Prenatal exposure to maternal voluntary exercise during pregnancy provides protection against mild chronic postnatal hypoxia in rat offspring

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Abstract: Postnatal hypoxia is a main cause of neuronal damage in newborn. However, our understanding of the possible preventive or therapeutic methods to reduce the harmful effects of hypoxia is still primary. Pregnant rats were provided with running wheels during their pregnancy. On PND4 (postnatal day 4)to PND8, the rat pups were exposed to postnatal chronic hypoxia (11% O_2, 89% N_2) in an air-tight plastic chamber for a period of six hours per day. The number of neurons and also angiogenesis in hippocampus were studied. Postnatal exposure to mild hypoxia decreased the number of the neurons in all studied regions of the hippocampus CA1, CA3(cornu ammonis), DG(dentate gyrus) and SUB(cubiculum) in rat pups. In other words the number of the neurons in rat pups born from voluntary exercise group was not significantly less than control group in CA1, CA3 and DG regions. So maternal Voluntary exercise during pregnancy increases the blood vessel density in the DG region of the hippocampus of the rat pups. In this study for the first time we provide evidences that show the protective effect of maternal voluntary exercise during pregnancy on rat offspring against postnatal hypoxia. We revealed that maternal exercise during pregnancy increases the hippocampal neuron number and angiogenesis in offspring.

Keywords: Physical activity, hippocampus, hypoxia, progeny.

INTRODUCTION

Transient reduction brain hypoxia frequently occurs during the neonatal period and may lead to neurological dysfunctions (Maneru 2001). In the early postnatal period, the developing rat brain is highly vulnerable to hypoxic damage (Ikonomidou 1989). Although several reports using different animal models have studied the results of hypoxia during early days of life (Schmitt 2007). Further studies are still needed to investigate the possible methods of preventing or diminishing these consequences. On the other hand, the effects of exercise and physical activity on cognitive function have been studied by several investigators (Carro 2001; Ding 2006 and Fabel 2003). Recently, it has been shown that prenatal exposure to maternal exercise during the time of pregnancy improved the neurogenesis, learning and memory in rat pups (Akhavan 2008, Kim 2007, Lee 2006 and Parnpiansil 2003). However, our knowledge regarding the mechanisms and the effects of prenatal exposure to maternal voluntary exercise on rat pups is still primary and more investigations are needed to clarify this phenomenon. Considering the findings given above, this study aimed to shed light on the potential protective effects of V.exercise in pregnancy on offspring and possible mechanisms through which maternal V. exercise benefits the cognitive function in offspring.

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MATERIALS AND METHODS

Animals

All animals were obtained from the breeding colony of pastor Institute, Tehran, Iran. All experimental procedures were carried out according to the guidelines of the national institute of health guide (1989 UNESCO animal rights) for the care and use of laboratory animals. Also, in each experiment; care was taken to use the minimum number of the animals and to minimize their suffering (according to the health guide). Male Wistar rats (210±10 g) were allowed to mate with female virgin Wistar rats (210±10 g) during a 24 hours period. Female rats were checked for the presence of a vaginal plug twice at midnight and at 5 a.m. the next day. Once the vaginal plug was observed the animal was considered as pregnant. The pregnant rats were randomly assigned to sedentary control (S. control) or voluntary (V. exercise groups)

(N=16 in each group) and were housed individually in cages with a 12 hour light/dark cycle at 22-24°C temperature, with food and water *ad libitum*.

Exercise paradigm

A detailed description of exercise model was reported (Akhavan, 2008). Briefly, in the group of V.exercise rats were given access to a running wheel (diameter=34.5cm, width=9.5cm) which was freely rotated against a resistance of 100g, until the end of their pregnancy. Each wheel was attached to a counter that shows its rotation. The rolling were recorded daily at 6 a.m. From the day 21 after mating, female rats were checked, at 9 a.m. and 6 p.m. The day that the rat pups were observed was as postnatal day 0 (PND0). After the delivery; each mother and its pups were transferred to a cage. In sedentary group; the pregnant rats were placed individually in similar cages without a running wheel. At PND0, seven rat pups from each group (one rat pup from each dam) were obtained .The brains were removed and used for angiogenesis measurement. The rest of the rat pups remained until PND29 when they were weaned.

Induction of hypoxia

On PND4, to PND8, 14 dams from V.exercise group were randomly selected to V.exercise-normoxia (V.Exer-Control) and V.exercise-hypoxia (V.Exer-Hypoxia) (N=7 in each group). Also 14 dams were randomly selected to S.control-normoxia (Sed-Control) and S.control-hypoxia (Sed-Hypoxia) groups (N=7 in each group). Both the rat pups and their mothers in the groups of V. Exer-Hypoxia and Sed-Hypoxia during the days (PND4-8), were exposed to postnatal chronic hypoxia (PCH) (11% O_2, 89% N_2) in an air-tight plastic chamber for a period of six hours per day (during the hypoxia periods; mothers were able to care for their pups) (Schmitt 2007). The control groups (V. Exer-Control and Sed-Control groups) were in a similar chamber without exposure to hypoxia. During hypoxia ; the concentration of O_2 was measured with an oxygen meter (model HI4421 sigma) which was placed inside the chamber.

Brain fixation and neuron counting

On PND36, all groups were consisting of 7 rat pups in each group from V.Exer-Hypoxia, V. Exer-Control, Sed-Hypoxia and Sed-Control (1 rat pup from each mother) then sacrificed and their brains were removed for cell count. We used PND36 as the measuring time point in this study to be similar with previous reports as to the effect of maternal V. exercise on offspring (Akhavan 2008 and Bick-Sander 2006).

As described earlier, rat pups from V.exercise and S.control groups (PND0) (N=7 for each group) and also rat pups from V.Exer-Hypoxia, V.Exer-Control, Sed-Hypoxia and Sed-Control groups (N=7 for each group) (PND36) were sacrificed and the brains were removed. The brains were placed in the (paraformaldehide 4% in 0.1 M phosphate buffer, pH 7.4 by immersion) for one week and then embedded in paraffin wax. Left hippocampus was sectioned coronally at 5-µm thick and stained with cresyl fast violet (Nissl) (Vanoye-Carlo, 2008 and Yan, 2007) by an experimenter blinded to the study codes for each sample. Ten sections were used for each animal. For cresyl fast violet staining, the sections were mounted on gelatin-coated slides. The mounted sections were re-hydrated in distilled water and submerged in 0.2% cresyl violet solution for about 5 min .The sections were rinsed in distilled water and dehydrated in graded series of ethanol and then immersed in xylene, mounted in DPX and cover slipped. Neurons in the dentate gyrus (granule cell layer), CA1, CA3 and subiculum (pyramidal cell layer) were counted using a light microscope with a 20× objective (from the rostral of the hippocampus, at bregma −3.30 mm, to the caudal end, at bregma −5.80 mm) with the counting frame of 100 x 100 µm, and the grid overlay of 200 x 200 µm. The optical fractionators' method (Stereo Investigator; MicroBrightField, Williston, VT) was used to estimate the neuron number and the average number of the neurons per square millimeter for each area was then derived for each animal.

Measurement of the angiogenesis

For measurement of angiogenesis, left hippocampus was sectioned coronally at 5-µm thick and stained with hematoxilin & eosin (H&E) as described by (Akhavan, 2008). Using a light microscope with a 10× objective with an eyepiece ; the number of blood vessels in 10 selected areas of CA1-CA2, CA3 or DG of the hippocampus was counted and the average number of countings was calculated for each slide. 5 slides from each animal were counted and the result was expressed as a number of blood vessels per square millimeter for each area of the hippocampus. All counts were conducted by an experimenter blind to treatment condition.

STATISTICAL ANALYSIS

Values represent the mean ± standard error of the mean (S.E.M.). A two-way ANOVA (analysis of variance) followed by a Tukey's test was conducted for data between multiple groups. Statistical differences were considered significant when $P<0.05$.

RESULTS

As it is presented in fig. 1 prenatal exposure to maternal V.exercise during pregnancy increases the neuron number in CA1, CA3 and DG but not in SUB of the rat pups on PND36. Statistical analysis indicated significant effects of regions ($F_{3, 96}=428.56$, $P<0.0001$), of groups ($F_{3, 96}=258.56$, $P<0.0001$) and a significant interaction between regions and groups ($F_{9, 96}=13.26$, $P<0.0001$).

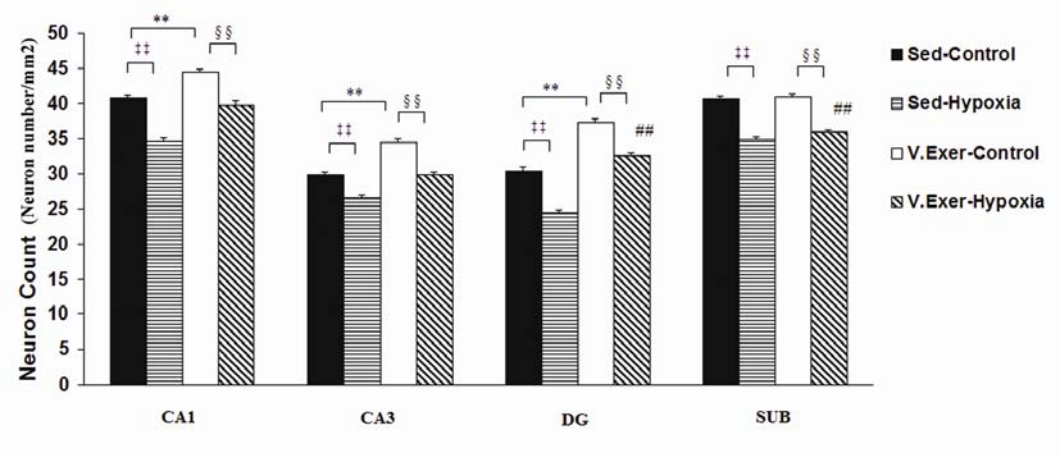

Fig. 1: Effect of maternal Voluntary (V. exercise) during pregnancy on neuron number in different regions of hippocampus CA1(cornu ammonis1), CA3 (cornu ammonis3) and DG (dentate gyrus) of the rat pups.
Data are expressed as the mean ± S.E.M. (N=7) * *P<0.05* and ** *P<0.01*). *
§ Represents the significant difference between (V.exer-hypoxia) and (V.exer-control) group.
‡ Represents the significant difference between sedentary (Sed. control) and (Sed. hypoxia) group.
# Represents the significant difference between (V.exer-hypoxia) and (Sed. control group).

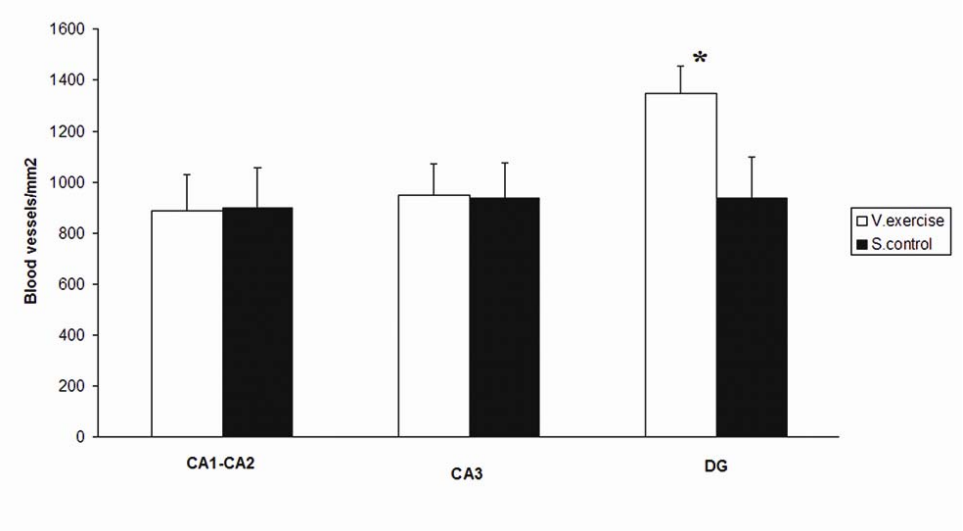

Fig. 2A: Effect of maternal V.exercise during pregnancy on blood vessel density in different regions of hippocampus CA1 (cornu ammonis1), CA3(cornu ammonis3) and DG(dentate gyrus) of the rat pups.
Data are expressed as the mean ± S.E.M. (N=8) * *P<0.05*).
*Represents the significant difference between voluntary (V. exercise) and sedentary (S. control) group.

Tukey's test indicated that there are significant differences in neuron counts in CA1 (pyramidal cell layer) (P<0.01), CA3 (P<0.01) and DG (granular cell layer) (P<0.01) between sedentary control and V.exercise groups. However, there was no any significant difference between the neuron count in SUB (subiculum) region between the groups. Postnatal exposure to mild hypoxia decreased the number of the neurons in some regions of the hippocampus such as (CA1, CA3, DG and SUB) in rat pups from S.control or V.exercise group (P<0.01 in all cases). On the other hand the, neuron count in V.exercise-hypoxia was not significantly less than S.control group in CA1, CA3 and DG regions.

On the other hand, as it is shown in fig. 2, prenatal exposure to maternal V.exercise during pregnancy increases the blood vessel density only in the DG region of the hippocampus and not in CA1-CA2 or CA3 regions of the rat pups on PND0. A two-way ANOVA indicated no-significant effect of regions ($F_{2, 42}$=1.802, P=0.178), of groups ($F_{1, 42}$=1.46, P=0.234) and of the interaction between regions and groups ($F_{2, 42}$=1.464, P=0.243). Tukey s test indicated that there are significant differences in blood vessels count in DG (granular cell layer) (P<0.05) between sedentary control and V.exercise groups. However, there was no any significant difference in the number of the blood vessels between sedentary control and V.exercise groups in any other region.

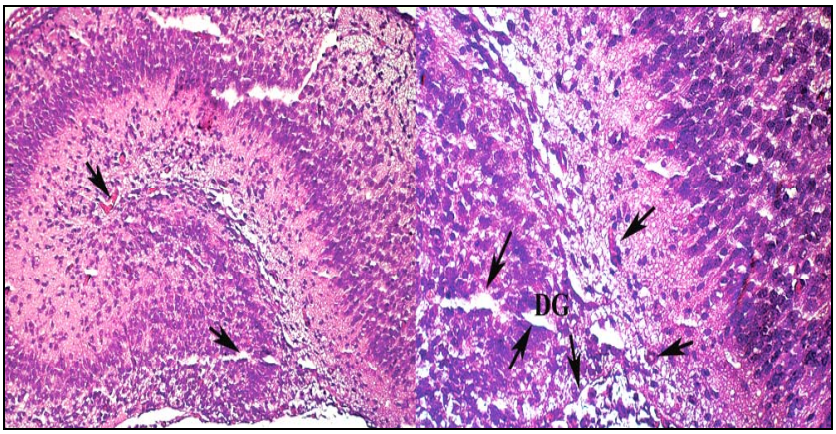

Fig. 2B: H&E-stained brain coronal plane (PND0), representative samples used to count the blood vessel density in voluntary (V. exercise) and sedentary (S. control groups). The arrows point to endothelial cells (left ×100 magnification and right ×200 magnification).

DISCUSSION

The main finding of this study is that maternal voluntary exercise during pregnancy has a protective effect on rat offspring against postnatal hypoxia. This protective effect is probably mediated via increasing the number of hippocampal neurons and blood vessels. The reverse association between voluntary exercise and neuronal damage caused by ischemia has been reported previously (Ding, 2004). However, to the best of our knowledge, this is the first report indicating that prenatal exposure to V.exercise during pregnancy causes protection of brain in rat pups against the PCH.

It is now well accepted that severe oxygen deprivation can induce brain cell death (Banasiak 2000). Previous studies have showed that transient cerebral hypoxia/ischemia triggers a series of pathophysiological changes that causes neuronal death in some brain regions including the hippocampus (Takagi 2000). The results of our study is in accordance with these suggestions as mild postnatal exposure to hypoxia decreased the number of the neurons in all examined areas of the hippocampus in sedentary-hypoxia group. Meanwhile, the number of the neurons in CA1, CA3 and DG of the hippocampus in the rat pups from V.exercise-hypoxia was at similar to sedentary-control group. This result shows a preventive effect for maternal V.exercise during pregnancy against the harmful effect of PCH on the rat pups hippocampal neurons.

Although the mechanism(s) of this protection is (are) not clear yet and probably include several factors, however, one explanation may simply be the enhancing effect of maternal exercise on the number of the neurons in pup's hippocampal areas rather than a real protective effect for the neurons. In contrast, there are evidences which lead to the hypothesis that V.exercise during pregnancy may exert a protecting effect on pup's hippocampal neurons through neurotrophic factors such as brain-derived neurotrophic factor (BDNF). It has been reported that maternal physical activity during pregnancy has an enhancing effect on hippocampal BDNF in the rat pups (Kim, 2007). BDNF has been shown to have a neuroprotective effect against ischemia (Sun 2008) and to decrease infarct size; whether infused into the ventricles beginning 1 day prior to ischemia (Schabitz 1997), or directly into the brain tissue immediately after permanent or transient focal ischemia (Yamashita 1997). More studies are needed to clarify the possible role of BDNF as another mechanism for the observed preventing effect of maternal V.exercise against PCH.

On the other hand a protecting effect could be the increased angiogenesis induced by maternal V.exercise during pregnancy. The enhancing effect of exercise on angiogenesis is not a new finding (Swan 2003). However, our results suggest that exposure to maternal exercise during pregnancy too, increases the blood vessel density in the DG of the rat pups hippocampus. It has been suggested that in an exercising animal, metabolic demand increased and this may lead to changes in the cortical and striatal vasculature density (Ding 2004). Increase in energy demand may require alterations by which sufficient oxygen and glucose can be delivered to active neurons and metabolic demands of functional activity may serve as a stimulant to angiogenesis (Ding 2004). However, the mechanism underling the region-specific effect of maternal exercise on angiogenesis is not clear and needs more investigation. Our finding is fully in accordance with another study which revealed that performing V.exercise on mice for 3-10 days increases the micro vessels density in DG but not in CA1 region of the hippocampus (Schmitt 2007). Also, an exercise-induced blood volume of DG (but not CA1/CA3 region) of the hippocampus in mice has been suggested (Pereira

2007). DG has been suggested as a region in the hippocampus in which production of new neurons continues in the adulthood (Abrous 2005). Since exercise stimulates neurogenesis in the DG (Wu 2008), this was accompanied by increase in energy demands which in turn stimulate angiogenesis in the DG. Effective great vascular surface for gas exchange is an important factor for the brain when oxygen supply is reduced as a result of ischemia/hypoxia. This may provide the neurons with better oxygen supply and/or bringing more neuro-protective agents as a result of reduced diffusion distance (Issacs 1992) which may play an important role as a protection against hypoxia.

Our current results indicate that maternal V.exercise during pregnancy may have a protective effect against postnatal exposure to PCH in rat offspring. Increasing the number of the neurons in the hippocampus of the rat offspring may play an important role in this protection. Also maternal V.exercise in pregnancy increased the angiogenesis in DG region of the hippocampus in the rat pups, which in turn can increase the blood supply to the cells as a part of another mechanism for neuroprotective effect of maternal exercise in pregnancy.

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