 0 s (A) and ITI of 30 s (B) in the T-maze. Data are mean + S.E. of all errors for training days of sham-lesioned (open circles) and lesioned groups (filled circles). Number of rats as indicated in Table 1. Differences between lesioned and sham-lesioned groups are indicated by asterisks (two-way ANOVA, post-hoc Tukey *t*-test; $^{*}P < 0.05$).

Table 1
Effect of neonatal and adult lesions in the medial prefrontal cortex on the total number of type I errors per training week in spatial continuous alternation with intertrial interval (ITI) of 0 s and ITI of 30 s

	Neonatal lesions		Adult lesions	
	Lesion	Sham-lesion	Lesion	Sham-lesion
ITI 0 s	$n = 19$	$n = 20$	$n = 10$	$n = 7$
week 1	26.1 ± 2.2	25.6 ± 1.4	25.2 ± 2.1	20.6 ± 3.5
week 2	23.7 ± 1.8	19.45 ± 1.6	17.8 ± 3.9	14.4 ± 0.9
ITI 30 s	$n = 22$	$n = 18$	$n = 8$	$n = 7$
week 1	30.5 ± 1.1	31.5 ± 1.3	33.4 ± 1.1*	26.4 ± 2.6
week 2	28.8 ± 1.3	26.6 ± 1.4	30.6 ± 1.1	28.4 ± 2.5
week 3	25.7 ± 1.2	23.2 ± 1.9	26.3 ± 0.9	29.1 ± 2.2

Data are mean+S.E. for the number of rats indicated. Differences between lesioned and sham-lesioned groups are indicated by asterisks (two-way ANOVA, post-hoc Tukey *t*-test; $^{*}P < 0.05$).

action between lesion and training week (ITI 30: $F_{2,50} = 5.437$, $P = 0.010$). Post-hoc testing revealed that lesioned rats made significantly more type I errors during the first week compared to sham-lesioned rats ($P < 0.05$) indicating a lesion effect on working memory in rats trained with delay but not in rats trained without delay (Table 1). In contrast to neonatal lesioned rats, adult lesioned rats did not differ from sham-lesioned rats in the number of type II errors. A two-way ANOVA showed a significant effect of week (ITI 0: $F_{1,29} = 5.858$, $P < 0.031$; ITI 30: $F_{2,50} = 25.017$, $P < 0.001$) but no effect of lesion status (ITI 0: $F_{1,29} = 0.816$, $P = 0.383$; ITI 30: $F_{1,50} = 0.924$, $P = 0.352$) and no interaction between these factors (ITI 0: $F_{1,29} = 1.107$, $P = 0.312$; ITI 30: $F_{2,50} = 1.059$, $P = 0.359$), indicating that adult lesions do not affect perseverative behaviour (Fig. 4B).

4.4.3. Reversal learning

In this task, neonatal lesioned rats performed worse than sham-lesioned rats. Analysis of the number of errors during reversal learning with a two-way ANOVA showed a significant effect of day ($F_{4,174} = 119.024$ $P < 0.001$) and of lesion status ($F_{1,174} = 9.623$, $P = 0.004$), while the interaction between day and lesion status was not significant ($F_{4,174} = 1.172$, $P = 0.326$). Additionally, post-hoc test for lesion status showed that except for day 4, lesioned rats made significantly more errors compared to sham-lesioned rats on all days ($P < 0.05$; Fig. 5).

4.5. Locomotor activity

4.5.1. Neonatal lesions

In the open-field lesions and/or dexamethasone-challenge had no effect under drug-free conditions. Apomorphine-injection enhanced locomotor activity, but this effect was reduced in dexamethasone-challenged groups while lesions had no effect. ANOVA showed a significant main effect of apomorphine ($F_{1,31} = 35.042$, $P < 0.001$). Additionally, the interaction between dexamethasone-challenge and apomorphine-treatment was significant ($F_{1,31} = 5.246$, $P = 0.029$) while there was no interaction between lesion and apomorphine-injection ($F_{1,31} = 0.002$, $P = 0.967$). There was also no interaction between all factors ($F_{1,31} = 0.001$, $P = 0.975$). Post-hoc testing revealed that apomorphine-injection significantly enhanced locomotor activity only in lesioned and sham-lesioned rats that were not challenged with dexamethasone ($P < 0.05$). Hence, the locomotor stimulating effect of apomorphine was reduced in dexamethasone-challenged groups (Fig. 6A).

Apomorphine also increased the number of rearings and percent time spent in the centre of the field in all groups. Here, we did not find any difference between groups. ANOVA showed a significant main effect of apomorphine (rearings: $F_{1,31} = 88.634$, $P < 0.001$, centre: $F_{1,31} = 25.191$, $P < 0.001$). There was no interaction between

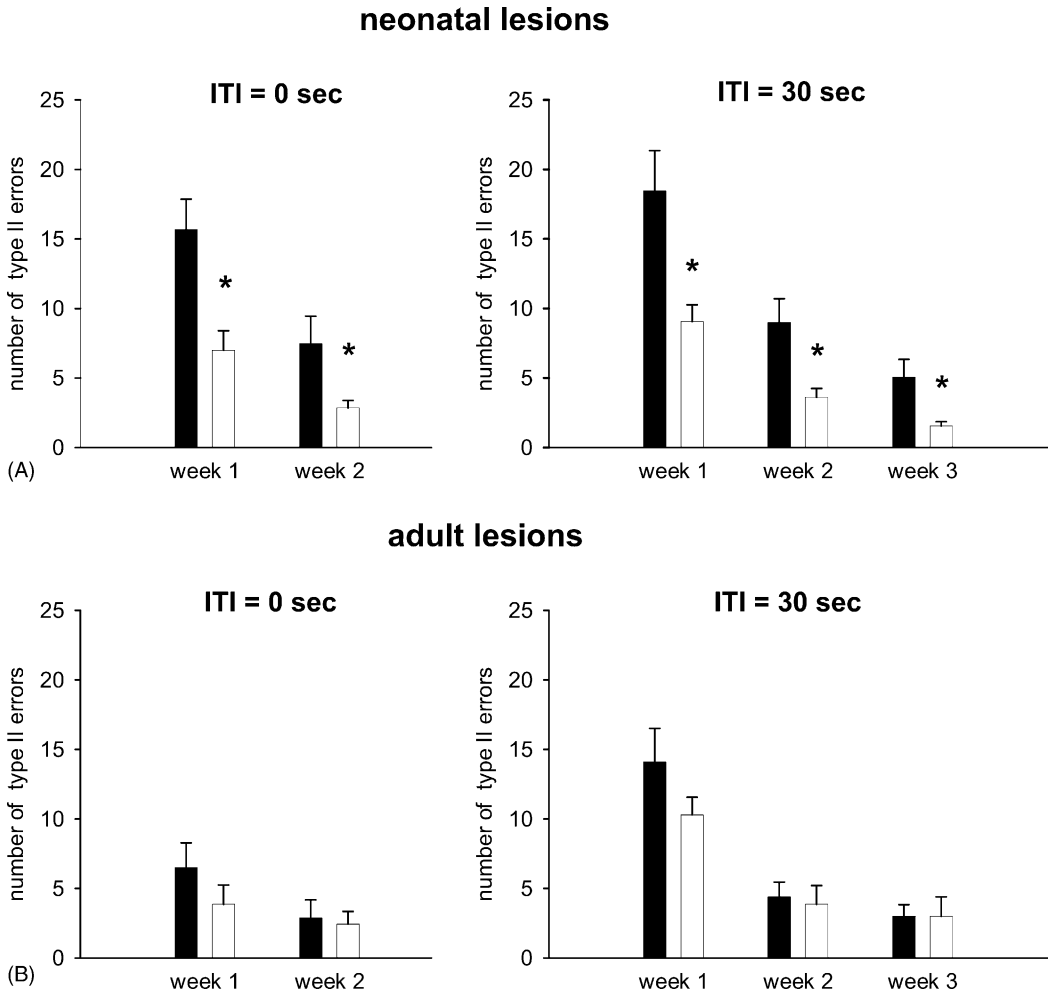

Fig. 4. Effect of neonatal (A) and adult (B) lesions in the medial prefrontal cortex on total number of type II errors per training week in spatial continuous alternation with intertrial interval (ITI) of 0 s and ITI of 30 s. Data are mean + S.E. for sham-lesioned (white bars) and lesioned groups (black bars). Number of rats as indicated in Table 1. Differences between lesioned and sham-lesioned groups are indicated by asterisks (two-way ANOVA, post-hoc Tukey *t*-test; $^{*}P < 0.05$).

dexamethasone-challenge and apomorphine-injection (rearings: $F_{1,31} = 0.037$, $P = 0.849$, centre: $F_{1,31} = 0.984$, $P = 0.329$) and between lesion and apomorphine-injection (rearings: $F_{1,31} = 0.463$, $P = 0.501$, centre: $F_{1,31} = 0.046$, $P = 0.831$). There was also no interaction between all factors (rearings: $F_{1,31} < 0.001$, $P = 0.983$, centre: $F_{1,31} = 0.272$, $P = 0.606$) (data not shown).

4.5.2. Adult lesions

In the open field, motor activity of adult lesions was similar as neonatal lesions. Total distance travelled did not differ between lesioned and sham-lesioned rats. Apomorphine-injection enhanced locomotor activity in both groups. Two-way ANOVA with lesion status as independent and apomorphine as dependent factor showed a significant effect of apomorphine ($F_{1,53} = 38.428$, $P < 0.001$), while lesion status ($F_{1,53} = 0.0221$, $P = 0.883$) and the interaction between these factors ($F_{1,53} = 0.0435$, $P = 0.836$) was not significant. Post-hoc testing revealed that apomorphine-injection significantly enhanced locomotor activity in lesioned and sham-lesioned rats ($P < 0.05$; Fig. 6B). Apomorphine also increased rearings in both groups. Two-way ANOVA showed a significant effect of apomorphine ($F_{1,53} = 16.115$, $P < 0.001$), but no effect of lesion status ($F_{1,53} = 0.255$ $P = 0.618$) and no interaction ($F_{1,53} = 1.825$, $P = 0.189$). The percent time spent in the centre of the field was also increased after apomorphine-injection, but this effect was not significant in both groups. Two-way ANOVA showed only a trend of enhancement by apomorphine ($F_{1,53} = 3.549$, $P = 0.071$), but no significant effect of treatment ($F_{1,53} = 1.006$, $P = 0.325$) and interaction ($F_{1,53} = 0.029$, $P = 0.865$; data not shown).

4.6. ASR and PPI

The statistical analysis with four-way ANOVA showed no effect of dexamethasone-challenge on ASR or PPI and no interaction between PPI after different prepulse-

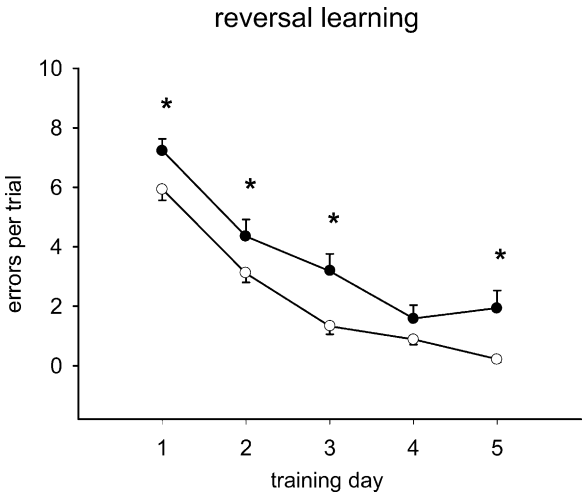

Fig. 5. Effect of neonatal lesions in the medial prefrontal cortex on the number of errors per day during acquisition of reversal learning in a T-maze. Data are mean + S.E. of sham-lesioned (open circles) and lesioned groups (filled circles). Differences between lesioned ($n = 17$) and sham-lesioned ($n = 18$) groups are indicated by asterisks (two-way ANOVA, post-hoc Tukey t-test; $^*P < 0.05$).

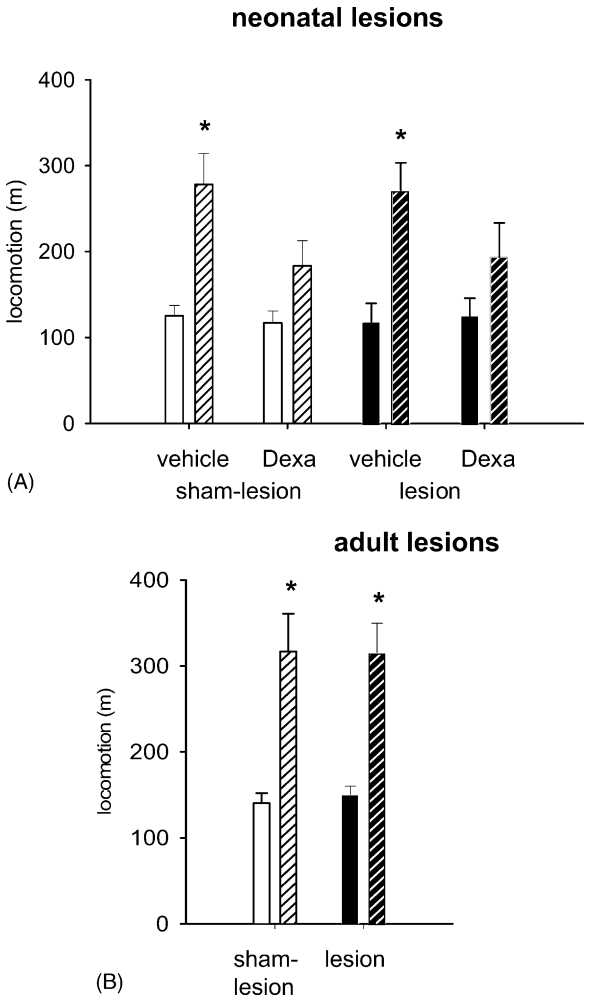

Fig. 6. Effect of neonatal lesions with dexamethasone-challenge ($n = 6$) or vehicle-challenge ($n = 9$) or sham-lesions with dexamethasone challenge ($n = 5$) or vehicle-challenge ($n = 8$; A) and adult lesions ($n = 13$) or sham-lesions ($n = 12$; B) on locomotor activity. Data are means + S.E. for sham-lesioned (white bars) and lesioned groups (black bars) for the total distance travelled (locomotion) within 35 min. Hatched bars show the effect of apomorphine-challenge. Significant differences between apomorphine- and vehicle-injection are indicated by asterisks (two-way ANOVA, post-hoc Tukey t-test; $^*P < 0.05$).

intensities and lesion ($P > 0.1$). Therefore, we grouped dexamethasone-challenged and vehicle-challenged rats for lesioned and sham lesioned groups and also pooled the PPI after different prepulse-intensities. We then evaluated the data using a two-way ANOVA with lesion as independent factor and apomorphine-treatment as within subject factor.

ASR magnitude was not affected by neonatal mPFC lesions. Apomorphine caused a decrease of ASR magnitude in neonatal sham-lesioned rats and an increase in ASR magnitude in lesioned rats, but this effect did not reach the level of significance. Two-way ANOVA with lesion as independent and apomorphine as dependent factor showed no effect of lesion ($F_{1,44} = 2.599$, $P = 0.122$), no effect of apomorphine ($F_{1,45} = 0.260$, $P = 0.615$) and only trend towards an interaction between these factors ($F_{1,45} = 3.779$, $P = 0.065$; ASR pulse-alone data not shown).

PPI of neonatal lesioned rats was enhanced compared to sham-lesioned rats, both after vehicle- and apomorphine-injection. Two-way ANOVA with lesion as independent and apomorphine as dependent factor showed a significant main effect of apomorphine ($F_{1,137} = 9.283$, $P < 0.001$), a significant effect of lesion status ($F_{1,137} = 9.283$, $P = 0.003$) while there was no interaction between these factors ($F_{1,137} < 0.001$, $P = 1.0$). Post-hoc testing revealed that apomorphine-injection significantly decreased PPI in neonatal lesions and sham-lesions ($P < 0.001$; Fig. 7).

5. Discussion

Main findings of our study were as follows. (1) Neonatal lesions of the mPFC led to a reduced extent of tissue and decreased number of parvalbumine-immunopositive—presumably GABAergic—interneurones in all mPFC-subregions. (2) Adult rats with neonatal lesions of the mPFC showed disturbed behavioural flexibility (enhanced perseveration and impaired reversal learning) while working memory was intact. In contrast similar lesions in adult rats induced a working memory deficit but no enhanced perseveration. (3) Pubertal dexamethasone-treatment reduced the locomotor-stimulating effect of apomorphine in all groups while neonatal lesions had no effect. (4) PPI of the ASR was increased after neonatal mPFC-lesions.

Excitotoxic lesions inflicted on PND 7 decreased the extent of mPFC-subregions and induced cytoarchitectural alterations. The mPFC is not a homogeneous structure and can be divided into at least three different subregions, the dorsal anterior cingulate cortex and the ventral prelimbic

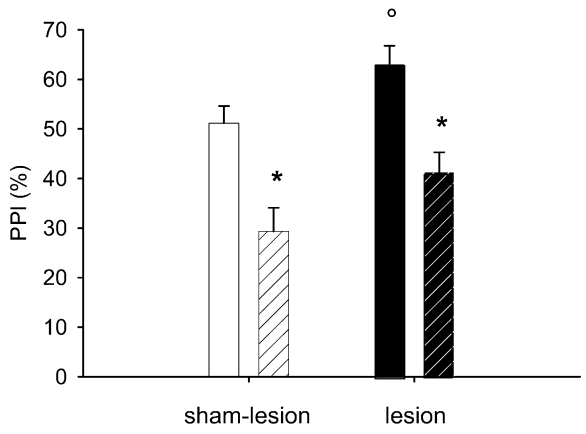

Fig. 7. Effect of neonatal lesions on prepulse inhibition (PPI) of startle. Data are means+S.E. for sham-lesioned ($n = 12$, white bars) and lesioned groups ($n = 11$, black bars). Hatched bars show the effect of apomorphine-challenge. Significant differences between apomorphine- and vehicle-injection are indicated by asterisks (∗), between lesion and sham-lesion by circles (two-way ANOVA, post-hoc Tukey *t*-test; $^{\circ}P < 0.05$).

and infralimbic cortices [8,50] which differ in their afferent and efferent projections [9,50]. Recent studies also suggest different functions of these PFC-subregions [15,21]. Neonatal lesions mainly comprised the ventral prelimbic and infralimbic cortices, while the dorsal anterior cingulate cortex was only marginally affected. The dorsal mPFC receives input mainly from the somatosensory cortex and projects to more caudal and lateral parts of the striatum and the nucleus accumbens core. It is therefore considered part of the premotor cortex. The ventral mPFC receives input from the ventral hippocampus, projects to the more rostral and medial parts of the striatum and the nucleus accumbens shell and is thus considered part of the limbic cortex [9,50]. Similar to findings of other groups [12,30,31], the morphology of the remaining mPFC after neonatal lesions was comparable to that of controls while the corresponding area after adult lesions consisted mainly of glia cells. The developing brain has apparently the capability of reorganisation and perhaps regeneration of neurones.

In addition to Nissl-staining, we performed an immunohistochemical staining for the calcium-binding protein parvalbumine, which is a reliable detector for a subset of GABAergic neurones, specifically wide-basket and chandelier subclasses of GABA neurones [46]. Both neurone-types are thought to have strong inhibitory control upon neuronal output since they synapse on the cell bodies and dendrites (wide basked neurones) or on the initial axon segments (chandelier neurones) of pyramidal cells [35,46]. We thus concentrated on parvalbumine-immunopositive neurones, although other inhibitory neurones may also be important. The total number of parvalbumine-immunopositive cells was reduced in all PFC-subregions after excitotoxic lesions. The number of these cells per area (density) was only reduced in the infralimbic cortex, which will probably reduce inhibitory control over the remaining mPFC excitatory neurones and the related subcortical networks. Particularly the striatothalamic pathway will be affected, leading to a decreased ability of the thalamus to filter of excessive or irrelevant stimuli as proposed for schizophrenia and related disorders [7]. In line with this, a reduced number of parvalbumine-immunopositive cells and other GABAergic neurones was found in the prefrontal cortex of patients with schizophrenia [3,4,35].

Neonatal lesions of the mPFC increased perseveration and disturbed behavioural flexibility in the T-maze while working memory was intact. In contrast, adult mPFC lesions led to a working memory deficit without increased perseveration. Our finding of spared working memory after neonatal lesions but deficits after adult lesions are in line with previous findings of other groups [11]. However, neonatal lesions in the present study increased perseveration in the continuous alternation task, an observation that was not found in an earlier comparable study by de Brabander et al. [11]. This finding of reduced behavioural flexibility was corroborated in a reversal learning task, where neonatal lesioned rats were also disturbed. In contrast, similar to the findings in the spatial alternation task, adult rats that were tested 2 weeks after excitotoxic mPFC lesions that were even larger than those induced in the present study, were not affected in this measure of behavioural flexibility (unpublished observations).

The finding of increased perseveration after neonatal lesions of the mPFC in this study, and the failure thereof in previous studies, may be explained by the exact localisation of the lesions. Lesions in de Brabander's study [11] affected the anterior cingulate cortex while lesions in our work mainly comprised the infralimbic-prelimbic region of the mPFC. Recent studies indicate that while deficits in working memory are consistently found after lesions of the anterior cingulate cortex [21], lesions of the prelimbic-infralimbic region are not specifically related to working memory impairment but affect more likely attentional processes [13,48].

According to Kennards' principle, the compensation for brain damage in infancy is better than later in life, because after damage the central nervous system has a greater developmental capability to compensate for loss of function than the adult brain. However, our observation that neonatal mPFC lesions have different effects on behaviour than adult lesions suggests that the juvenile brain not only has a greater capability to compensate for a loss of function but can also disturb development of other brain regions. While the working memory deficits seen after adult lesions of the mPFC will likely be due to the local lesion effects perseveration deficits after neonatal lesions are presumably mediated by disturbed mPFC functions together with developmental alterations of distant brain areas [19]. Compensatory changes, such as sprouting and rerouting of axons, or retrograde degeneration will probably compromise the functional integrity of distant brain areas and thus induce deficits that persist and even aggravate during brain maturation.

In this context, it is of interest, that dorsal hippocampus inactivation in rats caused a working memory deficit without increased perseveration while ventral hippocampus inactivation increased perseveration, but did not affect working memory [41]. The ventral hippocampus and the infralimbic-prelimbic region of the mPFC are strongly interconnected, suggesting a co-operation of these brain regions in behavioural control [27]. Therefore, it is reasonable to assume that neonatal lesions of the infralimbic-prelimbic region of the mPFC may have altered neuronal development of the ventral hippocampus. While our gross anatomical and morphological investigation of areas connected with the mPFC (ventral tegmental area, mediodorsal thalamus, hippocampus) did not reveal striking changes after mPFC lesions, the anatomical connections of the neonatally lesioned mPFC to these brain regions and the functional consequences of these connections will be investigated in future anatomical tract-tracing and electrophysiological studies.

The behavioural disturbances found after neonatal lesions do not result from generalised motor disturbances since neonatal lesioned rats did not differ in spontaneous activity from sham-lesioned animals. However, deficits in behavioural flexibility have to be partialled into the inability to learn a new arm-reward association and into the inability to suppress responding to the former rule. We cannot differentiate between these two alternatives with the paradigms used in this study.

We also investigated the effect of an additional challenge of brain development by a single pubertal treatment with the glucocorticoid receptor agonist dexamethasone. Pubertal dexamethasone-treatment did not affect working memory or perseverative behaviour, neither alone nor in combination with neonatal lesion of the mPFC. Previous studies [22,42] showed that a single injection of dexamethasone was sufficient to induce apoptotic cell death in striatal and hippocampal regions of adult rats. The authors suggested that this may have some bearings on cognitive deficits seen in psychiatric disorders, which was the rationale for using dexamethasone-challenge as second hit in our model. Their hypothesis was based on the following observations: (1) Acute single administration of dexamethasone (0.7–20 mg/kg) in rats induced apoptosis of enkephalin-immunopositive cells within the dorsomedial striatum [22], a brain region known to be involved in the control of locomotion and in certain cognitive processes [2]. (2) During the early stage of Huntington's disease an equivalent subpopulation of striatal neurones is affected [1,42]. (3) Cognitive deficits were seen following repeated administration of glucocorticoids to healthy volunteers [43,58] and steroid-induced psychosis is a well known clinical consequence of the administration of large doses of corticosteroids [23]. We did not find any behavioural effects induced by pubertal dexamethasone-treatment in the tests applied. However, we used only a single application of dexamethasone in order to replicate the treatment schedule used by Mitchell's group. Treatment at a different age, with a different dosage or with repeated injection may result in different findings.

The mPFC controls subcortical dopaminergical activity in two different ways: glutamatergic neurones project onto presynaptic dopaminergic terminals in the nucleus accumbens and via the pedunculopontine tegmental nucleus to the ventral tegmental area, the main source for dopaminergic input to the nucleus accumbens [8,14,28,50,54]. Loss of this excitatory influence by lesioning the mPFC is thought to reduce tonic accumbal dopamine release under basal conditions, which will increase subcortical dopamine receptor sensitivity ("denervation supersensitivity"). In line with this hypothesis neonatal and adult excitotoxic lesions of the rat mPFC have been associated with behavioural and biochemical evidence of increased subcortical dopaminergic activity, particularly within striatal and accumbens subregions [5,18,25,26]. Therefore, we hypothesised that behaviour which depends on subcortical dopaminergic activity, i.e. locomotor activity and PPI of ASR, would be altered by mPFC lesions and/or increase the sensitivity to the direct dopamine agonist apomorphine.

However, locomotor activity was not altered in our study neither by neonatal nor by adult lesions of the mPFC. Despite the hypothesis pointed out above, alteration in locomotor activity after neonatal or adult lesions of the mPFC is also not a consistent finding of other studies. While several groups found that the locomotor activity of adult rats with neonatal mPFC lesions was significantly enhanced in a novel environment and after D-amphetamine administration [5,18,25] other groups found no enhancement but rather an attenuated spontaneous or amphetamine-induced locomotor activity [37]. These differences may be explained by variations in the extent and type of lesion, but also by the environmental and testing conditions [34,37]. Similar to the extent of lesions in the study of Lipska et al. [37] our lesion comprised a more ventral portion of the mPFC, the prelimbic-infralimbic region. Instead, photomicrographs of the lesion described by Flores et al. [18] suggest that only more dorsal aspects of the mPFC were damaged, although the lesion boundaries in the schematic drawing showed lesions of the infralimbic-prelimbic region as well. Since the anterior cingulate cortex differs from the inframlimbic-prelimbic region with respect to anatomic connection to subcortical brain regions (see above), this may be one reason for the different findings with respect to locomotor activity.

Interestingly, dexamethasone-treatment during puberty reduced the locomotor-stimulating effect of apomorphine both in mPFC lesioned and sham-lesioned rats. Mitchell's group reported dexamethasone-induced apoptotic cell death almost exclusively in enkephalin-immunopositive medium spiny neurones, i.e. striatal neurones that belong to the indirect striatothalamic pathway and are therefore involved in the locomotor enhancing effect of dopaminergic activation [42]. Although we did not measure apoptosis in our study, a possible loss of enkephalinergic striatal projection neurones may explain the reduced response to apomorphine-challenge.

Another behaviour that is modulated by the mPFC and dopaminergic activity in subcortical brain regions is PPI of ASR. PPI is the reduction of the ASR to an intense acoustic stimulus when this stimulus is shortly preceded by a weaker stimulus [29,51]. It is considered to measure sensorimotor gating and reflects the ability to gate out irrelevant stimuli. The mPFC has been implicated in the regulation of PPI via tonical regulation of dopamine in the nucleus accumbens [6,24,51]. PPI is reduced by direct infusion of dopamine into the nucleus accumbens and has been used to detect denervation supersensitive dopamine receptors in the nucleus accumbens after 6-OHDA lesions of the dopaminergic system [52]. Therefore, we expected PPI to be enhanced under basal conditions but to be excessively disrupted after application of dopamine agonists because of a supersensitive dopamine system after neonatal mPFC lesions.

In line with these expectations PPI was increased in rats with neonatal lesions of the mPFC. Other groups also found an increase in PPI 2 weeks after large lesions of the adult mPFC [34] and similar, albeit not significant, effects were seen after neonatal mPFC lesions [53]. However, we did not see a pronounced sensitivity to apomorphine as reported in other studies [53]. It is possible that the dosage used in this study was not appropriate to detect changes in PPI after neonatal lesions. We also did not see differences in PPI in rats with large mPFC-lesions (lesioned as adults and tested 10 weeks after surgery) with and without apomorphine-injection [49]. It may be, that a pronounced sensitivity to apomorphine only appears within a limited time after mPFC lesions.

In summary, neonatal but not adult mPFC lesions induced an increase in perseverative behaviour, which may be explained by disturbed control of mPFC and related networks. Additionally, PPI was increased after neonatal lesions, probably due to the loss of excitatory influence onto dopaminergic activity in the nucleus accumbens. The important issue that remains concerns what has changed in the neuronal network anatomy and activity and how that can explain the observed behavioural effects. We want to address these questions by first determining the anatomic connections of the mPFC to its related network after neonatal lesions using anterograde tracer applications. We then plan to examine how altered neuronal input affects the physiological and pharmacological neuronal properties in these regions using electrophysiological techniques.

One of the aims of this study was to test a possible interaction effect of a maturational adverse treatment with neonatal lesions of the mPFC according to “two hit models” of schizophrenia. In none of the behavioural paradigms did we see an interaction between neonatal lesions and subsequent pubertal challenge with dexamethasone. Since this pharmacological challenge consisted of a single dose of dexamethasone given at just one time point in puberty, we can only conclude, that dexamethasone has no effect under the circumstances and in the paradigms used in this study. Since stress is a major precipitating factor in addition to early neurodevelopmental defects in the onset of schizophrenia, this approach may still be promising with more severe or long-lasting stressors during puberty.

Acknowledgements

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