Scientific Reports

https://doi.org/10.1038/s41598-026-48292-x

Article in Press

Maternal exercise enhances hippocampal plasticity and resilience against stress-induced depressive behaviors in adult offspring

Received: 13 October 2025

Accepted: 7 April 2026

Published online: 18 April 2026

Cite this article as: Iqbal Z., Rosa J.M., Yu J. *et al.* Maternal exercise enhances hippocampal plasticity and resilience against stress-induced depressive behaviors in adult offspring. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-48292-x

Zafar Iqbal, Julia Macedo Rosa, Jiasui Yu, Tong Cheng, Douglas Affonso Formolo, Akhlaq Hussain, Tak Pan Wong, Tatia M. C. Lee, Kwok-fai So & Suk-Yu Yau

Maternal exercise enhances hippocampal plasticity and resilience against stress-induced depressive behaviors in adult offspring

Zafar Iqbal[1,2], Julia Macedo Rosa[1,2], Jiasui Yu[1,2], Tong Cheng[1,2], Douglas Affonso Formolo[1,2], Akhlaq Hussain[1,2], Tak Pan Wong[3], Tatia MC Lee[4,5] , Kwok-fai So[6], Suk-Yu Yau[1,2*]

[1]Department of Rehabilitation Sciences, Faculty of Health and Social Sciences, The Hong Kong Polytechnic University, Hung Hom, Hong Kong SAR, China

[2]Mental Health Research Center, The Hong Kong Polytechnic University, Hung Hom, Hong Kong SAR, China

[3]Department of Psychiatry, McGill University, Montreal, Canada

[4]Department of Psychology, The University of Hong Kong

[5]Laboratory of Neuropsychology and Human Neuroscience, The University of Hong Kong

[6]Key Laboratory of CNS Regeneration (Ministry of Education), Guangdong-Hong Kong-Macau Institute of CNS Regeneration, Jinan University, Guangzhou, China

***Correspondence:** Suk-yu Yau; email: sonata.yau@polyu.edu.hk

Abstract:

Maternal physical activity during pregnancy has been shown to confer benefits on the brain functions of offspring. This study investigated the positive effects of maternal exercise during pregnancy on enhancing hippocampal synaptic plasticity and resilience to stress-induced depressive behavior in adult murine offspring. Using a mouse model with mother mice engaged in voluntary wheel running during pregnancy, we assessed changes in long-term potentiation (LTP) in the hippocampal dentate gyrus, synaptic protein expression, and behavioral responses to chronic stress in adult male and female offspring from exercised dams compared with those from sedentary dams. We found that maternal exercise enhanced LTP in offspring of both sexes. Western blot analysis of hippocampal synaptoneurosome extractions revealed significant main effects of maternal exercise on increasing the expression of brain-derived neurotrophic factor (BDNF), PSD-95, synaptophysin, and phosphorylation of N-methyl-D-aspartate receptor subunit GluN2A and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunit GluA1. Maternal exercise significantly increased synaptophysin levels in both male and female offspring, with sex-specific effects on increasing PSD-95 levels in male offspring and increased p-GluN2A levels in female offspring from exercised dams. Golgi staining revealed a significant increase in hippocampal dendritic spine density in female offspring only. Maternal exercise-induced improvements in hippocampal synaptic plasticity were associated with reduced depression-like behaviors in both male and female offspring exposed to chronic unpredictable stress. Additionally, male offspring displayed reduced anxiety-like behavior, while female offspring showed no significant anxiolytic changes. These findings elucidate the sex-specific effects of maternal exercise on enhancing hippocampal synaptic plasticity, which may contribute to increased resilience against stress-induced depressive behaviors in adult offspring.

Introduction

Depression is a leading cause of disability worldwide, imposing a significant economic burden globally [1-3]. It is well-documented that individuals with major depressive disorders often exhibit reduced volume of the hippocampus, a brain region crucial for emotion regulation and cognitive function [4-7]. Preclinical research has established that chronic stress impairs hippocampal neuroplasticity, manifesting as decreased adult neurogenesis, reduced dendritic complexity and spine density, impaired synaptic plasticity, and glial function [8-17]. Critically, the integrity of the hippocampal structure is linked to an individual's capacity for stress resilience [18]. Resilience to social stress in animal models is associated with increased hippocampal neurogenesis and larger hippocampal volume [19-21], a correlation was also observed in humans, where greater hippocampal volume predicts resilience to traumatic stress [22]. These collective findings suggest that strategies capable of enhancing hippocampal neuroplasticity could bolster stress resilience and mitigate maladaptive responses to stress-induced depressive disorders.

In this context, physical exercise emerges as a potent non-pharmacological intervention for treating depression. Preclinical studies have shown that physical exercise elicits antidepressant effects by enhancing hippocampal structural and functional plasticity [11-15, 23-27]. Notably, emerging evidence suggests that maternal exercise can exert long-lasting positive effects on both prenatal and postnatal brain development and improve neurobehavioral outcomes in offspring [28, 29]. Clinical studies indicate that maternal exercise during pregnancy not only supports fetal and placental growth but also enhances cerebral maturation and language development in children [29-33]. Likewise, animal studies reveal that maternal exercise during pregnancy reduces depressive phenotypes, improves hippocampal-dependent memory, and counteracts prenatal stress-induced

dendritic atrophy in offspring, potentially through increased hippocampal neurogenesis and elevated brain-derived neurotrophic factor (BDNF) levels [7, 34-41].

We have previously shown that maternal exercise promotes hippocampal adult neurogenesis and lowers depressive behavior in offspring [7]. Here, we further determine the effects of maternal exercise on promoting hippocampal synaptic plasticity in offspring, and examine whether adult offspring with maternal exercise show resilience to chronic stress-induced depression-like behavior. We hypothesized that maternal exercise during pregnancy confers stress resilience in adult offspring. The findings could inform the early-life intervention strategies to enhance stress resilience in adulthood.

Materials and Methods:

Animals and ethical approval

The study was conducted using six-week-old C57BL/6J male and female mice offspring, derived from mothers that either participated in voluntary exercise or remained sedentary during pregnancy. Mice of both sexes were housed separately in a controlled environment, maintained at a constant temperature of 22 ± 2 °C, with a 12:12-hour light-dark cycle. They had unrestricted access to food, chow, and water ad libitum. All experimental procedures were approved and performed in accordance with the relevant guidelines and regulations established by the Animal Subjects Ethics Sub-Committee at The Hong Kong Polytechnic University, viz., animal ethics approval number: 16-17/64-RS-R-HMRF, and the experiments conducted were also in compliance with the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments).

Experimental design

Two adult female mice were paired with one male following a two-day acclimation period. Pregnancy was confirmed by the presence of seminal plugs. Upon confirmation, we then perform pair-housing, with two dams sharing one running wheel per cage, to avoid the confounding effects of social isolation-induced stress in the dams, as previously described [7, 12, 42]. To minimize litter effects, we included only one to two offspring from each litter for behavioral testing, and only one offspring of each sex per litter for molecular and synaptic analyses. The running wheels were removed once the pups were born. The pups were weaned at three weeks of age and at four weeks, separated into cages according to sex without running wheels (Figure 4A).

Chronic unpredictable stressors (CUS)

To investigate whether maternal exercise enhances stress resilience in adult offspring, a cohort of male and female offspring at six weeks of age was subjected to 21 days of chronic unpredictable stressors (CUS). Non-stressed control offspring mice remained in their home cages without exposure to stressors. Mice were exposed daily to one of the following several aversive stressors, presented in a random and unpredictable order over three weeks (day 1 to day 21; See Table 1 for details). This well-established chronic stress protocol is known to induce depression-like behavior [43], successfully elevated blood corticosterone levels in our stressed animals compared to non-stressed controls (Supplementary Figure 1; $p<0.05$), thereby validating its efficacy in our study.

Behavioral tasks

Following the final stressor, male and female offspring at the age of 9 weeks were then subjected to a series of behavioral tests to assess various aspects of their behavior.

Forced swim test (FST)

To assess behavioral despair, each mouse was placed individually in a transparent cylinder (30 cm in height, 15 cm in diameter) filled with room temperature tap water to two-thirds of its height, as previously described [12, 13]. The session was video recorded for six minutes, and a trained researcher, blinded to the sample identities, analyzed the immobility time during the last four minutes. Immobility was defined as minimal movement necessary to keep the mouse's head above water. The immobility time served as an indicator of depression-like behavior, as previously described [12, 13, 44].

Sucrose splash test (SST)

Conducted under a red light (230 V, 15 W), this test involved squirting a 10% sucrose solution onto the dorsal coat of a mouse in its home cage. The viscosity of the sucrose solution dirties the fur, prompting grooming behavior. The time spent grooming was recorded over a five-minute period as an index of self-care and motivational behavior.

Open field test (OF)

This test evaluates exploratory behavior in a novel environment and screens for anxiety-like behavior in rodents. Mice were acclimated to the testing room for two hours prior to testing [42, 45]. Each mouse was then allowed to explore an open field (40 cm × 40 cm × 30 cm) for 10 minutes under dim lighting. Anxiety-like behavior and locomotor activity were analyzed using Any-maze software version 7.64 (Stoelting Co., IL, USA; https://www.any-maze.com/). Locomotor activity was quantified as the total distance traveled during the first five minutes.

Golgi staining and analysis

Golgi staining was performed according to the manufacturer's instructions using the FD Rapid Golgistain™ Kit (FD Neurotechnologies, MD). Briefly, mice were deeply anesthetized with isoflurane and perfused with 0.9% saline. The freshly dissected brains were immersed in pre-mixed impregnation solutions A and B, containing potassium dichromate and chromate, for two weeks at room temperature. Subsequently, the brains were transferred to solution C for 48 hours at 4°C. Brain tissues were sectioned into 150 µm slices from rostral to caudal (bregma −1.34 to −3.80 mm) using a vibratome (Leica Biosystems, Germany) and mounted on gelatin-coated slides. Pyramidal neurons were stained using a mixture of solutions D, E, and distilled water (1:1:2 ratio) for 10 minutes, followed by dehydration in 50%, 75%, 95%, and absolute ethanol. Finally, the brain sections were cleared in xylene and covered with a coverslip. For analysis, five neurons from each 150 µm-thick section were selected using Neurolucida (MicroBrightField, USA), following established protocol [11]. Only neurons located in the dentate gyrus of the hippocampus were chosen, ensuring they were relatively isolated from neighboring impregnated neurons to avoid analytical interference. Neurons were positioned in the middle part of the section thickness to minimize truncated branch segments and had to be consistently and darkly impregnated along the entire extent of all dendrites. Granule cells closer to the sub-granular zone typically exhibit simpler dendritic architecture with one primary dendrite, while those in the outer layer display multiple primary dendritic extensions and more complex branching. Single-branched granule cells were selected from the inner cell layer, and multiple-branched granule cells from the outer cell layer, as previously described [7, 42]. Spine density was measured by randomly selecting high-magnification tracings of terminal segments (>10 µm) of single and multiple dendritic branches. Six single and multiple-branched granule cells were selected from each sample. Six segments of

tertiary or quaternary dendritic branches (longer than 15 μm) were chosen from each granule cell using ×400 magnification under a light microscope [7, 42]. Quantification of dendritic spines was performed at ×630 magnification, with visible spines along the branch segment counted and expressed as number/10 μm.

Isolation of synaptoneurosome and western blotting

For synaptoneurosome extraction, six adult offspring from each group were rapidly sacrificed using isoflurane. The hippocampi were dissected and homogenized using the Syn-PER synaptic protein extraction reagent kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. The homogenates were centrifuged at 1200× g for 10 minutes at 4 °C to obtain the cytosolic fraction, which was further centrifuged at 15,000× g for 30 minutes at 4 °C. The resulting synaptosomal fraction was resuspended in Syn-PER reagent as previously performed [42].

Protein concentration of the hippocampal synaptoneurosome homogenates was determined using the Bradford assay. Samples were diluted to 0.5 μg/μl in a solution containing 0.25 M Tris–HCl (pH 6.8), 10% glycerol, 10% SDS, and 10 mM DTT, and linearized in 1× Laemmli buffer (Bio-Rad Laboratories, USA) with 5% β-mercaptoethanol (Abcam, USA) at 95 °C for 10 minutes. Each lane was loaded with 30 μg of synaptoneurosome protein and separated on a 10% SDS polyacrylamide gel (Bio-Rad Laboratories, USA), followed by transfer to polyvinylidene fluoride (PVDF) membranes (Bio-Rad Laboratories, USA). For blocking, 5% non-fat dry milk (Bio-Rad Laboratories, USA) was used for non-phosphorylated proteins, and 5% bovine serum albumin (Sigma, USA) in Tris-HCl buffer (pH 8.0) was used for phosphorylated proteins. Membranes were blocked for 1 hour at room temperature and then incubated overnight with primary antibodies diluted in blocking solution containing 0.05% Tween-20. The primary antibodies used were: rabbit

anti-PSD95, rabbit anti-Synaptophysin, rabbit anti-GluN2B, rabbit anti-phospho-GluN2B, rabbit anti-GluN2A, rabbit anti-phospho-GluN2A, rabbit anti-GluA1, rabbit anti-phospho-GluA1, and rabbit anti-α-tubulin (all 1:1000, Cell Signaling Technology, USA). After three washes with TBST, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hour at room temperature. Following a final set of three washes with TBST, bands were visualized using an enhanced chemiluminescent (ECL) detection kit (Santa Cruz Biotechnology, Inc., USA) and documented using a transilluminator (Bio-Rad Laboratories, USA). Densitometric analysis of the bands was performed using ImageJ software (USA).

Enzyme-linked immunosorbent assay (ELISA)

Blood samples were collected by cardiac puncture and centrifuged to harvest the serum. The serum corticosterone concentrations were then determined using a commercially available ELISA kit (Enzo, Cat. No. ADI-900-097).

***In vitro* electrophysiological field recordings**

Brain slices were prepared following the protocols described by previous studies [15] and [42]. Briefly, six-week-old male and female offspring were deeply anesthetized with isoflurane and immediately decapitated. The brains were rapidly immersed in oxygenated (95% O2/5% CO2) ice-cold artificial cerebrospinal fluid (aCSF), composed of 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaHPO4, 25 mM NaHCO3, 2 mM CaCl2, 1.3 mM MgCl2, and 10 mM dextrose, pH 7.3. Coronal hippocampal sections (350 μm thick) were cut using a vibratome (Leica, VT2000S) in well-oxygenated ice-cold aCSF. The slices were then gently transferred to a chamber and continuously incubated in oxygenated aCSF at 30°C for 1 hour to allow recovery before recordings.

Field excitatory postsynaptic potentials (fEPSPs) were recorded using a multi-electrode array system (Alpha MED Scientific Inc., Japan), as previously described [42]. A recording probe with extracellular electrodes (P515A, Alpha MED Scientific Inc., Osaka, Japan) was used to stimulate granule neurons in the middle molecular layer of the suprapyramidal blade of the hippocampal dentate gyrus. The slices were perfused at approximately 2 mL/min, and fEPSPs were acquired using MED-A64MD1 and MED-A64HE1S recording amplifiers (Alpha MED Scientific Inc., Osaka, Japan) and Mobius software. Stimulus intensity (20–30 μA) was adjusted for each slice to achieve 40–50% of the maximal response slope without population spikes. Stable baseline fEPSP measurements were obtained by delivering single-pulse stimulation at 15-second interstimulus intervals. After establishing a steady baseline for at least 30 minutes, high-frequency tetanic stimulation (HFS) was applied to induce NMDAR-dependent long-term potentiation (LTP) of fEPSPs in the DG. This was conducted in the presence of 5 μM bicuculline methiodide (Sigma-Aldrich, Canada) to inhibit γ-aminobutyric acid-A (GABAA) receptors and facilitate LTP induction [46]. Bicuculline methiodide was bath-applied before recording the baseline. The HFS protocol consisted of 4 trains of 50 pulses delivered at 100 Hz with a 30-second intertrain interval. Following field recordings, input/output curves were recorded with increasing stimulation intensity (10 μA steps) to measure basal dendritic excitation in response to increasing applied current in aCSF. This was performed using an increasing pulse width from 30 to 300 μs with 30 μs interstimulus intervals. Paired-pulse recordings were also conducted to assess the probability of presynaptic release, consisting of 5 sets of two pulses with a 50 ms inter-pulse interval and 20 seconds between paired stimuli, delivered in aCSF. All data were represented as percentage change from the initial average baseline fEPSP slope, defined as the average slope obtained from the last 20 minutes prior to HFS.

Statistical analyses

Statistical analyses were conducted using GraphPad Prism 11.0.0 (84) (GraphPad Software, USA; https://www.graphpad.com/) and SPSS software version 31.0.0 (IBM SPSS Statistics, USA; https://www.ibm.com/products/spss-statistics). Data were analyzed using one-way or two-way ANOVA followed by Tukey's post-hoc test when appropriate. Results are presented as means ± standard deviation (SD). A p-value of less than 0.05 was considered statistically significant.

Results:

Maternal exercise during pregnancy enhances hippocampal long-term potentiation formation in the adult offspring

We investigated the effects of maternal voluntary exercise on long-term potentiation (LTP) in the hippocampal dentate gyrus (DG) in adult offspring. Electrophysiological field recordings demonstrated the significant main effects of maternal exercise (Figure 1A-C; maternal exercise $F_{(1,\ 44)}$=54.90, p<0.0001), though no main effects of gender ($F_{(1,\ 44)}$=0.0063, p=0.9370) and interaction ($F_{(1,\ 44)}$=2.334, p=0.1337, two-way ANOVA). Post-hoc test revealed a significant increase of LTP formation in male and female adult offspring with exercise dams when compared to their counterparts with sedentary dams (Figure 1C; ***p=0.0009 for males, ****p<0.0001 for females).

We examined basal synaptic transmission using the input-output (I/O) function. There was significant main effect of maternal exercise (Figure 1D; maternal exercise $F_{(3,\ 108)}$=141.5, p<0.0001; gender $F_{(8,\ 108)}$=125.9, p<0.0001 and interaction $F_{(24,\ 108)}$=5.68, p<0.0001, two-way ANOVA). Results revealed an increase in I/O responses in female offspring with exercise dams compared to

sedentary controls (Figure 1D; $p<0.0001$). Short-term synaptic plasticity, assessed by measuring changes in paired-pulse ratio, showed no significant main effect of maternal exercise (Figure 1E-F; maternal exercise $F_{(1, 44)}=1.711$, $p=0.1976$; gender $F_{(1, 44)}=1.241$, $p=0.2713$, and interaction $F_{(1, 44)=0.055}$, $p=0.8155$, two-way ANOVA). Together, these results indicate that maternal exercise enhances LTP formation in males and females and increases basal synaptic transmission in female offspring only.

Maternal exercise enhances the expression of hippocampal synaptic proteins in adult offspring

We next examined whether enhanced hippocampal synaptic plasticity is associated with enhanced synaptic protein expression. Western blot analysis demonstrated a significant main effect of maternal exercise on the expression levels of BDNF (Figure 2A-B; $F_{(1, 15)}=7.238$, *$p=0.0168$), but no main effects of gender ($F_{(1, 15)}=0.1464$, $p=0.7074$) and interaction ($F_{(1, 15)}=0.3972$, $p=0.5380$, two-way ANOVA). However, maternal exercise did not show significant effects on BDNF expression in male ($p=0.6333$) and female ($p=0.2050$) offspring when compared to their counterparts with sedentary dams, respectively (Figure 2B).

There was a significant main effect of maternal exercise on the expression of postsynaptic density protein 95 (PSD-95) (Figure 2C-D; $F_{(1, 27)}=12.11$, **$p=0.0017$), with no significant main effects of gender ($F_{(1, 27)}=3.014$, $p=0.0939$) and interaction ($F_{(1, 27)}=3.268$, $p=0.0818$, two-way ANOVA). Post-hoc test showed that maternal exercise significantly upregulates PSD-95 expression in male offspring when compared to their counterparts with sedentary dams ($p=0.0062$). In contrast, there was no significant difference detected in female offspring (Figure 2D; $p=0.8062$).

Maternal exercise showed a significant main effect on enhancing expression levels of synaptophysin (Figure 2E-F; maternal exercise $F_{(1, 20)}$=65.17, ****p<0.0001; gender $F_{(1, 20)}$=7.878, p=0.0109; interaction $F_{(1, 20)}$=0.0113, p=0.9161, two-way ANOVA). Post-hoc test further revealed that maternal exercise significantly increased the levels of synaptophysin in adult male (****p<0.0001) and female offspring (****p<0.0001) when compared to their counterparts with sedentary dams (Figure 2F).

We further examined the impact of maternal exercise on the expression of hippocampal NMDA and AMPA receptor subunits in adult offspring. Maternal exercise did not affect the expression of the NMDA receptor subunit GluN2B in the hippocampus (Figure 2G-H; maternal exercise $F_{(1, 20)}$=2.397, p=0.1372; gender $F_{(1, 20)}$=2.102, p=0.1626, and interaction $F_{(1, 20)}$=1.175, p=0.2913, two-way ANOVA). Moreover, maternal exercise did not alter expression of GluN2B in either male (p=0.9997) or female offspring (p=0.3836) when compared to offspring with sedentary dams (Figure 2H). In contrast, there were significant main effects of maternal exercise on GluN2A expression (Figure 2I-J; maternal exercise $F_{(1, 20)}$=11.48, **p=0.0029), and interaction ($F_{(1, 20)}$=5.248, p=0.0330), though no main effects of gender observed ($F_{(1, 20)}$=2.611, p=0.1218, two-way ANOVA). Post-hoc test indicated that maternal exercise significantly increased the expression levels of phosphorylated GluN2A subunit in female offspring (**p=0.0041), but has no effect on male offspring (p=0.9715) when compared to their counterparts with sedentary dams (Figure 2J).

Western blot analysis also revealed a significant main effect of maternal exercise on phosphorylated GluA1, an AMPA receptor subunit (Figure 2K-L; maternal exercise $F_{(1, 20)}$=6.967,

*p=0.0157), but no significant main effect of gender ($F_{(1, 20)}$=0.8333, p=0.3722), and interaction $F_{(1, 20)}$=0.0298, p=0.8646, two-way ANOVA). Post-hoc tests showed that maternal exercise did not show a significant effect on the expression level of GluA1 (Figure 2L; p=0.4559 for male offspring; p=0.3128 for female offspring). These findings indicate that maternal exercise enhances neurotrophic support and synaptic protein expression in offspring hippocampi, with significant gender specific effects on enhancing PSD-95 and GluN2A expression levels in offspring.

Maternal exercise enhances spine density in female offspring

We further examined whether maternal exercise increases spine density in the offspring hippocampal neurons. We quantified the spine density of Golgi-stained granule neurons located in the outer or inner layer of the hippocampal dentate gyrus. There was no significant main effect of maternal exercise (Figure 3A-B; $F_{(1, 20)}$=3.178, p=0.0898) and Gender ($F_{(1, 20)}$=0.039, p=0.8454), but a significant interaction ($F_{(1, 20)}$=12.45, p=0.0021, two-way ANOVA). Post-hoc tests indicated that maternal exercise significantly increases spine density in granule neurons in the outer granule cell layer in adult female offspring (Figure 3A-B; **p=0.0074), but not male offspring (p=0.7936) when compared to female counterparts with sedentary dams. In contrast, no significant differences were observed between the sedentary and exercise dams, respectively

Similarly, there was no significant main effect of the maternal exercise (Figure 3C-D, $F_{(1, 20)}$=2.105, p=0.1623), gender ($F_{(1, 20)}$=0.6502, p=0.4295), and interaction ($F_{(1, 20)}$=13.02, p=0.0018, two-way ANOVA). However, post-hoc test revealed that maternal exercise significantly increased spine density in single-branched neurons in female offspring (Figure 3D; *p=0.0113), but not male offspring (p=0.6035) when compared to their sedentary counterparts. These findings suggest that maternal exercise has a gender-specific effect in enhancing spine density in female offspring only.

Maternal exercise prevents chronic stress-increased depression-like behavior in offspring

We next investigated whether maternal exercise confers resilience to chronic stress-increased depressive behaviors in offspring displaying enhanced hippocampal synaptic plasticity (Figure 4A). In the forced swim test, there were significant main effects of treatment (Figure 4B; male offspring: $F_{(2, 21)}$=26.74, p<0.0001; Figure 4F, female offspring: $F_{(2, 21)}$=9.151, p=0.0014, one-way ANOVA). Post-hoc test revealed that CUS increased depression-like behavior in male offspring (Figure 4B; *p=0.0116, Ctrl-Sed vs CUS-Sed), which could be prevented by maternal exercise (male offspring: **p=0.0014, ****p<0.0001, CUS-Sed vs CUS-MEx) and female offspring (Figure 4F; *p=0.0498, ***p=0.0010, CUS-Sed vs CUS-MEx). No difference in CUS-induced depressive phenotype was observed in female offspring (Figure 4F; p=0.2174, Ctrl-Sed vs CUS-Sed).

In the sucrose splash test, there were main effects of maternal exercise (Figure 4C, male offspring: $F_{(2, 21)}$=12.04, p=0.0003; Figure 4G, female offspring: $F_{(2, 21)}$=5.602, p=0.0112, one-way ANOVA). Post-hoc analysis revealed a significant effect of maternal exercise in restoring a decrease in grooming time in male offspring (Figure 4C; ***p=0.0003, CUS-Sed vs CUS-MEx), while CUS significantly reduced the grooming behavior in males with sedentary mothers (Fig. 4C; **p=0.0081, Ctrl-Sed vs CUS-Sed; p=0.3478, Ctrl-Sed vs CUS-MEx). CUS significantly reduced grooming time in female offspring with sedentary dams (Figure 4G; *p=0.0471, Ctrl-Sed vs CUS-Sed), which could be prevented by maternal exercise (Figure 4G; *p=0.0128, CUS-Sed vs CUS-MEx), indicating maternal exercise protects offspring against chronic stress-induced depression-like behavior. The grooming time didn't change significantly between control sedentary and stressed males with maternal exercise (Figure 4G; p=0.8198, Ctrl-Sed vs CUS-MEx).

In the open-field test, there was a main effect of maternal exercise on time spent in the centre (Fig 4D; male offspring: $F_{(2, 21)}$=10.36, p=0.0007, one-way ANOVA). Post-hoc test revealed that maternal exercise increased time spent in the center of stressed male offspring when compared to other groups, indicating reduced anxiety-like behavior (Figure 4D; **p=0.0026, CUS-Sed vs CUS-MEx; **p=0.0017, Ctrl-Sed vs CUS-MEx). While no significant difference was noticed between the control sedentary and CUS-Sed group (Figure 4D; p=0.9787, Ctrl-Sed vs CUS-Sed). In female offspring, there were no significant main effects of maternal exercise observed (Fig 4H; $F_{(2, 21)}$=0.9211, p=0.4136, p>0.05 for all comparisons; one-way ANOVA). Additionally, there was no significant main effect in total distance traveled, suggesting maternal exercise did not affect locomotor activity (Figure 4E; male offspring: $F_{(2, 21)}$=0.6812, p=0.5168; Fig 4I, female offspring: $F_{(2, 21)}$=0.9817, p=0.3912, one-way ANOVA).

Together, these data suggested that maternal exercise significantly prevented male and female offspring from chronic stress-increased depression behavior, suggesting positive effects in conferring resilience in offspring.

Discussion:

In this study, we report that maternal voluntary exercise during pregnancy enhances hippocampal synaptic plasticity in both male and female offspring, with sex-specific effects on synaptic protein expression and dendritic spine density. Building on our prior findings that maternal exercise promotes hippocampal neurogenesis and reduces depression-like behavior in both male and female offspring [7], current work strengthens the evidence for transgenerational benefits of gestational physical activity on offspring brain plasticity [34]. Additionally, our behavioral data indicated that

offspring with dams exercised during pregnancy confer stress resilience to prevent the effects of chronic stress-increased depression-like behavior, suggesting maternal exercise could be a strategy to enhance hippocampal plasticity and hence enhance stress resilience of adult offspring.

Voluntary running is well-established, which promotes hippocampal synaptic plasticity and neurogenesis in the hippocampus, contributing to improved cognitive functions and reduced depressive phenotypes [7, 47, 48]. Due to this reason, physical exercise can be used as a preventive measure as well as for the treatment of depression in both adults and adolescents [49-51]. Beyond mood and cognitive benefits, exercise during pregnancy exerts neuroprotective effects on offspring, potentially reducing the risk of neurological disorders [52]. Our results extend these benefits to the prenatal context, showing that maternal exercise upregulates the expression of synaptic proteins and neurotrophic support in offspring's hippocampi, as evidenced by significant main effects of physical exercise on enhancing BDNF and synaptic proteins such as PSD-95, synaptophysin, p-GluN2B, and GluA1 subunits. These findings align with previous reports linking maternal exercise to upregulated hippocampal BDNF levels in offspring [38-41, 53]. Maternal exercise during pregnancy increases BDNF levels and cell numbers in the hippocampal formation of offspring and subsequently enhances offspring cognitive function [38]. Similarly, Parnpiansil and collaborators [39] show that physical exercise during gestation in pregnant mothers can increase hippocampal BDNF mRNA expression of postnatal pups and result in an improvement in cognition in pups [39]. It was also noted that maternal running on a treadmill during pregnancy resulted in a significant increase in the expression of BDNF mRNA, enhanced hippocampal cell survival, and improved the short-term memory of offspring [40]. Additionally, it was shown that both forced and voluntary maternal exercise during pregnancy increased the level of BDNF protein in the

hippocampus of the rat pups, and inhibiting BDNF action abolished the exercise-induced cognitive improvements in offspring [41]. Other work demonstrated that the rat pups born from dams subjected to swimming exercise during pregnancy showed significantly increased BDNF mRNA expression, and enhanced hippocampal neurogenesis improved the memory performance in offspring [53]. In animal models, it has been further shown that increasing BDNF levels in the hippocampal CA3 or DG region is associated with stress resilience and prevent stress-induced depressive phenotypes [54, 55], possibly suggesting its function in promoting hippocampal neuronal survival, neurogenesis, dendritic branching, and synaptic function in both developing and adult brains [7, 35, 38-41, 53]. Conversely, BDNF knockdown in the hippocampal DG elevates corticosterone levels and induces depression-like behavior [55]. Therefore, it is reasonable to propose that increased BDNF expression by gestational exercise may enhance brain function in offspring [38-41, 53]. Dendritic spines are critical sites for the dynamic structural plasticity of excitatory transmission [56]. Numerous preclinical studies have shown that chronic stress exposure leads to spine loss in hippocampal neurons and impaired synaptic transmission, often accompanied by depressive behaviors [57-64]. We found that maternal exercise significantly increased hippocampal spine density in the dentate granule neurons of adult female offspring. Taken together, these findings underscore the positive effects of maternal exercise on enhancing synaptic changes in the hippocampi of adult offspring, suggesting that maternal exercise during pregnancy enhances offspring stress resilience that might be linked to up-regulated hippocampal synaptic plasticity in offspring from exercise dams. The observed synaptic protein upregulation and increased dendritic spine density in dentate granule neurons of female offspring further suggest structural adaptations underlying enhanced synaptic plasticity. However, hippocampal neurogenesis and long-term potentiation were not measured in the CUS-MEx, CUS-sedentary, and

control groups; therefore, we cannot directly correlate these specific hippocampal neural changes with the behavioral resilience observed in offspring exposed to chronic stress. Future studies should examine changes in hippocampal neurogenesis and synaptic plasticity to further elucidate the critical role these processes may play in enhancing stress resilience in both male and female offspring.

Moreover, since the pregnant dams were pair-housed with a shared running wheel, our study did not systematically record or analyze individual variability in maternal exercise behavior among dams within the exercise group. As a result, it is unknown whether variability in running activity may have contributed to differential neural or behavioral outcomes in the offspring. Future studies should incorporate more detailed monitoring and reporting of maternal daily running activity for each dam, ideally using single housing conditions, to better understand the potential impact on offspring neurodevelopment and behavior. Furthermore, as litter effects were not systematically examined in the current study, it is difficult to fully assess the independence of the observations. Future studies should address litter effects in conjunction with variation in maternal running activity to better understand their potential impact on offspring outcomes.

Behavioral data from the present study indicate that male and female offspring with exercised dams exhibited a significant reduction in immobility time in the forced swim test and increased grooming time during the sucrose splash test, suggesting that maternal exercise mitigated depressive-like behaviors induced by chronic, unpredictable stress in adult offspring. Additionally, young male mice with exercised dams spent more time in the center of the arena, indicating reduced anxiety-like behavior. Enhanced hippocampal synaptic plasticity may contribute to the observed improvements in depressive-like behavior in both male and female offspring. These

findings align with accumulating evidence from preclinical and clinical studies demonstrating that maternal physical activity enhances neurobehavioral functions in offspring during critical prenatal and postnatal periods [28, 29]. Clinical research has shown that gestational exercise not only optimizes fetal-placental development but also accelerates cerebral maturation in neonates and is associated with superior language acquisition in early childhood [29-33]. Preclinical studies further reveal that maternal exercise during pregnancy attenuates depressive phenotypes in offspring [7, 35, 65], by promoting hippocampal neurogenesis [7, 36, 40, 53], and increasing BDNF levels [38, 39, 41, 65], thereby enhancing mood [7, 35, 65] and cognitive functions in young adult mice [7, 39-41]. Moreover, maternal wheel running exercise during pregnancy ameliorates the deleterious effects of prenatal stress on dendritic morphology of pyramidal neurons in layer II/III of the parietal cortex and locomotor behavior in stressed mice [35]. Similarly, other studies reported that maternal exercise during pregnancy increases hippocampal neurogenesis in the adult pups and improves cognitive functions [34, 36, 53].

Our findings, along with previous research, suggest that physical exercise counteracts depression by enhancing hippocampal structural (neurogenesis and dendritic remodeling) and functional (synaptic plasticity) plasticity [11-15, 23, 24]. Notably, animals that are resilient to social stress exhibit higher levels of hippocampal neurogenesis [19, 20] and a larger hippocampal volume [21]. In humans, greater prefrontal cortex and hippocampal volumes are predictive of stress resilience to traumatic stress [22]. Collectively, these findings suggest that maternal exercise enhances hippocampal synaptic plasticity in adult offspring, which may be important for promoting long-lasting stress resilience. Of note, maternal exercise could reduce depression-like behavior in offspring via other biological and behavioral effects of maternal exercise, including alterations in

maternal behavior, direct neurochemical transfer across the placental or mammary barrier, and epigenetic mechanisms [37, 66].

In conclusion, our data suggest that maternal exercise is associated with enhanced hippocampal structural [7] and synaptic plasticity in offspring. This enhancement may contribute to increased resilience against chronic stress in adulthood. These findings support the potential beneficial effects of maternal exercise on offspring hippocampal plasticity. Future research should focus on elucidating the mechanistic insights underlying maternal exercise-induced epigenetic regulation, as well as determining whether structural and functional remodeling in the hippocampus of offspring from exercised dams is critical for conferring stress resilience.

Acknowledgments

The authors would like to thank the University Research Facility in Behavioral and Systems Neuroscience, Hong Kong Polytechnic University, for providing support during this study.

Funding declaration

This work was supported by the General Research Fund (GRF) of the Research Grants Council of Hong Kong (15104019) and the HKRGC Strategic Topics Grant (STG1/M-501/23-N).

Competing interest statement

The authors declare no competing interests.

Author contributions

Conceptualization, S.Y.Yau., K.F.So., and T.M.C Lee.; methodology, S.Y.Yau., Z.I., and J.M; validation, J.M. T.C.; investigation, Z.I., J.M., J.Y., T.C., D.F., A.H.; writing– original draft, Z.I. and S.Y.Yau; writing–review & editing, Z.I., J.M., J.Y., T.C., D.F., A.H., T.P W, K.F.So and T.M.C.Lee; funding acquisition, S.Y.Yau, K.F. So.; supervision, S.Y.Yau.

Data availability statement

The data obtained and analyzed in the current study have not been deposited in any public repository but are available from the corresponding author upon request.

References:

1. Friedrich, M.J., *Depression Is the Leading Cause of Disability Around the World.* Jama, 2017. **317**(15): p. 1517.
2. Kassebaum, N.J., et al., *Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015.* The Lancet, 2016. **388**(10053): p. 1603-1658.
3. Musazadeh, V., et al., *Probiotics as an effective therapeutic approach in alleviating depression symptoms: an umbrella meta-analysis.* Crit Rev Food Sci Nutr, 2023. **63**(26): p. 8292-8300.
4. Belleau, E.L., M.T. Treadway, and D.A. Pizzagalli, *The Impact of Stress and Major Depressive Disorder on Hippocampal and Medial Prefrontal Cortex Morphology.* Biol Psychiatry, 2019. **85**(6): p. 443-453.
5. Bisht, K., K. Sharma, and M. Tremblay, *Chronic stress as a risk factor for Alzheimer's disease: Roles of microglia-mediated synaptic remodeling, inflammation, and oxidative stress.* Neurobiol Stress, 2018. **9**: p. 9-21.
6. Milligan Armstrong, A., et al., *Chronic stress and Alzheimer's disease: the interplay between the hypothalamic-pituitary-adrenal axis, genetics and microglia.* Biol Rev Camb Philos Soc, 2021. **96**(5): p. 2209-2228.
7. Yau, S.-Y., et al., *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.* Frontiers in Neuroscience, 2019. **13**.
8. Zeng, J., et al., *Xiaoyaosan ethyl acetate fraction alleviates depression-like behaviors in CUMS mice by promoting hippocampal neurogenesis via modulating the IGF-1Rβ/PI3K/Akt signaling pathway.* J Ethnopharmacol, 2022. **288**: p. 115005.
9. Magariños, A.M. and B.S. McEwen, *Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors.* Neuroscience, 1995. **69**(1): p. 89-98.

10. Radley, J.J., et al., *Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex.* Cereb Cortex, 2006. **16**(3): p. 313-20.
11. Yau, S.Y., et al., *Hippocampal neurogenesis and dendritic plasticity support running-improved spatial learning and depression-like behaviour in stressed rats.* PLoS One, 2011. **6**(9): p. e24263.
12. Yau, S.Y., et al., *Physical exercise-induced hippocampal neurogenesis and antidepressant effects are mediated by the adipocyte hormone adiponectin.* Proc Natl Acad Sci U S A, 2014. **111**(44): p. 15810-5.
13. Yau, S.Y., et al., *Sustained running in rats administered corticosterone prevents the development of depressive behaviors and enhances hippocampal neurogenesis and synaptic plasticity without increasing neurotrophic factor levels.* Cell Transplant, 2014. **23**(4-5): p. 481-92.
14. Yau, S.Y., et al., *Chronic corticosterone administration reduces dendritic complexity in mature, but not young granule cells in the rat dentate gyrus.* Restor Neurol Neurosci, 2016. **34**(5): p. 849-57.
15. Yau, S.Y., et al., *Impaired bidirectional NMDA receptor dependent synaptic plasticity in the dentate gyrus of adult female Fmr1 heterozygous knockout mice.* Neurobiol Dis, 2016. **96**: p. 261-270.
16. Popoli, M., et al., *The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission.* Nat Rev Neurosci, 2011. **13**(1): p. 22-37.
17. Hardingham, G.E. and H. Bading, *Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders.* Nat Rev Neurosci, 2010. **11**(10): p. 682-96.
18. Surget, A. and C. Belzung, *Adult hippocampal neurogenesis shapes adaptation and improves stress response: a mechanistic and integrative perspective.* Mol Psychiatry, 2022. **27**(1): p. 403-421.
19. Lyons, D.M., et al., *Stress coping stimulates hippocampal neurogenesis in adult monkeys.* Proc Natl Acad Sci U S A, 2010. **107**(33): p. 14823-7.
20. Snyder, J.S., et al., *Adult hippocampal neurogenesis buffers stress responses and depressive behaviour.* Nature, 2011. **476**(7361): p. 458-61.
21. Tse, Y.C., et al., *A longitudinal study of stress-induced hippocampal volume changes in mice that are susceptible or resilient to chronic social defeat.* Hippocampus, 2014. **24**(9): p. 1120-8.
22. Chang, J., D. Song, and R. Yu, *The double-edged sword of the hippocampus-ventromedial prefrontal cortex resting-state connectivity in stress susceptibility and resilience: A prospective study.* Neurobiol Stress, 2023. **27**: p. 100584.
23. Wu, G., et al., *Understanding resilience.* Front Behav Neurosci, 2013. **7**: p. 10.
24. McEwen, B.S., *In pursuit of resilience: stress, epigenetics, and brain plasticity.* Ann N Y Acad Sci, 2016. **1373**(1): p. 56-64.
25. Horowitz, A.M., et al., *Blood factors transfer beneficial effects of exercise on neurogenesis and cognition to the aged brain.* Science, 2020. **369**(6500): p. 167-173.
26. Matinfar, P., M. Peeri, and M.A. Azarbayjani, *Swimming exercise attenuates anxiety-like behavior by reducing brain oxidative stress in type 2 diabetic mice.* Physiol Behav, 2021. **237**: p. 113449.
27. Otsuka, A., et al., *Voluntary exercise and increased food intake after mild chronic stress improve social avoidance behavior in mice.* Physiol Behav, 2015. **151**: p. 264-71.

28. Clapp, J.F., *Morphometric and neurodevelopmental outcome at age five years of the offspring of women who continued to exercise regularly throughout pregnancy.* The Journal of Pediatrics, 1996. **129**(6): p. 856-863.
29. Esteban-Cornejo, I., et al., *Maternal physical activity before and during the prenatal period and the offspring's academic performance in youth. The UP&DOWN study.* J Matern Fetal Neonatal Med, 2016. **29**(9): p. 1414-20.
30. Clapp, J.F., *Influence of endurance exercise and diet on human placental development and fetal growth.* Placenta, 2006. **27**(6-7): p. 527-34.
31. Jukic, A.M., et al., *Physical activity during pregnancy and language development in the offspring.* Paediatr Perinat Epidemiol, 2013. **27**(3): p. 283-93.
32. Jeon, Y.K. and C.H. Ha, *The effect of exercise intensity on brain derived neurotrophic factor and memory in adolescents.* Environ Health Prev Med, 2017. **22**(1): p. 27.
33. Labonte-Lemoyne, E., D. Curnier, and D. Ellemberg, *Exercise during pregnancy enhances cerebral maturation in the newborn: A randomized controlled trial.* J Clin Exp Neuropsychol, 2017. **39**(4): p. 347-354.
34. Dayi, A., et al., *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.* ScientificWorldJournal, 2012. **2012**: p. 429803.
35. Bustamante, C., et al., *Maternal exercise during pregnancy ameliorates the postnatal neuronal impairments induced by prenatal restraint stress in mice.* Int J Dev Neurosci, 2013. **31**(4): p. 267-73.
36. Bick-Sander, A., et al., *Running in pregnancy transiently increases postnatal hippocampal neurogenesis in the offspring.* Proc Natl Acad Sci U S A, 2006. **103**(10): p. 3852-7.
37. Robinson, A.M. and D.J. Bucci, *Maternal Exercise and Cognitive Functions of the Offspring.* Cogn Sci (Hauppauge), 2012. **7**(2): p. 187-205.
38. Gomes da Silva, S., et al., *Maternal Exercise during Pregnancy Increases BDNF Levels and Cell Numbers in the Hippocampal Formation but Not in the Cerebral Cortex of Adult Rat Offspring.* PLOS ONE, 2016. **11**(1): p. e0147200.
39. Parnpiansil, P., et al., *Exercise during pregnancy increases hippocampal brain-derived neurotrophic factor mRNA expression and spatial learning in neonatal rat pup.* Neurosci Lett, 2003. **352**(1): p. 45-8.
40. Kim, H., et al., *The influence of maternal treadmill running during pregnancy on short-term memory and hippocampal cell survival in rat pups.* Int J Dev Neurosci, 2007. **25**(4): p. 243-9.
41. M, M.A., et al., *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, 2013. **16**(9): p. 955-61.
42. Lee, T.H., et al., *Chronic AdipoRon Treatment Mimics the Effects of Physical Exercise on Restoring Hippocampal Neuroplasticity in Diabetic Mice.* Mol Neurobiol, 2021. **58**(9): p. 4666-4681.
43. Cheng, T., et al., *Rapid antidepressant effect of single-bout exercise is mediated by adiponectin-induced APPL1 nucleus translocation in anterior cingulate cortex.* Molecular Psychiatry, 2025. **30**(12): p. 5760-5776.

44. Yau, S.Y., et al., *Chronic minocycline treatment improves hippocampal neuronal structure, NMDA receptor function, and memory processing in Fmr1 knockout mice.* Neurobiol Dis, 2018. **113**: p. 11-22.
45. Lee, T.H., et al., *AdipoRon Treatment Induces a Dose-Dependent Response in Adult Hippocampal Neurogenesis.* Int J Mol Sci, 2021. **22**(4).
46. Yang, Q., et al., *Extrasynaptic NMDA receptor dependent long-term potentiation of hippocampal CA1 pyramidal neurons.* Sci Rep, 2017. **7**(1): p. 3045.
47. van Praag, H., et al., *Running enhances neurogenesis, learning, and long-term potentiation in mice.* Proc Natl Acad Sci U S A, 1999. **96**(23): p. 13427-31.
48. Patten, A.R., et al., *Long-term exercise is needed to enhance synaptic plasticity in the hippocampus.* Learn Mem, 2013. **20**(11): p. 642-7.
49. Bailey, A.P., et al., *Treating depression with physical activity in adolescents and young adults: a systematic review and meta-analysis of randomised controlled trials.* Psychol Med, 2018. **48**(7): p. 1068-1083.
50. Schuch, F.B., et al., *Physical Activity and Incident Depression: A Meta-Analysis of Prospective Cohort Studies.* Am J Psychiatry, 2018. **175**(7): p. 631-648.
51. Morres, I.D., et al., *Aerobic exercise for adult patients with major depressive disorder in mental health services: A systematic review and meta-analysis.* Depress Anxiety, 2019. **36**(1): p. 39-53.
52. Herring, A., et al., *Exercise during pregnancy mitigates Alzheimer-like pathology in mouse offspring.* Faseb j, 2012. **26**(1): p. 117-28.
53. Lee, H.H., et al., *Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups.* Brain Dev, 2006. **28**(3): p. 147-54.
54. Bergström, A., et al., *Stress sensitivity and resilience in the chronic mild stress rat model of depression; an in situ hybridization study.* Brain Res, 2008. **1196**: p. 41-52.
55. Taliaz, D., et al., *Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor.* J Neurosci, 2011. **31**(12): p. 4475-83.
56. Holtmaat, A. and K. Svoboda, *Experience-dependent structural synaptic plasticity in the mammalian brain.* Nat Rev Neurosci, 2009. **10**(9): p. 647-58.
57. Kassem, M.S., et al., *Stress-induced grey matter loss determined by MRI is primarily due to loss of dendrites and their synapses.* Mol Neurobiol, 2013. **47**(2): p. 645-61.
58. Qiao, H., et al., *Progressive alterations of hippocampal CA3-CA1 synapses in an animal model of depression.* Behav Brain Res, 2014. **275**: p. 191-200.
59. Krishnan, V. and E.J. Nestler, *The molecular neurobiology of depression.* Nature, 2008. **455**(7215): p. 894-902.
60. Franklin, T.B., B.J. Saab, and I.M. Mansuy, *Neural mechanisms of stress resilience and vulnerability.* Neuron, 2012. **75**(5): p. 747-61.
61. Chattarji, S., et al., *Neighborhood matters: divergent patterns of stress-induced plasticity across the brain.* Nat Neurosci, 2015. **18**(10): p. 1364-75.
62. Gold, P.W., *The organization of the stress system and its dysregulation in depressive illness.* Mol Psychiatry, 2015. **20**(1): p. 32-47.
63. McEwen, B.S., et al., *Mechanisms of stress in the brain.* Nature Neuroscience, 2015. **18**(10): p. 1353-1363.
64. Schoenfeld, T.J. and E. Gould, *Stress, stress hormones, and adult neurogenesis.* Exp Neurol, 2012. **233**(1): p. 12-21.

65. Aksu, I., et al., *Maternal treadmill exercise during pregnancy decreases anxiety and increases prefrontal cortex VEGF and BDNF levels of rat pups in early and late periods of life.* Neurosci Lett, 2012. **516**(2): p. 221-5.
66. Axsom, J.E. and J.R. Libonati, *Impact of parental exercise on epigenetic modifications inherited by offspring: A systematic review.* Physiol Rep, 2019. **7**(22): p. e14287.

Figure legends

Figure 1. Maternal Exercise Enhances Hippocampal Synaptic Plasticity in Adult Male and Female Offspring

(A) Representative traces of excitatory postsynaptic potentials (fEPSP) used for long-term potentiation (LTP) measurement. (B) LTP was induced by high-frequency stimulation, with maternal exercise significantly increasing the percentage of fEPSP in the hippocampal dentate gyrus, indicating enhanced LTP formation in offspring from exercised dams (N=4, n=3 slices/mice). (C) Maternal exercise significantly increased the average of the last 5-minute fEPSP in both male (***p=0.0009) and female offspring (****p<0.0001; N=4, n=3 slices/mice). (D) Basal synaptic transmission was recorded using input-output (I/O) curve functions, showing that adult female offspring from exercised dams exhibited significantly higher amplitudes with increasing stimulation intensity (p<0.0001; N=4, n=3 slices/mice). (E) Representative traces of paired-pulse ratio. (F) Maternal exercise did not affect the paired-pulse ratio in adult male and female offspring (N=4, n=3 slices/mice). Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test. Data are presented as mean ± SD. Sed = sedentary; MEx = maternal exercise; HFS = high-frequency stimulation.

Figure 2. Maternal Exercise Enhances Hippocampal Synaptic Protein Expression

(A, B) A main effect of maternal exercise was observed in increasing BDNF protein expression levels (p=0.0168; N=4-5/group). (C, D) Maternal exercise significantly upregulated the expression of the synaptic protein PSD-95 in adult offspring (p=0.0017; N=7-8/group). (E, F) It also significantly increased synaptophysin expression levels in both male and female offspring (p<0.0001; N=6/group). (G, H) No significant effects were found on the expression of the p-GluN2B subunit of the NMDA receptor (p=0.2913; N=6/group), but (I, J) there was an increase in

p-GluN2A expression levels in female offspring (p=0.0330; N=6/group). (K, L) Maternal exercise had a main effect on increasing the expression of GluA1, an AMPA receptor subunit (p=0.0157; N=6/group). Protein levels were normalized to α-tubulin, and results are presented as relative protein expression. Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test. Loading control in panels C and E was from the same gel. Data are presented as mean ± SD. Sed = sedentary; MEx = maternal exercise.

Figure 3. Maternal Exercise Increases Dendritic Spine Density in Female Offspring

(A) Representative Golgi-stained images of dendritic spines in granule neurons with multiple primary branches from male and female offspring. (B) Maternal exercise significantly increased spine density in granule cells with multiple primary dendritic branches in female offspring (p=0.0074) but had no effect in male offspring (p=0.7936; N=6/group). (C) Representative Golgi-stained images of dendritic spines in granule neurons with single primary branches from male and female offspring. (D) Maternal exercise significantly increased spine density in female offspring (p=0.0113) but showed no effect in male offspring (p=0.6035; N=6/group). Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test. Data are presented as mean ± SD.

Figure 4. Maternal Exercise Confers Stress Resilience In Offspring Exposed to Chronic Stress

(A) Schematic diagram illustrating the experimental timeline for maternal exercise and behavioral tasks in adult offspring. In male offspring, maternal exercise significantly decreased (B) immobility time in the forced swim test ($p<0.05$) and (C) increased grooming time in the sucrose splash test ($p<0.05$). (D) Maternal exercise significantly increased time spent in the center zone for male offspring exposed to chronic unpredictable stress compared to sedentary counterparts and

719 non-stressed controls ($p=0.05$). (E) No significant effect was observed on total distance traveled
720 in the open field test ($p=0.5168$). In female offspring, maternal exercise (F) prevented depressive-
721 like behavior induced by chronic unpredictable stress ($p=0.0014$) and (G) increased grooming time
722 in the sucrose splash test ($p<0.05$). Maternal exercise did not significantly affect total time spent
723 in the center ($p=0.4136$) (H) or total distance traveled (I) by female offspring under chronic
724 unpredictable stress ($p=0.3912$). Statistical analysis was performed using one-way ANOVA
725 followed by Tukey's post hoc test. Data are presented as mean ± SD.

726

727 **TablesTable 1: Schedule of chronic unpredictable stressors (CUS)**

Days	Type of stressors	Treatment frequency/duration
1	restraint stress	(0.5h)
2	inescapable foot shock	(1 s, 2mA every 30s for 10 min = 10 shocks)
3	exposure to the shock apparatus	(without shock- 10min)
4	restraint stress	(1.5h)
5	wet bedding	(maximum 24h)
6	food and water deprivation	(maximum 24h)
7	Empty bottle exposure	(1h)
8	inescapable foot shock	(1 s, 2mA every 30s for 10 min = 10 shocks)
9	exposure to the shock apparatus	(without shock- 10min)
10	restraint stress	(2h)
11	wet bedding	(maximum 24h)
12	food and water deprivation	(maximum 24h)
13	Empty bottle exposure	(1h)
14	inescapable foot shock	(1 s, 2mA every 30s for 10 min = 10 shocks)
15	exposure to the shock apparatus	(without shock- 10min)
16	wet bedding	(maximum 24h)
17	Cage tilt at an angle of 45°	cages were tilted at an angle of 45° for one hour
18	food and water deprivation	(maximum 24h)
19	inescapable foot shock	(1 s, 2mA every 30s for 10 min = 10 shocks)
20	exposure to the shock apparatus	(without shock- 10min)
21	restraint stress	(2.5h)

728