Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 77 (2004) 767–774
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Effects of postnatal anoxia on striatal dopamine metabolism and prepulse inhibition in rats

Karin Sandager-Nielsen[a,*], Maibritt B. Andersen[b], Thomas N. Sager[a,1], Thomas Werge[b], Jørgen Scheel-Krüger[a]

[a]Department of Behavioural Pharmacology, NeuroSearch A/S, Pederstrupvej 93, DK-2750 Ballerup, Denmark
[b]Research Institute of Biological Psychiatry, Sct. Hans Hospital, DK-4000 Roskilde, Denmark

Received 27 October 2003; received in revised form 16 January 2004; accepted 27 January 2004

Abstract

Various evidence indicate that schizophrenia is a neurodevelopmental disorder. Epidemiological observations point to oxygen deficiencies during delivery as one of the early risk factors for developing schizophrenia. The aim of the present study was to examine the effect of postnatal anoxia in rats. Anoxia was experimentally induced by placing 9-day-old rat pups for 6 min in a chamber saturated with 100% nitrogen (N_2). Exposure to anoxia on postnatal day (PND) 9 resulted in significantly reduced subcortical dopamine metabolism and turnover, as measured by striatal 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations. Furthermore, in the anoxic group only, striatal HVA concentrations were negatively correlated to prefrontal cortical *N*-acetylaspartate (NAA) levels. Similar findings of distorted prefrontal–subcortical interactions have recently been reported in schizophrenic patients. There was no effect of postnatal anoxia on either baseline or D-amphetamine-induced deficit in the prepulse inhibition (PPI) paradigm in adulthood. Accordingly, although oxygen deficiency early in life has been discussed as vulnerability factor in developing schizophrenia, exposure to postnatal anoxia in the rat does not show clear-cut phenomenological similarities with the disorder.

Keywords: Schizophrenia; Animal model; Anoxia; Prepulse inhibition; *N*-acetylaspartate; Dopamine

1. Introduction

Schizophrenia is one of the most devastating of the psychiatric diseases. One of the most influential hypothesis regarding the etiology and pathophysiology of this disorder is the neurodevelopmental hypothesis (Weinberger, 1987). It proposes that a ''lesion'' (due to, e.g., birth complications, infections, toxin exposure, maternal stress, etc.) occurring in uterus or in the peri/postnatal period disturbs the establishment of fundamental aspects of cerebral structure and function.

There is accumulating evidence that insults to the central nervous system very early in life may be related to the development of schizophrenia later on. (Akbarian et al., 1993a,b; Jacob and Beckmann, 1986, 1994; McNeil et al., 2000). Notably, deficiencies in oxygen during delivery have been stressed (Cannon, 1997; McNeil, 1995; Cantor-Graae et al., 2000) and it has been proposed that a common denominator of the various obstetrical complications may be oxygen deprivation (McNeil et al., 2000).

Several animal studies have addressed the outcome of early oxygen deprivation and found long-term changes in brain neurochemistry, especially dopamine and metabolite levels and behaviour (e.g., Bjelke et al., 1991; Brake et al., 1997; El-Khodor and Boksa, 1997, 1998; Nyakas et al., 1991; Speiser et al., 1983). These studies all exposed the pups to oxygen deprivation at the time around birth, i.e., just prior to delivery or up to the first 3 days postdelivery.

It is conceivable that the timing of the insult is of crucial importance to the outcome in order to affect the brain areas/systems of interest. The rodent brain is less mature at the

Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; HIP, hippocampus; HVA, homovanillic acid; NAA, *N*-acetylaspartate; mPFC, anteromedial prefrontal cortex; PND, postnatal day; PPI, prepulse inhibition; pp, prepulse; STR, striatum.

* Corresponding author. Tel.: +45-44-60-83-14; fax: +45-44-60-80-80.
E-mail address: ksn@neurosearch.dk (K. Sandager-Nielsen).
[1] Present address: H. Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen-Valby, Denmark.

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.01.017

time of birth as compared to the human brain and undergoes rapid brain growth postnatally, which is termed "the brain growth spurt," with a peak around postnatal days (PNDs) 7–10 (Dobbing and Sands, 1979). Furthermore, at PNDs 12–13 the development of the cerebral cortex in the rat corresponds roughly to the full-term newborn baby (Romijn et al., 1991). Accordingly, postponing the insult to around PNDs 7–12 in the rodent may be more comparable to the clinical situation.

Indeed, other types of insults inflicted on PNDs 7–9 in rat pups have been shown to induce changes in dopaminergic sensitivity and impair behaviours with relevance to schizophrenia. Ibotenic acid lesions of ventral hippocampus (HIP) at PND 7 impaired social behaviour and prepulse inhibition (PPI) and resulted in hyperlocomotion in adult rats (Lipska et al., 1995; Sams-Dodd et al., 1997). Furthermore, the animals showed hyperresponsiveness to dopamine agonists and decreased responsiveness to dopamine antagonists (Lipska and Weinberger, 1993). Moreover, separation of rat pups from their mothers for 24 h at PND 9 reduced PPI in adult rats (Ellenbroek et al., 1998).

The aim of the present study was to investigate in the adult rat the behavioural and biochemical effects of oxygen deprivation inflicted at PND 9. The behavioural consequences of postnatal anoxia were evaluated by PPI of the acoustic startle response in adulthood. Subcortical dopamine metabolism was evaluated by measuring striatal tissue concentrations of dopamine and its metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC). Furthermore, the concentration of *N*-acetylaspartate (NAA), a measure of neural dysfunction or loss (Cendes et al., 1997; Rubin et al., 1995; Tsai and Coyle, 1995), was analysed in the medial prefrontal cortex (mPFC) and the HIP.

2. Methods

2.1. Animals

All experimental procedures carried out in this study were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Danish Animal Welfare Committee, appointed by the Danish Ministry of Justice.

Female and male SPF Wistar rats (Taconic M and B, P.O. Box 1079, DK-8680 Ry, Denmark) were mated at the animal facilities at NeuroSearch and allowed to deliver normally. On PND 9, the anoxic insult was inflicted, consisting of exposure to 6 min of anoxia (see below). Prior experiments had shown that 6 min of anoxia resulted in a mortality rate of approximately 25%. In order to reach equal numbers of animals in each experimental group, around 2/3 of the males from each litter were allocated to the anoxia group, and the other 1/3 served as the sham group. The allocation to anoxia or sham treatment was random. PND 1 was defined as the day of delivery, or the following day after if the pups were born later than 6 p.m. Only males were used for the study, but female pups were allowed to stay in the litter until weaning. All rats were weaned at 4 weeks of age and the male rats were subsequently housed socially with two to three animals from the same litter belonging to the same experimental group. The animals used for the PPI experiment were transferred to the animal facilities at the Research Institute of Biological Psychiatry, Sct. Hans Hospital approximately 3 weeks prior to testing.

Two cohorts of rats were used in the present study. In the cohort of rats used for biochemical analysis, 3 out of 13 animals died during anoxia resulting in 10 animals in the anoxic group and a mortality rate of 23%. No rats died in the sham group, leaving 10 rats in this group. The animals were 4 months old at the time of decapitation. In the cohort of animals used for the PPI experiment, 7 out of 31 rats died during anoxia, leaving 24 animals in the anoxic group and a mortality rate of 23%. No rats died in the sham group, resulting in 23 animals in this group. They were approximately 3 months old at the beginning of the experiments.

2.2. Anoxia

Anoxia was carried out in an airtight chamber made of transparent plastic ($25.5 \times 16.0 \times 16.5$ cm) and equipped with an inlet to allow influx of 100% nitrogen (N_2). It was placed on a water-filled metal hotplate connected to a thermostat-controlled water bath in order to ensure a temperature of 37 °C in the chamber during the insult. The chamber was saturated with 100% N_2 immediately before introducing the pups. For each litter, all pups allocated to anoxia were exposed to oxygen deprivation at the same time. They were placed in the chamber together, left there for 6 min, and then quickly removed from the chamber and placed on a paper napkin on the hotplate. Spontaneous breathing was reinitiated by manual stimulation of the chest and mouth regions. When breathing stabilized and normal body colour returned, the pups were weighed, marked (see below) and returned to the dam. Animals, which had not initiated breathing within 1 min after the anoxia, were excluded (referred to as "died during anoxia," see above). The animals allocated to the sham group were placed in a chamber containing atmospheric oxygen, located on the hotplate, for the same period of time. Cutting the left digitus minor marked the animals exposed to anoxia. Cutting both the left and right digit minor identified the animals in the sham group.

2.3. Tissue concentrations of dopamine, HVA, DOPAC and NAA

The animals were decapitated under halothane anaesthesia and the brains were immediately removed. The striatum (STR), mPFC and HIP were removed from both hemispheres under macroscopic dissection. Only HIP tissue from

the left hemisphere was used in this study; HIP tissue from the right hemisphere was used for other purposes. STR was dissected according to the anatomical description in Heimer et al. (1995) and comprised the entire caudate–putamen lying within the ventromedially facing concavity of the external capsule, which is inserted between its dorsal, lateral and anterior margins and the neocortex and the corpus callosum. The lateral ventricles, bed nucleus of stria terminalis and globus pallidus comprised the medial borders. The ventral borders consisted rostrally of the ventral STR while the extended amygdala, including the bed nucleus of stria terminalis, sublenticular region and the amygdalostriatal area delimited the dissected tissue caudally. HIP tissue comprised the entire hippocampal formation including the dentate gyrus, the HIP proper and the subicular complex (Amaral and Witter, 1995), extending from the septal nuclei of the basal forebrain rostrodorsally, over and behind the diencephalon, to the incipient temporal lobe caudoventrally. mPFC tissue was dissected according to De Bruin (1994) including the frontal area 2, the dorsal anterior cingulated cortex, the prelimbic cortex and the infralimbic cortex. The agranular insular cortical areas were not included.

The tissues were placed in preweighed Eppendorf tubes on crunched dry ice, weighed and stored at −80 °C until further analysis.

2.3.1. Dopamine, DOPAC and HVA analysis

The tissue was dissolved in 1.0 ml 0.1 N perchloric acid (PCA) containing 5% EDTA and was homogenised. The homogenates were centrifuged at 14,000 × *g* for 30 min, and 200 μl of the supernatant was filtered through a 0.22-μm Millipore filter. The filtrate (20 μl) was collected in High Performance Lipid Chromatography (HPLC) vials, and analysed for DA, DOPAC and HVA contents using HPLC (ESA Coulochem II HPLC equipment) with UV detection at 214 nm. A Catecholamine HR-80 4.6 × 80 mm 3 μm Nucleosil C^{18} column was used to separate metabolites. The mobile phase consisted of 10.25 g NaH_2PO_4, 185 mg EDTA, 100 mg octansulphonic acid, 9% methanol (pH = 3.7). Five hundred milliliters of filtered (0.22 μm) MilliQ water was added. The detection limits was calculated as twice the baseline noise resulting in 0.15 fmol/μl (2 nA) for dopamine and 0.1 pmol/μl (20 nA) for DOPAC and HVA.

The detector sensitivities were 0.9996 (250 mV, 2 nA), 0.9993 (250 mV, 20 nA) and 0.9995 (250 mV, 20 nA) for dopamine, DOPAC and HVA, respectively. For calibration, 1 point calibration was used.

2.3.2. NAA analysis

The tissue was dissolved in 1.0 ml 0.1 N PCA and was homogenised. The homogenates were centrifuged at 20,000 × *g* for 10 min, the supernatants were filtered through a 0.2-μm Millipore filter, and 400 μl of the filtrates were collected in HPLC vials. The HPLC method described by Sager et al. (1995) was used for NAA determination with slight modifications. The mobile phase consisted of 0.1 N phosphate buffer (pH = 4.5), 20 ml 1 N KH_2PO_4 (pH = 4.5), 1.875 g/l KCL and 2000 ml distilled H_2O (Millipore) was added. A Partisil SAX K-45-168 anion exchange column was used to separate metabolites. NAA was detected by UV detection at 214 nm. Detection limit and sensitivity were 2 and 10 μmol, respectively.

2.4. Prepulse inhibition

The animals were housed in pairs belonging to the same experimental group, in a temperature- and humidity-controlled room, with free access to food and water. Animals were acclimatised to the experimental room for 1 h prior to testing. The experiments were conducted in two soundproof and ventilated startle chambers (San Diego Instruments), each containing a Plexiglas tube (diameter 8.2 cm, length 25 cm), and a speaker in the roof. The tubes were mounted on a platform under which a piezoelectric accelerometer was attached. The accelerometer detected and transduced motion of the tubes in response to the movements of the rats. The rats were placed in the startle chambers and allowed to habituate for 5 min. During the habituation period and throughout the experiment, animals were exposed to white background noise (70 dB[A]). The test session started and ended with five startle trials consisting of 120 dB[A] bursts of white noise each lasting 40 ms. These trials were not included in the calculation of PPI. Within a test session, each rat received 35 test trials. These 35 trials included 10 startle trials (120 dB[A], 20 ms) in order to measure basal startle response, 20 trials where the startling stimulus (120 dB[A], 20 ms) was preceded (100 ms) by a prepulse (pp) of varying intensity (2, 4, 8 or 16 dB[A] above background, 20 ms, each presented five times), and five trials with no stimulus present in order to measure basal activity. These 35 trials were presented in a semirandomized order preventing two identical trials in succession. The intertrial interval was variable between 10 and 20 s with a mean interval of 15 s. The entire session lasted approximately 20 min. The movements of the tubes were measured for 100 ms after onset of the startle stimulus (sampling frequency 1 kHz), amplified and fed into a computer where the peak response, the average response as well as the latency to the peak response were calculated. The peak response was used for further data analysis.

The effect of two doses of D-amphetamine (D-amphetamine sulphate, Nordisk Droge and Kemikalie, Denmark) was evaluated with approximately 1 week elapsing between each drug treatment. In order to prevent confounding effects of prior D-amphetamine exposure, half of the animals from each postnatal treatment group received vehicle (NaCl, 1 ml/kg sc) before both drug tests while the other half received D-amphetamine in a dose of 1.0 mg/kg during the first drug test and 3.0 mg/kg during the second drug test. D-amphetamine was administered subcutaneously in a dose volume of 1 ml/kg. Vehicle or D-amphetamine was injected

20 min before the animals were placed in the test chambers. The following parameters were calculated.

Percent PPI, calculated as:

$$100 - \frac{\text{Startle amplitude on prepulse + startle trial}}{\text{Startle amplitude on startle trial}} \times 100$$

Baseline startle amplitude was determined as the mean response of all startle trials. The startle amplitude for each pp intensity were averaged and used for statistical analysis.

2.5. Statistics

Group differences in STR dopamine, HVA and DOPAC levels and mPFC NAA levels were analysed by applying two-way ANOVA with group and hemisphere (left vs. right) as independent factors and dopamine, DOPAC, HVA or NAA as dependent factors. Because only HIP from the left hemisphere was available, a one-way ANOVA with group as independent and NAA levels as dependent factors was applied.

Correlations between mPFC and HIP NAA and STR metabolite concentration were performed by the Pearson product moment correlation.

Group and drug treatment differences in PPI were analysed by applying a three-way ANOVA with dose (vehicle, D-amphetamine 1.0, 3.0) as repeated measures and group (sham and anoxia) and pp intensity (pp 2, 4, 8 or 16) as factors.

Similarly, group and treatment differences in the no-stimulus situation or in basal startle response were analysed by a two-way ANOVA with dose (vehicle, D-amphetamine 1.0, 3.0) as repeated measures and group as factor. Post Hoc Fisher LSD Multiple Comparison Test was used when appropriate.

3. Results

3.1. Body weight

No differences were observed in body weight between sham- and anoxia-exposed animals in any of the cohorts used for the study, neither immediately after anoxia nor at the time of decapitation or test of PPI, respectively (data not shown).

3.2. Tissue concentrations of dopamine, HVA, DOPAC and N-acetylaspartate

Exposure of 9-day-old rat pups to 6 min of anoxia had no effect on the concentration of dopamine in either the right or left STR in adult rats [$F(1,35) = 0.35$, $P = .6$; Table 1]. In contrast, a highly significant group difference existed in the concentration of DOPAC [$F(1,33) = 18.5$, $P < .0001$], and post hoc testing showed that the DOPAC levels were significantly reduced in the right as well as the left STR in the anoxic group as compared to the sham group ($P < .01$ for both; Table 1). Likewise, the concentration of HVA was significantly reduced in animals exposed to anoxia [$F(1,35) = 15.7$, $P < .001$]. However, only HVA levels in the right STR reached significance ($P < .001$) in comparison to the sham group, although a tendency existed for HVA levels in the left STR ($P = .08$; Table 1).

Table 1
Tissue concentration of NAA, HVA, DOPAC and dopamine

Group	NAA (μM), mPFC	NAA (μM), HIP	DA (μg/g tissue), STR	HVA (μg/g tissue), STR	DOPAC (μg/g tissue), STR
	Mean (S.E.M.)				
Sham					
Right	7358.5 (294.1)		35.56 (2.27)	0.32 (0.02)	0.89 (0.06)
Left	7191.8 (149.5)	6827.4 (251.2)	32.57 (0.51)	0.35 (0.02)	0.89 (0.05)
Anoxia					
Right	6311.2 (585.0)		34.33 (0.49)	0.23*** (0.01)	0.68**
Left	7386.7 (323.5)	6810.8 (301.2)	32.14 (1.38)	0.30 (0.02)	0.64** (0.04)

Right: tissue concentration in the right hemisphere; left: tissue concentration in the left hemisphere.
** $P < .01$ as compared to the corresponding sham group.
*** $P < .001$ as compared to the corresponding sham group.

There was no significant difference between groups in the levels of NAA in the left HIP [$F(1,13) = 0.01$, $P = .9$] or in mPFC [$F(1,35) = 1.2$, $P = .3$; Table 1]. However, correlation analysis showed that tissue concentration of HVA in the right STR was significantly negatively correlated with the concentration of NAA in the right mPFC in animals exposed to postnatal anoxia ($r = -.67$, $P < .05$; Fig. 1A). Such a relationship was not observed in the sham group (Fig. 1B). Furthermore, there was no correlation between tissue concentration of HVA in the left STR and concentration of NAA in the left mPFC in either the sham group ($r = -.59$, $P = .09$) or the anoxia group ($r = -.19$, $P = .6$). Likewise, there were no correlations between striatal tissue concentrations of DOPAC and NAA concentrations in mPFC in either the left or the right side of the brain in the anoxic group ($r = .11$, $P = .78$ and $r = -.21$, $P = .56$, respectively) or the sham group ($r = -.17$, $P = .65$ and $r = -.08$, $P = .84$, respectively).

The weight of striatal tissue from the left hemisphere was significantly increased in animals exposed to anoxia as compared to the left hemisphere STR tissue from the sham group [$F(1,36) = 6.2$, $P < .05$; post hoc: $P < .05$; Table 2]. There was no group difference in the weight of either mPFC or HIP tissue [$F(1,36) = 0.2$ and $F(1,36) = 0.7$, respectively].

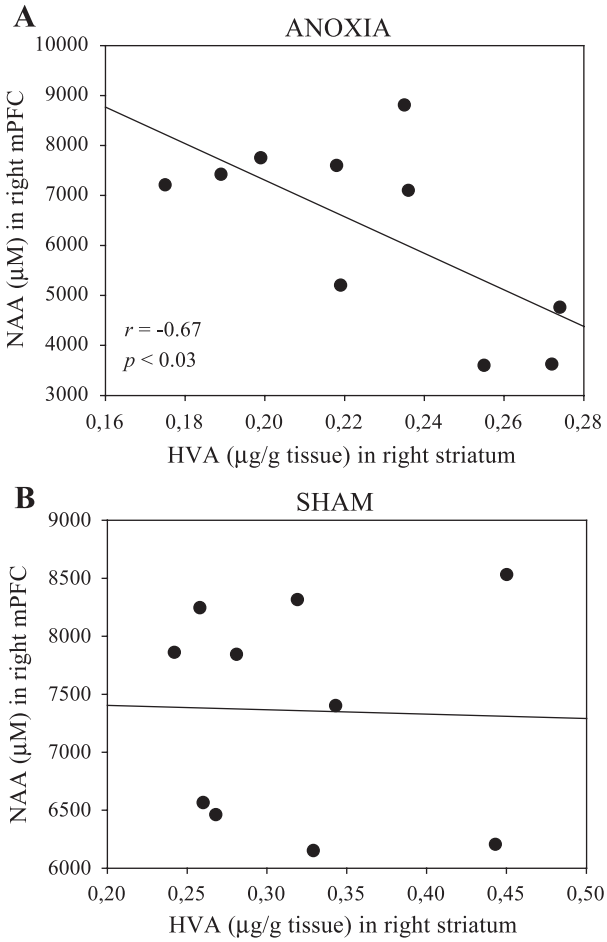

Fig. 1. Effects of postnatal anoxia on the relationship between NAA (µM) in right hemisphere mPFC and HVA (µg/g tissue) in right hemisphere STR. (A) In the anoxic group specifically, a significant negative correlation existed between the concentration of NAA in right hemisphere mPFC and HVA in right hemisphere STR ($r = -.67$, $P < .05$). (B) No such correlation was seen in the sham group. Data are presented as individual data points.

3.3. Prepulse inhibition

A three-way ANOVA revealed significant main effects of group [$F(1,156) = 10.2$, $P < .01$], pp [$F(3,156) = 96.5$, $P < .0001$] and drug treatment [$F(1,156) = 25.0$, $P < .0001$] and significant interaction between drug treatment and group [$F(1,156) = 8.1$, $P < .01$], but no Drug × Group × pp interaction [$F(1,156) = 0.7$]. Post hoc comparisons showed that in the sham as well as the anoxic group, 3.0 mg/kg of D-amphetamine caused a highly significant reduction in PPI as compared to the corresponding vehicle treated groups ($P < .0001$ and $P < .01$, respectively; Fig. 2B). Administration of the low dose of D-amphetamine (1.0 mg/kg) resulted in a tendency for a reduction of PPI in the anoxia group. However, the data just failed to reach significance ($P = .06$;

Table 2
Tissue weight of STR, HIP and mPFC

	STR right (g)	STR left (g)	HIP right (g)	HIP left (g)	PFC right (g)	PFC left (g)
	Mean (S.E.M.)					
Sham	0.023 (0.001)	0.024 (0.001)	0.044 (0.003)	0.043 (0.003)	0.029 (0.002)	0.026 (0.002)
Anoxia	0.025 (0.001)	0.028 (0.001) *	0.044 (0.003)	0.046 (0.003)	0.029 (0.003)	0.030 (0.003)

* $P < .05$ as compared to the sham group.

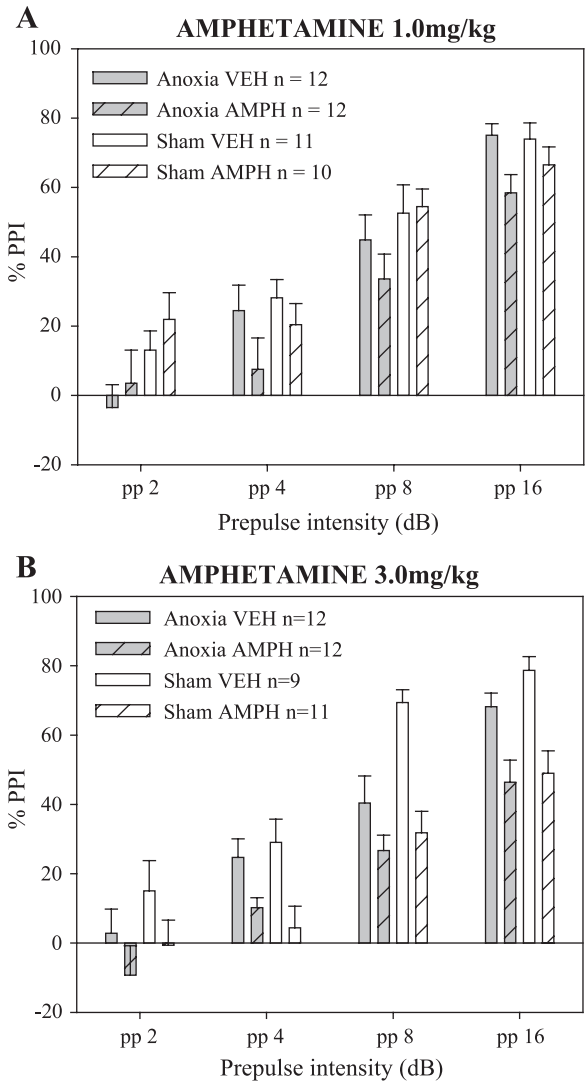

Fig. 2. Effect of postnatal anoxia on prepulse inhibition of the acoustic startle response. (A) There was no effect of 1.0 mg/kg of D-amphetamine on either the sham or the anoxic group as compared to saline treatment, although a trend existed in the anoxic group ($P = .9$ and $P = .06$, respectively). Intergroup comparisons showed that the low dose of D-amphetamine resulted in significantly reduced PPI in animals exposed to anoxia as compared to the effect of D-amphetamine in sham-treated animals ($P < .01$). There was no difference in baseline PPI, after vehicle administration, between the two groups ($P = .3$). (B) Animals exposed to anoxia as pups as well as sham animals displayed significantly reduced PPI after administration of the high dose of D-amphetamine (3.0 mg/kg, $P < .01$ and $P < .0001$, respectively). The effect of D-amphetamine did not depend on the pp intensity. Baseline PPI was, after vehicle administration, significantly reduced in the anoxic group as compared to the sham group ($P < .01$). AMPH 1.0: D-amphetamine 1.0 mg/kg. AMPH 3.0: D-amphetamine 3.0 mg/kg. Data are presented as mean and S.E.M.

Table 3
Amplitude of the response during startle trials and during no-stimulus trials

	Vehicle 1	Treatment 1	Vehicle 2	Treatment 2
	NaCl (1 ml/kg)	AMPH (1.0 mg/kg)	NaCl (1 ml/kg)	AMPH (3.0 mg/kg)
	Mean (S.E.M.)			
Anoxia				
Startle	3065.2 (358.8)	2807.4 (269.1)	2778.3 (412.3)	2295.6 (185.1)
No stimulus	5.0 (1.0)	8.0 (1.3)	6.9 (1.4)	11.3 (2.1)*
Sham				
Startle	3377.6 (388.6)	2724.2 (403.6)	3516.3 (413.1)	2766.1 (542.9)
No stimulus	4.4 (0.8)	6.8 (1.1)	5.6 (1.2)	8.3 (1.1)

AMPH: D-amphetamine (1.0 or 3.0 mg/kg sc, doses volume: 1 ml/kg).
* $P < .05$ as compared to Vehicle Treatment 2.

Fig. 2A). There was no effect of this dose of D-amphetamine in the sham group ($P=.9$).

Between-group comparisons showed that PPI was significantly more reduced in the anoxic group after 1.0 mg/kg D-amphetamine as compared to the same treatment in the sham group ($P < .01$; Fig. 2A). No such group difference existed when the animals were treated with 3.0 mg/kg of D-amphetamine ($P=.9$; Fig. 2B), in that case, the reduction in PPI was equal in both groups. Also, baseline PPI, after vehicle administration, was significantly reduced in the anoxic group during the second drug testing as compared to the sham group ($P < .01$; Fig. 2B). However, this was not the case during the first drug testing, where the degree of PPI was equal in both groups after vehicle administration ($P=.3$; Fig. 2A).

There was no main effect of group or drug treatment in basal startle response [$F(1,39) = 0.7$, $P=.4$ and $F(1,39) = 0.8$, $P=.4$, respectively; Table 3]. However, a significant main effect of drug treatment existed in the no-stimulus condition [$F(1,39) = 11.6$, $P < .01$], revealing that animals exposed to anoxia expressed higher activity levels during the no-stimulus condition when injected with 3.0 mg/kg of D-amphetamine as compared to saline treatment ($P < .05$; Table 3). There was no significant effect of either 1.0 or 3.0 mg/kg of D-amphetamine on the startle response in the sham group during the no-stimulus condition ($P=.6$ and $P=.1$, respectively; Table 3).

Repeated exposure to the PPI paradigm had no effect on baseline PPI, as shown by an equal degree of PPI after vehicle treatment during the first and second drug test sessions in the anoxic group as well as the sham group ($P=.3$ and $P=.6$, respectively; Fig. 2A and B).

4. Discussion

The main finding of this study was that exposing 9-day-old rat pups to 6 min of anoxia resulted in significantly reduced subcortical dopamine metabolism and turnover in adult rats, as measured by striatal DOPAC and HVA concentrations. Concomitantly, in the anoxic group only, a significant negative correlation existed between striatal HVA and NAA levels in the right mPFC. There was no effect of exposure to postnatal anoxia on baseline PPI and the response to D-amphetamine in the PPI paradigm, although a trend for enhanced sensitivity to the low dose of D-amphetamine (1.0 mg/kg) existed in the anoxic group.

The negative correlation between the concentration of NAA in mPFC and the dopamine metabolite, HVA in STR was found only in the anoxic group and only in right hemisphere tissue. No such correlation was evident in the sham group. Furthermore, exposure to postnatal anoxia resulted in highly significant reductions in striatal DOPAC and HVA levels in the adult animals.

It is well established that the prefrontal cortex has a prominent role in the regulation of striatal/nucleus accumbens dopamine function (Kolachana et al., 1995; Pycock et al., 1980). This regulatory role has been shown to be functionally lateralized with respect to regulation of stress response as well as regulation of subcortical dopamine function, and that it is primarily the mesocortical dopamine innervation in the right mPFC which is involved (Sullivan and Gratton, 1999; Sullivan and Szechtman, 1995). Moreover, exposure to anoxia during cesarean section has been shown to cause blunted stress-induced dopamine response in the right mPFC in the adult rat, without any effect in the left mPFC (Brake et al., 2000), supporting the findings of mainly right side effects in this study. Accordingly, postnatal anoxia may affect the functional integrity of the mesocortical dopamine system. However, the significance of the mainly right side effect in this study needs to be further explored.

Furthermore, it must be stressed that DOPAC and HVA levels are measures of dopamine metabolism and turnover and do not address whether the dopamine activity at the receptor is increased or decreased. Moreover, in the present study, there was no significant reduction of NAA in either right or left mPFC (or left hemisphere HIP) in animals exposed to anoxia as pups as compared to sham-treated animals. Reduced concentration of NAA in prefrontal cortex has been reported in a nonpharmacological rat model of schizophrenia (Bertolino et al., 2002). Likewise, reduced concentrations of NAA in dorsolateral prefrontal cortex have been reported in schizophrenic patients in most studies (e.g., Bertolino et al., 1996) but not in all, possibly depending on group size (Bertolino et al., 2000). Nevertheless, the data may indicate that postnatal anoxia do not affect single structures, such as the PFC or the HIP in isolation. Rather, an explanation could be that exposure to postnatal anoxia disturbs the interaction between structures, in particular, cortical–subcortical integration. Distorted prefrontal–subcortical dopamine interactions have been found repeatedly in schizophrenic patients (Bertolino et al., 1999).

Likewise, schizophrenic patients have frequently been reported to display deficient PPI (for a recent extensive

review, see Braff et al., 2001). In rats, deficits in PPI can be induced by psychotomimetic compounds, such as amphetamine and PCP (Mansbach and Geyer, 1989, 1991; Mansbach et al., 1988; for an extensive review, see Geyer et al., 2001) or nonpharmacological interventions, such as lesions of the ventral HIP at PND 7 (Lipska et al., 1995) or maternal separation at PND 9 (Ellenbroek et al., 1998).

In the present study, there was no difference in baseline PPI between saline-treated sham and anoxic rats during the first drug testing session. During the second drug testing session, in contrast, animals exposed to postnatal anoxia displayed significantly reduced PPI when injected with saline as compared to the sham group. However, the sham group showed a nonsignificant increase in PPI during the second test session as compared to the first vehicle session. Consequently, it is more likely that this group difference is due to increased PPI in the sham group rather than representing a real deficit in baseline PPI in the anoxic group. Thus, exposing rat pups to anoxia on PND 9 do not appear to affect baseline PPI.

Robust deficits in PPI were, as expected, seen in both groups after injection with the high dose of D-amphetamine as compared to vehicle injections. In contrast, no deficit was evident after injection with the low dose of D-amphetamine in either of the groups. However, it must be noted that a strong trend existed for reduced PPI after low dose of D-amphetamine as compared to saline injections in the anoxic group. This was further supported when an intergroup comparison was made, comparing the degree of PPI after 1.0 mg/kg of D-amphetamine in the two groups; the degree of PPI after low dose D-amphetamine treatment was significantly reduced in the anoxic group as compared to the effects in the sham group. Thus, exposing rat pups to anoxia on PND 9 do not appear to affect baseline PPI but may seem to render the animals more vulnerable to the deleterious effects of D-amphetamine. Parallel findings have been reported in schizophrenic patients, where administration of psychotomimetic compounds exacerbate or even induce psychotic symptoms in doses, which have no effect in normal subjects (e.g., Davis, 1974).

In conclusion, the present results demonstrate that exposing rat pups to anoxia on PND 9 results in long-lasting changes in subcortical dopamine metabolism tentatively caused by dysfunctional cortical–subcortical interaction. However, further studies need to verify and extend the nature of these findings. The early insult does not seem to affect baseline PPI in the adult rat, but may render the animals more vulnerable to the effect of D-amphetamine, although this was not statistically significant. Accordingly, although oxygen deficiency early in life has been discussed as a vulnerability factor in developing schizophrenia, it does not cause clear-cut phenomenological similarities with the disorder in the rat. However, the model may be interesting in exploring the effects of oxygen deficiency on the interaction between the prefrontal cortex and the subcortical dopamine systems.

Acknowledgements

The authors wish to thank Dr. Naheed Mirza, NeuroSearch, for helpful comments on the manuscript. Furthermore, the authors are grateful to Lis Johnsen and Pia Weikop, Neurosearch and Hanne Søndergaard, Research Institute of Biological Psychiatry, Sct. Hans Hospital, for excellent technical assistance with the biochemical analysis and PPI experiments, respectively. Part of the study was financed by the Danish National Psychiatric Research Foundation to T. Werge.

References

Akbarian S, Bunney WE, Potkin SG, Wigal SB, Hagman JO, Sandman CA, et al. Altered distribution of nicotinamide–adenine dinucleotide phosphate–diaphorase cells in frontal lobe of schizophrenics implies disturbances of cortical development. Arch Gen Psychiatry 1993a;50(3):169–77.

Akbarian S, Vinuela A, Kim JJ, Potkin SG, Bunney WE, Jones EG. Distorted distribution of nicotinamide–adenine dinucleotide phosphate–diaphorase neurons in temporal lobe of schizophrenics implies anomalous cortical development. Arch Gen Psychiatry 1993b;50(3):178–87.

Amaral DG, Witter MP. Hippocampal formation. In: Paxinos G, editor. The rat nervous system Second edition. California: Academic Press; 1995. p. 443–86.

Bertolino A, Nawroz S, Mattay VS, Barnett AS, Duyn JH, Moonen CT, et al. Am J Psychiatry 1996;153(12):1554–63.

Bertolino A, Knable MG, Saunders RC, Callicott JH, Kolachana B, Mattay VS, et al. The relationship between dorsolateral prefrontal *N*-acetylaspartate measures and striatal dopamine activity in schizophrenia. Biol Psychiatry 1999;45:660–7.

Bertolino A, Breier A, Callicott JH, Adler C, Mattay VS, Shapiro M, et al. The relationship between dorsolateral prefrontal neuronal A-acetylaspartate and evoked release of striatal dopamine in schizophrenia. Neuropsychopharmacology 2000;22(2):125–132.

Bertolino A, Roffman JL, Lipska BK, Van Geldern P, Olson A, Weinberger DR. Reduced *N*-acetylaspartate in prefrontal cortex of adult rats with neonatal hippocampal damage. Cereb Cortex 2002;12(9):983–90.

Bjelke B, Andersson K, Ögren SO, Bolme P. Asphyctic lesion: proliferation of tyrosine hydroxylase-immunoreactive nerve cell bodies in the rat substantia nigra and functional changes in dopamine neurotransmission. Brain Res 1991;543:1–9.

Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology 2001;156:234–58.

Brake WG, Noel MB, Boksa P, Gratton A. Influence of perinatal factors on the nucleus acumens dopamine response to repeated stress during adulthood: an electrochemical study in the rat. Neuroscience 1997;77(4):1067–76.

Brake WG, Sullivan RM, Gratton A. Perinatal distress leads to lateralized medial prefrontal cortical dopamine hypofunction in adult rats. J Neurosci 2000;20(14):5538–43.

Cannon TD. On the nature and mechanisms of obstetric influences in schizophrenia: a rewiew and synthesis of epidemiologic studies. Int Rev Psychiatry 1997;9:387–97.

Cantor-Graae E, Ismail B, McNeil TF. Are neurological abnormalities in schizophrenic patients and their siblings the result of perinatal trauma? Acta Psychiatr Scand 2000;101(2):142–7.

Cendes F, Andermann F, Dubeau F, Matthews PM, Arnold DL. Normalization of neuronal metabolic dysfunction after surgery for temporal lobe epilepsy. Evidence from proton MR spectroscopic imaging. Neurology 1997;49:1525–33.

Davis JM. A two factor theory of schizophrenia. J Psychiatr Res 1974;11: 25–9.

De Bruin JPC. Evolution of prefrontal cortex: comparative aspects of its behavioural functions. In: Greenspan RJ, Kyriacou CP, editors. Flexibility and constraints in behavioural systems. Wiley; 1994. p. 185–92.

Dobbing J, Sands J. Comparative aspects of the brain growth spurt. Early Hum Dev 1979;3(1):79–83.

El-Khodor BF, Boksa P. Long-term reciprocal changes in dopamine levels in prefrontal cortex versus nucleus accumbens in rats born by caesarean section compared to vaginal birth. Exp Neurol 1997;145:118–29.

El-Khodor BF, Boksa P. Birth insult increases amphetamine-induced behavioural responses in the adult rat. Neuroscience 1998;87:893–904.

Ellenbroek BA, van den Kroonenberg PT, Cools AR. The effects of an early stressful life event on sensorimotor gating in adult rats. Schizophr Res 1998;30:251–60.

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology 2001;156: 117–54.

Heimer L, Zahm DS, Alheid GF. Basal ganglia. In: Paxinos G, editor. The rat nervous system Second edition. California: Academic Press; 1995. p. 579–628.

Jacob J, Beckmann H. Prenatal developmental disturbances in the limbic allocortex in schizophrenics. J Neural Transm 1986;65:303–26.

Jacob J, Beckmann H. Circumscribed malformation and nerve cell alterations in the entorhinal cortex of schizophrenics. J Neural Transm [Gen. Sect.] 1994;98:83–106.

Kolachana BS, Saunders RC, Weinberger DR. Augmentation of prefrontal cortical monoaminergic acticity inhibits dopamine release in the caudate nucleus: an in vivo neurochemical assessment in the Rhesus monkey. Neuroscience 1995;69(3):859–68.

Lipska BK, Weinberger DR. Delayed effects of neonatal hippocampal damage on haloperidol-induced catalepsy and apomorphine-induced stereotypic behaviors in the rat. Brain Res Dev Brain Res 1993; 75(2):213–22.

Lipska BK, Swerdlow NR, Geyer MA, Jaskiw GE, Braff DL, Weinberger DR. Psychopharmacology 1995;122:35–43.

Mansbach RS, Geyer MA. Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. Neuropsychopharmacology 1989;2(4):299–308.

Mansbach RS, Geyer MA. Parametric determinants in prestimulus modification of acoustic startle: interaction with ketamine. Psychopharmacology 1991;105:162–8.

Mansbach RS, Geyer MA, Braff DL. Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology 1988;94:507–14.

McNeil TF. Perinatal risk factors and schizophrenia: selective review and methodological concerns. Epidemiol Rev 1995;17(1):107–12.

McNeil TF, Cantor-Graae E, Ismail B. Obstetric ocomplications and congenital malformation in schizophrenia. Brain Res Rev 2000;31: 166–78.

Nyakas C, Markel E, Schuurman T, Luiten PGM. Impaired learning and abnormal open-field behaviours of rats after early postnatal anoxia and the beneficial effect of the calcium antagonist nimodipine. Eur J Neurosci 1991;3:168–74.

Pycock CJ, Kerwin RW, Carter CJ. Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. Nature 1980;286: 74–7.

Romijn HJ, Hofman MA, Gramsbergen A. At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby? Early Hum Dev 1991;26:61–7.

Rubin Y, LaPlaca MC, Smith DH, Thibault LE, Lenkinski RE. The effect of *N*-acetyl-aspartate on the intracellular calcium concentration in Ntera2-neurons. Neurosci Lett 1995;198:209–12.

Sager TN, Laursen H, Hansen AJ. Changes in *N*-acetyl-aspartate content during focal and global grain ischemia of the rat. J Cereb Blood Flow Metab 1995;15:639–46.

Sams-Dodd F, Lipska BK, Weinberger DR. Neonatal lesions of the rat ventral hippocampus result in hyperlocomotion and deficits in social behaviour in adulthood. Psychopharmacology 1997;132(3): 303–10.

Speiser A, Korczyn AD, Teplitzky I, Gitter S. Hyperactivity in rats following postnatal anoxia. Behav Brain Res 1983;379–82.

Sullivan RM, Gratton A. Lateralized effects of medial prefrontal cortex lesions on neuroendocrine and autonomic stress responses in rats. J Neurosci 1999;19(7):2834–40.

Sullivan RM, Szechtman H. Asymmetrical influence of mesocortical dopamine depletion on stress ulcer development and subcortical dopamine systems in rats: implications for psychopathology. Neuroscience 1995; 3:757–66.

Tsai G, Coyle JT. *N*-acetylaspartate in neuropsychiatric disorders. Prog Neurobiol 1995;46:531–40.

Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 1987;44:660–9.