Acta Neuropsychiatrica

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Running from depression: the antidepressant-like potential of prenatal and pre-pubertal exercise in adolescent FSL rats exposed to an early-life stressor

Ashleigh J. Whitney[1], Zander Lindeque[2], Ruan Kruger[3,4] and Stephan F. Steyn[1]

[1]Centre of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa; [2]Human Metabolomics, Faculty of Natural and Agricultural Sciences, North-West University, Potchefstroom, South Africa; [3]Hypertension in African Research Team (HART), North-West University, Potchefstroom, South Africa and [4]MRC Research Unit for Hypertension and Cardiovascular Disease, North-West University, Potchefstroom, South Africa

Original Article

Cite this article: Whitney AJ, Lindeque Z, Kruger R, and Steyn SF. (2024) Running from depression: the antidepressant-like potential of prenatal and pre-pubertal exercise in adolescent FSL rats exposed to an early-life stressor. *Acta Neuropsychiatrica* **36**:363–377. doi: 10.1017/neu.2023.52

Received: 3 April 2023
Revised: 5 September 2023
Accepted: 7 October 2023
First published online: 16 November 2023

Keywords:
Adolescence; depression; flinders sensitive line; low-intensity exercise; oxidative stress

Corresponding author:
S. F. Steyn; Email: stephan.steyn@nwu.ac.za

Abstract

Objective: We aimed to answer the questions of whether early-life (perinatal and/or juvenile) exercise can induce antidepressant-like effects in a validated rodent model of depression, and whether such early-life intervention could prevent or reverse the adverse effects of early-life stress in their offspring. *Methods:* Male and female Flinders sensitive line rats born to a dam that exercised during gestation, or not, were either maternally separated between PND02 and 16 and weaned on PND17 or not. Half of these animals then underwent a fourteen-day low-intensity exercise regimen from PND22. Baseline depressive-like behaviour was assessed on PND21 and then reassessed on PND36, whereafter hippocampal monoamine levels, redox state markers and metabolic markers relevant to mitochondrial function were measured. *Results:* Pre-pubertal exercise was identified as the largest contributing factor to the observed effects, where it decreased immobility time in the FST by 6%, increased time spent in the open arms of the EPM by 9%. Hippocampal serotonin and norepinephrine levels were also increased by 35% and 26%, respectively, whilst nicotinic acid was significantly decreased. *Conclusion:* These findings suggest that pre-pubertal low-intensity exercise induces beneficial biological alterations that could translate into antidepressant behaviour in genetically susceptible individuals.

Significant outcomes

- Prenatal exercise may alter coping behaviours in adolescent Flinders sensitive line (FSL) rats offspring.
- Prenatal exercise has long-term beneficial effects on hippocampal redox state.
- Pre-pubertal low-intensity exercise reduces depressive-like behaviour in adolescent FSL rats.
- Low intensity exercise alters hippocampal redox state, pointing to mitochondrial involvement.

Limitations

- The metabolic profile sample collection method, specifically the use of the buffer, may have influenced the results and therefore our findings require confirmation and validation.
- We did not measure any neuro- and biochemical markers on PND21, therefore the behavioural findings of the TST require confirmation.
- Pregnant dams exercised for 13 ± 5 days, which may have influenced the results of the prenatal exercise group.
- No stress-related markers were measured in animals born to an exercised dam, which would elaborate on the behavioural interpretations of these pups.

Introduction

The human brain accounts for only 2% of a person's weight yet consumes 20% of total glucose and oxygen (Rolfe & Brown, 1997; Manji *et al.*, 2012; Pei & Wallace, 2018), explaining the

particular high energy demand of brain neurons (80–90% of the total brain demand). In fact, the human brain uses ~ 5.7 kg of ATP/day at rest, of which the majority is utilised by cortical neurons (Zhu *et al.*, 2012). These energy demands are significantly increased by stress (Bryan, 1990) and sets off structural and functional changes in surrounding cells to match the required behaviour in response to stress (Picard *et al.*, 2014). Early-life trauma has been shown to negatively affect central and peripheral mitochondrial function (Hoffmann & Spengler, 2018; Ruigrok *et al.*, 2021), thereby adversely altering stress-response pathways (Zitkovsky *et al.*, 2021), and contributing to the underlying pathophysiology of depression (Sharma & Akundi, 2019; van Rensburg *et al.*, 2022) and other psychiatric conditions (van Rensburg *et al.*, 2022). Clinical findings have also reported dysfunctional mitochondria in depressed patients (reviewed by Caruso *et al.* (2019)) and the diverse effects of approved psychotropic drugs on mitochondrial function (Emmerzaal *et al.*, 2021).

With this considered, mitochondrial (dys)function may be a promising target to treat depression (Allen *et al.*, 2018; Sharma & Akundi, 2019; Wu *et al.*, 2019) and is of particular importance and value in vulnerable populations, such as pregnant women and pre-pubertal children, in which the currently approved treatment options are limited to selective serotonin reuptake inhibitors (Kimmel *et al.*, 2018; Viswanathan *et al.*, 2020). Moreover, the use of these (and other) antidepressants during these vulnerable developmental periods is often questioned (Bérard *et al.*, 2017; Molenaar *et al.*, 2018; Hengartner, 2020) because of the uncertainty surrounding their long-term effects and overall safety profiles. In fact, all antidepressants require a "black box" warning for increased suicidal behaviour in juvenile patients (U.S. Food & Drug Administration, 2004). It is for these reasons that alternative treatment options, especially in these vulnerable populations, are necessary. One promising non-pharmacological treatment option is exercise. The antidepressant effects of exercise have been widely established (Carter *et al.*, 2016; Kandola *et al.*, 2019), yet the exact mechanisms through which it exerts its antidepressant effects remain unknown (Schuch *et al.*, 2016). Numerous mechanisms have been proposed (Kandola *et al.*, 2019; de Oliveira *et al.*, 2022) and include (but are not limited to) increased monoamine neurotransmission (Lin & Kuo, 2013) and neuroplasticity (El-Sayes *et al.*, 2019) and decreased inflammation and oxidative stress (Eyre & Baune, 2012), all of which have been implicated in the pathophysiology of depression and as alluded to earlier, linked to mitochondrial function (Allen *et al.*, 2018; Sharma & Akundi, 2019; van Rensburg *et al.*, 2022). Additionally, exercise also induces bio-energetic enhancing effects (i.e., improved mitochondrial functioning (Aguiar *et al.*, 2014; Wu *et al.*, 2019)). In contrast, physical inactivity has been linked to adverse health outcomes, including depression (Kandola *et al.*, 2019) and metabolic disorders, such as diabetes and cardiovascular diseases (Katzmarzyk *et al.*, 2022), which are often co-morbid with depression. Exercise during the prenatal period is known to be safe and beneficial to the mother and offspring (Davenport, *et al.*, 2018; Davenport, *et al.*, 2018; Moyer *et al.*, 2016). Also, increased physical activity during juvenile development is inversely associated with depressive symptoms (Biddle *et al.*, 2019; Dale *et al.*, 2019). Therefore, physical exercise may not only be a promising treatment option for depression but also provide additional health benefits, and even reduce the risk to develop depression in children with a genetic predisposition to develop depression.

Early-life development is a sensitive period, characterised by extensive growth and plasticity, that makes the developing brain sensitive to external influences (Heim *et al.*, 2010; Scattolin *et al.*, 2022). For instance, early-life adversity, such as neglect or abuse, can have detrimental developmental effects with long-lasting consequences (Andersen & Teicher, 2008; Obi *et al.*, 2019) to such an extent that one-third of mental disorders, including depression and anxiety can be ascribed to early-life adversity (Kessler *et al.*, 2010; McLaughlin *et al.*, 2019). In the USA, it is estimated that 4.4% and 9.4% of children aged 3–17 years suffer from depression and anxiety, respectively, with 33% of children who suffer from anxiety also experiencing depressive symptoms (Centers for Disease Control and Prevention, 2021). Importantly, neurodevelopment does not only occur during childhood and the adolescent period but begins as early as the embryonic stage (Scattolin *et al.*, 2022) and is therefore also influenced by the perinatal environment (Schuurmans & Kurrasch, 2013). To this extent, it is worth noting that prenatal stress (including maternal depression and family adversity) is not only associated with offspring depression later in life but is also a significant risk factor for childhood trauma (Liu *et al.*, 2022). It is for this reason that maternal health during the perinatal and post-partum period is significant for a developing child. Importantly, maternal depression during the perinatal and or post-partum period significantly increases the offspring's risk to develop depression later in life by as much as 70% (Tirumalaraju *et al.*, 2020) and therefore, by simply being born into a family with a history of depression, increases the risk for developing depression (Fihrer *et al.*, 2009; Thompson *et al.*, 2018; Tirumalaraju *et al.*, 2020). Still, due to ethical and practical reasons, whether prenatal exercise can protect or even reverse the adverse effects of early-life adversity, remains unexplored. Therefore, to mimic this increased risk of developing depression both through a combination of genetic and environmental influences, the current study applied an early-life stressor (i.e. maternal separation and early weaning; MSEW) to an approved genetic rodent model of depression (i.e. Flinders sensitive line (FSL) rat) (Overstreet & Wegener, 2013), as this strain has been reported to already display depressive-like behaviour during juvenile development (Malkesman & Weller, 2009; Whitney *et al.*, 2023).

Considering the above, we hypothesised that exercise during the prenatal and pre-pubertal periods would induce antidepressant-like behaviour by improving hippocampal mitochondrial function, monoaminergic neurotransmission and redox state in adolescent FSL rats (representing a juvenile patient, predisposed to develop depression). Moreover, we hypothesised that these effects would prevent and/reverse the depressogenic effects of the early-life stressor.

Materials and methods

Study layout

A similar study layout, except for the exercise interventions, was used as before to build on our previous findings (Whitney *et al.*, 2023). Briefly, pregnant FSL dams were either subjected to a chronic low-intensity exercised regimen or not. Next, the offspring of these dams were then further divided into MSEW and non-MSEW groups. On PND21, animals were subjected to the open field (OFT) and tail suspension tests (TST) to determine early-life depressive-like behaviour. Next, 50% of the animals underwent a 14-day low-intensity exercise regimen, whereafter all animals were subjected to the OFT and forced swim tests (FST) on PND36,

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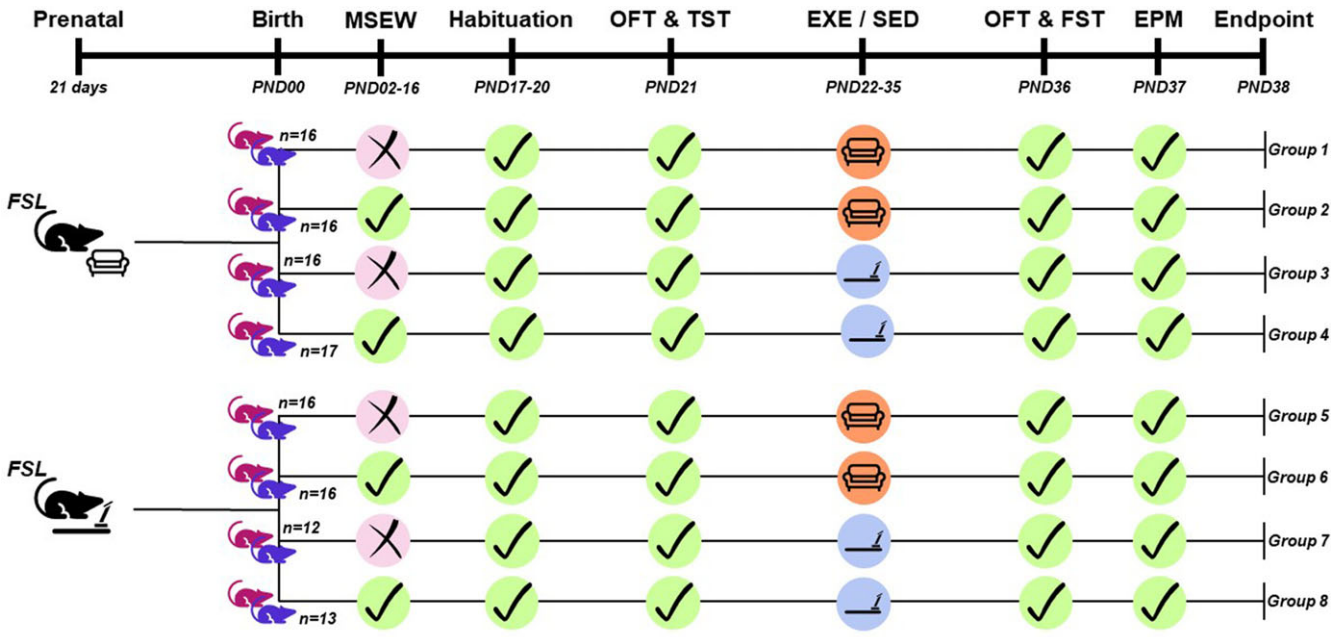

Figure 1. Graphical summary of the study layout. Pregnant FSL dams were either subjected to a prenatal sedentary or low-intensity exercise regimen. Animals were either subjected to early-life stress (MSEW) between PND02 and 17 or not. Early-life behavioural testing took place on PND21 to determine the effects of prenatal exercise. To investigate the bio-behavioural effects of juvenile exercise (with and without prenatal exercise), a 14-day low-intensity exercise (or sedentary) regimen was introduced on PND22, whereafter behavioural testing took place on PNDs36 and 37, followed by decapitation and brain dissection on PND38. Tissue was frozen at −80°C until neurochemical analyses were performed. Couch icon: sedentary group. Treadmill icon: exercise group. Pink rat icon: female rats. Purple rat icon: male rats. EPM, elevated plus maze; EXE, low-intensity exercise; FRL, flinders resistant line; FSL, flinders sensitive line; FST, forced swim test; MSEW, maternal separation with early weaning; OFT, open field test; PND, postnatal day; SED, sedentary; TST, tail suspension test.

followed by the elevated plus maze test on PND37. The sequence of the behavioural analysis, specifically in terms of performing the forced swim test before the elevated plus maze test, has been carried out by others (Neumann *et al*., 2011; Rea *et al*., 2014; Bay-Richter *et al*., 2019). Moreover, to minimise the risk for potential habitual learning, depressive-like behaviour was analysed by two different behavioural tests (i.e. TST and FST) at different time points. To ensure normal initial foraging and activity of nocturnal animals, testing only commenced one hour after the start of the dark cycle. Tests were carefully spaced to allow 30 min between each test for animals to habituate to the environment. Automated tracking software (Ethovision XT14 Software; Noldus information Technology BV, Wageningen, NLD) was used to track behaviour in the OFT and EPM. TST and FST behaviour was manually scored by a researcher blind to the experimental group details, from recordings of the behavioural tests, recorded with a camera mounted in front of the test apparatus.

Animals and justification of group sizes

Building on previous findings, where we investigated the effects of MSEW on FSL rats (Whitney *et al*., 2023), male and female FSL ($n = 122$) rats were divided into eight experimental groups, consisting of sixteen rats (50:50 females:males) per group (Fig. 1). Male and female rats were grouped together as sex differences is not expected in pre-pubertal animals. Still, results were visually inspected to identify any obvious sex differences. Smaller groups were, however, sometimes employed due to the exclusion of non-runners and/or when the breeding programme failed to supply the adequate number of animals. Group sizes were calculated with a predicted effect size F of 0.403 ($\eta^2_p = 0.14$), α error (0.05) and 80% power. Rats were group housed (3–4 rats/cage), according to sex, with corncob bedding changed weekly and the environmental temperatures maintained at 22 ± 1°C in a relative humidity of 55 ± 10%. A 12 h light/dark cycle was followed with food and water provided *ad libitum*.

Maternal separation and early weaning

As described by George and colleagues (2010), pups in the MSEW groups were left in their home cages, while the dams were relocated to new, clean cages with *ad libitum* access to food and water for 3 h per day from PND02 to 16. In contrast, the non-MSEW pups were left, undisturbed with the dam, with all pups remaining with their littermates. Finally, MSEW animals were also weaned on PND17, opposed to the standard PND21 when the non-MSEW pups were weaned.

Exercise regimen

Forced exercise was implemented using a custom-built, programmable treadmill, comprising of a single running belt six shocking grids installed at the back of the treadmill. The shocking grid delivered an electrical shock of 1 mA (3 Hz), and the shocking intensity was selected to be uncomfortable but not painful or harmful. Sedentary animals were removed from their home cages and placed on a mock (still standing) treadmill. All exercise interventions were performed during the animal's active dark cycle (i.e. from 18:00 in the evening until latest 03:00 in the morning, depending on animal numbers). All animals were familiarised to the treadmill using a 10-min routine to reduce injury risk and identify any 'non-runners'. As described previously (Kregel *et al*., 2006), animals were classified as 'non-runners' when they were unable to keep up with the speed of the treadmill (i.e. shocked three

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Table 1. Summarised protocol for prenatal and juvenile familiarisation and exercise. Adapted from Aksu *et al.*, 2012 and Seo *et al.*, 2013

Intervention period	Protocol
Prenatal exercise	
Familiarisation	5m/min for 10 min/day for 7 days
Pregnancy	**Exercise group:** 30 min/ day at 2m/min for 5 min followed by 5m/min for 5 min and then 8m/min for 20 min 7days a week until birth
Juvenile exercise	
Familiarisation	All pups (*regardless of experimental group*) from PND 17-20 were subjected to a daily 10-min routine (*except for PND 19 where it is a 9 min routine*) with gradual increases in treadmill speed.
	PND 17: 2m/min for 5 min followed by gradual 0.1m/min increases every 1 min until a final speed of 2.5m/min in the last min.
	PND 18: 2m/min for 3 min followed by 2.5m/min for 2 min. Then a 1 min rest period. 2.5m/min for 1 min followed by 3m/min for 3 min.
	PND 19: 2m/min for 3 min followed by 3m/min for 3 min with an increase to 4m/min for 3 min and a final speed of 4.5m/min for 1 min.
	PND 20: 2.5m/min 3 min, then 3.5m/min for 3 min followed by 4.5m/min for 3 min.
PND22-PND35	**Exercise group:** 30 min/day at 2m/min for 5 min followed by 5m/min for 5 min and then 12m/min 7 days a week for 14 days.
	Sedentary group: placed in a mock treadmill for 30 min/day for 7 days a week for 14 days

times within 1 min) during the familiarisation period. Pups identified as 'non-runners' were used as control rats; however, those identified as 'non-runners' during the exercise intervention were removed from the study. We observed a small number of rats (< 10%) that displayed 'non-runner' behaviour. Pregnant FSL dams allocated to the exercised group were familiarised with the treadmill two days after being paired to ensure that dams exercise for the full term of their pregnancy. The protocol approach for the familiarisation and low-intensity exercise differed between prenatal exercise and juvenile exercise and is therefore summarised in Table 1.

Behavioural analyses

Open field test

The OFT was used to measure general locomotor activity, and consisted of a 1 m^2 test arena, surrounded by opaque black, vertical walls. As previously (Steyn *et al.*, 2020) described, each rat, on the day of testing, was placed in the centre of the arena and allowed to freely explore the arena for 5 min under red light. Total distance moved was interpreted as a measure of general activity.

Tail suspension test

In the current study, the TST was used to screen baseline juvenile depressive-like behaviour on PND21. As before (Castagné *et al.*, 2010; Cryan, *et al.*, 2005), on the day of testing, each rat was suspended by the tail with adhesive tape, positioned three-quarters of the distance from the base of the tail from a suspension hook for 6 min. To avoid injury, the suspension hook went through the adhesive tape as close as possible to the tail to ensure the animal hangs with its tail in a straight line (Castagné *et al.*, 2010). The total time spent immobile was recorded and interpreted as an indication of depressive-like behaviour.

Forced swim test

The FST was performed on PND36, as previously described in our laboratories (Brand & Harvey, 2017; Steyn *et al.*, 2020), without a pre-conditioning swim trial, 24 hr prior to the testing trial (Overstreet *et al.*, 2005; Overstreet & Wegener, 2013). Briefly, during the dark cycle, animals were placed in an inescapable Perspex® cylinder filled with 30 cm of water at a temperature of 25 ± 1°C for 6 min. Behaviour was scored manually by an experimenter blind to the experimental group, with the first minute of the test ignored (Roets *et al.*, 2023). Behaviour scored included immobility (floating with no active movements made, except those necessary to keep the rat's head above water), swimming (horizontal movements throughout the cylinder that included crossing into another quadrant) and struggling (upward-directed movements of the forepaws along the inside of the swim cylinder) (Cryan *et al.*, 2002; Cryan, *et al.*, 2005). Increased immobility was considered an indication of depressive-like behaviour.

Elevated plus maze

The EPM is plus shaped Perspex maze that consists of two closed and two open arms, elevated approximately 50 cm above the floor with a 1 cm transparent Plexiglas border to prevent animals from falling. As described previously (Regenass *et al.*, 2018), rats were placed in the centre zone of the maze, facing the open arm opposite the investigator, and allowed to freely explore the maze for 5 min under red light. Increased time spent in the closed arms was interpreted as anxiety-like behaviour, with entrance into an arm considered when the centre point, as defined by the automated scoring program, entered the arm.

Bio-analyses

Tissue collection and storage

Animals were euthanised by decapitation on PND38, whereafter brain and heart samples were harvested and weighed. Following the decapitation, right and left hippocampi were dissected on an ice-cooled dissection slab and stored separately. The right hippocampi were used for neurochemical analysis via LC–MS and snap-frozen in liquid nitrogen and stored at -80°C. The left hippocampi were removed and immediately placed into an isolation buffer (mannitol 200 mM, sucrose 50 mM, potassium phosphate 5 mM, EGTA 1 mM, 3-(N-morpholino)propanesulfonic acid 5 mM and bovine serum albumin 0.10% pH 7.2) (Kim *et al.*, 2016), whereafter it was also stored at -80°C.

Quantitative analyses of hippocampal monoamines, GSH and GSSG

Quantitative monoaminergic, GSH (glutathione) and GSSG (glutathione disulphide) concentrations were analysed via LC–MS, as before (Whitney *et al.*, 2023). A detailed description of the method is available as Supplementary data.

Metabolic profiling via GC-TOF-MS analysis

Untargeted gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) was performed as previously described (Lindeque *et al.*, 2013; Terburgh *et al.*, 2019) on the left hippocampi of FSL rats (Whitney *et al.*, 2023). Briefly, a stepwise Bligh–Dyer extraction

https://doi.org/10.1017/neu.2023.52 Published online by Cambridge University Press

method (Wu *et al.*, 2008) was performed resulting in biphasic separation. Furthermore, all samples were derivatised via oximation and silylation as previously decribed (Lindeque *et al.*, 2013; Terburgh *et al.*, 2019) prior to GC-TOF-MS analysis. For data acquisition and extraction, the LECO Corporation ChromaTOF® software (v 4.5x) was utilised. The NIST MS search program (v 0.2) using AMDIS (National Institute of Standards and Technology) was used to compare measured spectra to the NIST 11 mass spectral library to identify all the detected components and validate relevant metabolites. A detailed description of the method is available as Supplementary data.

Statistical analyses

Statistical analyses were performed in IBM® SPSS® Statistics (version 28), assisted by Laerd Statistics® (https://statisticslaerd.com) and the NWU statistical consultation services. Effect magnitude indicators were calculated in Exploratory Software for Confidence Intervals (Cumming, 2014). All graphical representations were created in GraphPad Prism® (version 10) with the initial power analysis performed in G*Power (version 3; Universität Kiel, GER).

The Grubb's test was used to identify outliers and are reported in figure and table legends. Normality of distribution and homogeneity of variances were determined with the Shapiro-Wilk and Levene's tests, respectively. Only instances where these assumptions were not true are reported in the text. As for the metabolomic screening, normality of data was not analysed due to the number of measurements. Instead, data were simply log transformed and analysed with the appropriate statistical tests (Lindeque *et al.*, 2013) and normalised using the MSTUS normalisation method (Warrack *et al.*, 2009). First, normal two-way ANOVAs (analysis of variances) were used for PND21 analysis, with prenatal activity (EXE and SED) and early-life adversity (MSEW and non-MSEW) set as variables. Next, normal three-way ANOVAs were performed on PND36 parameters, with juvenile activity (SED and EXE), prenatal activity (SED and EXE) and early-life adversity (MSEW and non-MSEW) considered. Where locomotor activity was expected to influence results, appropriate ANCOVAs (analysis of co-variances) were performed to correct for this expected influence. A 5% confidence limit for error in all cases was accepted as statistically significant and reported as a Bonferroni-adjusted value. The mean differences between groups are reported with 95% confidence interval. For the GC–MS data, an independent *t*-test was performed in MetaboAnalyst version 5 (www.metaboanalyst.ca)

Statistical analyses were followed up by effect magnitude calculations (Cumming et al., 2007; Lakens, 2013). Partial eta squared (ηp^2) and the unbiased Cohen's d (dunb) values (Cumming, 2014) were used to calculate effect magnitude of interactions and intergroup differences, respectively. Large effect sizes were accepted as $\eta p^2 \geq 0.14$ (Ellis, 2010) and $d \geq 0.8$ (Sullivan & Feinn, 2012). Importantly, to facilitate the interpretation of these findings, the effect magnitude values of the different behavioural parameters were calculated to identify the largest and statistically non-zero contributing factor (i.e. main effect), which was subsequently used to guide further analyses (see Section 3.1).

Results

Effects of prenatal activity and maternal separation and early weaning

In Fig. 2a, there was no significant interaction between prenatal activity (PRE) and early-life adversity (ELA) ($F_{1,117} = 0.74$, $p = 0.39$, $\eta_p^2 = 0.006$), nor any significant main effect ($p>0.05$) for distance moved in the OFT. Nonetheless, this parameter was used as a covariant in the analysis of the TST below.

Locomotor activity did not significantly influence immobility time in the TST ($F_{1,116} = 2.19$, $p = 0.14$, $\eta_p^2 = 0.019$), yet a significant PRE*ELA interaction was identified (Fig. 2b; $F_{1,116} = 5.02$, $p = 0.027$, $\eta_p^2 = 0.041$), with PRE also contributing as an independent factor ($F_{1,116} = 9.53$, $p = 0.003$, $\eta_p^2 = 0.076$). In terms of this effect, pups born to an EXE dam (regardless of ELA) were 27 s [10; 44 s] more immobile than their SED counterparts. More specifically, this effect only reached statistical significance in MSEW animals ($p \leq 0.0005$, $d_{unb} = 1.1$ [0.5; 1.7]).

Effects of pre-pubertal low-intensity exercise

The overall effect of the various factorial interactions and main effects of the behavioural parameters, including those discussed below, are presented in Fig. 3. Based on these results, juvenile activity (JUV) was the largest and statistically non-zero contributing factor that was used to guide further analyses and simplify the interpretation thereof. Still, all other significant findings are available as supplementary data.

FST behaviour on PND36, after correcting for locomotor differences

In Fig. 4a, a significant three-way interaction ($F_{1,114} = 6.89$, $p = 0.01$, $\eta_p^2 = 0.06$; *Supplementary data*), as well as a significant PRE*ELA interaction ($F_{1,114} = 6.77$, $p = 0.01$, $\eta_p^2 = 0.06$), existed for distance moved in the OFT on PND36. Despite narrowly missing significance ($F_{1,114} = 3.74$, $p = 0.056$, $\eta_p^2 = 0.03$), JUV independently trended to influence distance moved, so that pups that exercised (regardless of PRE and ELA) covered 235 cm [6; 476 cm] more than sedentary controls ($d_{unb} = 0.3$ [−0.1; 0.7]). These differences were subsequently used as a covariant in the FST analyses.

Distance moved in the OFT had no significant effect on any of the FST behavioural parameters ($p > 0.05$ in all instances; Table 2). After correcting for distance moved, there were no significant three-way interactions for time spent immobile ($F_{1,113} = 0.239$, $p = 0.63$, $\eta_p^2 = 0.002$), swimming ($F_{1,113} = 0.14$, $p = 0.71$, $\eta_p^2 = 0.001$) or struggling ($F_{1,113} = 0.55$, $p = 0.46$, $\eta_p^2 = 0.005$).

In addition to the significant PRE*ELA interaction (*Supplementary data;* $F_{1,\ 113} = 4.85$, $p = 0.03$, $\eta_p^2 = 0.04$;), JUV independently also influenced time spent immobile in the FST (Fig. 4b; $F_{1,113} = 5.94$, $p = 0.02$, $\eta_p^2 = 0.05$). Pups that exercised (regardless of PRE and ELA) were 13 s [2; 24 s] less immobile than their sedentary controls ($d_{unb} = 0.5$ [0.1; 0.9]).

For time spent swimming (Fig. 4c), three significant two-way interactions were identified (*Supplementary data*), with PRE*ELA considered the largest ($F_{1,113} = 18.64$, $p \leq 0.0005$, $\eta_p^2 = 0.14$). However, after correcting for distance moved, JUV, as independent factor, did not influence time spent swimming in the FST ($F_{1,\ 113} = 2.19$, $p = 0.14$, $\eta_p^2 = 0.02$).

Only JUV influenced struggling behaviour (Fig. 4d) in the FST ($F_{1,113} = 4.20$, $p = 0.04$, $\eta_p^2 = 0.04$), so that pups that exercised (regardless of PRE or ELA) struggled 8.6 s [0.3; 17 s] longer than sedentary controls ($d_{unb} = 0.4$ [0.1; 0.8]).

EPM behaviour on PND36

In Fig. 5, there was no significant three-way interaction ($F_{1,109} = 0.89$, $p = 0.35$, $\eta_p^2 = 0.01$), nor any two-way interactions

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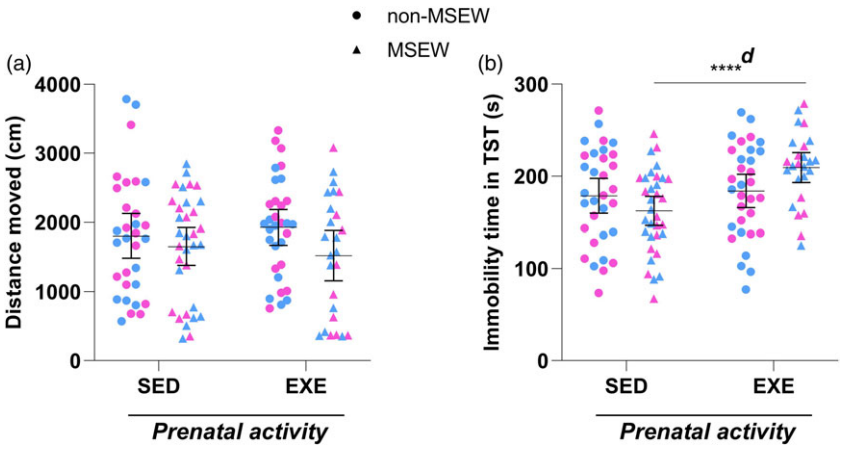

Figure 2. PND21 effects of prenatal exercise on FSL offspring either exposed to early-life adversity or not. (***a***) distance moved (over 5 min) in the OFT[a,b] and (***b***) time spent immobile in the TST on PND21. Data points represent the mean ± 95% CI, with male and female indicated in blue and pink, respectively. Statistical analyses are reported in the text. [a)] not all data sets were normally distributed. [b)] outlier identified and excluded from analysis. EXE, pre-natal low-intensity exercise; MSEW, maternal separation and early weaning; SED, sedentary. TST: tail suspension test.

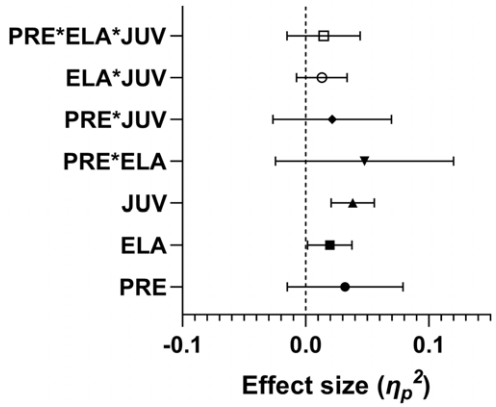

Figure 3. Forest plot of the overall behavioural effects of the contributing factors. ELA, early-life adversity; JUV, juvenile activity; PRE, prenatal activity.

identified for percentage time spent in the open arm of the EPM. However, JUV, independently influenced time spent in the open arms ($F_{1,109} = 6.22$, $p = 0.01$, $\eta_p^2 = 0.05$), so that pups that exercised (regardless of PRE and ELA) spent 9% [2; 15%] more time in the open arms, compared to sedentary controls ($d_{unb} = 0.5$ [0.1; 0.8]).

Anatomical markers

For whole brain (Fig. 6a; $F_{1,107} = 0.62$, $p = 0.43$, $\eta_p^2 = 0.006$) and heart (Fig. 6b; $F_{1,109} = 0.26$, $p = 0.62$, $\eta_p^2 = 0.002$) weight, there were no significant three-way interactions, nor any two-way interactions. However, PRE, ELA (*Supplementary data*) and JUV independently influenced whole brain ($F_{1,107} = 5.23$, $p = 0.02$, $\eta_p^2 = 0.02$) and heart ($F_{1,109} = 6.237$, $p = 0.014$, $\eta_p^2 = 0.05$) weights, so that the brains and hearts of pups that exercised (regardless of PRE and ELA), respectively, weighed 0.08% [0.02; 0.1%] ($d_{unb} = 0.3$ [−0.02; 0.7]) and 0.03% [0.01; 0.06%] ($d_{unb} = 0.5$ [0.1; 0.9]) more than that of their sedentary controls.

Hippocampal monoamine levels and redox state

In addition to the significant three-way interaction ($F_{1,102} = 18.033$, $p \leq 0.0005$, $\eta_p^2 = 0.15$; *Supplementary data*), hippocampal norepinephrine levels were also influenced by JUV (Fig. 7a; $F_{1,102} = 12.97$, $p \leq 0.0005$, $\eta_p^2 = 0.11$), independently, so that the levels of pups that exercised (regardless of PRE and ELA) were 233.75 ng/g [105; 363 ng/g] higher than that of sedentary controls ($d_{unb} = 0.4$ [0.0; 0.8]).

Hippocampal serotonin levels were also significantly influenced by a three-way interaction ($F_{1,106} = 13.05$, $p \leq 0.0005$, $\eta_p^2 = 0.110$; *Supplementary data*) and independently by JUV (*Table 3;* $F_{1,106} = 5.13$, $p = 0.026$, $\eta_p^2 = 0.046$). In line with the latter, pups that exercised (regardless of PRE and ELA) had 9.86 ng/g [4; 16 ng/g] more serotonin than sedentary controls ($d_{unb} = 0.3$ [-0.1; 0.7]). Despite these differences, hippocampal serotonin turnover (Fig. 7b) was comparable across groups.

In terms of hippocampal redox state, there was no significant three-way interaction ($F_{1,106} = 1.66$, $p = 0.20$, $\eta_p^2 = 0.015$), nor any significant two-way interactions ($p>0.05$ in all instances; *Supplementary data*). However, JUV independently affected GSH/GSSG values (Fig. 7c; $F_{1,106} = 35.25$, $p \leq 0.0005$, $\eta_p^2 = 0.25$), so that this ratio (regardless of PRE and ELA) was 4.52 [3; 6] higher in pups that exercised, compared to sedentary controls ($d_{unb} = 0.9$ [0.5; 1.3]).

Metabolic markers relating to mitochondrial function

Summarised in Table 3, the effects of the largest identified behavioural influencing factor (i.e. juvenile activity; JUV) were significant in the following metabolic markers: palmitic acid (or hexadecenoic acid), stearic acid (or octadecanoic acid), oleic acid, 1-monopalmitin, and 1-monostearin and nicotinic acid (or niacin). Specifically, JUV (regardless of PRE and ELA) significantly ($p<0.05$) decreased all these markers, relative to pups that did not exercise during pre-pubertal development (i.e. SED).

Discussion

In this work, we investigated the interaction between prenatal activity, an early-life stressor in the form of chronic MSEW, and pre-pubertal low-intensity exercise on the behavioural profile of an approved rodent model for depression. Importantly, the characteristic behavioural profile of the juvenile FSL rat was investigated and reported elsewhere (Whitney *et al.*, 2023). Briefly, juvenile FSL rats (regardless of sex) displayed increased immobility and decreased escape-directed behaviour in the FST on PND36, together with increased hippocampal norepinephrine and serotonin turnover (5-HIAA/5-HT), and decreased GSH/GSSG values. In terms of the effect of an early-life stressor, we previously found that MSEW induced lasting behavioural deficits, as measured in the FST (Whitney *et al.*, 2023). Here, both FSL and FRL rats (regardless of

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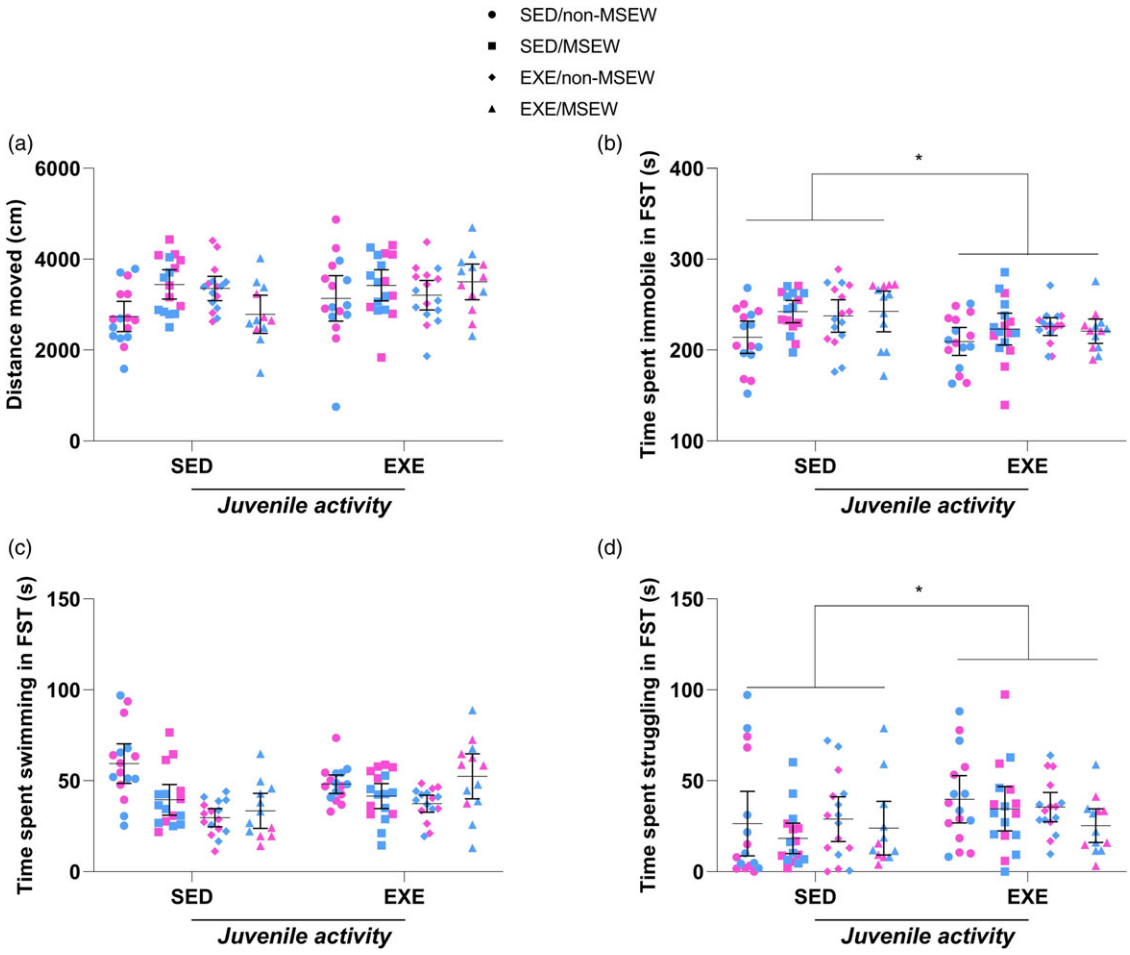

Figure 4. Behavioural effects on PND36. (***a***) Distance moved in the OFT. (***b***) Time spent immobile[a,b,c], (***c***) swimming[a,b,c] and (***d***) struggling[a,b,c] in the FST. Data points represent the mean ± 95% CI, with male and female indicated in blue and pink, respectively. Statistical analyses are reported in the text. [a)] Not all data sets were normally distributed. [b)] Outlier identified but not excluded. [c)] Heterogeneity of variances. FST, forced swim test; EXE, juvenile low-intensity exercise; MSEW, maternal separation and early weaning; SED, sedentary.

sex) exposed to MSEW displayed increased depressive-like behaviour at PND36. Based on these findings, we could investigate whether prenatal and/or pre-pubertal low- intensity exercise could reverse or at least prevent the adverse effects caused by MSEW in a subject with a predisposed susceptibility to develop depression.

According to recent meta-analyses, exercise interventions induce an overall moderate beneficial effect in terms of childhood and adolescent depressive symptoms (Hu *et al.*, 2020; Wegner *et al.*, 2020; Axelsdottir *et al.*, 2021). Although the exact mechanism of action remains unknown (Schuch *et al.*, 2016), exercise has been linked to improved neuroplasticity (Gourgouvelis *et al.*, 2017; El-Sayes *et al.*, 2019), decreased neuro-inflammation (Eyre & Baune, 2012; Kandola *et al.*, 2019) and oxidative stress damage (Eyre & Baune, 2012; Schuch *et al.*, 2014; Lu *et al.*, 2021), enhanced monoaminergic neurotransmission (Lin & Kuo, 2013; da Costa Daniele *et al.*, 2017) and improved mitochondrial function (Aguiar *et al.*, 2014). Contrastingly, traumatic experiences during early-life development can negatively affect these pathways, eventually leading to impaired mood and/or increased anxiety levels (Palmier-Claus *et al.*, 2016; LeMoult *et al.*, 2020). We therefore aimed to determine whether prenatal low- intensity exercise could induce protective mechanisms against the adverse effects of early-life stress, and whether pre-pubertal exercise, with or without prenatal exercise, could induce antidepressant effects during juvenile development.

The early-life effects of prenatal activity and an early-life stressor (i.e. MSEW)

The beneficial effects of prenatal exercise are well established and proven to be safe for both the mother and foetus (Davenport *et al.*, 2018; Davenport *et al.*, 2018; Moyer *et al.*, 2016). Several groups have reported on the beneficial metabolic effects of maternal exercise in the rodent offspring (reviewed by Kusuyama *et al.* (2020)), with others also observing cardiovascular (May *et al.*, 2014) and even neuro-behavioural benefits in new-born babies (Clapp *et al.*, 1999). Here, pregnant FSL dams were subjected to a low intensity, treadmill exercise regimen, for an average of 13 ± 5 days. Pups born to these dams (regardless of sex and ELA) were more immobile in the TST on PND21, compared to those born to a sedentary dam. Interestingly, this effect was more prominent in animals exposed to MSEW (Fig. 2b). Others have suggested prenatal exercise to improve cognitive function and induce anxiolytic effects in rodent offspring, in part by increasing hippocampal neuroplasticity (Aksu *et al.*, 2012; Ji *et al.*, 2020). However, a limitation of the current study is that no neurochemical markers were measured at this age (i.e. PND21), leading to any explanation of these behavioural differences to be speculative. Still, that prenatal exercise can influence the hypothalamic-adrenal axis (Carlberg *et al.*, 1996) suggests that prenatal exercise could alter

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Table 2. Original and ANCOVA-adjusted parameters of the TST and FST

			TST immobility (s)		FST immobility (s)	
PRE	ELA	JUV	Original *mean ± SD (n)*	Adjusted *mean ± SEM (n)*	Original *mean ± SD (n)*	Adjusted *mean ± SEM (n)*
Sedentary	***Normal***	***SED***	178.69 ± 52.28 (*32*)	179.52 ± 8.37 (*32*)	214.18 ± 33.42 (*16*)	211.86 ± 7.54 (*16*)
		EXE			209.48 ± 28.84 (*16*)	209.13 ± 7.30 (*16*)
	MSEW	***SED***	162.35 ± 44.43 (*33*)	161.68 ± 8.23 (*33*)	242.35 ± 23.07 (*16*)	243.53 ± 7.35 (*16*)
		EXE			223.13 ± 33.95 (*17*)	224.19 ± 7.13 (*17*)
Exercise	***Normal***	***SED***	183.93 ± 50.34 (*32*)	184.92 ± 9.38 (*32*)	237.51 ± 33.61 (*16*)	238.24 ± 7.31 (*16*)
		EXE			225.88 ± 18.42 (*16*)	225.89 ± 7.29 (*16*)
	MSEW	***SED***	209.39 ± 39.29 (*25*)	207.93 ± 9.52 (*25*)	242.53 ± 35.27 (*12*)	240.45 ± 8.59 (*12*)
		EXE			220.76 ± 22.21 (*13*)	222.21 ± 8.17 (*13*)
			FST swimming (s)		FST struggling (s)	
PRE	ELA	JUV	Original *mean ± SD (n)*	Adjusted *mean ± SEM (n)*	Original *mean ± SD (n)*	Adjusted *mean ± SEM (n)*
Sedentary	***Normal***	***SED***	59.44 ± 20.43 (*16*)	60.16 ± 3.77 (*16*)	26.43 ± 33.24 (*16*)	28.07 ± 5.85 (*16*)
		EXE	48.15 ± 9.57 (*16*)	48.26 ± 3.65 (*16*)	39.82 ± 24.48 (*16*)	40.06 ± 5.66 (*16*)
	MSEW	***SED***	39.51 ± 15.74 (*16*)	39.14 ± 3.68 (*16*)	18.29 ± 15.78 (*16*)	17.46 ± 5.71 (*16*)
		EXE	41.55 ± 13.28 (*17*)	41.22 ± 3.57 (*17*)	34.62 ± 23.69 (*17*)	33.87 ± 5.53 (*17*)
Exercise	***Normal***	***SED***	29.66 ± 9.36 (*16*)	29.43 ± 3.66 (*16*)	28.92 ± 23.09 (*16*)	28.41 ± 5.68 (*16*)
		EXE	37.38 ± 8.77 (*16*)	37.38 ± 3.65 (*16*)	35.51 ± 15.17 (*16*)	35.50 ± 5.66 (*16*)
	MSEW	***SED***	33.44 ± 15.13 (*12*)	34.08 ± 4.30 (*12*)	23.94 ± 23.27 (*12*)	25.40 ± 6.67 (*12*)
		EXE	52.42 ± 20.51 (*13*)	51.97 ± 4.09 (*13*)	25.29 ± 15.30 (*13*)	24.27 ± 6.35 (*13*)

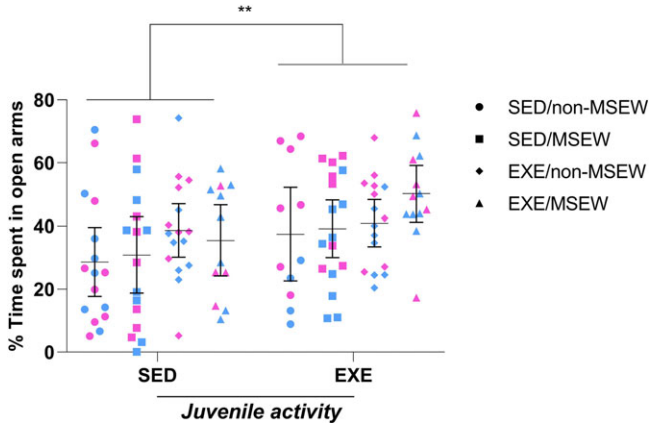

Figure 5. Percentage time spent in the open arm of the EPM. Data points represent the mean ± 95% CI, with male and female indicated in blue and pink, respectively. Statistical analyses are reported in the text. EXE: juvenile low-intensity exercise. MSEW: maternal separation and early weaning. SED: sedentary.

behaviour and stress responses. Consequently, the behaviour observed in the TST on PND21 would benefit from corticosterone analyses as a recent meta-analysis concluded that although cortisol release is blunted in children and adolescents who suffered ELA, these effects were more prominent in adults (Bunea *et al.*, 2017). Conversely, an earlier report found that prenatal exercise increased foetal corticosterone levels (Carlberg *et al.*, 1996), while another found that offspring of women who regularly exercised during pregnancy scored better on the Bayley psychomotor scale (Clapp *et al.*, 1998). Considered together, although on face value, the TST behaviour may represent improved depressive-like behaviour, and further neurochemical analyses are required to confirm such interpretation. Later in life, pups born to an exercised dam also displayed increased immobility time and decreased swimming behaviour (*Figure 4 and Supplementary data*) in the FST, compared to those born to a sedentary dam. These behaviours again point towards a depressogenic effect yet considering the reduced hippocampal serotonin turnover, improved redox state, and increased brain weight on PND38 (*Figure 7 and Supplementary data*), it may be that prenatal exercise may actually induce a more resilient behaviour. In this regard, although the FST is an accepted screening tool for antidepressant-like effects, this simplified interpretation of time spent immobile as an indicator of depressive-like behaviour has been challenged (Boccia *et al.*, 2007; Commons *et al.*, 2017). Therefore, pending confirmation, our results may suggest that prenatal exercise does indeed induce significant behavioural changes in adolescent FSL rats, and that these effects may (or may not) resemble improved coping behaviour.

As to whether prenatal exercise could prevent the adverse effects of MSEW, it is first worth noting that we have previously shown that MSEW worsens depressive-like behaviour in the Flinders line rat, with more prominent effects in the resistant (i.e. FRL) strain (Whitney *et al.*, 2023). Similarly, in the current study, MSEW also increased time spent immobile in the FST on PND36 and decreased the whole brain weight of FSL rats (*Supplementary data*), thereby supporting a depressogenic effect, caused by an

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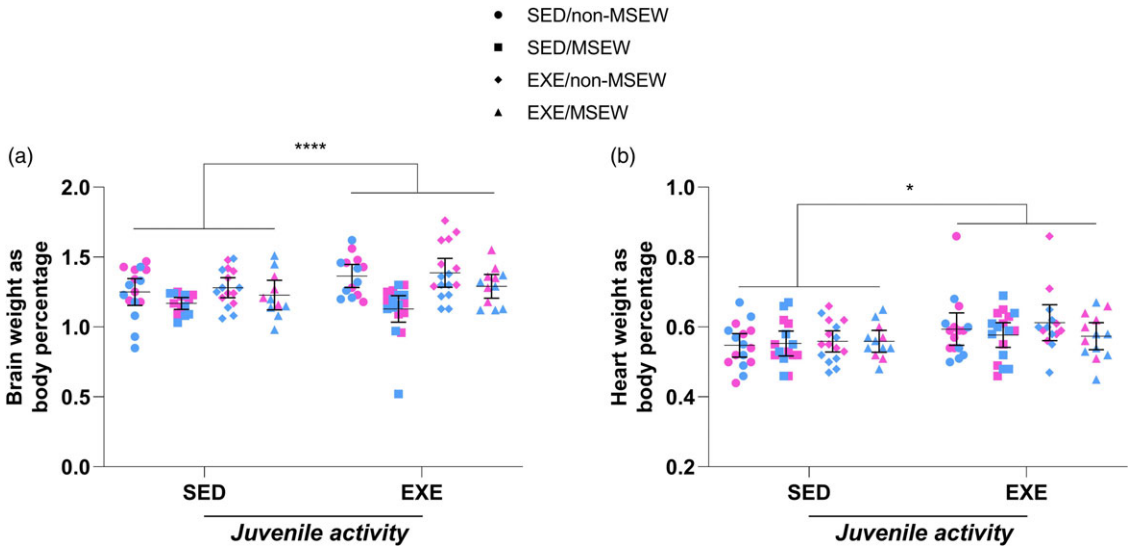

Figure 6. Heart and whole brain weight of male and female FSL rats. (***a***) Brain[a,b] and (***b***) heart[b,c] weight of FSL rats, expressed as a percentage of body weight. Data points represent the mean±95 %CI, with male and female indicated in blue and pink, respectively. Statistical analyses are reported in the text. [a)] Not all data-sets were normally distributed. [b)] Outlier identified and excluded. [c)] Outliers identified but not excluded. EXE, juvenile low-intensity exercise; MSEW, maternal separation and early weaning; SED, sedentary.

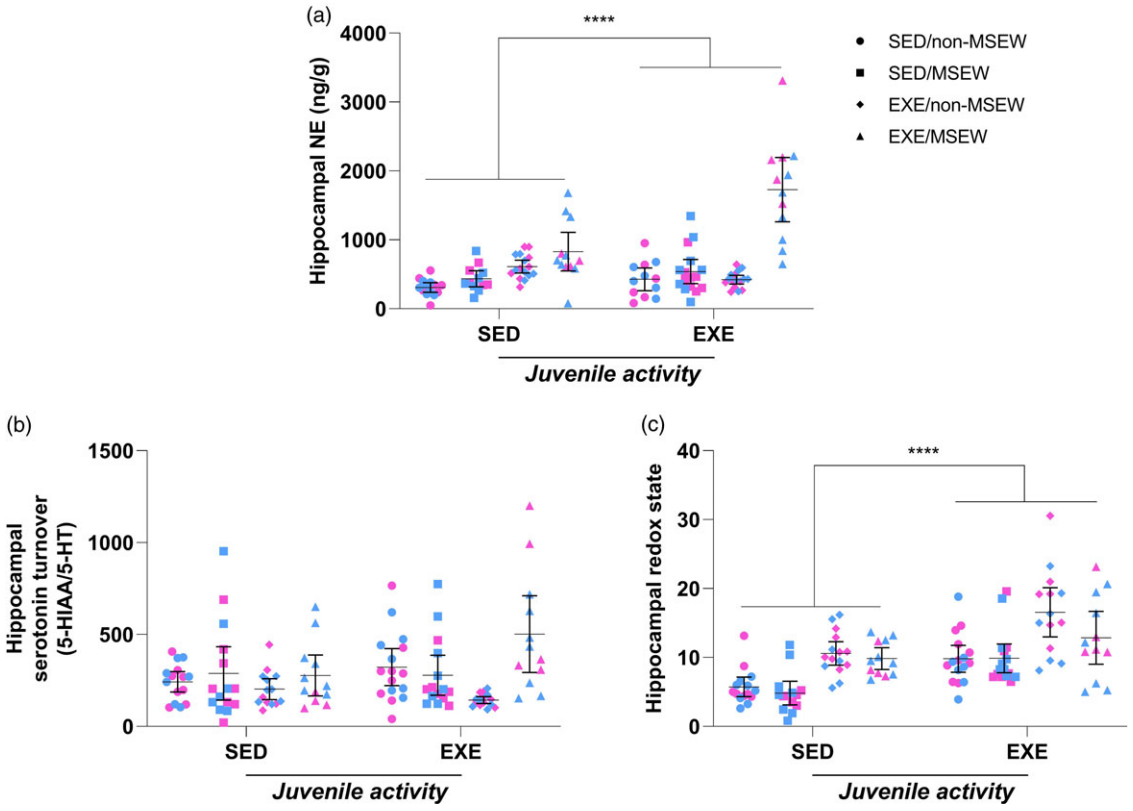

Figure 7. Hippocampal monoamine levels and redox state markers. (***a***) Norepinephrine levels[a,c], (***b***) serotonin turnover (5-HIAA/5-HT)[a,b,c], (***c***) redox state (GSH/GSSG)[a,b,c] on PND38. Data points represent the mean±95% CI, with male and female indicated in blue and pink, respectively. Statistical analyses are reported in the text. [a)] Outliers identified and excluded. [b)] Not all data-sets are normally distributed. [c)] Heterogeneity of variances. EXE, juvenile low-intensity exercise; MSEW, maternal separation and early weaning; SED, sedentary.

early-life stressor. However, none of the parameters where PRE and MSEW interacted to influence the outcome, showed any statistical evidence to accurately answer this question. Still, that prenatal activity (regardless of ELA and JUV) beneficially altered hippocampal redox state and serotonin turnover during pubertal onset, at least hints towards a protective effect that was not inhibited nor prevented by an early-life stressor (*Supplementary data*). Further investigation into this aspect is however required.

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Table 3. Metabolic markers in the hippocampus of FSL rats that relate to mitochondrial function

PRE	ELA	JUV	Palmitic acid *mean ± SD (n)*	Stearic acid *mean ± SD (n)*	Oleic acid *mean ± SD (n)*
Sedentary	***Normal***	***SED***	4.40 ± 0.76 (*3*)	12.31 ± 3.71 (*3*)	2.54 ± 1.21 (*3*)
		EXE	1.92 ± 0.74 (*10*)	8.09 ± 2.15 (*10*)	1.71 ± 0.62 (*10*)
	MSEW	***SED***	3.50 ± 0.63 (*7*)	10.23 ± 0.79 (*7*)	1.69 ± 0.20 (*7*)
		EXE	2.45 ± 0.84 (*9*)	8.15 ± 2.18 (*9*)	1.46 ± 0.37 (*9*)
Exercise	***Normal***	***SED***	2.45 ± 0.51 (*10*[a])	7.54 ± 1.79 (*10*[a])	1.50 ± 0.47 (*11*)
		EXE	2.19 ± 0.70 (*9*)	7.09 ± 1.89 (*9*)	1.24 ± 0.31 (*9*)
	MSEW	***SED***	2.72 ± 0.65 (*9*)	8.68 ± 1.14 (*9*)	1.40 ± 0.14 (*9*)
		EXE	2.90 ± 0.66 (*8*)	9.05 ± 2.17 (*8*)	1.34 ± 0.27 (*8*)
PRE	ELA	JUV	1-monopalmitin *mean ± SD (n)*	1-monostearin *mean ± SD (n)*	Nicotinic acid *mean ± SD (n)*
Sedentary	***Normal***	***SED***	0.64 ± 0.07 (*3*)	1.34 ± 0.001 (*2*[a])	101.75 ± 1.81 (*2*[a])
		EXE	0.27 ± 0.13 (*10*)	0.59 ± 0.19 (*10*)	12.52 ± 13.10 (*10*)
	MSEW	***SED***	0.47 ± 0.14 (*7*)	0.86 ± 0.20 (*7*)	77.00 ± 46.57 (*7*)
		EXE	0.31 ± 0.12 (*9*)	0.63 ± 0.19 (*9*)	30.99 ± 18.56 (*9*)
Exercise	***Normal***	***SED***	0.33 ± 0.14 (*11*)	0.69 ± 0.21 (*10*)	33.67 ± 18.69 (*11*)
		EXE	0.29 ± 0.08 (*9*)	0.51 ± 0.11 (*9*)	26.62 ± 9.05 (*5*[a])
	MSEW	***SED***	0.30 ± 0.03 (*8*[a])	0.57 ± 0.09 (*9*)	34.62 ± 15.54 (*8*)
		EXE	0.29 ± 0.05 (*8*)	0.57 ± 0.12 (*8*)	55.39 ± 46.05 (*8*)

ELA, early-life adversity; **JUV**, juvenile activity; **PRE**, prenatal activity.
[a]Outlier identified and removed from analysis.
The values presented here are all log transformed and therefore contain no SI unit. Group sizes differ from behavioural analyses and could be explained by the storage buffer used. Because of the significant influence of main effects, all statistical findings are reported in text.

Table 4. Hippocampal serotonergic and redox state markers

PRE	ELA	JUV	5-HT (*ng/g*) *mean ± SD (n)*	5-HIAA (*ng/g*) *mean ± SD (n)*	5-HIAA/5-HT *mean ± SD (n)*
Sedentary	***Normal***	***SED***	19.8 ± 7.9 (*15*)	242.5 ± 101.3 (*15*)	13.9 ± 6.5 (*15*)
		EXE	23.3 ± 15.4 (*16*)	332.0 ± 188.5 (*16*)	19.7 ± 17.8 (*16*)
	MSEW	***SED***	19.0 ± 7.5 (*15*)	288.1 ± 262.3 (*15*)	16.3 ± 13.2 (*15*)
		EXE	20.8 ± 9.6 (*15*)	278.1 ± 195.9 (*15*)	14.2 ± 6.9 (*15*)
Exercise	***Normal***	***SED***	23.2 ± 10.7 (*14*)	202.6 ± 97.9 (*14*)	10.0 ± 5.2 (*14*)
		EXE	13.6 ± 3.8 (*15*)	143.4 ± 34.2 (*15*)	11.1 ± 3.4 (*15*)
	MSEW	***SED***	24.6 ± 13.8 (*12*)	277.0 ± 175.6 (*12*)	13.0 ± 7.0 (*12*)
		EXE	54.8 ± 36.1 (*12*)	501.3 ± 328.6 (*12*)	10.8 ± 6.5 (*12*)
Redox state markers					
PRE	**ELA**	**JUV**	**GSH (*μg/g*) *mean ± SD (n)***	**GSSG (*μg/g*) *mean ± SD (n)***	**GSH/GSSG *mean ± SD (n)***
Sedentary	***Normal***	***SED***	42.3 ± 7.3 (*15*)	8.4 ± 2.8 (*15*)	5.7 ± 2.6 (*15*)
		EXE	58.8 ± 25.1 (*16*)	6.3 ± 2.4 (*16*)	9.8 ± 3.7 (*16*)
	MSEW	***SED***	41.8 ± 21.8 (*14*)	9.7 ± 4.4 (*14*)	4.8 ± 3.0 (*14*)
		EXE	56.5 ± 11.5 (*16*)	6.2 ± 1.8 (*16*)	9.9 ± 3.9 (*16*)
Exercise	***Normal***	***SED***	50.5 ± 11.4 (*15*)	5.1 ± 1.5 (*15*)	10.6 ± 3.1 (*15*)
		EXE	33.0 ± 3.9 (*14*)	2.2 ± 0.8 (*14*)	16.6 ± 6.2 (*14*)
	MSEW	***SED***	77.2 ± 31.5 (*12*)	7.9 ± 2.4 (*12*)	9.8 ± 2.5 (*12*)
		EXE	138.9 ± 55.5 (*12*)	12.7 ± 7.0 (*12*)	12.8 ± 6.0 (*12*)

The mean values of the specific markers are presented here to promote transparency and were used to calculate the serotonin turnover and redox state reported in the results section. Because of the significant influence of main effects, all statistical findings are reported in text.

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The influence of pre-pubertal low-intensity exercise

As mentioned earlier, exercise is a recognised non-pharmacological treatment strategy for depression (Carter *et al.*, 2016; Kandola *et al.*, 2019) and importantly, can be implemented across all ages, making it a promising treatment option for childhood depression (Hu *et al.*, 2020; Wegner *et al.*, 2020; Axelsdottir *et al.*, 2021). Therefore, the current study investigated whether a 14-day low-intensity exercise regimen during pre-pubertal development could attenuate the depressive-like phenotype of the adolescent FSL rat. It must however be noted here that although all three factors (early-life adversity, and prenatal and juvenile exercise) were considered and controlled for in the study design, the delayed effect findings are interpreted and discussed only in terms of the most robust and statistically non-zero factor (Fig. 3),that is, pre-pubertal low-intensity exercise. All other statistical findings are available as supplementary data.

On PND36, animals (regardless of sex) that were exposed to low-intensity exercise during pre-pubertal development (PND22 to 35) displayed decreased depressive-like behaviour (i.e. time spent immobile) in the FST, compared to their sedentary controls (Fig. 4b), irrespective of PRE and ELA. Moreover, this behaviour was also accompanied by increased time spent struggling (Fig. 4d) – indicative of antidepressive-like and/or increased coping behaviour (Lucki, 1997). Our findings of exercise exerting antidepressant-like effects are in line with pre-clinical (Steyn *et al.*, 2020; Gruhn *et al.*, 2021; de Oliveira *et al.*, 2022; Sohroforouzani *et al.*, 2022) and clinical (Oberste *et al.*, 2020) findings. Although the FSL rat is not known to display increased anxiety-like behaviour (Overstreet & Wegener, 2013), pre-pubertal low-intensity exercise appeared to induce anxiolytic-like effects in FSL rats on PND37 (Fig. 5). It must be mentioned that this effect was only relative to sedentary FSL, and not FRL controls, and therefore requires confirmational studies. Regardless, clinical (Stubbs *et al.*, 2017) and pre-clinical (Cevik *et al.*, 2018) studies that have also reported on the anxiolytic effect of exercise further validates our findings and supports the efficacy of low-intensity exercise as a treatment option for childhood depression.

To confirm our behavioural findings and shed further light on the probable mechanisms involved, we considered anatomical markers, and measured hippocampal monoamine levels, together with markers of oxidative stress and mitochondrial function. First, pre-pubertal low-intensity exercise (regardless of PRE and ELA) increased the brain and heart weights of male and female FSL rats, relative to sedentary control groups (Fig. 6a, b). This is a noteworthy finding, as we have previously reported, that the adolescent FSL rat has lower whole brain and heart weights than its age matched FRL counterpart (Whitney *et al.*, 2023). Therefore, that chronic low-intensity exercise increased brain weight, suggests neuroplasticity mechanisms to be at play (Gourgouvelis *et al.*, 2017; El-Sayes *et al.*, 2019). These findings however warrant confirmation by means of analysing appropriate markers, such as brain-derived neurotrophic factor – a neurotrophin known to be decreased in depressed patients (Brunoni *et al.*, 2008) and increased by exercise (Luo *et al.*, 2019; Naghibi *et al.*, 2021). Exercise further directly affects autonomic outflow, benefitting cardiovascular functioning (Gademan *et al.*, 2007), and although no cardiac tissue biomarkers were measured, our finding of exercise-induced hypertrophy is at least supported by clinical studies (Xiang *et al.*, 2020). That autonomic dysfunction has been shown to be altered in depressed patients (Hartmann *et al.*, 2019; Herbsleb *et al.*, 2020), further emphasises the value of our findings in a genetic rodent model of depression.

As an indirect indicator of mitochondrial function, pre-pubertal low-intensity exercise also beneficially influenced the hippocampal redox state (GSH/GSSG; Fig. 7c), suggesting antioxidant defences to be increased. The GSH/GSSG ratio is a valuable biomarker of cellular redox state (Enns & Cowan, 2017), with lower levels indicating increased oxidative stress (Chai *et al.*, 1994). Our findings are in agreement with others (Higashi, 2016) showing that regular low- to moderate-intensity exercise induces beneficial effects. To this point, that a dysfunctional redox state has previously been observed in the juvenile FSL strain (relative to FRL controls; (Whitney *et al.*, 2023), allow us to conclude that pre-pubertal exercise can reverse this deficit. That this improved redox state was observed together with increased brain weight (Fig. 6a), suggests that oxidative stress damage, specifically in the hippocampus was mitigated, potentially via improvement of mitochondrial function (Memme *et al.*, 2021) and enhanced neuroplasticity and neurogenesis (Park *et al.*, 2018; Park *et al.*, 2019), thereby supporting increasing evidence describing depression as a bio-energetic disorder. To this point, that pre-pubertal low-intensity exercise decreased hexadecanoic acid (palmitic acid), octadecanoic acid (stearic acid), oleic acid (octadecenoic acid), 1-monopalmitin (a monoacylglycerol with hexadecanoic acid), and 1-monostearin, which points towards improved mitochondrial function. These metabolic markers are generally observed in patients with metabolic syndrome and insulin resistance, both conditions strongly associated with mitochondrial dysfunction (Pari & Venkateswaran, 2004; Zeng *et al.*, 2009; Ma *et al.*, 2015). In these patients, a skewed ATP:ADP ratio stimulates lipolysis, which leads to the breakdown of triacylglycerols into monoacylglycerol and free fatty acids (especially hexadecanoic acid and octadecanoic acid). That these levels were decreased in the hippocampi of animals that exercised during pre-pubertal development in the current study, which is likely indicative of increased energy metabolism (and consequently mitochondrial function). Moreover, that these animals also had lower nicotinic acid levels than their sedentary counterparts, which may suggest a lower breakdown of nicotinamide adenine dinucleotide (NAD^+) and/or better utilisation of nicotinic acid in the formation of NAD^+. Briefly, nicotinic acid is a precursor of NAD^+, which acts as an electron carrier in the electron transport chain, where it regulates the redox state of the mitochondria and contributes to ATP production (Crowley *et al.*, 2000; Sauve, 2008). Although clinical and pre-clinical research differs in terms of the effect of exercise on NAD^+ levels (White & Schenk, 2012), decreased levels are generally associated with age-associated pathologies (reviewed by (Imai & Guarente, 2014)). However, considered with the behavioural and neurochemical alterations reported here, and the known mitochondrial enhancing effects of exercise (Memme *et al.*, 2021), pre-pubertal low-intensity exercise may have increased the nicotinic acid to NAD^+ conversion, thereby decreasing the available nicotinic levels and potentially increasing the NAD^+/NADH ratio. This is at least partly supported by our previous finding that pubertal FSL rats have higher hippocampal nicotinic concentrations than FRL controls (Whitney *et al.*, 2023). Still, we invite confirmatory investigations.

Finally, pre-pubertal low-intensity exercise (regardless of PRE and ELA) also increased hippocampal norepinephrine and serotonin, relative to sedentary controls (Fig. 7a and Table 4), without affecting serotonin turnover (Fig. 7b). These increases support the decrease in depressive-like behaviour and the increase in escape-directed behaviour observed in the FST. As mentioned earlier, one of the mechanisms through which exercise exerts its antidepressant-like effects is by increasing

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monoamine neurotransmission and considered together with the improved hippocampal redox state and mitochondrial markers, our findings reaffirm this effect and again show that exercise can mimic currently approved pharmacological treatment options. Further research is, however, needed into whether the apparent antidepressant-like effects of pre-pubertal exercise are indeed unrelated to ELA and PRE.

Conclusion

The current study explored three factors influencing depressive-like behaviour in an approved genetic rodent model of depression to demonstrate how environmental and genetic influences can alter this behaviour. Our findings show that pre-natal exercise induces beneficial long-term neurochemical alterations that is unaffected by an early-life stressor. Pre-pubertal low intensity was effective in reducing depressive-like behaviour and oxidative stress in a rodent model of depression, whilst also increasing monoaminergic levels, and in doing so, implicating improved mitochondrial function. Taken together, our findings highlight the need to further investigate the role of mitochondrial function in depression and support the use of pre-pubertal low-intensity exercise as an effective treatment strategy for childhood depression.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/neu.2023.52.

Acknowledgements. The authors would like to acknowledge and thank Dr Francois Viljoen for his assistance with neurochemical analyses. We would also like to acknowledge Mr Cor Bester, Mrs Antoinette Fick, Mr Kobus Venter and Sr Irene Serage for their support and assistance in overseeing the welfare of the animals and their technical support. This work emanated from a Masters (MSc) degree (AJW).

Author contribution. SFS conceptualised and designed the layout of the study. AJW performed all the experimental work and analysed and interpreted the data with SFS. AJW and SFS also wrote the original draft of the manuscript, with ZL and RK collating and finalising the paper for submission. All authors contributed to the various sections of this paper. The authors would like to acknowledge Dr Laneke Luies for her assistance and support with the GC-TOM-MS system.

Financial support. This work was funded by internal North-West University research grants awarded to SFS.

Competing interests. None.

Animal welfare. All animal procedures were approved by the Animal Care, Health and Safety Research Ethics Committee of the North-West University (NWU-AnimCareREC; ethics approval number: NWU-00419-21-A5) and in accordance with the relevant code of ethics. All procedures complied with national legislation that pertains to experimental animal welfare (including the Department of Health's Ethics in Health Research: Principles, Processes and Structures and the South African National Standard: The Care and Use of Animals for Scientific Purposes (SANS 10,386:2008)). This work also complied with the ARRIVE guidelines ensuring that all experimental data are reproducible, transparent, accurate comprehensive and logically ordered to promote well written manuscripts.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the South African National Standards and institutional guides on the care and use of laboratory animals.

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https://doi.org/10.1017/neu.2023.52 Published online by Cambridge University Press