Intermittent Hypobaric Hypoxia during Development – Morphological and Functional Changes in the Neocortex

Langmeier M., Marešová D.
Department of Physiology of the First Faculty of Medicine,
Charles University in Prague, Czech Republic

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Mailing Address: Prof. Miloš Langmeier, MD., DSc. Department of Physiology of the First Faculty of Medicine, Charles University, Albertov 5, 128 00 Prague 2, Czech Republic, Phone: +420 224 968 401, Fax: +420 224 918 816, e-mail: milos.langmeier@lf1.cuni.cz

Abstract: Infant rats, together with their mother, were exposed to the simulated altitude of 7,000 m for 8 hours per day since birth to the age of 17 days. Animals were studied the 25th day, 8 days after the last exposure to hypoxia.
The experimental and control animals were sacrificed the 25th day by the transaortic perfusion with 4% buffered neutral formaldehyde under ether anaesthesia. Brains were processed for classical neurohistological analysis (Nissl staining), Fluoro-Jade B and Hoechst. Cortical area in the AP plane 3 mm posterior to bregma was subjected to quantification and "laminar analysis" of the neurones count. The findings were as follows: a) The cytoarchitectonics of the brain in animals exposed to hypoxia was not severely damaged. b) The thickness of neocortex is in the experimental animals lower than that in the controls.
c) The "laminar analysis" of neocortex showed a relative increase of neuronal density in layers I., II., V. and VI. of the cortex. d) The electrical stimulation of sensorimotor cortex 8 days after the end of hypoxia brought about prolongation of evoked cortical after discharges. These results demonstrate that the intermittent hypobaric hypoxia has a profound effect on morphological maturation of the central nervous system in infant rats. Hypoxia influenced the excitation – inhibition mechanisms of cortical neurones.

Introduction

The ontogenetic development of neuronal structures is long-term process, which included the proliferation, migration and differentiation of neurones and glial cells. Particularly the period of differentiation is crucial for the structural and functional formation of neuronal circuits. The above-mentioned processes act in different time of the development nervous system as well as in the development of the individual neurones [1, 2, 3].

The stimulus with high biological efficacy as is the long-term hypoxia can create serious changes in the structure and function of the central nervous system [4, 5, 6]. Histological analyses of the central nervous system just after the end of intermittent long-term hypobaric hypoxia demonstrated fine morphological damage of neurones in the cortex, changes of the arborisation of dendrites and changes in the number of dendrite spines [7]. The influence of the hypoxia on development of the dendrite arborisation was demonstrated similarly in the hippocampus [8, 9, 10, 11]. The retardation of the development of cortical synapses [12, 13, 14] and time-lag of the maturation and myelination of the corpus callosum was established [15, 16, 17].

Many of the structural changes in experimental animals exposed to hypoxia have a correlate with functional alterations of the central nervous system [18]. Changes in the duration of cortical after-discharges after the end of the intermittent hypobaric hypoxia were registered [19, 20].

Our study analyses late morphological and functional changes of the cortex in rats exposed to long-term intermittent hypobaric hypoxia.

To avoid effects of hypoxia on the level of tissue hydration, the process of neocortical maturation was studied eight days after the last exposure to hypoxia.

Materials and methods

Infant rats, together with their mother, were exposed to the hypobaric hypoxia (simulated altitude of 7,000 m for 8 hours per day since birth to the age of 17 days without 6. and 7., 13. and 14 day) in the special barometric chamber (barometric pressure 307.8 mmHg, constant temperature 24 ± 1° C, constant composition of the atmosphere – N_2 78 vol. %, O_2 21 vol. %, CO_2 0.035 vol. %, pO_2 64.4 mmHg). Animals were studied the 25th day, 8 days after the last exposure to hypoxia. As controls were used rats not exposed to hypoxia.

For histological analyses, 6 males from experimental group and 5 male rats from control group were used. The experimental (E) and control (C) animals were killed the 25th day by the transaortic perfusion with 4% buffered neutral formaldehyde under deep thiopental anaesthesia. The brains were removed from the skull, post fixed in the same solution for 24h in a refrigerator. 40 μm thick serial cryostat sections were stained with Fluoro-Jade B and DNA-specific dye bis-benzimide (Hoechst nuclei staining). Paraffin sections 10 μm thick were stained according Nissl. The stained sections were dehydrated, cleared with xylene and mounted in DPX (Fluka). Material was examined under the microscope Olympus Provis AX-70 with epifluorescence.

Cortical area in the AP plane 3 mm posterior to bregma was subjected to quantification and "laminar analysis" of the neuron count (Figure 1). Number of neurons was counted in the 6th adjacent square fields 50 μm wide. The width of the cortex also was measured.

Functional study: experiments were performed on freely moving 25-day-old rats. Test of the excitability were studied by evoked cortical after-discharges – ADs (Figure 2). Repeated stimulation (5times with 1 min interval between the end of ADs and next stimulation) of sensorimotor cortex was used [21, 20].

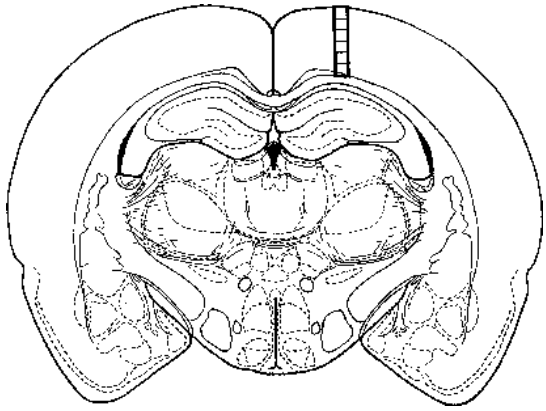

Figure 1 – Scheme of evaluation of the number of neurons in the 6th adjacent square fields 50 μm wide in the cortical area in the AP plane 3 mm posterior to bregma.

ANOVA and t-test were used for evaluation of results. Significance was set on 5% level.

Results

Histological study

a) The cytoarchitectonics of the brain in animals exposed to hypoxia was not severely damaged (Colour figure 6a–f).

b) The thickness of the cortex in the experimental animals was 6.1% lower than that in the controls – in experimental animals 1136 ± 10.08 μm, in controls 1210 ± 14.54 μm (Figure 3).

c) The "laminar analysis" showed a relative increase of neuronal density in layer I (by 40%), layer II (by 31.3%), layer V (by 11.7%) and layer VI (by 11.5%) of the cortex (Figure 4a). The density of neurones in cortical layers III and IV was not changed.

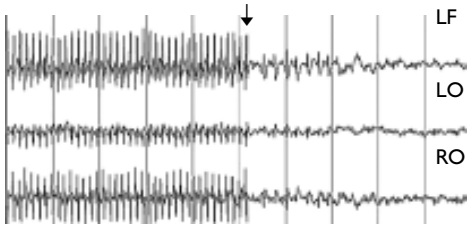

Figure 2 – Electrocorticogram from left frontal (LF), left occipital (LO) and right occipital area (RO) in unipolar connection. Arrow indicates the end of the stimulation.

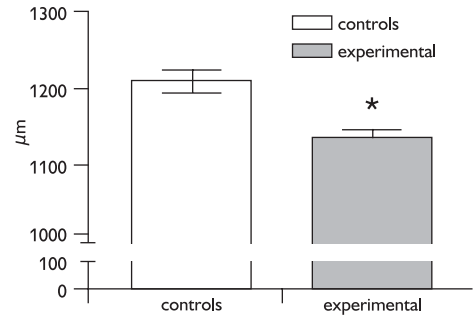

Figure 3 – Thickness of cerebral cortex.
** Significance level of differencs at p ≤ 0.05*

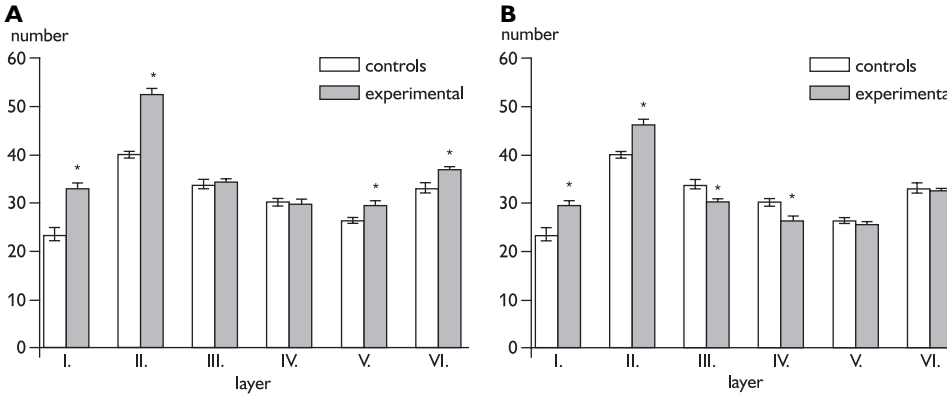

Figure 4a – Number of cortical neurons per field (2500 mm^2); 4b – Number of cortical neurons per field (2500 mm^2) with correction on the reduced neocortical area (0.88).
** Significance level of differences at p ≤ 0.05*

d) However, after "correction" on the reduced neocortical area was the findings somewhat different. The numbers of neurones increased in layer I (by 25.7%) and layer II (by 15.6%), but decreased in the layer III (by 11%) and layer IV (by 13%) of the cortex. No significant differences were found between experimental and control animals in the layers V and VI (Figure 4b, Table 1).

Functional study

Electrical stimulation of sensorimotor cortex confirmed our results from previous study [22]. Repeated stimulation of the cortex in control animals lasting 15 seconds evoked periods of postictal depression – the duration of after-discharges were shorted after the 2nd, 3rd and 4th stimulation. In animals exposed to hypoxia a marked prolongation of evoked cortical after-discharges after the first four stimulations was observed (Table 2), but the shortening between the first three ADs in this group had the same course as was in control rats.

Repeated exposition to intermittent hypoxia retarded the somatic development – animals had significantly lower body weight [22].

Discussion

Repeated exposition of young animals together with their mothers to hypoxia represents a complex stimulus influencing the whole organisms. It brings retardation of the somatic development, changes in circulation and in other systems, including alteration of the brain structure and function [4, 23, 24].

Our results confirmed the serious influence of prolonged intermitted hypoxia during the early postnatal period on the development of the neocortex [7].

Table 1 – Number of cortical neurones after correction in controls (C) and in experimental animals (E) per section area

Cortical layer	Number of neurones per section area after correction C	E
I.	23.5 ± 1.13	29.55 ± 1.04*
II.	40.0 ± 0.77	46.2 ± 1.16*
III.	34.0 ± 0.93	30.3 ± 0.63*
IV.	30.2 ± 0.63	26.3 ± 0.94*
V.	26.4 ± 0.69	25.5 ± 0.69
VI.	33.1 ± 1.03	32.5 ± 0.66

* Significance level of differences at $p \leq 0.05$

Table 2 – The duration (mean ± S.E.M. in s) of cortical after discharges (ADs) in controls (C) and in animals 8 days after exposure to intermittent hypobaric hypoxia (E)

	The duration of cortical afterdischarges (s) C	E
1. AD	6.03 ± 0.64	19.0 ± 6.4**
2. AD	0.41 ± 0.2 ***	3.8 ± 1.9**
3. AD	1.65 ± 0.5***	7.7 ± 2.1**
4. AD	2.7 ± 0.6***	6.7 ± 1.5**
5. AD	4.9 ± 1.4	5.4 ± 1.5
6. AD	5.8 ± 1.1	4.8 ± 1.6*

*** Significance level of differences at $p \leq 0.05$–0.001

The organization of cortical layers was altered. The described reduction of the cortical volume was not induced by a lower hydration as experiments were performed 8 days after the last exposition to hypoxia [25, 26]. We presume that the described changes result from the reorganisation of the neuropil [27].

The increased number of neurones in superficial layers compared to deeper layers confirmed results of Fischer et al. [7], who used similar model of hypoxia.

The different sensitivity of individual cortical layers on the repeated intermittent hypoxia can result from the sequence of the development: Neurones are generated prenatally between the day 16 and 21 and than they are arranged into the laminar pattern [28]. The first migrating cells are pressed from the superficial layers by the newly formed cells [29].

Differences in the sensitivity of cortical layers can also result from the different afferent input. In the second postnatal day cortical layers V and VI have already an established afferent input, which does practically not exist in the more superficial layers [28]. Together with later maturation of neurones in layers I and II it can explain the higher number of neurones in superficial layers after the rather not specific stimulus as represented by hypobaric hypoxia.

Another possible explanation of the different sensitivity of cortical neurons is related to their natural "pruning" during the development. It was described that the process of apoptosis varied in different parts of the brain – the number of neurones can decrease from 15 to 75% [30, 31, 32, 33, 34, 35]. It is possible that changes of internal milieu either induce an activation or inhibition of this mechanism [36, 37, 38].

Hypoxia is probably not the only factor which influenced the brain development in our experiments – at least the possible nutritional or social influences should be considered [4, 39]. The first three postnatal weeks is the period of morphological and functional maturation of ion channels and receptors for neurotransmitters like for the GABA system and the system of excitatory amino acids [40, 41, 42, 43]. The hypoxic stimulus brings formation of oxygen free radicals during the phase of reperfusion and induces metabolic changes that interact with the processes of organization of mediators and modulators, with changes of the blood-brain barrier permeability and thus interfere with the genetically programmed brain development [44]. Resulting morphological changes bring about the functional alteration – the shift in the balance between excitation and inhibition towards the processes of excitation [4, 19, 22].

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