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Maternal physical activity-induced adaptive transcriptional response in brain and placenta of mothers and rat offspring

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Abstract

Maternal physical activity induces brain functional changes and neuroplasticity, leading to an improvement of cognitive functions, such as learning and memory in the offspring. This study investigated the effects of voluntary maternal physical activity on the gene expression of the neurotrophic factors (NTFs): BDNF, NTF4, NTRK2, IGF-1 and IGF-1r in the different areas of mother's brain, placenta and foetus brain of rats. Female Wistar rats (n = 15) were individually housed in voluntary physical activity cages, containing a running wheel, for 4 weeks (period of adaptation) before gestation. Rats were classified as inactive (I, n = 6); active (A, n = 4) and very active (VA, n = 5) according to daily distance spontaneously travelled. During gestation, the dams continued to have access to the running wheel. At the 20th day of gestation, gene expression of NTFs was analysed in different areas of mother's brain (cerebellum, hypothalamus, hippocampus and cortex), placenta and the offspring's brain. NTFs gene expression was evaluated using quantitative PCR. Very active mothers showed upregulation of IGF-1 mRNA in the cerebellum (36.8%) and NTF4 mRNA expression in the placenta (24.3%). In the cortex, there was a tendency of up-regulation of NTRK2 mRNA ($p = 0.06$) in the A and VA groups when compared to I group. There were no noticeable changes in the gene expression of NTFs in the offspring's brain. Our findings suggest the existence of a developmental plasticity induced by maternal physical activity in specific areas of the brain and placenta representing the first investment for offspring during development.

Introduction

Human and animal studies have shown that environmental stimuli, such as maternal physical activity, influence the brain development and function of both mother and offspring.[1–3] In humans, infants from active mothers during pregnancy (three times per week, at least 20 min/day at 55% of their maximal aerobic capacity) showed a better response to sound discrimination and auditory memory as measured by electroencephalography.[2] In rats, young pups born from dams which were active throughout pregnancy showed an increased amount of neuronal and non-neuronal cells in the hippocampus, improved cognitive functions (habituation behaviour and spatial learning) and enhanced memory as tested using a novel object recognition paradigm.[1,4] The underlying mechanism of this neuroplasticity can be related to adaptive changes in the expression of neurotrophic and growth factors.

Neurotrophic factors (NTFs) are a family of peptides involved in the control of growth, survival and differentiation of neurons. NTFs include neurotrophins, glial cell-line-derived neurotrophic factor family ligands and neuropoietic cytokines.[5] Brain-derived neurotrophic factor (BDNF) and its receptor neurotrophic tyrosine kinase receptor type 2 (NTKR2, or TrkB), insulin-like growth factor 1 (IGF-1), insulin-like growth factor 1 receptor (IGF-1r) and neurotrophin-4 (NTF4/NT-4) are expressed in different areas of the brain such as the hippocampus, hypothalamus, cerebellum and cortex.[6–10] BDNF, IGF-1 and their respective receptors are survival factors for sympathetic and sensory neurons, mediators for synaptogenesis, neuronal growth and differentiation in the peripheral and central nervous systems. These NTFs are also important for cognitive functions, such as memory and learning.[5,11,12] In addition, these NTFs and growth factors can regulate the development and efficiency of the placenta.[13–16] A previous study showed that BDNF is able to increase the growth of blastocysts (embryonic cells), *in vitro*,

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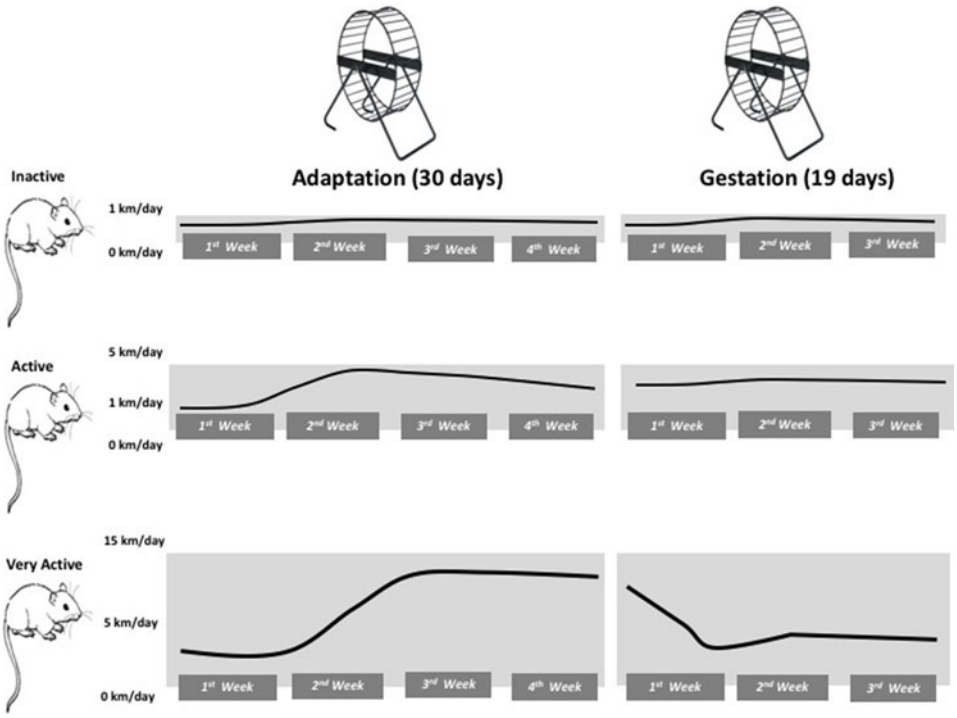

Fig. 1. Graphical representation of the maternal voluntary physical activity during the period of adaptation and gestation of inactive, active and very active rats.

during the pre-implantation period.[14] Similarly, it was demonstrated that IGF-1 stimulates the migration of placental trophoblastic cells, regulating foetus-placental growth.[17]

The efficiency in the transport of oxygen and nutrients through the placenta is essential for the growth and development of the foetus.[18,19] Previous studies have shown that regular exercise during pregnancy enhances foetus-placental growth.[18,20,21] Babies from exercising women (intensity between 55% and 60% of the preconception maximum aerobic capacity) showed higher body weight and length at birth than those born from sedentary women.[20] Likewise, regular exercise during pregnancy increased intervillous space blood volume, cardiac output and placental function.[18] Conversely, recent meta-analyses have shown multiple studies converged on the finding that: (i) overweight or obese women performing an exercise regimen during pregnancy reduced their own gestational weight as well as the risk of developing gestational diabetes[22]; and (ii) prenatal physical exercise reduced odds of macrosomia without any adverse side effect.[23] In rats, pups from active dams throughout pregnancy showed morphological changes in the placenta at the 19th day of gestation.[24] Since the placental development and efficiency are regulated by growth factors (IGF-1/IGF-1r) and NTFs (BDNF and NTF4), the hypothesis that maternal physical activity can alter gene expression of NTFs is plausible.

In our previous studies, we demonstrated that maternal voluntary physical activity was able to alter the growth and developmental trajectory of the offspring.[25,26] Voluntary maternal physical activity increased the indicators of somatic growth (laterolateral axis of skull, longitudinal axis and tail length) of the offspring during lactation.[26] Moreover, there was an earlier occurrence of the day of ear opening, internal auditory conduct opening, eruption of lower incisors and the palmar grasp reflex in pups from the very active dams during lactation.[26] In addition, maternal voluntary physical activity attenuated the effects of maternal low-protein diet (8% protein) on patterns of locomotor activity (distance travelled, average power, total energy and time of immobility) of the offspring at 60 days old.[25] The underlying mechanisms may be related to the uterine blood flow and enhanced placental transfer of oxygen and diffusible substrates.[18] Herein, we have used the same experimental model of voluntary maternal physical activity to investigate the physical activity–induced neuroplasticity on transcriptional gene expression of NTFs.

In the present study, we tested the hypothesis that voluntary physical activity performed by mothers before and during gestation modulates the expression of some trophic factors in the brain and placenta of dams, while having less readily detectable effects on the expression of NTFs in the progeny. Thus, the main goal of the present study was to evaluate the effects of voluntary maternal physical activity on gene expression of IGF-1, IGF-1r, BDNF and NTRK2 in the brain of mothers and foetus and gene expression of IGF-1, IGF-1r, BDNF and NTF4 in the placenta.

Material and methods

The experimental protocol was approved by the Ethical Committee of the Biological Sciences Centre (protocol no 23076.015984/2015-30), Federal University of Pernambuco, Brazil, and followed the Guidelines for the Care and Use of Laboratory Animals.

Animals

Fifteen virgin female albino Wistar rats (*Rattus norvegicus*) aged 85–95 days were obtained from the Department of Nutrition, Federal University of Pernambuco, Brazil. Animals were maintained at a temperature of 22 ± 1 °C with a controlled light–dark cycle (dark 06.00 am–6.00 pm). Food and water were given *ad libitum* throughout the experiment. The rats were individually housed in voluntary physical activity cages (cages equipped with a

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Table 1. Primers sequence used to perform qRT-PCR

Gene	Forward /Reverse	T	Sequence 5′-3′	Amplicon size
Igf1	F R	60°C	GCTCTTCAGTTCGTGTGTGG GCAACACTCATCCACAATGC	108 bp
Igf1r	F R	60°C	CTGGTCTCTCATCTTGGATGC GCTTCCCACACACACTTGG	197 bp
Bdnf	F R	60°C	GAGTGAAGATACCATCAGCA ATCTAGGCTACGTGAAGTCT	117 bp
Ntf4	F R	65°C	CTGAGATGTCAGGGAGGAGA ATGGCTTTGCACACCTGTCA	115 bp
Ntrk2	F R	60°C	GTGGTGATTGCCTCTGTGG TTGGAGATGTGGTGGAGAGG	149 bp
RPL19	F R	58°C	CTGAAGGTCAAAGGGAATGTG GGACAGAGTCTTGATGATCTC	195 bp
Actb	F R	60°C	AGCCATGTACGTAGCCATCC TCCCTCTCAGCTGTGCTGGTGAA	231 bp

running wheel) for 4 weeks. After this period, females were placed into a standard cage and mated (one female for one male) for a period of 1–5 days. Females had no access to the running wheel during mating. The day on which spermatozoa were present in a vaginal smear was designated as day 0 of gestation. Pregnant dams were then transferred back to their original cages with free access to the running wheel throughout gestation. At day 20 of gestation, dams were anesthetised with xylazine (10 mg/kg, *ip*.) and ketamine (80 mg/kg, *ip*.) prior to decapitation after a 6 h fasting period. Experimental analyses were performed in specific brain areas of mothers (cerebellum, hypothalamus, hippocampus and cortex), placenta and the entire brain of male offspring. The tissues collected were stored at −80 °C until RNA extraction.

Voluntary physical activity measurements

Female Wistar rats were individually housed in voluntary physical activity cages (with running wheels – 27 cm diameter) for a 4-week period of adaptation. A wireless cyclocomputer (Cataye, model CC-VL820, Colorado, USA) was attached in the wheel to calculate and display information related to physical activity, such as distance travelled, duration of activity and estimated calorie burned. These parameters were used to classify the rats according to their level of daily physical activity: inactive (I, n = 6), active (A, n = 4) or very active (VA, n = 5) according to previous studies.[25,26] After mating, rats continued to have access to the running wheel during gestation (Fig. 1).

Body weight and food intake

Mother's body weight was recorded every 3 days throughout the experiment. Maternal food consumption was determined by the difference between the amount of food provided at the onset of the dark cycle (06.00 h) and the amount of food remaining 48 h later. Body weight of the foetus and the placental weight were measured at the day of sacrifice (day 20 of gestation). Body weight was recorded using a Marte Scale (AS-1000) with 0.01 g accuracy.

Blood glucose measurements

Fasting glycaemia levels were evaluated in the last day of adaptation and weekly during gestation using blood samples from the tail vein of the rats, using a glucometer (Accu Check Advantage and Accutrend GCT) and the glucose oxidase method. The animals were fasted 6 h prior to glycaemia measurement.

RNA extraction

Total RNA was extracted from brain regions of mothers (cerebellum, hypothalamus, hippocampus and cortex), placenta and brain of offspring with the TRI reagent® (SIGMA-ALDRICH T9424, St. Quentin Fallavier, France) according to the manufacturer's instructions. Briefly, 1 ml of TRI reagent® was added per 50–100 mg of tissue, and the resulting suspension was homogenised and incubated at room temperature for 5 min. Thereon, 0.2 ml of chloroform was added, and samples were vortexed for 15 s, incubated for 5 min at room temperature and centrifuged at 12,000 × *g* for 15 min at 4 °C. The upper aqueous phase was transferred to a fresh tube, and 0.5 ml of isopropanol was added to precipitate RNA. Samples were incubated for 10 min at room temperature and centrifuged at 12,000 × *g* for 15 min at 4°C. The supernatant was removed, and RNA-containing pellets were washed sequentially with 75% and 100% ethanol and dissolved in 100 µl RNase-free water. RNA concentration and purity (defined by a 260/280 nm absorbance ratio >1.8) was determined using a Nanodrop 2000 (Thermofisher).

Reverse transcription

Reverse transcription was performed using an PrimeScript RT reagent Kit-Perfect Real Time (TAKARA) using 0.5 µg of RNA for brain of mothers (cerebellum, hypothalamus, hippocampus and cortex) and 1 µg of RNA for placenta and brain of offspring following the manufacturer's instructions. RNase-free water (3 µl), PrimeScript Buffer 5× (4 µl), Oligo dT – 50 µM (1 µl), Random hexamers – 100 µM (1 µl) and of PrimeScript RT Enzyme Mix (1 µl) were sequentially added, followed by a 15-min incubation at 37°C and for 15 s at 85°C. Reverse transcription reactions were brought to 200 µl final volume by adding RNase-free water and stored at −20°C.

Quantitative PCR (qPCR)

Real-time quantitative PCR amplification (qPCR) was performed using a Rotor-Gene Real-Time PCR System (Labgene Scientific Instruments, Archamps, France). The sequences of primers used in this study are reported in Table 1. Reactions were incubated at 95°C for 10 min, followed by 40 cycles of denaturation (95°C, 10s), annealing (58–65°C depending on the primer sets, 30s) and elongation (72°C, 30 s). We measured gene expression levels of IGF-1, IGF-1R, BDNF, NTF4 and NTRK2, or TrkB. qPCR results from each gene (including the housekeeping genes) were expressed as arbitrary units derived from a standard calibration curve derived from a reference sample. Reference samples for the tissues were generated by mixing 5 µl aliquots from multiple cDNA samples (three from the Inactive group, four from the Active group and three from the Very Active group). qPCR for each sample was carried out in duplicate. The mRNA levels of the analysed genes were normalised using the mRNA levels of ribosomal protein L19 (RPL19) and beta actin (Actb).

Statistical analyses

The Kolmogorov–Smirnov test was performed to determine if the data were normally distributed. Measurements of distance travelled, time of activity and estimated calorie burned were analysed by two-way ANOVA (using day and physical activity as factors)

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Table 2. Maternal and foetal physiological parameters during adaptation (30 days before pregnancy) and gestation. Values expressed as mean and S.E.M

	Inactive		Active		Very Active		
	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	*P* values
Adaptation							
Initial BW (g)	223.5	4.0	223.0	4.7	221.6	5.0	0.952
Final BW (g)	233.2	5.2	236.5	7.1	231.6	8.2	0.890
Gain of BW (g)	9.7	3.7	13.5	3.6	10.0	4.4	0.784
Food intake (g/day)	13.6	0.6	14.1	1.1	16.0	0.3	0.070
Fasting Glycaemia at day 30 (mg/dl)	103.2	5.9	98.7	3.4	101.2	2.4	0.808
Gestation							
Initial BW (g)	255.5	8.1	256.3	7.3	245.4	7.8	0.582
Final BW (g)	324.3	13.3	318.5	11.8	350.6	5.5	0.150
Gain of BW (g)	68.8	7.0	62.2	13.8	105.2[a,b]	4.9	0.008
Food intake (g/day)	17.0	0.7	16.7	0.4	20.5[a,b]	0.7	0.003
Fasting glycaemia at day 20 (mg/dl)	63.2	3.5	69.5	4.4	66.8	4.2	0.546
Number of foetuses	11.0	0.7	11.2	0.6	13.4[a]	0.2	0.022
Number of female foetuses	3.3	0.5	3.5	0.9	5.4	0.5	0.055
Number of male foetuses	7.7	0.7	7.7	0.5	8.0	0.5	0.925
Foetus weight (g)	3.6	0.2	3.0	0.3	4.2	0.5	0.092
Placenta weight (g)	0.6	0.08	0.5	0.02	0.5	0.03	0.191

Mothers and placenta: Inactive (n = 6), Active (n = 4) and Very Active (n = 5).
Foetus: Inactive (n = 12), Active (n = 8) and Very Active (n = 10). [a]$p < 0.05$ *vs* Inactive and [b]$p < 0.05$ *vs* Active using one-way ANOVA with Tukey's post-hoc.
BW = body weight

followed by the Bonferroni post-hoc test. For body weight, food intake, blood glycaemia, placental weight, number of foetus and gene expression, statistical analyses were performed by one-way ANOVA followed by the Tukey's post-hoc test. All data are presented as mean ± S.E.M. Significance was set at $p < 0.05$. Data analysis was performed using the statistical programme GraphPad Prism 5® (GraphPad Software Inc., La Jolla, CA, USA).

Results

Data on maternal voluntary physical activity parameters during the period of adaptation are schematically presented in Fig. 2. After the adaptation period (30 days), rats were classified as inactive, active or very active according to the daily level of physical activity. During adaptation, inactive dams performed less than 1 km/day in the running wheel. Active dams performed a constant amount of distance travelled, while the very active dams presented a progressive increase of distance travelled. In this period, body weight, food intake and fasting glycaemia did not change among groups (Table 2).

During gestation, dams remained in the special cages with the running wheel. The inactive dams kept the distance travelled less than 1 km/day in the running wheel. However, active and very active dams reduced the distance travelled between 1 and 3 km/day (Fig. 3). Body weight variation (initial and final), fasting glycaemia, number of offspring (males and females) and placental weight were similar when active dams were compared to inactive dams (Table 2). However, the very active dams presented an increase in body weight gain, food intake and number of foetus (Table 2).

Very active mothers showed increased IGF-1 mRNA in the cerebellum when compared to inactive mothers (Fig. 4). In response to physical activity, there were no changes in IGF1, IGF1r, BDNF and NTRK2 mRNA expression in different areas of the mother's brain: hypothalamus, hippocampus and cortex. In the cortex, there was a tendency of up-regulation of NTRK2 mRNA ($p = 0.06$) in the active and very active groups when compared to the inactive group (Fig. 4).

Voluntary maternal physical activity also induced statistically significant changes in the mRNA expression in the placenta. Very active mothers showed upregulation of NTF4 mRNA in relation to inactive mothers (Fig. 5). In the brain of foetus, voluntary maternal physical activity did not alter mRNA expression of the NTFs (Fig. 5).

Discussion

In the present study, a spontaneous active phenotype was observed in a subset of rats. Individual animals presented different levels of physical activity that allowed the categorisation of rats as inactive, active and very active groups. This categorisation is aligned with previous studies.[25,26] Earlier literature has also shown that such behavioural traits, i.e. the propensity or not for spontaneous physical activity, can be regulated by central (mRNA expression in the central nervous system) and/or peripheral (mRNA expression in skeletal muscle) mechanisms.[27–29] In order to test the hypothesis that physical activity induces changes in the gene expression of NTFs in different areas of the brain, we evaluated the expression of IGF-1, IGF-1r, BDNF and NTRK2 mRNA. Previous studies in rats have shown that physical activity on running wheels

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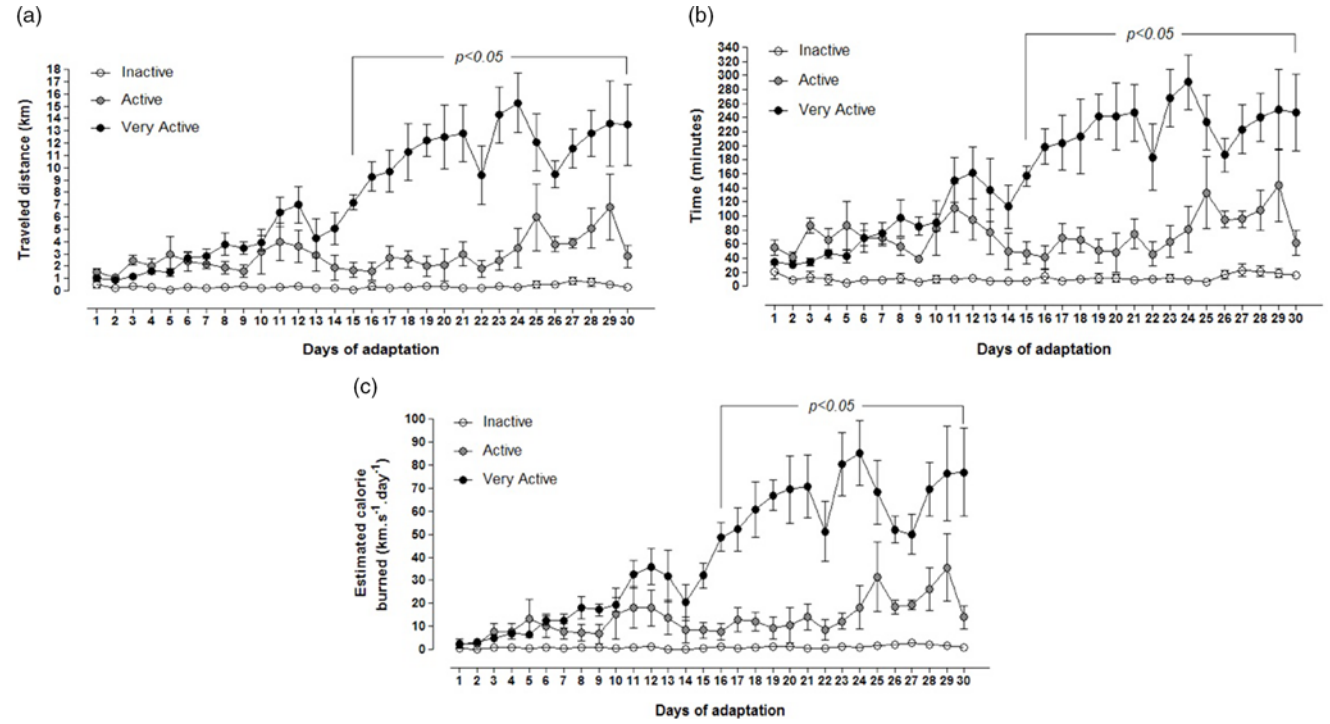

Fig. 2. Parameters of voluntary physical activity for Inactive (n = 6), Active (n = 4) and Very Active dams (n = 5). Travelled distance (A), time of activity (B) and estimated calorie burned (C) were recorded during the period of adaptation. Values are presented as mean ± S.E.M. *Statistical analysis was performed using two-way ANOVA with Bonferroni post-hoc test.

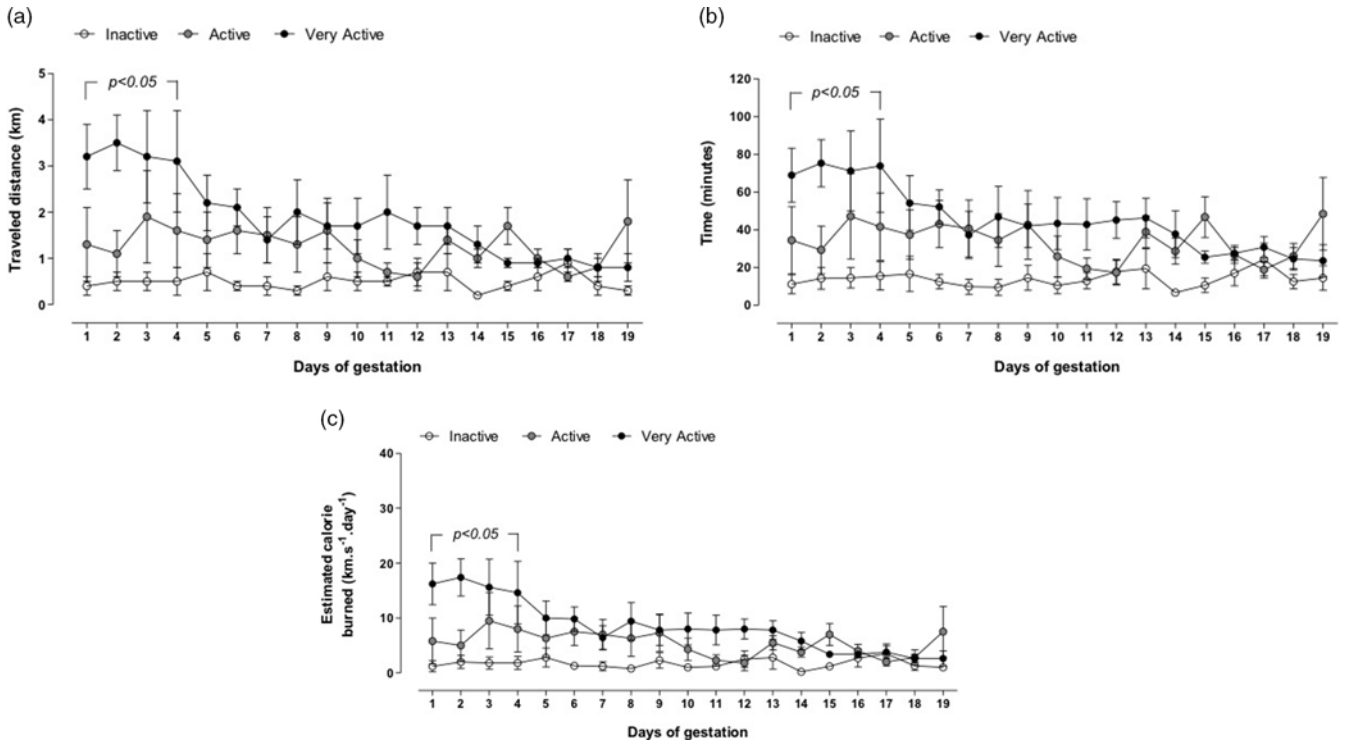

Fig. 3. Parameters of voluntary physical activity for Inactive (n = 6), Active (n = 4) and Very Active dams (n = 5). Travelled distance (A), duration of physical activity (B) and estimated calorie burned (C) were recorded during the period of gestation. Values are presented as mean ± S.E.M. *Statistical analysis was performed using two-way ANOVA with Bonferroni post-hoc test.

increased the expression of IGF-1 mRNA, BDNF and TrkB in the hippocampus, but without alteration of IGF-1r expression.[6,9] Another study showed that physical activity increased the expression of BDNF in the cerebellum.[7] In the present study, the expression of IGF-1 mRNA in the cerebellum was increased in response to physical activity on running wheels. In the placenta,

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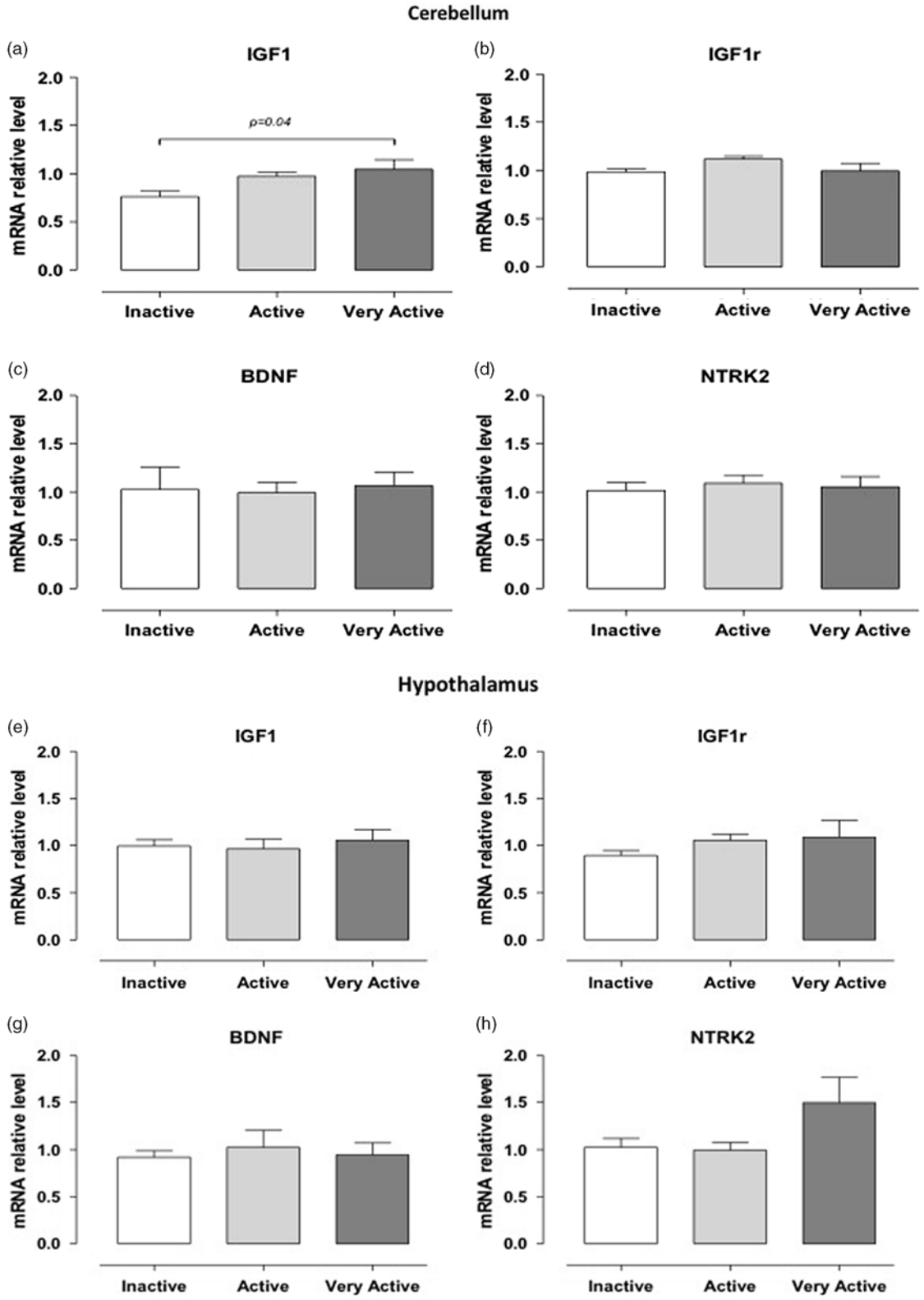

Fig. 4. mRNA expression of IGF1, IGF1r, BDNF and NTRK2 in the cerebellum (A–D), hypothalamus (E–H), hippocampus (I–L) and cortex (M–P). The groups were constituted by Inactive (n = 6), Active (n = 4) and Very Active (n = 5). Values are presented as mean ± S.E.M. Statistical analysis was performed using one-way ANOVA with Tukey's post-hoc test.

there was an increase in NTF4, but there were no changes in mRNA of the NTFs in the offspring's brain.

During gestation, active and very active rats continued to perform physical activity on the running wheel, but with a substantial reduction in the distance travelled in the very active dams (from 12 km/day to 1.9 km/day). The reduction in physical activity levels on the running wheel may be due to a switch in maternal behaviour to favour the disposal of nutrients for the development of the

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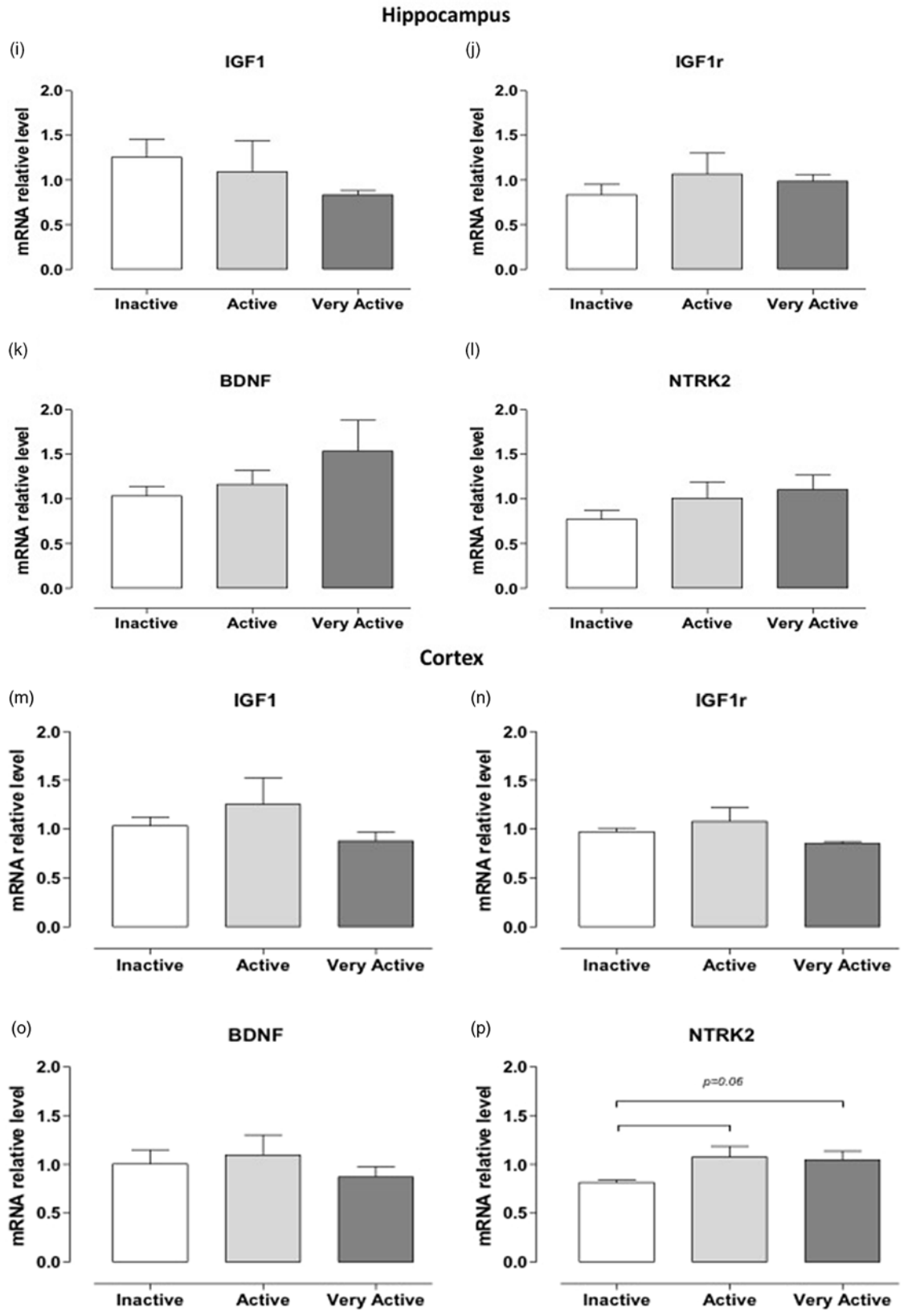

Fig. 4. Continued.

offspring.[30,31] Interestingly, very active dams showed increased number of foetuses, but there was no difference in the body weight of foetuses. It is probable that the increase in the number of foetuses in very active dams influenced the increased food intake to ensure energy supply for the developing foetuses. This result is aligned with our previous observations.[25] The number of foetuses

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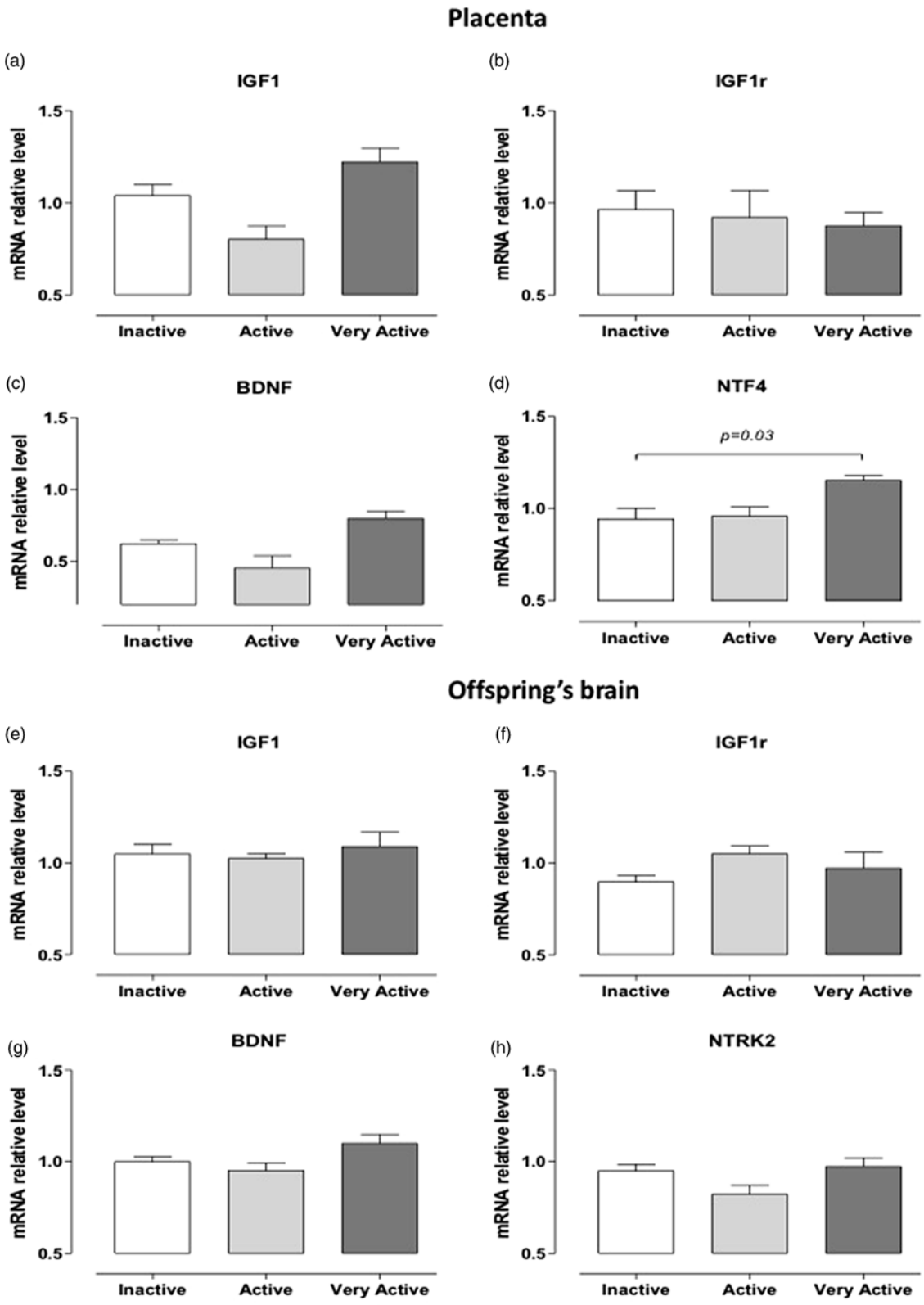

Fig. 5. mRNA expression of IGF1, IGF1r, BDNF and NTF4 in the placenta (A–D). The groups were constituted by Inactive (n = 6), Active (n = 4) and Very Active (n = 5). mRNA expression of IGF1, IGF1r, BDNF and NTRK2 in the brain of offspring (E–H). The groups were constituted by Inactive (n = 12), Active (n = 8) and Very Active (n = 10). Values are presented as mean ± S.E.M. Statistical analysis was performed using one-way ANOVA with Tukey's post-hoc test.

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represents one of the variables of the maternal reproductive ability, which is dependent on the quality of the environment.[32] Indeed, mothers can establish different reproductive strategies depending on the environmental context.[32] Thus, the increase in the number of offspring can be considered as a reproductive strategy in response to physiological mechanisms that allow, for example, a high availability of nutrients in the rats that performed physical activity.

In the present study, the expression levels of IGF-1, BDNF, IGF-1r and NTRK2 mRNA did not change in the cortex, hypothalamus and hippocampus from active and very active rats. In our study, we used an experimental model of spontaneous physical activity since forced exercise could induce stress and thus influence the expression of NTFs, thereby masking the naturally occurring benefits arising from a non-coercive induction of physical activity.[33] This experimental approach has a translational relevance in the context of the application of physical activity protocols to human subjects.

One of the most well-established brain metabolic changes evoked by exercise is indeed the increase of BDNF and its receptor NTRK2 mRNA.[34] Our data showed that 6 weeks of voluntary physical activity on running wheel induced a tendency to upregulate NTRK2 mRNA in the cortex. The increase of NTRK2 is related to neuronal development, differentiation and angiogenesis.[35] The regulation of BDNF receptors in the brain can be also regulated by oestrogen and progesterone.[36] These observations suggest that the mechanism by which maternal physical activity affects NTFs may have a neuroendocrine basis.

In the present study, very active dams showed an upregulation of IGF-1 mRNA in the cerebellum. The upregulation of IGF-1 mRNA may be likely responsible for the improvement in motor behaviour and increased travelled distance as seen in very active dams. Previous study performed on male rats showed, on the contrary, that chronic forced resistance physical training induced reduction of IGF-1 mRNA in the cerebellum.[37] Forced and voluntary exercise may differently affect brain and behaviour.[38] Forced exercise involves a slower, more constant pace for longer periods of time, whereas voluntary wheel running is characterised by rapid pace and short duration.[38] However, throughout pregnancy, active and very active dams arrest their physical activity. It is possible, however, that the observed increase of IGF-1 mRNA in response to earlier physical activity on running wheel may act as a neuroprotective factor on the maternal brain.[39–41]

The connectivity between mother and placenta (and consequently the foetus) may be adaptable according to the demands of different environmental stimuli.[42] It has been demonstrated that the maternal environment determines the structure and function of the placenta, with consequences on the development of the foetus.[43,44] In the present study, we observed an increase in the expression of NTF4 in the placenta of very active mothers as an adaptive response to maternal physical activity. NTF4 is related to placental growth and inhibition of apoptosis promoting the survival of placental cells.[14] This function contributes to the maintenance of villi improving placental transfer of substrates.[18] In contrast, the present study showed that the expression of IGF-1 and BNDF and their receptors (IGF1r and NTRK2) were not altered in the foetus' brain in response to maternal physical activity on running wheel. Accordingly, a recent study has demonstrated that exercise during pregnancy did not change BDNF levels in brain of mice offspring at 21 days old.[3] Absence of gene expression modulation in the foetus' brains could be related to technical difficulties, as the whole brain, and not specific regions, were analysed, due to the foetus' brain small size. Alternatively, it is possible that the underlying mechanism to improve neuroplasticity in foetus from exercised mothers possibly is not related to changes in neurotrophic gene expression.

Conclusion

In the present study, we demonstrated that maternal voluntary physical activity induced a tendency to upregulate NTRK2 mRNA in the cortex, upregulation of IGF-1 mRNA in the cerebellum and NTF4 mRNA in the placenta. However, there were no detectable modifications in the brain of foetus. Our findings suggest the existence of a developmental plasticity induced by physical activity in specific areas of the brain and placenta representing the first investment for offspring during development.

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