CHARLES UNIVERSITY IN PRAGUE
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DEPARTMENT OF ANATOMY AND BIOMECHANICS

Changes in motor function and seizure susceptibility after photothrombic ischemic stroke in immature rat

Doctoral dissertation Summary

Supervisor:
Assoc. Prof. MUDr. Jakub Otáhal, Ph.D.

Author:
Tufikameni Brima, Bc. MSc.

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AUTOREFERÁT

Změny motorických funkcí a citlivosti k vyvolání epileptických záchvatů po fototrombní mozkové ischemii u nezralého potkana

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Školitel:

Doc. MUDr. Jakub Otáhal, Ph.D.

Vypracovala:

Mgr. Tufikameni Brima

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SUMMARY

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Mgr. Tufikameni Brima

Perinatal stroke is a common cerebrovascular disorder that affects 1 in every 4000 births and is associated with epilepsy, motor and cognitive deficits. Determing the effect of perinatal induced stroke on motor function and seizure susceptibility is paramount. To this end, we made use of a photothrombotic model of stroke in rat at postnatal day 7. Ischemic lesions of different extends were induced, to assess the consequences of stroke on motor function, locomotion and its correlation to morphological changes after stroke. Paradigms sensitive to sensorimotor changes were used and histological changes were analyised. In addition the use of pure cortical lesions assisted in the analysis of seizure susceptibility in PTZ elicited models of epileptic seizures. For seizure occurrence, latency and severity, two different concentrations of PTZ (60 and 100 mg/kg) were administered subcutaneously in two different age groups at P 12 and P 25. Episodes of rhythmic EEG activity were also registered at P 25 following successive 20- and 40-mg/kg doses of PTZ administered interperitonealy.

A clear relationship between motor impairments and lesion extend was observed; indicating that brain injuries greatly affect motor function in rats. Cortical ischemic lesion during early development also had an impact on the sensitivity PTZ; decreasing thresholds and increasing susceptibility to PTZ-induced seizures, just 5 and 18 days post photothrombotic insults.

Změny motorických funkcí a citlivosti k vyvolání epileptických záchvatů po fototrombní mozkové ischemii u nezralého potkana

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Perinatální mozková ischemie je časté crebrovaskulární onemocnění, které se vyskytuje při každém 4000 porodu a u novorozence se typicky projevuje motorickým a kognitivním deficitem a dlouhodobými komorbiditami včetně epilepsie. Cílem práce bylo zjistit efekt perinatálně indukované mozkové ischemie na motorické funkce a citlivost k vyvolání epileptických záchvatů u potkana v průběhu života.

Fototrombní mozková ischemie byla vyvolána u nezralých potkanů sedmý den po narození. Indukovali jsme ischemické léze různých rozsahů, abychom mohli posoudit vliv ischemie na motorické funkce, pohyb a korelovat je s morfologickými změnami po ischemii. Byly použity motorické behaviorální testy citlivé na senzomotorické poruchy. V druhém experimentu jsme vyvolali čistě korovou ischemickou lézi a hodnotili citlivost k vyvolání epileptických záchvatů pomocí PTZ. Pro zhodnocení výskytu, latence a charakteru záchvatů dvě různé dávky PTZ (60 a 100mg/kg) byly aplikovány subkutáně ve dvou věkových skupinách P12 a P25. Rytmická EEG aktivita byla hodnocena na EEG záznamu u 25denních potkanů při dvou additivních intraperitoneálních dávkách PTZ (20 a 40 mg/kg po 20min.)

V naší studii jsme popsali jasný vztah mezi rozsahem léze a výsledným motorickým deficitem v dospělosti. Korová léze vyvolaná během časného vývoje jedince měla dále signifikantní vliv na citlivost k PTZ, snížila práh a zvýšila citlivost k vyvolání záchvatů pomocí PTZ pátý a osmnáctý den po ischemickém insult.

INTRODUCTION

Perinatal ischemic stroke is the leading cause of cerebrovascular disorder in infants occurring around the time of birth with pathological evidence of focal arterial infarction (de Vries et al. 1997). Up to 27% of infants with these brain injuries later in life develop, numerous consequent pathologies including behavioral and functional deficits, speech delays, epilepsy, hemi-paresis and hemi-sensory impairments (Westmacott et al. 2009; Vinay et al. 2005). Clinically, little is known concerning long-term behavioral outcome after neonatal stroke (Westmacott et al. 2009), this fact holds true also for experimental studies. Existing work of this nature documented a correlation between the degree of brain injury in neonatal rats and functional deficits such as asymmetries of limb placing, foot-faults and abnormality in the postural reflex tests (Bona et al. 1997). Although neonatal stroke is increasingly being studied, most of the foundation of our understanding on functional and behavioral end points comes from research done in adult animal models.

Bengal Rose model of photothrombotic stroke used in this study, is based on thrombus-producing photochemical reaction, producing ischemic infarcts of specified location and size (Karhunen et al. 2007; Brant D et al. 2009). Presently, two ischemic lesions of different extends were induced, at postnatal day (P) 7. Four simple behavioral tests (bar holding, rotarod, inclined grid, and ladder rung walking) were used to assess the consequences of stroke on motor function responses in adulthood. Moreover, locomotor activity expressed as distance moved in the open field (OF) was monitored.

Epilepsy is a neurological condition characterized by spontaneous recurrent epileptic seizures. These seizures often appear as a consequence of numerous pathologies including stroke, resulting in brain damage and neuronal hyperexcitability (Sahin et al. 2003). Initial

brain damage that leads to epilepsy is frequent in the early stages of brain development and initial seizure induction is highest in the first month of life (Hauser et al. 1993).

We applied photothrombosis in immature rats at postnatal day 7, to analyze seizure susceptibility in Pentylentetrazol (PTZ) elicited models of epileptic seizures as a consequence of stroke, five and eighteen days after induction of stoke. Three models of epileptic seizures facilitated by a systemic administration of PTZ, namely, minimal clonic seizures (mS), generalized tonic-clonic seizures (GTCS) (Browning & Nelson 1985) and nonconvulsive seizures - rhythmic EEG spike-and-wave activity (Snead, III 1992) were assessed as described by (Tchekalarova et al., 2010; Velisek et al. 1992). Rat pups were studied at two different ages - 12 and 25 days corresponding to human early postnatal stages and school going periods respectively (Dobbing 1970).

AIMS OF RESEARCH

The purpose of the present dissertation is to investigate the effect of photothromosis on motor and cognitive functions of adult rats after focal cerebral ischemia induced in the early postnatal periods in rats. In addition, seizure susceptibility in a PTZ elicited model of epileptic seizures, as a consequence of stroke was also analysed. The specific aims were:

1. Assess the effect of stroke using simple motor and cognitive functional responses, such as gross motor coordination in adulthood.
2. To investigate the relationship between morphological and behavioural change after stroke.
3. To analyze changes of post stroke susceptibility to PTZ–induced seizures in immature rats as early as five and eighteen days after photothrombosis.

EXPERIMENTS

EXPERIMENT 1

The effect of early postnatal stroke on motor performance in adult rats and it correlation to the accompanying morphological changes

Materials And Methods

Animals

Experiments were performed in male albino Wistar rats (n = 139), provided by the Institute of Physiology of the Academy of Sciences of Czech Republic. At postnatal day (P) 7 the animals were brought to the experimental room and divided into four groups: animals (n=30) exposed to laser light for 5 min. (BR_5 min); animals (n=38) exposed to laser light for 30 sec. (BR_30 s); sham-operated controls (Sham) treated with saline (n=31) and intact control group (C, n=40). Behavioral tests were performed at P 67. A group of seventy-eight rats (BR_5 min = 20, BR-30 s = 19, Sham = 19 and C = 20) were submitted to rotarod, bar holding and inclined grid tests. A second group of sixty-one rats (BR_5 min = 10, BR_30 s = 19, Sham = 12 and C = 20), were submitted to ladder rung walking tests and open field (OF). A subset of twenty-one animals, n = 7 from each experimental group, were used to confirm the ischemic injury. Rats were housed in standard plastic cages in a temperature-controlled environment of 22±1˚C, humidity 50-60% with a 12-h light/dark cycle (lights on at 6 a.m.) with free access to food and water. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Results

Our data depicted two kinds of lesions with different shapes and sizes relative to laser illumination. Exposure to laser light for 30 sec resulted in small lesion restricted to the sensorimotor cortex leaving the subcortical structures intact. While exposure for 5 min resulted in large lesions that involved all cortical layers including the underlying white matter and striatum of affected hemisphere. The performance of rats submitted to stroke was porr as compaired to controls. In the bar holding test, one of the most demanding tests of motor coordination, impairments of motor performance were more expressed. Specifically, the animals with larger lesions were only able to grasp the bar for a very short time. They also walked a shorter distance in the open field tests, suggesting a marked inhibition in locomotion. Surprisingly, in our animals, no signs of ataxia or loss in balance where observed. A clear relationship between sensorimotor impairments and lesion extend as confirmed.

EXPERIMENT 2

Increased susceptibility to pentetrazol-induced seizures in developing rats after cortical photothrombotic ischemic stroke at p7

Materials and methods

Animals

A total of eighty-nine rats were used in this study. The photothrombotic stroke was induced in immature male albino Wistar rats at P7 (the day of birth was defined as day 0). Sham-operated animals of the same age served as controls. Two experiments were performed: experiment 1 for the induction of motor seizures by PTZ in a set of rat at P 12 and P 25 while in experiment 2, rats underwent video EEG monitoring at P25. The animals (n=65) in experiment 1, were divided into two basic groups–rats with photothrombotic lesion (n=33) and surgical controls (n=32). These groups were sub divided; half of these were administered with the 60 mg/kg dose of PTZ and the other half was injected with a dose of 100 mg/kg both at P 12 and P 25 i.e. 5 or 18 days after ischemic insult respectively. In experiment 2, rats (n=24) were divided into two groups - surgical controls (n=15) and rats with photothrombotic lesion (n=9), all of them were injected with two successive doses (20 mg/kg and 40 mg/kg) of PTZ to elicit EEG spike-and-wave rhythm.

All rats were housed in standard plastic cages, nests of ten pups per mother, in a temperature-controlled environment of 22±1˚C, humidity 50-60% with a 12-h light/dark cycle (lights on at 6 am) with free access to food and water.

Results

Cortical photothrombotic lesions induced in immature rats in this study, affected seizures elicited by pentetrazol, later during postnatal development. Major changes were found in a model of human absences induced by a low dose of PTZ and an easy transition from EEG spike-and-wave rhythm into minimal clonic seizures; while convulsive seizures remained almost unaffected. These changes were observed in 25-day-old rats, i.e. 18 days after lesion induction, indicating permanent or at least long lasting changes in selective seizure susceptibility.

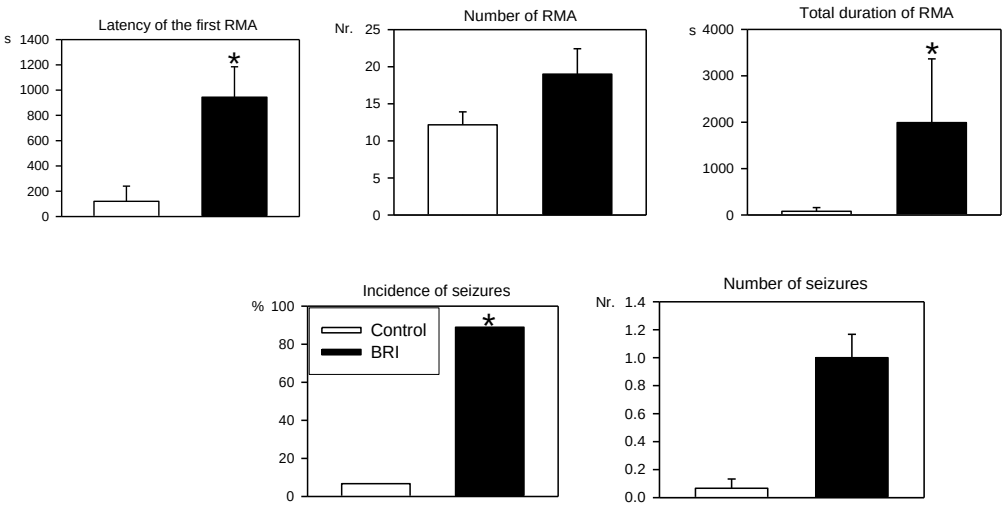

Figure 12: Graphs from left to right: latency of the first rhythmic activity (RMA); number of RMA; total duration of RMA; incidence of minimal clonic seizures; average number of seizures. Asterisks denote significant difference between controls (white columns) and phototrombotic animals (black columns).

CONCLUSION

Results from the studies that make up the back bone of the present thesis, demonstrated that photothrombotic cerebral ischemic stroke induced in the early postnatal period and tested in adult rats, indeed influenced functional task performance governed by the affected cortical area. A further analysis of one of the major consequences of stroke, epilepsy, revealed that these cortical lesions had an effect on the sensitivity to PTZ-induced seizures, shortly after stroke. Despite the fact that all animals subjected to stroke performed poorly as compared to the controls, they were all able to fulfil the given tasks. No signs of ataxia or loss in balance where observed. The only exception was that at least half the animals were not able to cross the ladder with irregular pattern arrangement within 60 sec. This might be brought about by the brain's ability to compensate for the loss functions; possibly due to plasticity of the immature brain.

Lourenco et al. (2010) states that motor performance variables has a potential to better identify motor control deficits than clinical outcome measures alone. Future considerations may therefore be aim to explore motor patterns used for task performance post stroke. Kinematic variables were shown to provide detailed measures of movement quality, such as reaching movements - joint ranges of motion, grasping and supination, postural adjustments – change of body angle or shoulder and trunk movement. It will be of great interest to extend this study to analyze kinematic changes and task performance such as lime placing, foot faults and postural motor function. Task performance analysis of animals submitted to stroke during adulthood compared to those submitted to stroke during perinatal periods might shed light to our current results.

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