IBRO Neuroscience Reports 17 (2024) 263–279

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

journal homepage: www.sciencedirect.com/journal/IBRO-Neuroscience-Reports

Research paper

Spontaneous running wheel exercise during pregnancy prevents later neonatal-anoxia-induced somatic and neurodevelopmental alterations

Vitor Yonamine Lee [a], Aline Vilar Machado Nils [b], Bruna Petrucelli Arruda [c], Gilberto Fernando Xavier [b], Maria Inês Nogueira [a], Lívia Clemente Motta-Teixeira [a,b,d,1], Silvia Honda Takada [a,c,*,1]

[a] Departamento de Anatomia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 2415, Sao Paulo, SP 05508-900, Brazil
[b] Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, R. do Matão, Travessa 14, 101, Sao Paulo 05508-900, Brazil
[c] Centro de Matemática, Computação e Cognição, Universidade Federal do ABC, Alameda da Universidade, s/n, Bloco Delta, São Bernardo do Campo, SP 09606-070, Brazil
[d] Departamento de Ciências Fisiológicas, Faculdade de Ciências Médicas da Santa Casa de São Paulo, R. Jaguaribe, 155 - Vila Buarque, Sao Paulo, SP 01224-001, Brazil

ARTICLE INFO

Keywords:
Perinatal asphyxia
Gestational exercise
Reflexes ontogeny
Somatic development
Growth factors
Myelination

ABSTRACT

Introduction: About 15–20 % of babies that suffer perinatal asphyxia die and around 25 % of the survivors exhibit permanent neural outcomes. Minimization of this global health problem has been warranted. This study investigated if the offspring of pregnant female rats allowed to spontaneously exercise on running wheels along a 11-day pregnancy period were protected for somatic and neurodevelopmental disturbs that usually follow neonatal anoxia.

Methods: spontaneous exercise was applied to female rats which were housed in cages allowing free access to running wheels along a 11-day pregnancy period. Their offspring were submitted to anoxia 24–36 h after birth. Somatic and sensory-motor development of the pups were recorded until postnatal day 21 (P21). Myelin basic protein (MBP)-stained areas of sensory and motor cortices were measured at P21. Neuronal nuclei (NeuN)-immunopositive cells and synapsin-I levels in hippocampal formation were estimated at P21 and P75.

Results: gestational exercise and / or neonatal anoxia increased the weight and the size of the pups. In addition, gestational exercise accelerated somatic and sensory-motor development of the pups and protected them against neonatal-anoxia-induced delay in development. Further, neonatal anoxia reduced MBP stained area in the secondary motor cortex and decreased hippocampal neuronal estimates and synapsin-I levels at P21; gestational exercise prevented these effects. Therefore, spontaneous exercise along pregnancy is a valuable strategy to prevent neonatal-anoxia-induced disturbs in the offspring.

Conclusion: spontaneous gestational running wheel exercise protects against neonatal anoxia-induced disturbs in the offspring, including (1) physical and neurobehavioral developmental impairments, and (2) hippocampal and cortical changes. Thus, spontaneous exercise during pregnancy may represent a valuable strategy to prevent disturbs which usually follow neonatal anoxia.

Abbreviations: 5-HT, serotonin; A, anoxia group; ABC, avidin-biotin complex; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BrdU, bromodeoxyuridine; CA1, Cornu Ammonis 1 subfield of hippocampus; CA2-3, Cornu Ammonis 2 and Cornu Ammonis 3 subfields of hippocampus; DAB, 3,3'-diaminobenzidine; DG, dentate gyrus; E, exercise group; ECL, enhanced chemiluminescence; EGF, epidermal growth factor; E-NA, exercise and non-anoxia group; Gx, gestational day x; HIE, hypoxic-ischemic encephalopathy; HPA, hypothalamic-pituitary-adrenocortical axis; IGF, insulin-like growth factor; MBP, myelin basic protein; M1, primary motor cortex; M2, secondary motor cortex; NDS, normal donkey serum; NE-A, non-exercise and anoxia group; NE-NA, non-exercise and non-anoxia group; NeuN, neuronal nuclei protein; NGF, nerve growth factor; NHI, neonatal hypoxia-ischemia; NT-3, neutrophin-3; PA, perinatal asphyxia; PBS, phosphate-buffered saline; PLP, proteolipid protein; Px, postnatal day x; S1, primary sensory cortex; S1Dz, dysgranular region of the primary sensory cortex; S1Tr, trunk region of the primary sensory cortex; SDS, sodium dodecyl sulfate; TBS, tris-buffered saline; TBST, tris-buffered saline with Tween® 20 detergent; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

* Corresponding author at: Departamento de Anatomia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 2415, Sao Paulo, SP 05508-900, Brazil.
E-mail address: silvia.honda@ufabc.edu.br (S.H. Takada).
[1] Both senior authors.

https://doi.org/10.1016/j.ibneur.2024.08.008
Received 1 October 2023; Accepted 26 August 2024
Available online 31 August 2024

Introduction

Hypoxic-ischemic encephalopathy (HIE) is the brain injury resultant from Perinatal Asphyxia (PA) (Hakobyan et al., 2019; Greco et al., 2020). The incidence of HIE is 2/1000 in term births and 9/1000 in preterm births in developed countries and more than 10 times higher in developing countries. Around 15–20 % of affected infants die in the neonatal period and 25 % of survivors exhibit permanent neurologic outcomes (Schmidt and Walsh, 2010; Odd et al., 2017). Neural cell death that follows HIE is triggered by a range of interconnected phenomena, including inflammation, oxidative stress, and excitotoxicity (Millar et al., 2017).

PA disrupts proliferation, migration, differentiation, and synaptogenesis in brain neurodevelopment (Schneider and Miller, 2019). Depending on the intensity and the moment of PA, different brain regions may suffer damage, such as hippocampus, dentate gyrus (DG), cerebral cortex, basal ganglia, thalamus, and white matter (Millar et al., 2017). These disruptions lead to impairments related to learning and memory, as well as other neurobehavioral deficits, such as increased anxiety-like behavior, sexually dimorphic sensorimotor development and vocalization alterations during lactation period, increased nociceptive response and impairments on risk assessment behavior and in the auditory and contextual fear conditioning (Takada et al., 2011, 2015, 2016; Kumar et al., 2017, 2022; Millar et al., 2017; Cruz-Ochoa et al., 2019; Schneider and Miller, 2019; Helou et al., 2021, 2022; Matsuda et al., 2021). Several studies also show PA-related changes in body growth, body composition, metabolism and somatic and sensory-motor development in the pups (Dell'anna et al., 1991; Raff et al., 2001; Grojean et al., 2003; Ten et al., 2003; Lubics et al., 2005; Tang and Nakazawa, 2005; Chen et al., 2007; Yan et al., 2016; Kumar et al., 2017, 2022; Menshanov et al., 2017; Cruz-Ochoa et al., 2019).

Maternal physical exercise along pregnancy, on the other hand, induces beneficial effects in the offspring's brain and body in development (Gomes da Silva and Arida, 2015; Kusuyama et al., 2020). It enhances brain-derived neurotrophic factor (BDNF) expression and growth factors (Parnpiansil et al., 2003; Akhavan et al., 2013; Ferrari et al., 2018), increases hipocampal neurogenesis and neuroprotection (Lee et al., 2006; Kim et al., 2007; Dayi et al., 2012; Choi et al., 2013), and improves learning, memory and sensory-motor function (Parnpiansil et al., 2003; Lee et al., 2006; Kim et al., 2007; Dayi et al., 2012; Akhavan et al., 2013; Choi et al., 2013; Robinson and Bucci, 2014). Additionally, it induces energy-related metabolic changes such as leptin expression, body weight and body composition (Dayi et al., 2012; Rosa et al., 2013; Ferrari et al., 2018).

Protective effects of maternal physical exercise along pregnancy against latter offspring PA-induced dysfunctions have also been reported. For instance, Akhavan et al. (2012) demonstrated that gestational exercise along pregnancy prevents neonatal hypoxia-ischemia (NHI)-induced reduction in number of hippocampal neurons. Sanches et al. (2017, 2020, 2021) observed that swimming along pregnancy protects against NHI-induced disturbs of the offspring's maturation of reflexes, levels of BDNF, learning, cell surviving, locomotion, hippocampus integrity and mitochondrial function. Finally, Gorgij et al. (2021) reported that treadmill exercise during pregnancy decreases NHI-induced pro-inflammatory agent levels, infarction volume in the brain, edema, reaction time in negative geotaxis and cliff drop avoidance tests. Thus, these studies clearly show positive influences of gestational exercise in offspring submitted to NHI models of PA that mimic hypoxia/ischemia in either term or near-term born subjects.

Takada et al. (2011) developed neonatal-anoxia-induced model for rats that mimics metabolic and physiological changes observed in human neonates exposed to acute anoxia. The changes, observable in rats exposed to 100 % N2 for 25 min at P2, include (1) body growth and somatic and sensory-motor ontogeny disturbs, (2) decrease of neural cells in hippocampus and DG, adult neurogenesis and hippocampal volume, (3) performance impairments in the Morris' water maze and elevated plus maze tasks, and (4) and alterations in pups' vocalization and nociception (Takada et al., 2015, 2016; Kumar et al., 2017, 2022; Cruz-Ochoa et al., 2019; Helou et al., 2021, 2022; Matsuda et al., 2021).

In the present study we investigated if 11 days of spontaneous gestational exercise (from G6 to G16) in rats prevent somatic and neurodevelopment disturbs which follow their pups exposure to the neonatal anoxia model described by Takada et al. (2011). The offspring observations included (1) body growth and somatic and sensory-motor development (reflex ontogeny) assessed from P2 to P21, (2) myelination in primary and secondary motor cortices (M1 and M2) and primary sensory cortex (S1) assessed at P21 and P75, (3) neuronal estimates in the hippocampus and DG at P21 and P75, and (4) synapsin-I integrity in the hippocampus. The somatic development was assessed by the somatic milestones, including the auditory conduit opening, the pinna detachment, the eyes opening, the superior incisor eruption and the inferior incisor eruption. The reflexes observed included palmar grasp, cliff avoidance, surface righting, negative geotaxis, vibrissae placing, acoustic startle response and free-fall righting reflex.

Material and methods

Ethics statement

All experimental procedures adhered to the Ethical Principles of Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethics Committee in Animal Experimentation (CEEA) of the Institute of Biomedical Sciences of the University of Sao Paulo (CEUA Protocol Nº. 190, page 139, book 2).

Experimental design

The experimental design of the present study is illustrated in Fig. 1.

Mating

Each of the 8 male Wistar rats (*Rattus norvegicus*) was allocated in standard cages for mating with either 1 or 2 females. They were maintained in the vivarium of the Laboratory of Neurosciences and Behavior, Institute of Biosciences, at the University of São Paulo, under environmental controlled conditions, temperature at 22 ± 2°C, a dark-light cycle of 12,12 h and food and water *ad libitum*.

The day of the appearance of a vaginal plug or detection of spermatozoids in the female vagina was recorded as gestational day one (G1). The presence of spermatozoids in the vagina was assessed using vaginal washing, according to Marcondes et al. (2002). A maximum of eight pups, including mostly males but also females, were kept in one litter. Although male and female offspring were housed together for nestling purposes until P21, only male pups (n = 90) were included in the experiments, due to the previously observed sexual dimorphism in several parameters by our research group, as outlined by Kumar et al. (2017). In other words, since the present study aimed at evaluating potential protective effects of gestational exercise on pups exposed to neonatal anoxia, it seemed appropriate to select male subjects, that exhibit greater disturbs following the anoxic event, because in principle there would be a "greater window" for revealing protective effects of spontaneous gestational exercise.

Spontaneous exercise in running wheel during pregnancy

Since gestational period from G6 to G16 in rodents corresponds to a critical period of increasing neurogenesis and preparation for the subsequent developmental events, such as synaptogenesis, synaptic pruning and myelination (Zeiss, 2021), pregnant females were exposed to the running wheels during this period. In addition, maintenance of pregnant females in these running wheel cages in the final stages of gestation

would be less comfortable as compared to their maintenance in standard home cages.

Thus, from G6 to G16 pregnant females were individually maintained in cages (46 × 25 × 38 cm) with either a free running wheel (exercise – E) or with a locked running wheel (non-exercise – NE). Thus, while pregnant females exposed to the free running wheel (n = 10) could spontaneously exercise in the wheel by running in it, pregnant females exposed to the locked running wheel (n = 6) had no opportunity of running in the wheel. Each wheel, measuring 30 cm in diameter and 10 cm width, was connected to a microprocessor programed to record the number of rotations all along the period. Six out of 10 pregnant females exposed to the exercise ran more than 7500 m per day, thus having their pups included in the exercise groups. At G16 all pregnant females that reached this criterion were transferred to individual standard cages (49 ×34 x 16 cm), where they were maintained until delivery and later until weaning of the pups.

Groups constitution

After delivery, all male pups of each litter were randomly selected to either the anoxia (A) or non-anoxia (NA) treatments, thus resulting in 4 groups, E-A (exercise and anoxia) (n = 24), E-NA (exercise and non-anoxia) (n = 27), NE-A (non-exercise and anoxia) (n = 20) and NE-NA (non-exercise and non-anoxia) (n = 19). Each group included pups from multiple litters, to minimize possible litter effects. The pups were housed with the dams until weaning at P21.

Neonatal anoxia

The neonatal anoxia model used in this study was validated by our research group (Takada et al., 2011, 2015, 2016; Motta-Teixeira et al., 2016; Kumar et al., 2017, 2022; Helou et al., 2021, 2022; Matsuda et al., 2021). At P1-P2, about 24–36 h-old pups weighing 6–8 g were exposed to 100 % nitrogen gas inside a non-hermetic polycarbonate chamber (31.0 ×14.0 ×19.5 cm) partially immersed in water at a temperature of 36 ± 1°C. This particular timepoint was chosen for the anoxia induction because some processes of the development of the rodent nervous system, such as myelination, correspond to an extreme premature human baby at about 24–26 weeks of gestation (Semple et al., 2013). Once closed, the chamber received a constant flux of nitrogen of 3 L/min at a pressure of 101.7 kPa for 25 min. Pups from NA groups were submitted to the same procedures, except that instead of nitrogen they were exposed to the environmental air.

Evaluation of body growth, somatic development, and reflexes ontogeny

Body growth evaluation of the pups included measurements of weight gain using an electronic scale, and body length, mediolateral head axis gain, anteroposterior head axis gain using a Vernier caliper [following Kumar et al. (2017) guidelines].

The same subjects were submitted to evaluation of somatic and sensory-motor (reflexes) development following the guidelines by Campos et al. (2021) and Kumar et al. (2017). The day of appearance of somatic and sensory-motor developmental milestones were recorded for each subject. The somatic developmental milestones observed included the auditory conduit opening, the pinna detachment, the eyes opening, the superior incisor eruption and the inferior incisor eruption. The reflexes observed included palmar grasp, cliff avoidance, surface righting, negative geotaxis, vibrissae placing, acoustic startle response and free-fall righting reflex. The reflex was considered to occur when it appeared within 10 s after the eliciting stimulus presentation. All these developmental milestones and procedures are described in Kumar et al. (2017).

All measurements and observations were carried out once a day, between 8,00–12,00 a.m., along the lactation period, except at P1, to avoid stress immediately after delivery. Each group included 5 subjects.

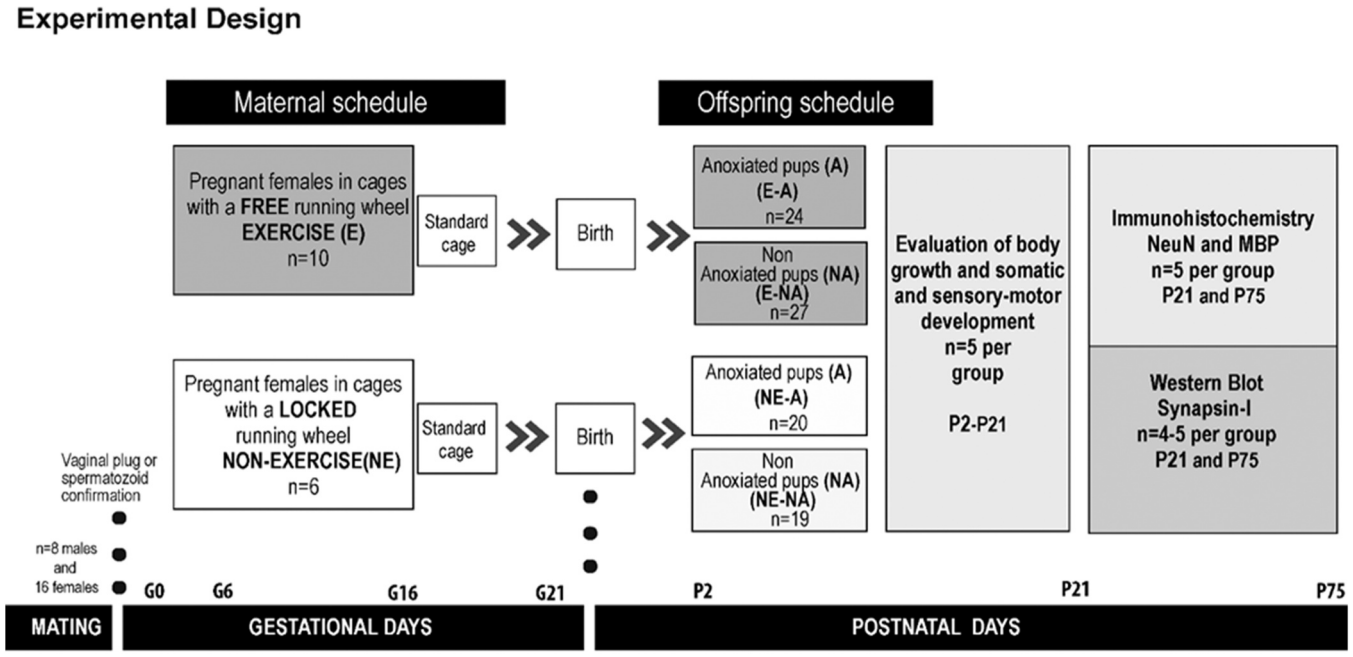

Fig. 1. Schematic representation of the experiments time-course. Each of 8 male rats was allocated for mating with either 1 or 2 females. The day of appearance of a vaginal plug or spermatozoids in the female vagina was considered the gestational day 1 (G1). From G6 to G16, 10 pregnant females were maintained in cages with a free running wheel (**E** groups). Similarly, 6 pregnant females were maintained in cages with a locked running wheel (**NE** groups). At G16, all females were transferred to standard cages without running wheels where they were maintained until delivery at G21. The day of birth was considered the postnatal day 1 (P1). When pups reached 6–8 g, at P2, they were submitted to either anoxia (**A** groups) or to a non-anoxia control procedure (**NA** groups). Thus, there were 4 groups, NE-NA (non-exercise and non-anoxia), E-NA (exercise and non-anoxia), NE-A (non-exercise and anoxia) and E-A (exercise and anoxia). From P2 to P21, body growth and somatic and sensory-motor development were assessed in 5 pups per group. At P21 and P75, rats were processed for immunohistochemistry and western blot.

Transcardial perfusion and immunohistochemistry

At P21, after evaluation of body growth and somatic and sensory-motor development, five animals of each group were anesthetized using intraperitoneal injections of ketamine (100 mg/kg) and xylazine (7 mg/kg), and transcardially perfused with saline 0.9 % (pH 7.4) and formaldehyde 4 % (pH 7.4, at 4 °C). Similarly, five animals of each group were perfused at P75. After perfusion, the brains were dissected, postfixed in formaldehyde 4 % overnight and cryoprotected in 30 % sucrose solution, and then sectioned in 40-μm-thick coronal sections, sequentially distributed in 12 wells of a corning costar plate.

The sections of one of these wells were processed for Nissl staining. The sections of two other wells were submitted to immunohistochemistry for anti-NeuN antibody (P21 and P75 animals) and to immunohistochemistry for anti-MBP antibody (P21 animals), respectively. The sections of the remaining wells were stored for replacement, if required. For immunohistochemistry, the sections were washed with 0,1 % phosphate-buffered saline (PBS), incubated for 30 min in 0.3 % hydrogen peroxide solution, washed again with 0,1 % PBS, incubated in blocking solution (normal donkey serum [NDS] 1333 [Vector Laboratories, Burlingame, CA, USA] + Triton X-100 0.3 % [Solon, OH, USA] + PBS) for 40 min and then incubated for 24 h at room temperature with mouse monoclonal primary antibody anti-NeuN (MAB 377, Millipore Corporate Headquarters, Billerica, MA, USA) (1,1000) or anti-MBP (Abcam Inc., Cambridge, MA, USA) (1100), both with NDS 1333. Then, the sections were washed, incubated with biotinylated secondary donkey anti-mouse antibody (Abcam Inc., Cambridge, MA, USA) (1,1000) for 90 min, washed, incubated in solution with avidin-biotin complex (ABC) 1500 from VECTASTAIN® Elite ABC Kit Standard (Vector Laboratories, Burlingame, CA, USA) for 90 min, washed, and then immersed in a 3,3'-diaminobenzidine (DAB) (Abcam Inc., Cambridge, MA, USA) (DAB 0.05 % + hydrogen peroxide 0.01 % + PBS) solution for the staining reaction, which was visually controlled. The sections were mounted on slides, dehydrated, and then covered with coverslips using DPX (Sigma-Aldrich Inc., UK).

Estimates of NeuN-immunopositive cells

The estimates of NeuN-immunopositive cells included the pyramidal cell layers of the Cornu Ammonis 1 (CA1) and CA2–3 subfields, and the DG granule cell layer of the right dorsal hippocampal formation. The number of NeuN-immunopositive cells was estimated using unbiased stereological principles and cell counts (West et al., 1991) using the Stereo Investigator program (MBF Biosciences, Williston, VT, USA). The images were captured using an optical microscope with a motorized stage (Ludl Electronic Products, Hawthorne, NY, USA), connected to a high-resolution camera (Nikon Instruments Inc., Melville, NY, USA).

The estimates of NeuN-immunopositive cells in each region of each subject were obtained using counting frames measuring 1600 μm^2 (40 μm × 40 μm), sampling grid area measuring 24,860 μm^2 (226 μm × 110 μm), dissector height (*Z*) measuring 10 μm, guard zone distance of 1 μm and section evaluation interval of 12, in 4 sections of each subject. The coefficient of error (Gundersen, m = 1) varied between 0.04 and 0.09 for all estimates.

Cortical myelination

The percentage of cortical myelination was calculated for M1, M2, S1Tr and S1Dz in one section of each subject. The percentage of cortical myelination corresponded to the MBP-immunopositive stained area in each cortical region divided by the total area of the same region, multiplied by 100 (van Tilborg et al., 2017). Photomicrographs of the sections were taken in a light microscope (DM5500B, Leica Microsystems, Wetzlar, Germany) and measurements of areas were performed using the Image-J program (NIH, Bethesda, Maryland).

Synapsin-I levels

At P21, Subjects were anesthetized and At P21 (NE-NA = 4, NE-A = 4, E-NA = 5, E-A = 5) and P75 (NE-NA = 4, NE-A = 4, E-NA = 5, E-A = 5) the hippocampal formations were bilaterally dissected and homogenized in 700 μl of mammalian cell lysate buffer supplemented with benzonase (Merck KgaA, Darmstadt, HE, Germany) and protease inhibitor (Qproteome Mammalian Protein Prep Kit – Qiagen Group). The homogenate was centrifuged at 4 °C for 10 min (14,000 x g) and the supernatant was separated. The total protein quantity was obtained using the Bradford method (Bradford, 1976), which employs Coomassie Brilliant Blue G250 dye and spectrometry. After the quantification, 75 μg of protein was diluted in Laemmli buffer with dithiothreitol 100 mM. This solution was transferred to acrylamide gels of 8 % sodium dodecyl sulfate (SDS) (Bio-Rad, Hercules, CA, USA) for electrophoresis, which was run using a constant current of 25 mA. After the electrophoresis, the proteins were transferred to 0.2 % Ponceau S nitrocellulose membranes. The membranes were washed with Tris-buffered saline (TBS), incubated in blocking solution (skimmed milk 5 % [Molico, Nestlé] + TBS) for 1 h, washed with TBST (Tween® 20 0.1 % [Amresco, Solon, OH, USA] + TBS) and incubated with primary monoclonal rabbit antibody against synapsin-I (Abcam, Cambridge, MA, USA) (1,1000) in TBST at 4 °C overnight. The membranes were then washed again with TBST (3 ×10 min), incubated with peroxidase-labeled secondary antibody against rabbit protein (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) (1,1000) in TBST for 2 h, washed again and immersed in enhanced chemiluminescence (ECL) kit (Amersham Biosciences) for chemiluminescence. Signals of the bands were captured as the optical density of the immunoreactivity using the scanner SCAN-C (LI-COR Biosciences) and were analyzed using the Image Studio Digits software (LI-COR Biosciences). An antibody against β-actin (1,10,000) (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) was used as the internal loading control. The synapsin-I optical density values were normalized using β-actin as internal control. The statistical analysis was run using the average of each group, in each age.

Statistical analysis

Data of body growth were analyzed using repeated-measures analysis of variance (ANOVA). Spontaneous gestational exercise in running wheel (Exercise) and neonatal anoxia (Anoxia) were included in the model as between-subjects factors, and Postnatal day was included as a within-subjects factor. Post-hoc analysis was run using Tukey-Kramer Test.

Data of the developmental milestones were analyzed using non-parametric Kruskal-Wallis test followed by Dunn's multiple comparisons post-hoc test.

Estimates of NeuN-immunopositive cells and levels of synapsin-I both at P21 and P75 and percentage of cortical myelination at P21 were analyzed using two-way ANOVA. Exercise and Anoxia were included as between-subjects factors. Post-hoc analysis involved Tukey-Kramer Test. Differences were considered significant when the p-value was less than 0.05.

Results

Body growth

Fig. 2 shows the body growth as expressed by body length (Fig. 2A), anteroposterior head axis (Fig. 2B), mediolateral head axis (Fig. 2C), and body weight (Fig. 2D).

Independent ANOVAs for data of body length, anteroposterior head axis, mediolateral head axis and body weight revealed significant main effects for (1) **Exercise** (body length, $F_{(1,\ 160)} = 233.2$, $p < 0.0001$; anteroposterior head axis, $F_{(1,\ 160)} = 226.6$, $p < 0.0001$; mediolateral head axis, $F_{(1,\ 160)} = 60.79$, $p < 0.0001$ and body weight, $F_{(1,\ 160)} =$

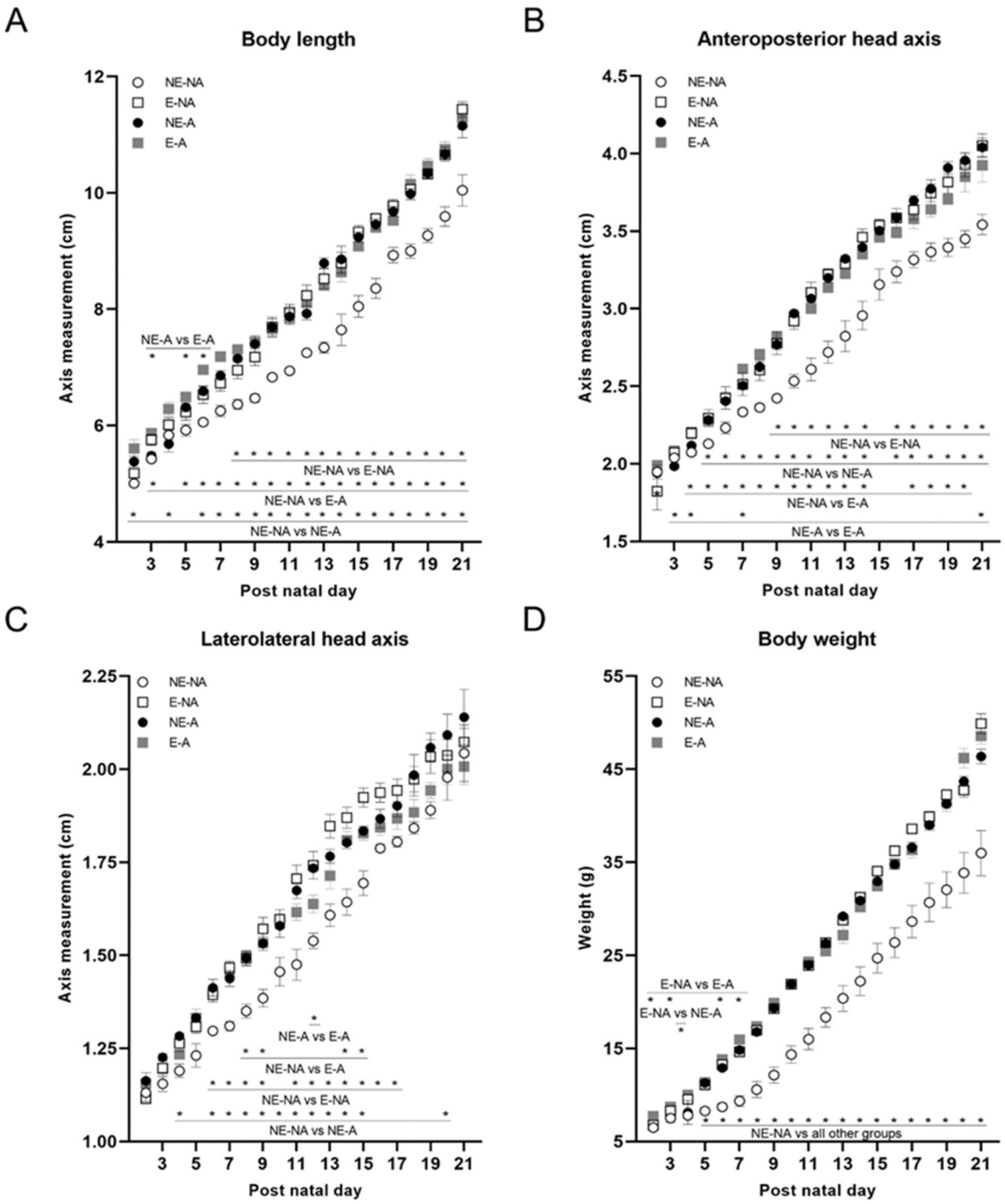

Fig. 2. Body growth as expressed by (A) body length, (B) anteroposterior head axis, (C) mediolateral head axis and (D) body weight. n = 5 in each group. Post-hoc Tukey-Kramer Test revealed groups differences as indicated by the asterisks. * $p < 0.05$. Data are presented as mean ± S.E.M.

328.5, $p < 0.0001$), (2) **Anoxia** (body length, $F_{(1, 160)} = 312.3$, $p < 0.0001$; anteroposterior head axis, $F_{(1, 160)} = 125.7$, $p < 0.0001$; mediolateral head axis, $F_{(1, 160)} = 30.63$, $p \leq 0.0001$ and body weight, $F_{(1, 160)} = 435.4$, $p \leq 0.0001$), and (3) **Postnatal day** (body length, $F_{(19, 160)} = 914.2$, $p < 0.0001$; anteroposterior head axis, $F_{(19, 160)} = 465.1$, $p < 0.0001$; mediolateral head axis, $F_{(19, 160)} = 338.4$, $p < 0.0001$; body weight, $F_{(19, 160)} = 1266$, $p < 0.0001$).

ANOVA also revealed significant interaction effects for (4) **Exercise and Anoxia** (body length, $F_{(1, 160)} = 143.8$, $p < 0.0001$; anteroposterior head axis, $F_{(1, 160)} = 332.8$, $p < 0.0001$; mediolateral head axis, $F_{(1, 160)} = 198.00$, $p \leq 0.0001$ and body weight, $F_{(1, 160)} = 254.7$, $p \leq 0.0001$), (5) **Postnatal day and Exercise** (anteroposterior head axis, $F_{(19, 160)} = 2.303$, $p = 0.0027$; mediolateral head axis, $F_{(19, 160)} = 2.830$, $p = 0.0002$; body weight, $F_{(19, 160)} = 3.459$, $p < 0.0001$), (6) **Postnatal day and Anoxia** (mediolateral, $F_{(19, 160)} = 2.238$, $p = 0.003$; body weight, $F_{(19, 160)} = 5.127$, $p < 0.0001$), and (7) **Postnatal day, Exercise and Anoxia** (body length, $F_{(19, 160)} = 4.563$, $p < 0.0001$; anteroposterior head axis, $F_{(19, 160)} = 8.349$, $p < 0.0001$; mediolateral head axis, $F_{(19, 160)} = 2.173$, $p = 0.004$; body weight, $F_{(19, 160)} = 4.447$, $p < 0.0001$). Non-significant data were not reported.

Thus, these results show that both maternal spontaneous exercise and neonatal anoxia, either separated or associated, promoted increase of somatic growth from P2 to P21 in the offspring (see relevant specific statistical comparisons in Fig. 2).

Somatic development

Fig. 3 shows the postnatal day when pinna detachment appeared (Fig. 3A), auditory conduit opened (Fig. 3B), superior incisor erupted (Fig. 3C), inferior incisor erupted (Fig. 3D) and eyes opened (Fig. 3E).

The Kruskal-Wallis Test revealed significant groups' differences for pinna detachment ($H(3) = 15.47$, $p = 0.001$), auditory conduit opening ($H(3) = 10.89$, $p = 0.012$) and eye opening ($H(3) = 11.91$, $p = 0.007$), but not for superior incisor eruption ($H(3) = 4.04$, $p = 0.25$) and inferior incisor eruption ($H(3) = 3.95$, $p = 0.26$).

As Fig. 3 shows that anoxia accelerated pinna detachment (Fig. 3A) and postponed auditory conduit opening (Fig. 3B). This latter effect was prevented by the maternal spontaneous exercise (Fig. 3B). Data also show that maternal spontaneous exercise accelerated eye opening of the offspring (Fig. 3E).

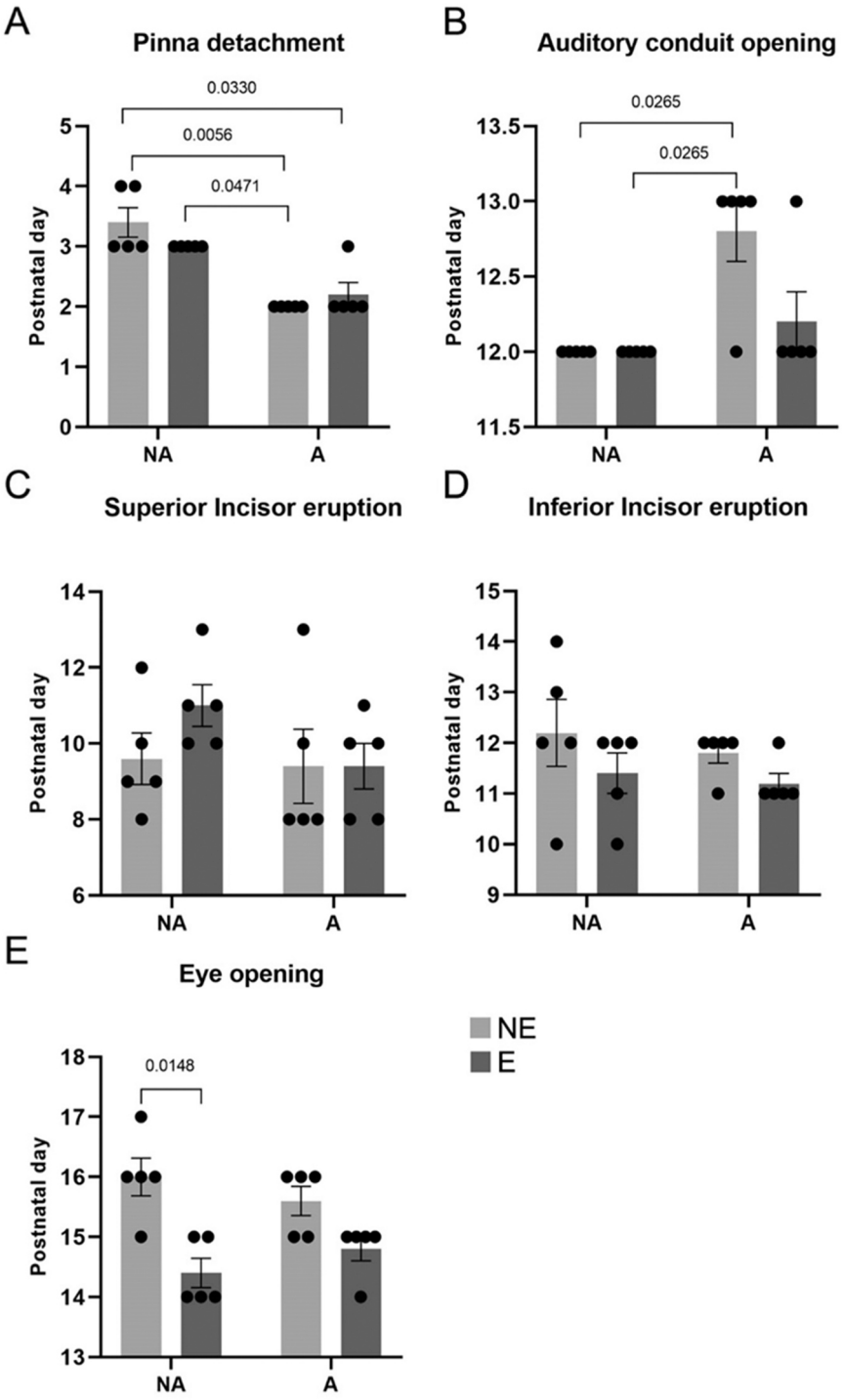

Fig. 3. Somatic development as expressed by the Postnatal day of (A) pinna detachment, (B) auditory conduit opening, (C) superior incisor eruption, (D) inferior incisor eruption and (E) eye opening. Individual scores for each subject are plotted. Horizontal lines represent the medians. Groups' data were analyzed using Kruskal-Wallis Test. Post-hoc Dunn's multiple comparisons test revealed significant groups differences, as indicated by the p-values and horizontal bars indicating the compared groups. n = 5 per group.

Reflex ontogeny

The postnatal day of emergence of developmental reflexes are presented in Fig. 4, including palmar grasp (Fig. 4A), surface righting (Fig. 4B), cliff avoidance (Fig. 4C), vibrissae placing (Fig. 4D), negative geotaxis (Fig. 4E), auricular startle response (Fig. 4F), and free-fall righting (Fig. 4F).

The Kruskal-Wallis test revealed significant groups' differences for palmar grasp ($H(3) = 15.83$, $p = 0.0012$), surface righting ($H(3) = 15.22$, $p = 0.001$), vibrissae placing ($H(3) = 10.89$, $p = 0.012$), cliff avoidance ($H(3) = 10.03$, $p = 0.018$), negative geotaxis ($H(3) = 12.82$, $p = 0.005$) and free-fall righting ($H(3) = 8.60$, $p = 0.035$). In addition, the Kruskal-Wallis test revealed lack of significant difference for acoustic startle response ($H(3) = 5.698$, $p = 0.12$).

Thus, neonatal anoxia postponed the emergence of palmar grasp (Fig. 4A), surface righting (Fig. 4B) and negative geotaxis (Fig. 4E) reflexes. These anoxia-induced postponing effects were prevented by prior exposure of the subjects' dams to spontaneous exercise along pregnancy (see Figs. 4A, 4B and 4E, respectively). In contrast, while neonatal anoxia significantly accelerated the emergence of the free-fall righting

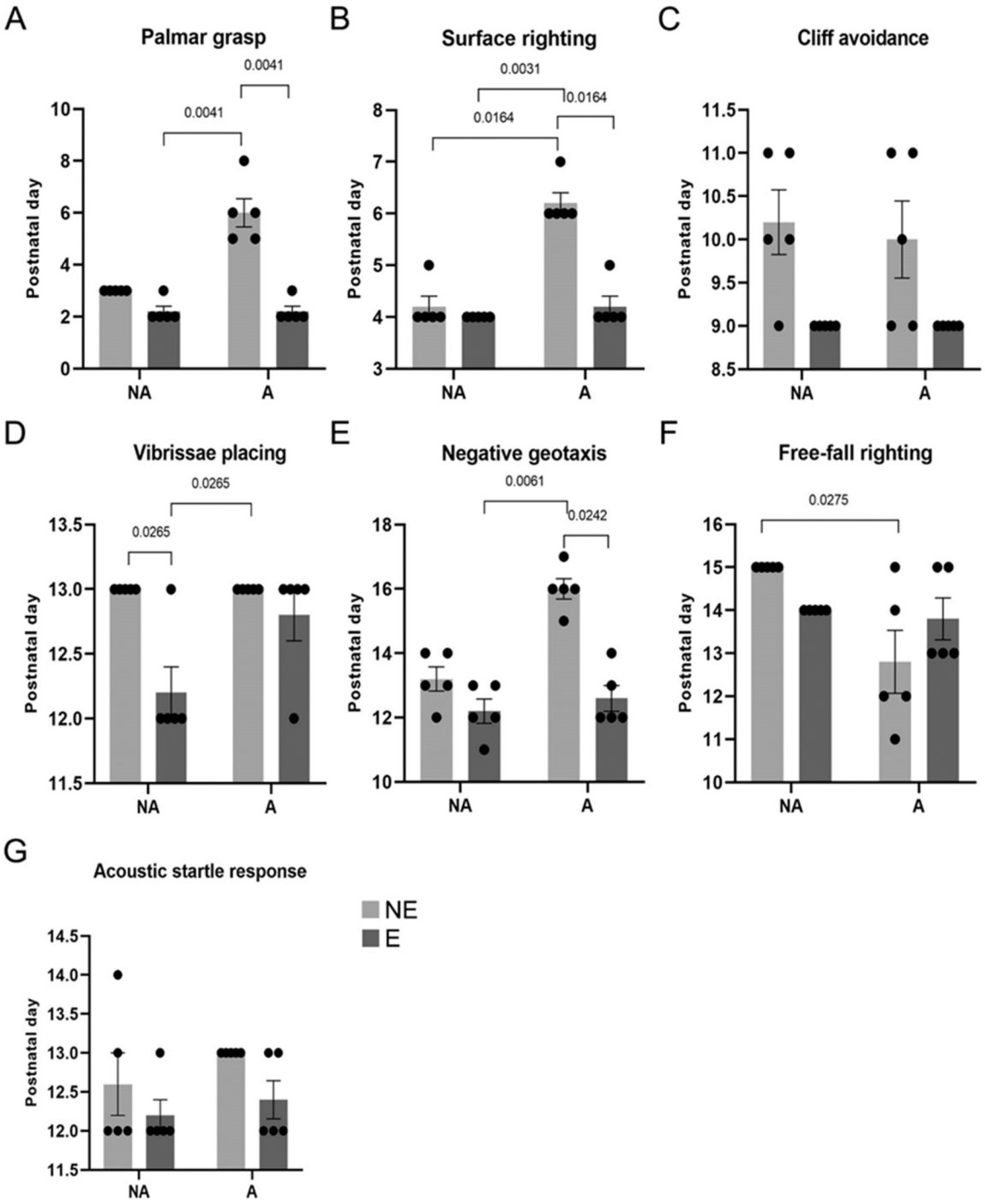

Fig. 4. Postnatal day of emergence of (A) palmar grasp, (B) surface righting, (C) cliff avoidance, (D) vibrissae placing, (E) negative geotaxis, (F) free-fall righting and (G) auricular startle response reflexes. Horizontal lines represent the medians. Groups' data were analyzed using Kruskal-Wallis Test. Post-hoc Dunn's multiple comparisons test revealed significant groups differences, as indicated by the p-values and horizontal bars indicating the compared groups. n = 5 per group.

reflex (Fig. 4F), spontaneous gestational exercise significantly accelerated the emergence of the vibrissae placing reflex (Fig. 4D). The Kruskal-Wallis test also revealed that mothers' exercise during pregnancy anticipates the emergence of cliff aversion of the offspring (Fig. 4C).

Extent of cortical myelination

Relative to cortical myelination in M2 (Fig. 5A), two-way ANOVA revealed significant main Anoxia ($F_{(1, 13)} = 12.21$, $p = 0.004$) and Exercise ($F_{(1, 13)} = 5.223$, $p = 0.039$) effects, and an almost significant Exercise and Anoxia interaction effect ($F_{(1, 13)} = 10.90$, $p = 0.057$). As Fig. 5A shows, NE-A subjects exhibited a significant smaller percentage of MBP-immunopositive stained areas as compared to subjects of the E-A groups, indicating that mothers' spontaneous exercise in running wheel along pregnancy prevents anoxia-induced reduction of the MBP-immunopositive stained areas in the offspring.

In relation to cortical myelination in M1 (Fig. 5B), two-way ANOVA detected significant effect of Exercise ($F_{(1, 13)} = 5.233$, $p = 0.039$), but not for Anoxia ($F_{(1, 13)} = 1.254$, $p = 0.283$) nor for Anoxia and Exercise interaction ($F_{(1, 13)} = 1.472$, $p = 0.283$). Despite the lack of statistical significance of differences between groups it is possible to note that anoxia reduces cortical myelination in M1, and exercise counteracts this anoxia-induced effect.

Relative to myelination in S1Tr (Fig. 5C) and S1Dz (Fig. 5D), two-way ANOVA revealed lack of significant Exercise ($F_{(1, 13)} = 0.805$, $p = 0.385$; $F_{(1, 13)} = 2.666$, $p = 0.126$, respectively) and Anoxia ($F_{(1, 13)} = 0.947$, $p = 0.348$; $F_{(1, 13)} = 0.085$, $p = 0.775$, respectively) main effects, and lack of significant Exercise and Anoxia interaction effects ($F_{(1, 13)} = 0.139$, $p = 0.715$; $F_{(1, 13)} = 0.109$, $p = 0.746$, respectively).

Fig. 6 shows representative photomicrographs of MBP-immunopositive stained coronal sections of the right medial cortex for each of the groups, collected at P21. They clearly show that neonatal anoxia reduced myelination of M2, and that this effect is prevented by maternal spontaneous exercise. These effects were not seen both in M1 and S1 cortices.

Estimates of the number of NeuN-immunopositive cells

Fig. 7 shows estimates of the number of NeuN-immunopositive cells in the CA1 and CA2–3 hippocampal subfields, and DG, at P21 and P75.

Relative to the estimated number of NeuN-immunopositive cells in CA1 at P21 (Fig. 7A), ANOVA revealed a significant main effect for Exercise ($F_{(1,16)} = 5.046$; $p \leq 0.05$) and for Exercise versus Anoxia interaction effect ($F_{(1,16)} = 15.73$; $p \leq 0.01$), but not for Anoxia ($F(1,16) = 0.2030$; $p = 0.6583$). Post-hoc analysis indicates differences between NE-NA versus NE-A ($p=0.0300$) and NE-A versus E-A ($P = 0.0023$).

Relative to the estimated number of NeuN-immunopositive cells in CA2–3 at P21 (Fig. 7C), ANOVA revealed significant main Exercise ($F_{(1,16)} = 10.51$; $p \leq 0.01$) and Anoxia ($F_{(1,16)} = 5.698$; $p \leq 0.05$) effects, and a significant Exercise versus Anoxia ($F_{(1,16)} = 44.47$; $p \leq 0.01$) interaction effect. Similarly, relative to the estimated number of NeuN-immunopositive cells in DG at P21 (Fig. 7E), ANOVA revealed significant main Exercise ($F_{(1,16)} = 48.51$; $p \leq 0.0001$) and Anoxia ($F_{(1,16)} = 58.44$; $p \leq 0.0001$) effects, and a significant Exercise and Anoxia interaction effect ($F_{(1,16)} = 33.26$; $p \leq 0.0001$) (see relevant statistical comparisons in Fig. 7). Together, these figures indicate that anoxia

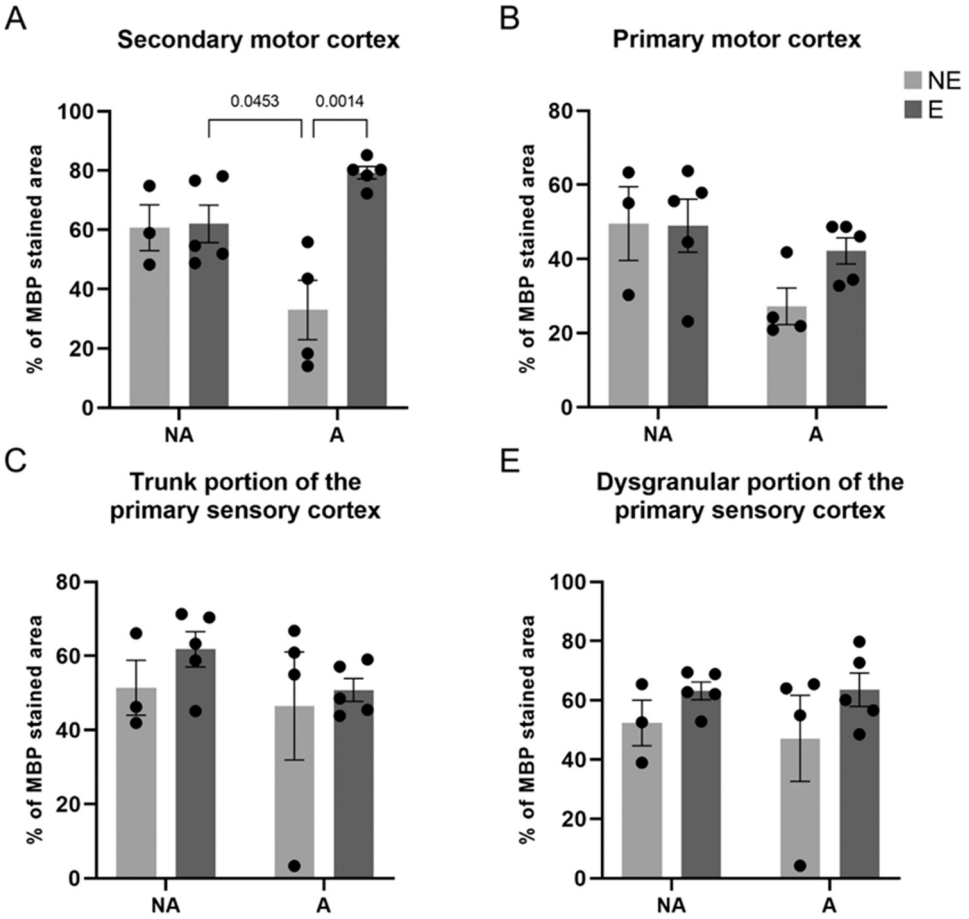

Fig. 5. Percentage of myelination in (A) secondary motor cortex, (B) primary motor cortex, (C) trunk portion of the primary sensory cortex and (D) dysgranular portion of the primary sensory cortex. Data were analyzed using two-way ANOVA. The Tukey-Kramer post-hoc test revealed differences between groups indicated by the p-values and horizontal bars. n = 5 in all groups. Data are presented as mean ± S.E.M.

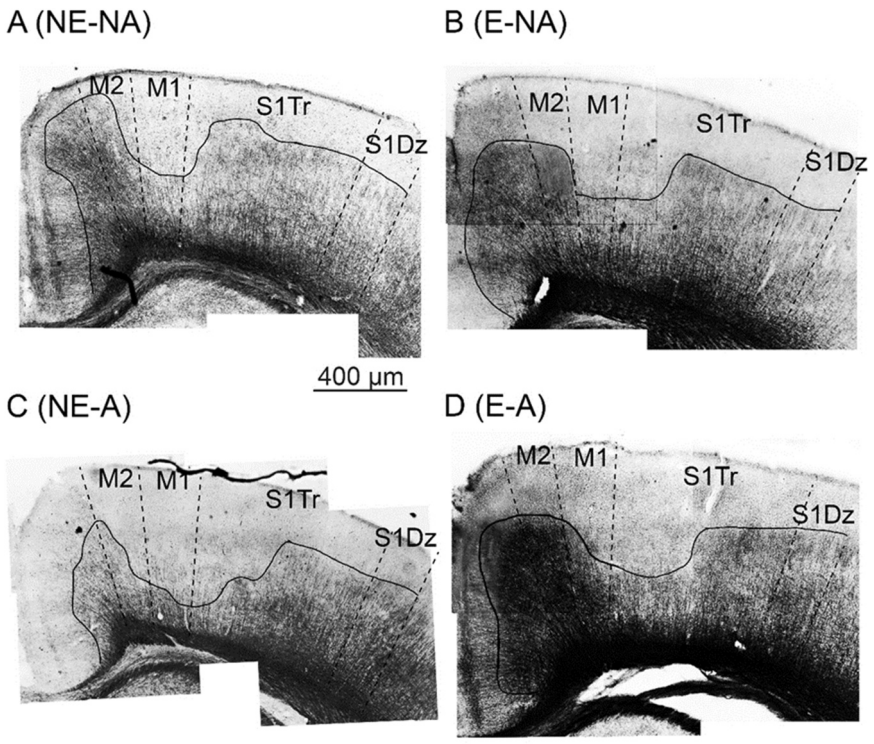

Fig. 6. Representative photomicrographs of MBP-immunopositive stained coronal sections of the right medial cortex at P21, including one exemplar of (A) NE-NA, (B) E-NA, (C) NE-A and (D) E-A groups. M2, Secondary motor cortex. M1, Primary motor cortex. S1Tr, Trunk portion of the primary sensory cortex. S1Dz, Dysgranular portion of the primary sensory cortex. The darkest areas delimited by the lines contain most of MBP-immunostained fibers.

reduced the number of NeuN-immunopositive cells and that this effect was prevented by gestational exercise.

Relative to the estimated number of NeuN-immunopositive cells in CA1 at P75 (Fig. 7B), ANOVA revealed significant main Exercise ($F_{(1,16)} = 22.57$; $p \leq 0.001$) and Anoxia ($F_{(1,16)} = 12.11$; $p \leq 0.01$) effects, and lack of a significant interaction effect for Exercise versus Anoxia ($F_{(1,16)} = 0.5728$; $p = 0.4601$). Similarly, relative to the estimated number of NeuN-immunopositive cells in CA2–3 at P75 (Fig. 7D), ANOVA revealed significant main effects for Exercise ($F_{(1,16)} = 30.53$; $p \leq 0.0001$) and Anoxia ($F_{(1,16)} = 7.811$; $p \leq 0.05$), but lack of significant interaction effects for Exercise versus Anoxia ($F_{(1,16)} = 1.398$; $p = 0.2543$). These figures indicate that while the mother's exercise increase the number of NeuN-immunopositive cells in the CA1 and CA2–3, anoxia decreases them. Relative to the estimated number of NeuN-immunopositive cells in DG (Fig. 7F), ANOVA revealed a significant main Anoxia effect ($F_{(1,16)} = 17.53$; $p \leq 0.001$) and significant Exercise versus Anoxia interaction effect ($F_{(1,16)} = 9.879$; $p \leq 0.01$), but lack of significant main Exercise effect ($F_{(1,16)} = 0.0044$; $p \leq 0.9474$) (see relevant statistical comparisons in Fig. 7). Together, these figures indicate that anoxia reduces the number of NeuN-immunopositive cells in dentate gyrus and that gestational exercise partially prevent this reduction.

Thus, results showed that neonatal anoxia induced a significant reduction in the number of neurons in the CA1 (Figs. 7A and 7B), in the CA2–3 (Figs. 7C and 7D) and DG (Figs. 7E and 7F), at both P21 and P75. Results also showed that spontaneous gestational exercise prevented anoxia-induced reduction in the number of neurons in the offspring, except for the DG at P75 (Fig. 7F).

Fig. 8 shows representative photomicrographs of coronal sections immuno-stained with antibody against NeuN-immunopositive cells at the level of the right dorsal hippocampal formation; each photomicrograph shows the image of one subject of each group, at P21 and P75.

Synapsin-I levels

Fig. 9 shows scores of optical density of synapsin-I/β-actin ratio in the hippocampal formation as a function of group, at P21 (Fig. 9A) and P75 (Fig. 9C), and representative electrophoresis bands of synapsin-I and β-actin in the hippocampal formation of each group, at P21 (Fig. 9B) and P75 (Fig. 9D).

ANOVA for synapsin-I/β-actin optical densities at P21 revealed a significant main Anoxia effect ($F_{1,16} = 5.930$, $p \leq 0.05$), but lack of significant main Exercise ($F_{1,16} = 0.8440$, $p = 0.371$) and Anoxia versus Exercise interaction ($F_{1,16} = 0.234$, $p = 0.6347$) effects (Fig. 9A and B). Post-hoc multiples' comparison test did not reveal any significant difference between specific pairs of groups. However, as can be seen in Fig. 7A, subjects exposed to anoxia exhibited reduced optical density for synapsin-I/β-actin ratio, in the hippocampal formation at P21, as compared to subjects not exposed to anoxia. Differently, at P75, ANOVA revealed lack of significant main Exercise ($F_{1,16} = 1.469$, $p = 0.2431$) and main Anoxia ($F_{1,16} = 0.006925$, $p = 0.9347$) effects, and lack of a significant Exercise versus Anoxia interaction effect ($F_{1,16} = 0.09022$, $p = 0.7678$) for synapsin-I/β-actin ratio (Fig. 9C).

These results indicate that subjects exposed to anoxia exhibited, at P21, a decreased synapsin-I expression and that this effect was not prevented by spontaneous gestational exercise (Fig. 9A). In contrast to the significant anoxia-induced reduction in the expression of synapsin-I at P21, at P75 there were no significant anoxia-induced differences, suggesting that synapsin-I levels returned to normal levels along development after P21 (Fig. 9C).

Discussion

The present study evaluated the potential preventive effects of spontaneous gestational exercise in a running wheel on the offspring's somatic and neurodevelopmental disturbs which follow PA, using a rodent model of neonatal anoxia that mimics the premature condition.

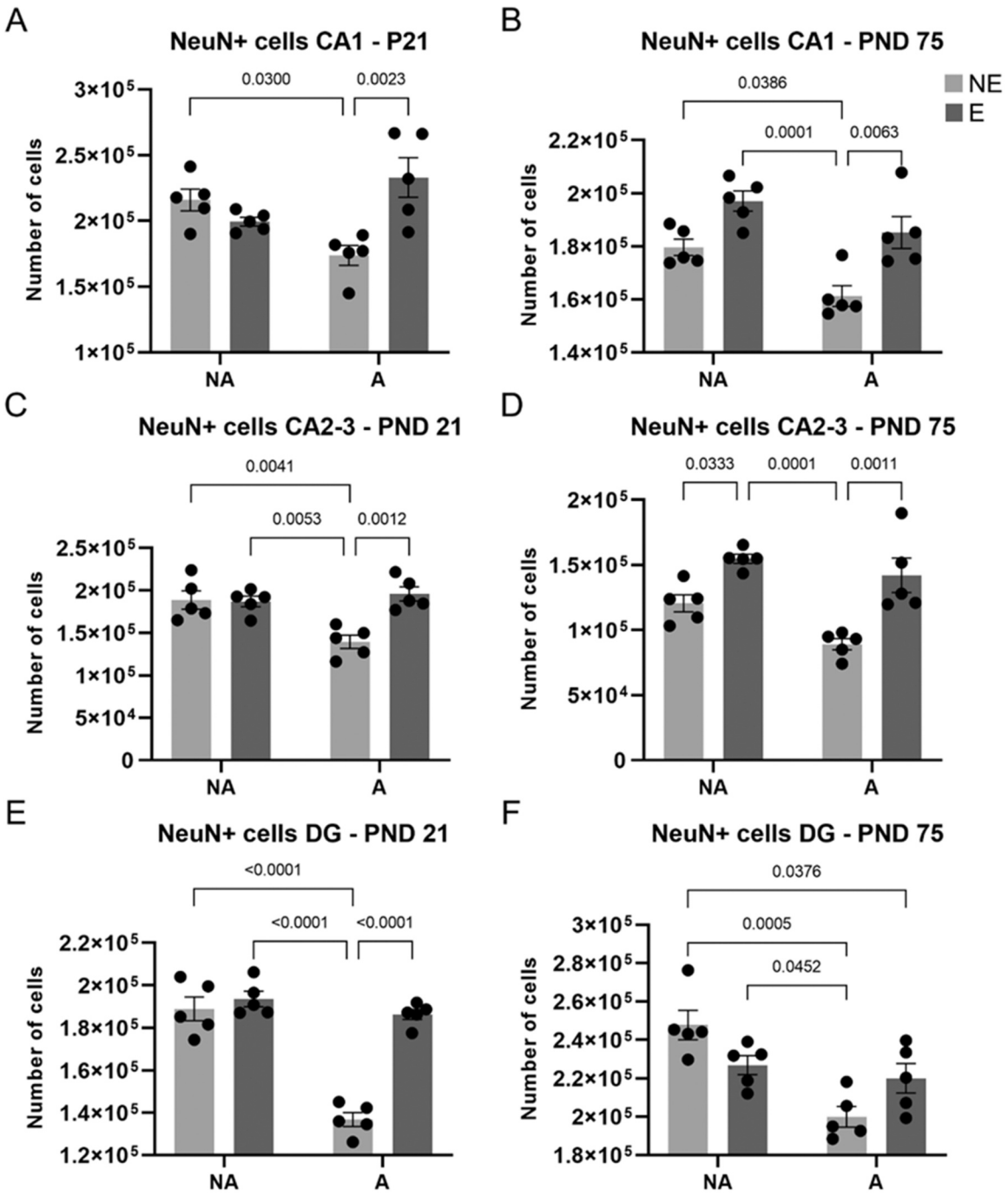

Fig. 7. Estimates of the number of NeuN-immunopositive cells in the CA1 at P21 (A) and P75 (B), in the CA2–3 at P21 (C) and P75 (D), and in the dentate gyrus at P21 (E) and P75 (F). Data were compared using ANOVA and Tukey-Kramer as post-hoc test. n = 5 in each group. * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$. Data are expressed as mean ± S.E.M.

Confirming previous observations of our group, neonatal anoxia induced several disturbs in somatic maturation (Kumar et al., 2017, 2019, 2022; Matsuda et al., 2021), expressed by smaller body length, smaller anteroposterior head axis, slower growth of mediolateral head axis, smaller body weight, later auditory conduit opening, later palmar grasp, later surface righting, later negative geotaxis, and earlier free-fall righting, with long-term effects on motor cortex myelination and hippocampal cell death.

These disturbs have also been reported by other laboratories employing different anoxia/hypoxia models (e.g., Dell'anna et al., 1991; Raff et al., 2001; Grojean et al., 2003; Ten et al., 2003; Fan et al., 2005; Lubics et al., 2005; Tang and Nakazawa, 2005; Chen et al., 2007; Yan et al., 2016; Menshanov et al., 2017).

Corroborating our working hypothesis, maternal spontaneous exercise during pregnancy prevented neonatal-anoxia-induced disturbs in their offspring, including (i) somatic and neurodevelopmental delays, (ii) reduction of cortical myelination in the secondary motor cortex and (iii) decreased NeuN-immunopositive cells in CA1 and CA2–3 hippocampal subfields, and in the DG.

All these alterations were prevented by allowing the pregnant females of the offsprings latter anoxic subjects to perform spontaneous running wheel exercise along 11 days (G6 to G16) during pregnancy. In this context, it seems interesting to note that not all neonatal-induced changes were prevented by spontaneous exercise; for instance, anoxia-

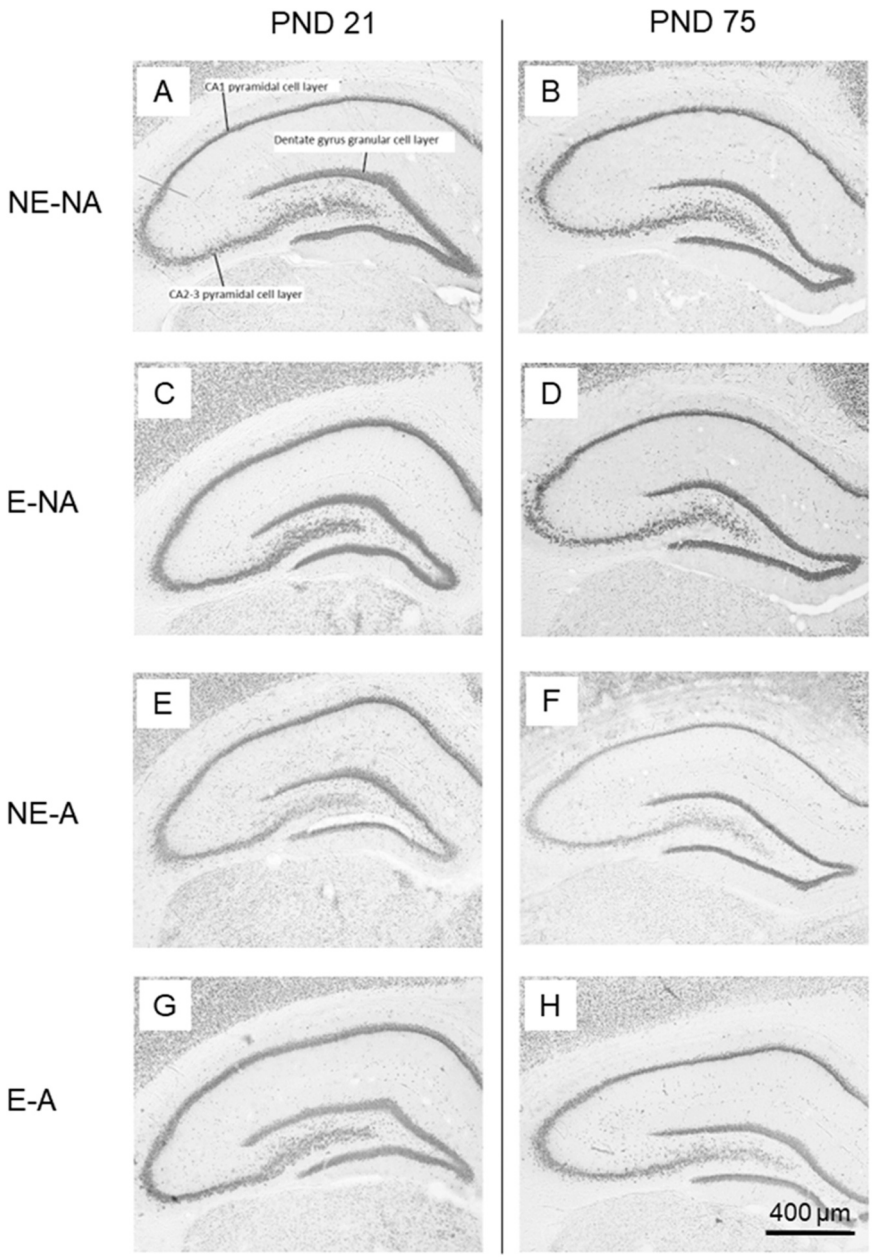

Fig. 8. Representative photomicrographs of coronal sections at the level of the right dorsal hippocampal formation immuno-stained with NeuN-antibody at P21 (Figures A, C, E and G) and P75 (B, D, F and H). Fig. 8A shows indications of the DG granule cell layer, CA2–3 pyramidal cell layer and CA1 pyramidal cell layer.

induced earlier pinna detachment was not prevented by the maternal spontaneous exercise. On the other hand, maternal spontaneous exercise, by itself, promoted earlier eye opening and earlier vibrissae placing of their offspring, as compared to the offspring of the dam not exposed to spontaneous exercise.

These wide range of neonatal-anoxia-induced effects on development seem to be related to changes in distinct brain processes at different brain regions. There have been reports that neonatal anoxia affects both the white and gray matters, at cortical and subcortical regions, including spinal cord, and nerves (Herrera-Marschitz et al., 2015; Marriott et al., 2017; Millar et al., 2017). There has also been reports that neonatal anoxia impairs blood-brain barrier permeability, induces energy failure, promotes loss of cellular ion homeostasis, increases acidosis, increases intracellular calcium, and thus excitotoxicity increases free radical-mediated toxicity, induces epigenetic dysregulation, and activates inflammatory cascades, thus inducing damage in the immature brain (Millar et al., 2017; Bustelo et al., 2020).

Moreover, it has already been described that neonatal anoxia leads to the downregulation/upregulation of neurotropic and growth factors in the earlier neonatal days and weeks, with an increased apoptotic activity that has a negative influence on neurodevelopmental outcomes (Lima et al., 2018; van Bel and Groenendaal, 2020). Subtle changes in the expression pattern of growth factors can modify neurodevelopment and somatic growth (Calamandrei and Alleva, 1989; Santucci et al., 1994; Futamura et al., 2003; Kiss et al., 2012; Chen et al., 2016; Aloe et al., 2019).

Consistent with our previous findings (Kumar et al., 2017), the data from the present study indicate that neonatal anoxia leads to increased weight gain and size of male pups during the lactation period. This observation aligns with clinical studies reporting higher serum levels of leptin and insulin in neonates suffering from HIE compared to healthy neonates, both at birth and in the early postnatal period (El-Mazary et al., 2015; Hagag et al., 2020). Considering the critical roles of leptin and insulin in growth and development, it is plausible to hypothesize

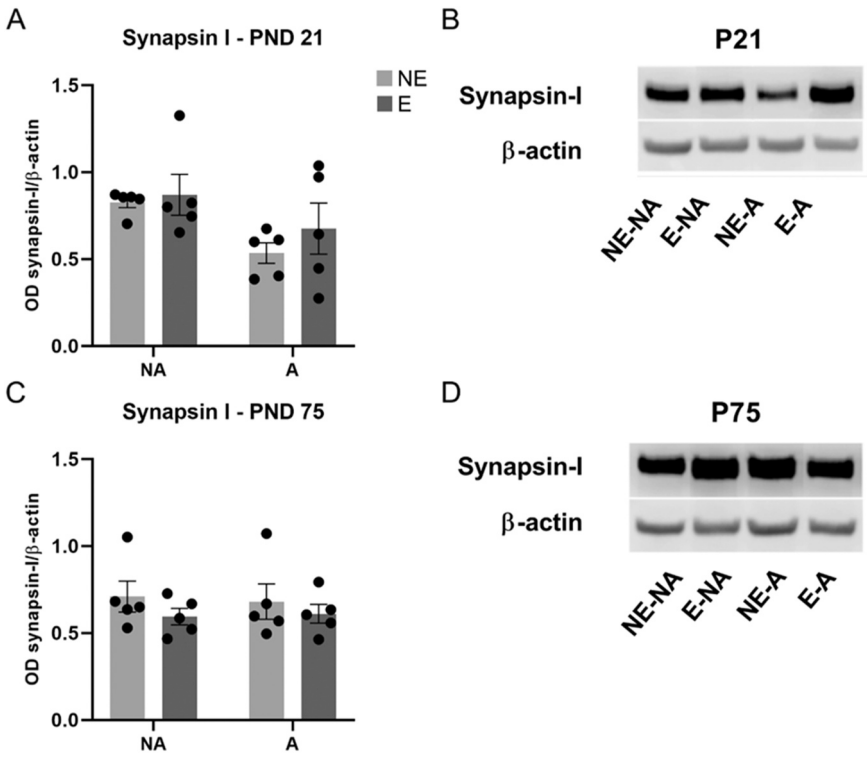

Fig. 9. Optical density of synapsin-I/β-actin ratio in the hippocampal formation at P21 (A) and P75 (C), and representative electrophoresis bands of synapsin-I and β-actin in the hippocampal formation at P21 (B) and P75 (D). Data were analysed using ANOVA followed by Tukey-Kramer post-hoc test. n = 4 in NE-NA and NE-A groups at P21 and P75, and n = 5 in E-NA and E-A groups, at P21 and P75. Data are expressed as mean ± S.E.M.

that the observed greater weight gain in subjects exposed to neonatal anoxia during the pre-weaning period is a consequence of dysregulation in these hormones and energy metabolism, accompanied by alterations in the development of hypothalamic neurocircuits involved in appetite regulation.

Insulin-like growth factors (IGFs) are key molecule involved in the control of body weight, somatic growth, and neurodevelopment (Stratikopoulos et al., 2008; Nieto-Estévez et al., 2016; Yoshida and Delafontaine, 2020). Previous studies have shown that the birth weight of IGFs-knockout mice is about 60 % of their wild-type littermates (DeChiara et al., 1990; Baker et al., 1993; Liu et al., 1993; Woods and Savage, 1996; Lupu et al., 2011). In contrast, over-expression of IGF-1 in mice increases the body weight by 30 % and is often associated with somatic overgrowth and increased lean mass (Morison and Reeve, 1998; Biagetti and Simó, 2021). Altered serum IGFs levels have been detected in newborns with HIE (Satar et al., 2004; Umran et al., 2016) and in HIE models (Radom-Aizik et al., 2013). IGF-I plays a vital role in controlling growth and metabolism. Reduced secretion of IGF-I can lead to growth restriction during early life and contribute to dysregulated energy metabolism, cardiovascular disease, and diabetes in adulthood (Yumani et al., 2015). Thus, it is plausible to hypothesize that neonatal anoxia may disrupt the expression of transcription factors, levels of insulin growth factors, or both, thereby contributing to the observed effects on growth and development.

There have been demonstrations that gestational exercise upregulates the placental IGF system and enhances the offspring's insulin sensitivity, improves glucose handling, increases lean mass, decreases fat mass and body weight as compared to offspring of sedentary mothers (Laker et al., 2014; Stanford et al., 2015; Quiclet et al., 2016, 2017; Mangwiro et al., 2018a; Kusuyama et al., 2020; Son et al., 2020; Zheng et al., 2020). Furthermore, clinical reports that aerobic exercise during pregnancy is associated with increased infant head circumference, birth weight, and birth length and contribute to the maintenance of metabolic health (Clark et al., 2019). Besides that, previous studies have shown that gestational exercise induced upregulation of growth factors in the serum and placenta during pregnancy (Son et al., 2019). The enhancement of placental and IGF signaling system could be one possible mechanism related to the beneficial effects of maternal physical activity on body weight, somatic growth, and physical development.

The results of the present study show that gestational exercise had no effect on offspring birth weight and increased body weight and somatic growth during the pre-weaning period, in relation to the offspring of non-exercised dams. The current data is consistent with previous reports (Santana Muniz et al., 2014; Fragoso et al., 2017) showing that maternal voluntary physical activity during gestation/lactation was able to alter the offspring's growth trajectory leading to increased indicators of somatic growth (laterolateral axis of skull, longitudinal axis, and tail length) and body weight gain during pre-weaning period. These results align with clinical reports that aerobic exercise during pregnancy is associated with increased infant head circumference, birth weight, and birth length and contributes to the maintenance of metabolic health (Clark et al., 2019).

The beneficial effects of gestational exercise against anoxia involve multiple pathways that impact the offspring's growth, development, and brain function. These mechanisms include alterations in maternal behavior, the direct transfer of neurochemicals across the placental or mammary barrier, and, importantly, epigenetic modifications and upregulating essential neurotrophins and growth factors, such as IGF-1, brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (BFGF) (Bhattacharjee et al., 2021;Mangwiro et al., 2018b; Che et al., 2022; Son et al., 2019; Niño Cruz et al., 2018; Fragoso et al., 2020).

Moreover, IGF-1 and BDNF play significant roles in regulating glucose and lipid metabolism, as well as energy homeostasis, and they also play a crucial role in postnatal growth (Colardo et al., 2021; Iu and Chan, 2022). Therefore, the upregulation of the placental insulin-like growth factor (IGF) and BDNF signaling system induced by maternal physical activity, can alter the offspring's insulin sensitivity, glucose

handling, body weight, somatic growth, and physical development. Taken together, the interplay of these mechanisms may be the underlying processes through which gestational exercise impacts offspring development and metabolism.

Regarding physical features maturation, neonatal anoxia and gestational exercise affected pinna detachment, auditory conduit opening and eye opening. In accordance with our previous studies (Kumar et al., 2017, 2019; Matsuda et al., 2021), neonatal anoxia delayed auditory conduit opening and promoted acceleration in the postnatal appearance of the pinna detachment. The maturation of physical features is controlled by several growth and transcription factors signalling pathways that could be disturbed by oxygen deprivation (Huang et al., 2004). It is likely that neonatal anoxia downregulates the expression of epidermal growth factor (EGF), alpha transforming growth factor (TGF)-α, and IGF that play critical roles in optimal epithelial and ear development.

Consistent with previous studies (Santana Muniz et al., 2014; Fragoso et al., 2017), data of the present study showed that spontaneous gestational exercise led to an earlier occurrence of the day of eye opening. There is ample evidence that gestational exercise upregulates cell growth-related genes and growth factor levels (Niño Cruz et al., 2018; Fragoso et al., 2020). IGF-1, nerve growth factor (NGF) and BDNF strongly affect visual system development (Huang et al., 1999; Hanif et al., 2015; Baroncelli et al., 2017; Mui et al., 2018; Zhang et al., 2019; Rajala et al., 2022), thus, earlier eye opening in exercised offspring could be a direct result of enhanced levels of these growth factors.

Besides the wide range neonatal-anoxia-induced effects on development already mentioned, developmental disruptions in 5-HT levels can influence brain development and the function. 5-HT network is among the earlier systems to develop in the brain (Oberlander, 2012; Brummelte et al., 2017). It modulates critical neurodevelopmental processes including neurogenesis, neuroapoptosis, dendritic refinement, cell migration and differentiation, and synaptic plasticity. Thus, it plays a critical modulation role in the development of reflexes, related to hyperinnervation of 5-HT in the primary sensory neocortex and α motor neurons (Gaspar et al., 2003; Whitaker-Azmitia, 2010; Labonte-Lemoyne et al., 2017; Lozano et al., 2021). In addition, 5-HT secreted by the gut and peripheral organs, plays endocrine roles. Both, central and peripheral 5-HT participate in regulation of energy metabolism. While central 5-HT, interacting with neuropeptides and other neurotransmitters, modulates appetite and energy metabolism, peripheral 5-HT interacts with liver, adipose tissues, pancreas, and muscle, leading to alterations of hormones and thus energy metabolism (Donovan and Tecott, 2013; Martin et al., 2017). There have been reports that neonatal anoxia leads to disruption of the serotonergic system (Carneiro et al., 2022). It seems possible that physical, somatic, and developmental changes could be related to modifications of the serotoninergic signalling induced by neonatal anoxia. Neonatal anoxia delayed the maturation of palmar grasp, surface righting, negative geotaxis, and earlier free-fall righting reflexes. Maternal spontaneous running during pregnancy prevented, either completely or partially, changes in the ontogenesis of the reflexes of the offspring.

During embryonic brain development, placenta is an important source of hormone and neurotransmitters, which is important to fetal brain neurodevelopment (Rosenfeld, 2020). 5-HT might be implicated in placental development and placental 5-HT is a transient source of this neurotransmitter for fetal brain (Rosenfeld, 2020). It also acts as a potent trophic factor during development and regulates neurodevelopmental processes (Narboux-Nême et al., 2013). It has been recognized that disturbances in placental and fetal 5-HT homeostasis impair neurodevelopment and can be related to metabolic disorders (Paquette et al., 2013). Therefore, it seems possible that gestational exercise prevents neonatal anoxia-induced neurodevelopmental delays by enhancing 5-HT expression in the placenta thereby manipulating fetal 5-HT levels.

Reflexes are sensorimotor responses of the central nervous system to either internal or external stimuli that are critical for survival in the extrauterine environment (Fox, 1965). These sensorimotor responses serve as indicators of brain development, and their maturation is intricately connected to processes such as neurogenesis, myelination, synapses, and neurotransmitter activity (Fox, 1965; Melo et al., 2019). That is, they indicate the maturation of sensory (including vestibular function), development of orientation and motor coordination, mediated by the cortex, cerebellum, basal ganglia, brain stem, and spinal cord, besides the skeletal muscle (Buller et al., 2012; Secher et al., 2006). Thus, it seems likely that neonatal anoxia insult promotes changes in maturation of these systems, thus reflecting on the appearance of these reflexes along development, by changes in the functional organization of the complex synaptic circuitries underlying these reflexes. Myelination of axons by oligodendrocytes is an important aspect of neural development. It is stimulated both by sensorial experience and by the passage of action potentials through the axon (Turner, 2019). There is growing consensus that myelin is not only critical for normal motor and sensory functions, but that it also contributes for functioning of brain regions underlying higher-order functions such as cognition, learning, and memory (Fletcher et al., 2018).

In fact, data of the present study showed that neonatal anoxia induced a significant decrement in MBP stained area in M2 and that spontaneous gestational exercise during pregnancy prevented this effect. M2 is a cortical associative region that receives afferents from various cortical and thalamic regions and sends efferences to regions associated with motor control including the striatum, caudate-putamen, and spinal cord (Barthas and Kwan, 2017). Previous studies have shown that white matter injuries (including oligodendrocyte) which follow hypoxia in rats are accompanied by increased activation of microglia and inflammation (Kaur et al., 2013; Li et al., 2017). HIE in rats reduces the volume of cerebral white matter; myelination is decreased, radial glia is disrupted, microglia is activated, and gliotic scares are created, inducing neurons to enter a state of cellular death and promoting sensory-motor impairment (Schneider and Miller, 2019; Jung et al., 2020; Shao et al., 2021). Sizonenko et al. (2003) showed that neonatal rats exposed to hypoxia and ischemia at P3 exhibited reduction of myelination in cortical areas that comprise M1 and M2 as evaluated at P21. In the present study, involving exposure to anoxia at P1 and evaluation at P21, it was shown greater damage at M2, as compared to M1, suggesting greater vulnerability of M2 when using this anoxia model.

Even though research focusing on specific effects of gestational exercise during pregnancy on myelination of M1 and M2 areas of the offspring are scarce, it is assumed that exercise-induced enhancement of neurotrophic factors can contribute for neuroprotection and myelination in cortical in hypoxic states (Gavrish et al., 2022). Neurotrophic factors regulate proliferation, migration, differentiation of oligodendrocyte precursor cells and myelination (Park et al., 2001; Assanah et al., 2009; Bergles and Richardson, 2016). There have been reports that adult mice that spontaneously exercise in running wheels exhibit increments in myelination in the motor cortex; exercise also promotes oligodendrogenesis in vitro (Alvarez-Saavedra et al., 2016; Zheng et al., 2019). Similarly, treadmill exercise by young mice leads to enhancement in Wnt/β-catenin signaling pathways and stimulates neurogenesis and myelin repair in the brain of juvenile rats after cerebral ischemia/reperfusion (Cheng et al., 2020). It seems possible that gestational exercise-induced enhancement of postnatal neurogenesis, synaptic plasticity, and myelination mediated by neurotrophic factors, plays a crucial role in protecting the immature brain against neonatal anoxia.

In this context, previous research has indicated that maternal spontaneous wheel running promotes hippocampal cell proliferation, neuronal differentiation, and cognitive functions in offspring (Bick-Sander et al., 2006; Toda et al., 2019; Yau et al., 2019; Kim et al., 2021). It is plausible to hypothesize that besides increasing the number of hippocampal cells in the offspring, gestational exercise also contributes to preventing anoxia-induced impairments. Neonatal anoxia in rats

has been associated with reduced numbers of Nissl-stained cells in hippocampal subfields and the granule cell layer at various stages of development (Takada et al., 2016; Kumar et al., 2017). This study's findings support the hypothesis, as maternal spontaneous exercise during pregnancy prevented anoxia-induced hippocampal cell loss in rats, suggesting that factors exchanged between the fetus and mother during early life intrauterine environment may promote long-lasting neuroplasticity in the offspring and protect against hippocampal cell loss after neonatal anoxia (Akhavan et al., 2013). The results further extend our understanding by demonstrating that maternal physical activity can also reduce hippocampal cell loss induced by neonatal anoxia.

In fact, previous studies from our group showed that neonatal anoxia in rats reduces the number of Nissl-stained cells at P41 both in the CA1 and CA2–3 dorsal hippocampal subfields and in the dorsal DG (Takada et al., 2016; Kumar et al., 2017). It also reduced the number of NeuN/Bromodeoxyuridine (BrdU) labeled cells in the dorsal DG, at P60 (Takada et al., 2016; Kumar et al., 2017). The present study showed that neonatal anoxia in rats reduces NeuN-immunopositive cells in the CA1 and CA2–3 hippocampal subfields, and in DG granule cell layer at both P21 and P75. Confirming the above-mentioned hypothesis, these anoxia-induced effects were prevented by maternal spontaneous exercise during pregnancy, suggesting that early life intrauterine environment and/or fetus-mother exchange of factors may promote long-lasting neuroplasticity in the offspring, as well as to prevent hippocampal cell loss following neonatal anoxia. Akhavan et al. (2013) also showed that gestational exercise during pregnancy prevents neonatal-hypoxia-ischemia-induced decrease in the number of hippocampal neurons in offspring, in rats. Results of the present study extended these findings by showing that maternal physical activity also reduced hippocampal cell loss induced by neonatal anoxia.

Synapsin-I, primarily found in the inner surface of axon terminals, bounds neurotransmitter vesicles to the cytoskeleton and regulates the release of neurotransmitters, thus playing an important role in neuronal development, influencing the formation of neurites, axons, and synapses (Fornasiero et al., 2010). Data of the present study showed that neonatal anoxia reduces synapsin-I levels at P21 but not at P75, thus suggesting that there may be important connectivity limitations early in life following neonatal anoxia and that the brain in this condition finds ways to compensate for production of this molecule along development.

It seems likely that the initial neuroinflammation induced by neonatal anoxia could strongly influence early synaptic plasticity and myelination. Microglia plays a role in immunosurveillance and, also, regulates synaptic formation, shaping synaptic contacts and adult neurogenesis (Salter and Stevens, 2017). Moreover, activated microglia is one of the main sources of IGF-1 in the brain (Wlodarczyk et al., 2014), a gene related to important neurodevelopmental processes, such as myelination and neurogenesis. The depletion of $CD11c^{+}$ microglial IGF-1 leads to impairment of primary myelination (Wlodarczyk et al., 2017). Anoxia-induced oxidative stress induces neuroinflammation, demyelination, microglial activation, and neuronal death (Peng et al., 2022). Although not statistically significant, gestational exercise seems to have partially prevented the reduction of synapsin-I expression at P21. Therefore, it is possible that gestational exercise could contribute for regulation of synaptic plasticity by inhibiting overreactive neuroinflammation, increasing neurotrophins, and by transcriptional regulation of synaptic-plasticity-related essential genes.

In conclusion, results of this study show that neonatal anoxia delayed both physical maturation and neurobehavioral development. Neonatal anoxia also induced both long-term disturbs in motor cortex myelination and hippocampal cell death. Maternal spontaneous exercise during pregnancy prevented neonatal-anoxia-induced physical and neurodevelopmental changes. Spontaneous wheel running during pregnancy also prevented reduction of MBP stained area in the secondary motor cortex, as well as the synapsin-I levels at P21, and decreased NeuN-immunopositive cells in the hippocampus. One limitation of the present research is that we excluded the female pups, due to previous sexually dimorphic results presented in other studies of the group, showing the increased severity of sequelae in male rats (Kumar et al., 2017; Arruda et al., 2024). Further studies using both male and female rats are necessary, as well as a deep investigation of their different mechanisms of injury and commitment.

Spontaneous gestational exercise poses an opportunity for preventing neonatal-hypoxia-induced neural disruption of the offspring, thus providing a promising preventive approach to minimize both neurological and cognitive disturbs that usually follow hypoxia-ischemia episodes. The specific mechanisms underlying these maternal-exercise-induced beneficial effects on the offspring development require additional investigations.

Author contributions

Conception and design of the study, V.Y.L., S.H.T., A.V.M., L.C.M.T., G.F.X., M.I.N.; Acquisition and analysis of data, V.Y.L., A.V.M.N., L.C.M.T.; Drafting a significant portion of the manuscript or figures, V.Y.L., S.H.T., B.P.A., A.V.M., L.C.M.T., G.F.X., M.I.N.

CRediT authorship contribution statement

Silvia Honda Takada: Writing – review & editing, Supervision, Project administration, Funding acquisition. **Vitor Yonamine Lee:** Writing – original draft, Methodology, Data curation, Conceptualization. **Aline Vilar Machado Nils:** Writing – original draft, Methodology, Formal analysis, Data curation. **Lívia Clemente Motta Teixeira:** Writing – review & editing, Project administration, Formal analysis, Conceptualization. **Maria Inês Nogueira:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Gilberto Fernando Xavier:** Writing – review & editing, Formal analysis, Conceptualization. **Bruna Petrucelli Arruda:** Visualization, Methodology, Formal analysis, Data curation.

Ethical Statement

I have read and have abided by the statement of ethical standards for manuscripts submitted to Neuroscience.

Funding

This study was supported by grants of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil CAPES/DS [grant number 1128123] and of the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) [grant numbers 2010/01488-9, 2011/19747-3, 2011/03960-0, 2019/17239-2, 2020/16268-6 and 2023/14779-1].

Conflicts of interest

Nothing to report.

Acknowledgements

The authors thank Prof. Luiz Roberto G. de Britto and the technician Adilson da Silva Alves, for allowing the use of the western blot equipment. We thank Prof. Jackson Cioni Bittencourt and his team for allowing the use of stereological equipment. The authors especially acknowledge the laboratory technician Kelly Patricia Borges and Rosana Prisco for help with statistics.

References

Akhavan, M.M., Foroutan, T., Safari, M., Sadighi-Moghaddam, B., Emami-Abarghoie, M., Rashidy-Pour, A., 2012. Prenatal exposure to maternal voluntary exercise during pregnancy provides protection against mild chronic postnatal hypoxia in rat offspring. Pak. J. Pharm. Sci. 25, 233–238.

Akhavan, M.M., Miladi-Gorji, H., Emami-Abarghoie, M., Safari, M., Sadighi-Moghaddam, B., Vafaei, A.A., Rashidy-Pour, A., 2013. Maternal voluntary exercise during pregnancy enhances the spatial learning acquisition but not the retention of memory in rat pups via a TrkB-mediated Mechanism, the role of hippocampal BDNF Expression. Iran. J. Basic Med. Sci. 16, 955–961.
Aloe, L., Alleva, E., Ricceri, L., 2019. Mast Cells, NGF and neurobehavioural regulations in developing and adult mice. In: Husband, A.J. (Ed.), Psychoimmunology CNS-Immune Interactions, 1st ed. CRC Press, New York, pp. 51–64.
Alvarez-Saavedra, M., De Repentigny, Y., Yang, D., O'Meara, R.W., Yan, K., Hashem, L. E., Racacho, L., Ioshikhes, I., Bulman DE, Parks, R.J., Kothary, R., Picketts, D.J., 2016. Voluntary running triggers VGF-mediated oligodendrogenesis to prolong the lifespan of Snf2h-null ataxic mice. Cell Rep. 17, 862–875.
Assanah, M.C., Bruce, J.N., Suzuki, S.O., Chen, A., Goldman, J.E., Canoll, P., 2009. PDGF stimulates the massive expansion of glial progenitors in the neonatal forebrain. Glia 57, 1835–1847.
Baker, J., Liu, J.P., Robertson, E.J., Efstratiadis, A., 1993. Role of insulin-like growth factors in embryonic and postnatal growth. Cell 75, 73–82.
Baroncelli, L., Cenni, M.C., Melani, R., Deidda, G., Landi, S., Narducci, R., Cancedda, L., Maffei, L., Berardi, N., 2017. Early IGF-1 primes visual cortex maturation and accelerates developmental switch between NKCC1 and KCC2 chloride transporters in enriched animals. Neuropharmacology 113, 167–177.
Barthas, F., Kwan, A.C., 2017. Secondary motor cortex, where 'sensory' meets 'motor' in the rodent frontal cortex. Trends Neurosci. 40, 181–193.
van Bel, F., Groenendaal, F., 2020. Birth asphyxia-induced brain damage, the long road to optimal reduction and prevention! Pediatr. Med. 3.
Bergles, D.E., Richardson, W.D., 2016. Oligodendrocyte development and plasticity. Cold Spring Harb. Perspect. Biol. 8, a020453.
Bhattacharjee, J., Mohammad, S., Adamo, K.B., 2021. Does exercise during pregnancy impact organs or structures of the maternal-fetal interface? Tissue Cell 72, 101543.
Biagetti, B., Simó, R., 2021. GH/IGF-1 abnormalities and muscle impairment, from basic research to clinical practice. Int. J. Mol. Sci. 22, 415.
Bick-Sander, A., Steiner, B., Wolf, S.A., Babu, H., Kempermann, G., 2006. Running in pregnancy transiently increases postnatal hippocampal neurogenesis in the offspring. Proc. Natl. Acad. Sci. USA 103, 3852–3857.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Brummelte, S., Mc Glanaghy, E., Bonnin, A., Oberlander, T.F., 2017. Developmental changes in serotonin signaling, Implications for early brain function, behavior and adaptation. NeuroSci 342, 212–231.
Bustelo, M., Barkhuizen, M., van den Hove, D.L.A., Steinbusch, H.W.M., Bruno, M.A., Loidl, C.F., Gavilanes, A.W.D., 2020. Clinical implications of epigenetic dysregulation in perinatal hypoxic-ischemic brain damage. Front. Neurol. 11, 483.
Calamandrei, G., Alleva, E., 1989. Epidermal growth factor has both growth-promoting and growth-inhibiting effects on physical and neurobehavioral development of neonatal mice. Brain Res. 477, 1–6.
Campos, R.M.M., Toscano, A.E., Gouveia, H.J.C.B., Lacerda, D.C., Pereira, S. da C., Paz, I. A.A. de S.G., Dantas Alves, J.S., Manhães-de-Castro, R., 2021. Neonatal fluoxetine exposure delays reflex ontogeny, somatic development and food intake similarly in male and female rats. Can. J. Physiol. Pharmacol. 99, 490–498.
Carneiro, I.B.C., Toscano, A.E., da Cunha, M., de, S.B., Lacerda, D.C., Pontes, P.B., de Castro, R.M., de Jesus Deiró, T.C.B., Medeiros, J.M.B., 2022. Serotonergic mechanisms associated with experimental models of hypoxia, A systematic review. Int. J. Dev. Neurosci. 82, 668–680.
Chen, X.Q., Dong, J., Niu, C.Y., Fan, J.M., Du, J.Z., 2007. Effects of hypoxia on glucose, insulin, glucagon, and modulation by corticotropin-releasing factor receptor type 1 in the rat. Endocrinol 148, 3271–3278.
Chen, J., Zeng, F., Forrester, S.J., Eguchi, S., Zhang, M.-Z., Harris, R.C., M-z, Z., 2016. Expression and function of the epidermal growth factor receptor in physiology and disease. Physiol. Rev. 96, 1025–1069.
Cheng, J., Shen, W., Jin, L., Pan, J., Zhou, Y., Pan, G., Xie, Q., Hu, Q., Wu, S., Zhang, H., Chen, X., 2020. Treadmill exercise promotes neurogenesis and myelin repair via upregulating Wnt/β-catenin signaling pathways in the juvenile brain following focal cerebral ischemia/reperfusion. Int. J. Mol. Med. 45, 1447–1463.
Choi, J.-H., Kim, T.-S., Park, J.-K., Sim, Y.-J., Kim, K., Lee, S.-J., 2013. Short-term treadmill exercise preserves sensory-motor function through inhibiting apoptosis in the hippocampus of hypoxic ischemia injury rat pups. J. Exerc. Rehabil. 9, 457–462.
Clark, E., Isler, C., Strickland, D., McMillan, A.G., Fang, X., Kuehn, D., Ravisankar, S., Strom, C., May, L.E., 2019. Influence of aerobic exercise on maternal lipid levels and offspring morphometrics. Int. J. Obes. 43, 594–602.
Colardo, M., Martella, N., Pensabene, D., Siteni, S., Bartolomeo, S.D., Pallottini, V., Segatto, M., 2021. Neurotrophins as key regulators of cell metabolism, implications for cholesterol homeostasis. Int. J. Mol. Sci. 22 (11), 5692.
Cruz-Ochoa, N.A., Ochoa-Amaya, J.E., Pulecio, S.L., Xavier, G.F., Nogueira, M.I., 2019. Neonatal anoxia impairs long-term energy metabolism and somatic development of Wistar rats. Int. J. Dev. Neurosci. 79, 76–85.
Dayi, A., Agilkaya, S., Ozbal, S., Cetin, F., Aksu, I., Gencoglu, C., Cingoz, S., Pekcetin, C., Tugyan, K., Kayatekin, B.M., Uysal, N., 2012. Maternal aerobic exercise during pregnancy can increase spatial learning by affecting leptin expression on offspring's early and late period in life depending on gender. Sci. World J. 2012, 429803.
DeChiara, T.M., Efstratiadis, A., Robertsen, E.J., 1990. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature 345, 78–80.
Dell'anna, M.E., Calzolari, S., Molinari, M., Iuvone, L., Calimici, R., 1991. Neonatal anoxia induces transitory hyperactivity, permanent spatial memory deficits and CA1 cell density reduction in developing rats. Behav. Brain Res. 45, 125–134.
Donovan, M.H., Tecott, L.H., 2013. Serotonin and the regulation of mammalian energy balance. Front. Neurosci. 7.
El-Mazary, A.A.M., Nasif, K.A., Abdel-Hakeem, G.L., Sherif, T., Farouk, E., El-Gezawy, E. M., 2015. Adiponectin, leptin and insulin levels at birth and in early postnatal life in neonates with hypoxic ischemic encephalopathy. J. Diabetes Metab. Disord. 14, 87.
Fan, L.W., Lin, S., Pang, Y., Lei, M., Zhang, F., Rhodes, P.G., Cai, Z., 2005. Hypoxia-ischemia induced neurological dysfunction and brain injury in the neonatal rat. Behav. Brain Res. 165, 80–90.
Ferrari, N., Bae-Gartz, I., Bauer, C., Janoschek, R., Koxholt, I., Mahabir, E., Appel, S., Alejandre Alcazar, M.A., Grossmann, N., Vohlen, C., Brockmeier, K., Dötsch, J., Hucklenbruch-Rother, E., Graf, C., 2018. Exercise during pregnancy and its impact on mothers and offspring in humans and mice. J. Dev. Orig. Health Dis. 9, 63–76.
Fletcher, J.L., Murray, S.S., Xiao, J., 2018. Brain-derived neurotrophic factor in central nervous system myelination, A new mechanism to promote myelin plasticity and repair. Int. J. Mol. Sci. 19, 4131.
Fornasiero, E.F., Bonanomi, D., Benfenati, F., Valtorta, F., 2010. The role of synapsins in neuronal development. Cell Mol. Life Sci. 67, 1383–1396.
Fox, W.M., 1965. Reflex-ontogeny and behavioural development of the mouse. Anim. Behav. 13, 234–IN5.
Fragoso, J., Carvalho Jurema Santos, G., Thomaz da Silva, H., Oliveira Nogueira, V., Loizon, E., Vidal, H., Costa-Silva, J.H., da Silva Aragão, R., Pirola, L., Leandro, C.G., 2020. Maternal physical activity-induced adaptive transcriptional response in brain and placenta of mothers and rat offspring. J. Dev. Orig. Health Dis. 11, 108–117.
Fragoso, J., Lira, A., de, O., Chagas, G.S., Lucena Cavalcanti, C.C., Beserra, R., de Santana-Muniz, G., Bento-Santos, A., Martins, G., Pirola, L., da Silva Aragão, R., Leandro, C.G., 2017. Maternal voluntary physical activity attenuates delayed neurodevelopment in malnourished rats. Exp. Physiol. 102, 1486–1499.
Futamura, T., Kakita, A., Tohmi, M., Sotoyama, H., Takahashi, H., Nawa, H., 2003. Neonatal perturbation of neurotrophic signaling results in abnormal sensorimotor gating and social interaction in adults, implication for epidermal growth factor in cognitive development. Mol. Psychiatry 8, 19–29.
Gaspar, P., Cases, O., Maroteaux, L., 2003. The developmental role of serotonin, news from mouse molecular genetics. Nat. Rev. Neurosci. 4, 1002–1012.
Gavrish, M.S., Urazov, M.D., Mishchenko, T.A., Turubanova, V.D., Epifanova, E.A., Krut', V.G., Babaev, A.A., Vedunova, M.V., Mitroshina, E.V., 2022. Overexpression of Brain- and Glial Cell Line-Derived Neurotrophic Factors Is Neuroprotective in an Animal Model of Acute Hypobaric Hypoxia. Int. J. Mol. Sci. 23, 9733.
Gomes da Silva, S., Arida, R.M., 2015. Physical activity and brain development. Expert Rev. Neurother. 15, 1041–1051.
Gorgij, E., Fanaei, H., Yaghmaei, P., Shahraki, M.R., Mirahmadi, H., 2021. Treadmill exercise during pregnancy decreased vulnerability to neonatal hypoxia-ischemia through reducing inflammation and increasing antiapoptotic gene expressions and antioxidant capacity in rats. Stroke Res. Treat. 2021, 1–10.
Greco, P., Nencini, G., Piva, I., Scioscia, M., Volta, C.A., Spadaro, S., Neri, M., Bonaccorsi, G., Greco, F., Cocco, I., Sorrentino, F., D'Antonio, F., Nappi, L., 2020. Pathophysiology of hypoxic-ischemic encephalopathy, a review of the past and a view on the future. Acta Neurol. 120 (2), 277–288.
Grojean, S., Schroeder, H., Pourié, G., Charriaut-Marlangue, C., Koziel, V., Desor, D., Vert, P., Daval, J.-L., 2003. Histopathological alterations and functional brain deficits after transient hypoxia in the newborn rat pup, a long term follow-up. Neurobiol. Dis. 14, 265–278.
Hagag, A.A., El Frargy, M.S., Abd El-Latif, A.E., 2020. Study of cord blood erythropoietin, leptin and adiponectin levels in neonates with hypoxic ischemic encephalopathy. Endocr. Metab. Immune Disord. Drug Targets 20, 213–220.
Hakobyan, M., Dijkman, K.P., Laroche, S., Naulaers, G., Rijken, M., Steiner, K., van Straaten, H.L.M., Swarte, R.M.C., ter Horst, H.J., Zecic, A., Zonnenberg, I.A., Groenendaal, F., 2019. Outcome of infants with therapeutic hypothermia after perinatal asphyxia and early-onset sepsis. Neonatology 115, 127–133.
Hanif, A.M., Lawson, E.C., Prunty, M., Gogniat, M., Aung, M.H., Chakraborty, R., Boatright, J.H., Pardue, M.T., 2015. Neuroprotective effects of voluntary exercise in an inherited retinal degeneration mouse model. Invest Ophthalmol. Vis. Sci. 56, 6839.
Helou, A.Y., Martins, D.O., Arruda, B.P., Souza, M.C., Cruz-Ochoa, N.A., Nogueira, M.I., Chacur, M., 2021. Neonatal anoxia increases nociceptive response in rats, Sex differences and lumbar spinal cord and insula alterations. Int. J. Dev. Neurosci. 81, 686–697.
Helou, A.Y., Ochoa, N.A.C., Felicio, L.F., Nogueira, M.I., 2022. Neonatal anoxia impacts rats' maternal behavior and offspring development due to ultrasonic vocalization's impairment. Dev. Neurosci. 44, 629–642.
Herrera-Marschitz, M., Neira-Peña, T., Leyton, L., Gebicke-Haerter, P., Rojas-Mancilla, E., Morales, P., Bustamante, D., 2015. Short- and long-term consequences of perinatal asphyxia, looking for neuroprotective strategies. Adv. Neurobiol. 169–198.
Huang, Z.J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M.F., Maffei, L., Tonegawa, S., 1999. BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98, 739–755.
Huang, S. -T.J., Vo, K.C.T., Lyell, D.J., Faessen, G.H., Tulac, S., Tibshirani, R., Giaccia, A. J., Giudice, L.C., 2004. Developmental response to hypoxia. FASEB J. 18, 1348–1365.
Iu, E.C.Y., Chan, C.B., 2022. Is brain-derived neurotrophic factor a metabolic hormone in peripheral tissues? Biology 11 (7), 1063.
Kaur, C., Rathnasamy, G., Ling, E.-A., 2013. Roles of Activated Microglia in Hypoxia Induced Neuroinflammation in the Developing Brain and the Retina. J. Neuroimmune Pharmacol. 8, 66–78.
Kim, T.-W., Ko, Y.J., Youn, K.-H., Hwang, B.-G., Bang, H.-S., Lee, S.-J., 2021. Treadmill exercise improves spatial learning ability by increasing cell proliferation in offspring

born to maternal rats receiving stress during pregnancy. J. Exerc. Rehabil. 17, 88–95.
Kim, H., Lee, S., Kim, S., Yoo, J., Kim, C., 2007. The influence of maternal treadmill running during pregnancy on short-term memory and hippocampal cell survival in rat pups. Int. J. Dev. Neurosci. 25, 243–249.
Kiss, A.C.I., Woodside, B., Felício, L.F., Anselmo-Franci, J., Damasceno, D.C., 2012. Impact of maternal mild hyperglycemia on maternal care and offspring development and behavior of Wistar rats. Physiol. Behav. 107, 292–300.
Kumar, A.J., Helou, A.Y., Petrucelli, B.A., Xavier, G.F., Martins, D.O., Chacur, M., Nogueira, M.I., 2022. Sensorimotor development of male and female rats subjected to neonatal anoxia. Dev. Psychobiol. 64, e22291.
Kumar, A.J., Motta-Teixeira, L.C., Takada, S.H., Yonamine-Lee, V., Machado-Nils, A.V., Xavier, G.F., Nogueira, M.I., 2019. Behavioral, cognitive and histological changes following neonatal anoxia, Male and female rats' differences at adolescent age. Int. J. Dev. Neurosci. 73, 50–58.
Kumar, A.J., Takada, S.H., Motta-Teixeira, L.C., Lee, V.Y., Xavier, G.F., Nogueira, M.I., 2017. Sex differences in somatic and sensory motor development after neonatal anoxia in Wistar rats. Behav. Brain Res. 333, 242–250.
Kusuyama, J., Alves-Wagner, A.B., Makarewicz, N.S., Goodyear, L.J., 2020. Effects of maternal and paternal exercise on offspring metabolism. Nat. Metab. 2, 858–872.
Labonte-Lemoyne, E., Curnier, D., Ellemberg, D., 2017. Exercise during pregnancy enhances cerebral maturation in the newborn, A randomized controlled trial. J. Clin. Exp. Neuropsychol. 39, 347–354.
Laker, R.C., Lillard, T.S., Okutsu, M., Zhang, M., Hoehn, K.L., Connelly, J.J., Yan, Z., 2014. Exercise prevents maternal high-fat diet–induced hypermethylation of the *Pgc-1α* gene and age-dependent metabolic dysfunction in the offspring. Diabetes 63, 1605–1611.
Lee, H.-H., Kim, H., Lee, J.-W., Kim, Y.-S., Yang, H.-Y., Chang, H.-K., Lee, T.-H., Shin, M.-C., Lee, M.-H., Shin, M.-S., Park, S., Baek, S., Kim, C.-J., 2006. Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups. Brain Dev. 28, 147–154.
Li, B., Concepcion, K., Meng, X., Zhang, L., 2017. Brain-immune interactions in perinatal hypoxic-ischemic brain injury. Prog. Neurobiol. 159, 50–68.
Lima, J.P.M., Rayêe, D., Silva-Rodrigues, T., Pereira, P.R.P., Mendonca, A.P.M., Rodrigues-Ferreira, C., Szczupak, D., Fonseca, A., Oliveira, M.F., Lima, F.R.S., Lent, R., Galina, A., Uziel, D., 2018. Perinatal asphyxia and brain development, mitochondrial damage without anatomical or cellular losses. Mol. Neurobiol. 55, 8668–8679.
Liu, J.P., Baker, J., Perkins, A.S., Robertson, E.J., Efstratiadis, A., 1993. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75, 59–72.
Lozano, A.F.Q., Moura, M.S., Tavares, B.M., Kempinas, W.D.G., 2021. Exposure of pregnant rats to stress and/or sertraline, Side effects on maternal health and neurobehavioral development of male offspring. Life Sci. 285, 119960.
Lubics, A., Reglodi, D., Tamás, A., Kiss, P., Szalai, M., Szalontay, L., Lengvári, I., 2005. Neurological reflexes and early motor behavior in rats subjected to neonatal hypoxic-ischemic injury. Behav. Brain Res. 157, 157–165.
Lupu, M., Khalil, M., Iordache, F., Andrei, E., Pfannkuche, K., Spitkovsky, D., Baumgartner, S., Rubach, M., AbdelRazik, H., Buzila, C., Brockmeier, K., Simionescu, M., Hescheler, J., Maniu, H., 2011. Direct contact of umbilical cord blood endothelial progenitors with living cardiac tissue is a requirement for vascular tube-like structures formation. J. Cell Mol. Med. 15, 1914–1926.
Mangwiro, Y.T.M., Cuffe, J.S.M., Briffa, J.F., Mahizir, D., Anevska, K., Jefferies, A.J., Hosseini, S., Romano, T., Moritz, K.M., Wlodek, M.E., 2018a. Maternal exercise in rats upregulates the placental insulin-like growth factor system with diet- and sex-specific responses, minimal effects in mothers born growth restricted. J. Physiol. 596, 5947–5964.
Mangwiro, Y.T.M., Cuffe, J.S.M., Briffa, J.F., Mahizir, D., Anevska, K., Jefferies, A.J., Hosseini, S., Romano, T., Moritz, K.M., Wlodek, M.E., 2018b. Maternal exercise in rats upregulates the placental insulin-like growth factor system with diet- and sex-specific responses, minimal effects in mothers born growth restricted. J. Physiol. 596, 5947–5964.
Marcondes, F.K., Bianchi, F.J., Tanno, A.P., 2002. Determination of the estrous cycle phases of rats, some helpful considerations. Braz. J. Biol. 62, 609–614.
Marriott, A.L., Rojas-Mancilla, E., Morales, P., Herrera-Marschitz, M., Tasker, R.A., 2017. Models of progressive neurological dysfunction originating early in life. Prog. Neurobiol. 155, 2–20.
Martin, A.M., Young, R.L., Leong, L., Rogers, G.B., Spencer, N.J., Jessup, C.F., Keating, D. J., 2017. The diverse metabolic roles of peripheral serotonin. Endocrinology 158, 1049–1063.
Matsuda, V.D.V., Tejada, M.B., Motta-Teixeira, L.C., Ikebara, J.M., Cardoso, D.S., Machado-Nils, A.V., Lee, V.Y., Diccini, I., Arruda, B.P., Martins, P.P., Dias, N.M.M., Tessarotto, R.P., Raeisossadati, R., Bruno, M., Takase, L.F., Kihara, A.H., Nogueira, M.I., Xavier, G.F., Takada, S.H., 2021. Impact of neonatal anoxia and hypothermic treatment on development and memory of rats. Exp. Neurol. 340, 113691.
Melo, M.F.F.T., Pereira, D.E., Moura, R.L., Silva, E.B., Melo, F.A.L.T., Dias, C.C.Q., Silva, M.C.A., Oliveira, M.E.G., Viera, V.B., Pintado, M.M.E., Santos, A.G., Soares, J. K.B., 2019. Maternal supplementation with avocado (Persea americana mill.) Pulp and oil alters reflex maturation, physical development, and offspring memory in rats. Front. Neurosci. 23 13, 9.
Menshanov, P.N., Bannova, A.V., Dygalo, N.N., 2017. Anoxia ameliorates the dexamethasone-induced neurobehavioral alterations in the neonatal male rat pups. Horm. Behav. 87, 122–128.
Millar, L.J., Shi, L., Hoerder-Suabedissen, A., Molnár, Z., 2017. Neonatal hypoxia ischaemia, mechanisms, models, and therapeutic challenges. Front. Cell Neurosci. 11, 78.
Morison, I.M., Reeve, A.E., 1998. Insulin-like growth factor 2 and overgrowth, molecular biology and clinical implications. Mol. Med. Today 4, 110–115.
Motta-Teixeira, L.C., Takada, S.H., Machado-Nils, A.V., Nogueira, M.I., Xavier, G.F., 2016. Spatial learning and neurogenesis, Effects of cessation of wheel running and survival of novel neurons by engagement in cognitive tasks. Hippocampus 26, 794–803.
Mui, A.M., Yang, V., Aung, M.H., Fu, J., Adekunle, A.N., Prall, B.C., Sidhu, C.S., Park, H. na, Boatright, J.H., Iuvone, P.M., Pardue, M.T., 2018. Daily visual stimulation in the critical period enhances multiple aspects of vision through BDNF-mediated pathways in the mouse retina. PLoS One 13, e0192435.
Narboux-Nême, N., Angenard, G., Mosienko, V., Klempin, F., Pitychoutis, P.M., Deneris, E., Bader, M., Giros, B., Alenina, N., Gaspar, P., 2013. Postnatal growth defects in mice with constitutive depletion of central serotonin. ACS Chem. Neurosci. 4, 171–181.
Nieto-Estévez, V., Defterali, Ç., Vicario-Abejón, C., 2016. IGF-I, A key Growth Factor that Regulates Neurogenesis and Synaptogenesis from Embryonic to Adult Stages of the Brain. Front Neurosci. 10, 52.
Niño Cruz, G.I., Ramirez Varela, A., da Silva, I.C.M., Hallal, P.C., Santos, I.S., 2018. Physical activity during pregnancy and offspring neurodevelopment, a systematic review. Paediatr. Perinat. Epidemiol. 32, 369–379.
Oberlander, T.F., 2012. Fetal serotonin signaling, setting pathways for early childhood development and behavior. J. Adolesc. Health 51, S9–S16.
Odd, D., Heep, A., Luyt, K., Draycott, T., 2017. Hypoxic-ischemic brain injury, Planned delivery before intrapartum events. J. Neonatal Perinat. Med. 10, 347–353.
Paquette, A.G., Lesseur, C., Armstrong, D.A., Koestler, D.C., Appleton, A.A., Lester, B.M., Marsit, C.J., 2013. Placental *HTR2A* methylation is associated with infant neurobehavioral outcomes. Epigenetics 8, 796–801.
Park, S.-K., Solomon, D., Vartanian, T., 2001. Growth factor control of CNS myelination. Dev. Neurosci. 23, 327–337.
Parnpiansil, P., Jutapakdeegul, N., Chentanez, T., Kotchabhakdi, N., 2003. Exercise during pregnancy increases hippocampal brain-derived neurotrophic factor mRNA expression and spatial learning in neonatal rat pup. Neurosci. Lett. 352, 45–48.
Peng, X., Wang, J., Peng, J., Jiang, H., Le, K., 2022. Resveratrol improves synaptic plasticity in hypoxic-ischemic brain injury in neonatal mice via alleviating SIRT1/NF-κB signaling–mediated neuroinflammation. J. Mol. Neurosci. 72, 113–125.
Quiclet, C., Dubouchaud, H., Berthon, P., Sanchez, H., Vial, G., Siti, F., Fontaine, E., Batandier, C., Couturier, K., 2017. Maternal exercise modifies body composition and energy substrates handling in male offspring fed a high-fat/high-sucrose diet. J. Physiol. 595, 7049–7062.
Quiclet, C., Siti, F., Dubouchaud, H., Vial, G., Berthon, P., Fontaine, E., Batandier, C., Couturier, K., 2016. Short-term and long-term effects of submaximal maternal exercise on offspring glucose homeostasis and pancreatic function. Am. J. Physiol. Endocrinol. Metab. 311, E508–E518.
Radom-Aizik, S., Zaldivar, F.P., Nance, D.M., Haddad, F., Cooper, D.M., Adams, G.R., 2013. Growth inhibition and compensation in response to neonatal hypoxia in rats. Pediatr. Res. 74, 111–120.
Raff, H., Bruder, E.D., Jankowski, B.M., Colman, R.J., 2001. Effect of neonatal hypoxia on leptin, insulin, growth hormone and body composition in the rat. Horm. Metab. Res. 33, 151–155.
Rajala, A., Teel, K., Bhat, M.A., Batushansky, A., Griffin, T.M., Purcell, L., Rajala, R.V.S., 2022. Insulin-like growth factor 1 receptor mediates photoreceptor neuroprotection. Cell Death Dis. 13, 613.
Robinson, A.M., Bucci, D.J., 2014. Physical exercise during pregnancy improves object recognition memory in adult offspring. NeuroSci 256, 53–60.
Rosa, B.V., Blair, H.T., Vickers, M.H., Dittmer, K.E., Morel, P.C.H., Knight, C.G., Firth, E. C., 2013. Moderate exercise during pregnancy in Wistar rats alters bone and body composition of the adult offspring in a sex-dependent manner. PLoS One 8, e82378.
Rosenfeld, C.S., 2020. Placental serotonin signaling, pregnancy outcomes, and regulation of fetal brain development. Biol. Reprod. 102, 532–538.
Salter, M.W., Stevens, B., 2017. Microglia emerge as central players in brain disease. Nat. Med. 23, 1018–1027.
Sanches, E.F., Dos Santos, T.M., Odorcyk, F., Untertriefallner, H., Rezena, E., Hoeper, E., Avila, T., Martini, A.P., Venturin, G.T., da Costa, J.C., Greggio, S., Netto, C.A., Wyse, A.T., 2021. Pregnancy swimming prevents early brain mitochondrial dysfunction and causes sex-related long-term neuroprotection following neonatal hypoxia-ischemia in rats. Exp. Neurol. 339, 113623.
Sanches, E.F., Durán-Carabali, L.E., Tosta, A., Nicola, F., Schmitz, F., Rodrigues, A., Siebert, C., Wyse, A., Netto, C., 2017. Pregnancy swimming causes short- and long-term neuroprotection against hypoxia–ischemia in very immature rats. Pediatr. Res. 82, 544–553.
Sanches, E.F., Fabres, R.B., Mazeron, L.S., Rezena, E., Tassinari, I.D., Carvalho, A.S., Andrade, M.K., de Fraga, L.S., Netto, C.A., 2020. Previous adaptation triggers distinct molecular pathways and modulates early and long-term neuroprotective effects of pregnancy swimming preventing neonatal hypoxia-ischemia damage in rats. Brain Res. 1733, 146722.
Santana Muniz, G., Beserra, R., da Silva, G., de, P., Fragoso, J., Lira, A., de, O., Nascimento, E., Manhães de Castro, R., Leandro, C.G., 2014. Active maternal phenotype is established before breeding and leads offspring to align growth trajectory outcomes and reflex ontogeny. Physiol. Behav. 129, 1–10.
Santucci, D., Cagiano, R., Calamandrei, G., 1994. IGF-I and IGF-I24–41 but not IGF-I57–70 affect somatic and neurobehavioral development of newborn male mice. Brain Res. Bull. 35, 367–371.

Satar, M., Özcan, K., Yapıcıoğlu, H., Narlı, N., 2004. Serum insulin-like growth factor 1 and growth hormone levels of hypoxic-ischemic newborns. Neonatology 85, 15–20.
Schmidt, J.W., Walsh, W.F., 2010. Hypoxic-ischemic encephalopathy in preterm infants. J. Neonatal Perinat. Med. 3 (4), 277–284.
Schneider, J., Miller, S.P., 2019. Preterm brain Injury, White matter injury. Handb. Clin. Neurol. 155–172.
Semple, B.D., Blomgren, K., Gimlin, K., Ferriero, D.M., Noble-Haeusslein, L.J., 2013. Brain development in rodents and humans, Identifying benchmarks of maturation and vulnerability to injury across species. Prog. Neurobiol. 106-107, 1–16.
Shao, R., Sun, D., Hu, Y., Cui, D., 2021. White matter injury in the neonatal hypoxic-ischemic brain and potential therapies targeting microglia. J. Neurosci. Res. 99, 991–1008.
Sizonenko, S.V., Sirimanne, E., Mayall, Y., Gluckman, P.D., Inder, T., Williams, C., 2003. Selective cortical alteration after hypoxic-ischemic injury in the very immature rat brain. Pediatr. Res. 54, 263–269.
Son, J.S., Liu, X., Tian, Q., Zhao, L., Chen, Y., Hu, Y., Chae, S.A., Avila, J.M., Zhu, M., Du, M., 2019. Exercise prevents the adverse effects of maternal obesity on placental vascularization and fetal growth. J. Physiol. 597, 3333–3347.
Son, J.S., Zhao, L., Chen, Y., Chen, K., Chae, S.A., de Avila, J.M., Wang, H., Zhu, M.-J., Jiang, Z., Du, M., 2020. Maternal exercise via exerkine apelin enhances brown adipogenesis and prevents metabolic dysfunction in offspring mice. Sci. Adv. 6, eaaz0359.
Stanford, K.I., Lee, M.-Y., Getchell, K.M., So, K., Hirshman, M.F., Goodyear, L.J., 2015. Exercise Before and During Pregnancy Prevents the Deleterious Effects of Maternal High-Fat Feeding on Metabolic Health of Male Offspring. Diabetes 64, 427–433.
Stratikopoulos, E., Szabolcs, M., Dragatsis, I., Klinakis, A., Efstratiadis, A., 2008. The hormonal action of IGF1 in postnatal mouse growth. Proc. Natl. Acad. Sci. USA 105, 19378–19383.
Takada, S.H., Motta-Teixeira, L.C., Machado-Nils, A.V., Lee, V.Y., Sampaio, C.A., Polli, R. S., Malheiros, J.M., Takase, L.F., Kihara, A.H., Covolan, L., Xavier, G.F., Nogueira, M. I., 2016. Impact of neonatal anoxia on adult rat hippocampal volume, neurogenesis and behavior. Behav. Brain Res. 296, 331–338.
Takada, S.H., Sampaio, C.A.G., Allemandi, Wilma, Ito, P.Hiromi, Takase, L.F., Nogueira, M.I., 2011. A modified rat model of neonatal anoxia, Development and evaluation by pulseoximetry, arterial gasometry and Fos immunoreactivity. J. Neurosci. Methods 198, 62–69.
Takada, S.H., dos Santos Haemmerle, C.A., Motta-Teixeira, L.C., Machado-Nils, A.V., Lee, V.Y., Takase, L.F., Cruz-Rizzolo, R.J., Kihara, A.H., Xavier, G.F., Watanabe, I.S., Nogueira, M.I., 2015. Neonatal anoxia in rats, Hippocampal cellular and subcellular changes related to cell death and spatial memory. NeuroSci 284, 247–259.
Tang, A.C., Nakazawa, M., 2005. Neonatal novelty exposure ameliorates anoxia-induced hyperactivity in the open field. Behav. Brain Res. 163, 1–9.
Ten, V.S., Bradley-Moore, M., Gingrich, J.A., Stark, R.I., Pinsky, D.J., 2003. Brain injury and neurofunctional deficit in neonatal mice with hypoxic-ischemic encephalopathy. Behav. Brain Res. 24, 67–87.
van Tilborg, E., van Kammen, C.M., de Theije, C.G.M., van Meer, M.P.A., Dijkhuizen, R. M., Nijboer, C.H., 2017. A quantitative method for microstructural analysis of myelinated axons in the injured rodent brain. Sci. Rep. 7, 16492.
Toda, T., Parylak, S.L., Linker, S.B., Gage, F.H., 2019. The role of adult hippocampal neurogenesis in brain health and disease. Mol. Psychiatry 24, 67–87.
Turner, R., 2019. Myelin and modeling, bootstrapping cortical microcircuits. Front. Neural Circuits 13, 34.
Umran, R., Al-Tahir, M., Jagdish, D., Chouthai, N., 2016. Insulin-Like growth factor-1 Levels in term newborns with hypoxic–ischemic encephalopathy. Am. J. Perinatol. 33, 640–645.
West, M.J., Slomianka, L., Gundersen, H.J.G., 1991. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat. Rec. 231, 482–497.
Whitaker-Azmitia, P.M., 2010. Serotonin and Development. Handb. Behav. Neurosci. 309–323.
Wlodarczyk, A., Holtman, I.R., Krueger, M., Yogev, N., Bruttger, J., Khorooshi, R., Benmamar-Badel, A., Boer-Bergsma, J.J., Martin, N.A., Karram, K., Kramer, I., Boddeke, E.W., Waisman, A., Eggen, B.J., Owens, T., 2017. A novel microglial subset plays a key role in myelinogenesis in developing brain. EMBO J. 36, 3292–3308.
Wlodarczyk, A., Løbner, M., Cédile, O., Owens, T., 2014. Comparison of microglia and infiltrating CD11c+ cells as antigen presenting cells for T cell proliferation and cytokine response. J. Neuroinflamm. 11, 57.
Woods, K.A., Savage, M.O., 1996. Laron syndrome, typical and atypical forms. Baillieres Clin. Endocrinol. Metab. 10, 371–387.
Yan, F., Zhang, M., Meng, Y., Li, H., Yu, L., Fu, X., Tang, Y., Jiang, C., 2016. Erythropoietin improves hypoxic-ischemic encephalopathy in neonatal rats after short-term anoxia by enhancing angiogenesis. Brain Res. 1651, 104–113.
Yau, S.-Y., Lee, T.H.-Y., Formolo, D.A., Lee, W.-L., Li, L.C.-K., Siu, P.M., Chan, C.C.H., 2019. Effects of maternal voluntary wheel running during pregnancy on adult hippocampal neurogenesis, temporal order memory, and depression-like behavior in adult female and male offspring. Front. Neurosci. 13, 470.
Yoshida, T., Delafontaine, P., 2020. Mechanisms of IGF-1-mediated regulation of skeletal muscle hypertrophy and atrophy. Cells 9, 1970.
Yumani, D.F.J., Lafeber, H.N., Weissenbruch, M.M., 2015. Dietary proteins and IGF I levels in preterm infants, determinants of growth, body composition, and neurodevelopment. Pediatr. Res. 77 (1-2), 156–163.
Zeiss, C.J., 2021. Comparative milestones in rodent and human postnatal central nervous system development. Toxicol. Pathol. 49 (8), 1368–1373.
Zhang, X., Girardot, P.E., Sellers, J.T., Li, Y., Wang, J., Chrenek, M.A., Wu, W., Skelton, H., Nickerson, J.M., Pardue, M.T., Boatright, J.H., 2019. Wheel running exercise protects against retinal degeneration in the I307N rhodopsin mouse model of inducible autosomal dominant retinitis pigmentosa. Mol. Vis. 25, 462–476.
Zheng, J., Alves-Wagner, A.B., Stanford, K.I., Prince, N.B., So, K., Mul, J.D., Dirice, E., Hirshman, M.F., Kulkarni, R.N., Goodyear, L.J., 2020. Maternal and paternal exercise regulate offspring metabolic health and beta cell phenotype. BMJ Open Diabetes Res. Care 8, e000890.
Zheng, J., Sun, X., Ma, C., Li, B., Luo, F., 2019. Voluntary wheel running promotes myelination in the motor cortex through Wnt signaling in mice. Mol. Brain 12, 85.