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Long-lasting effects of fluoxetine and/or exercise augmentation on bio-behavioural markers of depression in pre-pubertal stress sensitive rats

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HIGHLIGHTS

- Maximal exercise intensity (as estimated by VO2max) in rats increases with age and should be adapted accordingly.
- Pre-pubertal fluoxetine and fluoxetine plus low intensity exercise exerts early antidepressant-like effects.
- Pre-pubertal low intensity exercise or low dose fluoxetine exerts lasting antidepressant-like effects into early adulthood.
- The combination of fluoxetine with low intensity exercise does not exert any lasting effects on depressive-like behaviour.
- Exercise, fluoxetine and the combination thereof increased hippocampal superoxide dismutase in early-adulthood.

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ABSTRACT

Juvenile depression is of great concern with only limited treatment currently approved. Delayed onset of action, low remission and high relapse rates, and potential long-lasting consequences further complicates treatment and highlights the need for new treatment options. Studies reporting on long-lasting effects of early-life treatment have reported conflicting results, with the pre-adolescent period mostly overlooked. The anti-depressive effect of exercise, as a possible treatment option or augmentation strategy, is dependent on age and exercise intensity. We investigated the immediate (i.e. postnatal day 35 (PND35)) and lasting (PND60 to PND61) effects of pre-pubertal (PND21 to PND34) fluoxetine and/or exercise on bio-behavioural markers of depression and oxidative stress in stress sensitive Flinders Sensitive Line rats. Low, but not moderate, intensity exercise or 5, but not 10, mg/kg/day fluoxetine displayed anti-depressant-like properties at PND35. Pre-pubertal treatment with 5 mg/kg/day fluoxetine or low intensity exercise exerted lasting anti-depressive-like effects into adulthood, whereas the combination of these two treatments did not. Furthermore, the combination of fluoxetine plus exercise *reduced* hippocampal BDNF levels as compared to exercise alone, which may explain the latter findings. In all treatment groups hippocampal SOD activity was significantly increased at PND61, suggesting an increased anti-oxidant capacity in adulthood. In conclusion, the data confirm the anti-depressant-like properties of both early-life fluoxetine and exercise in a genetic animal model of depression. However, optimal lasting effects of early-life interventions may require adjustment of antidepressant dose and/or exercise intensity to developmental age, and that a combination of antidepressant and exercise may not necessarily be augmentative.

1. Introduction

Major depressive disorder (MDD) is one of the most challenging mental health problems of our time, affecting an estimated 350 million people worldwide, at any given time point [1]. Children are also affected and due to increased awareness and a rise in the number of juveniles diagnosed with MDD, it has become the most common mental health disorder in this age group [1]. In fact, MDD has an estimated prevalence of 2–5% in children, associated with a fourfold enhanced risk of enduring or reoccurring in adulthood [2]. Also, severe depression often leads to suicide [3], making it the fourth leading cause of death in pre-adolescent children [4]. An increase in the prescription rate of antidepressants in this age group has also been documented, further highlighting the need for safe and effective treatment options in this age group.

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During pre-adolescence the serotonin pathway is already matured, whereas the noradrenergic and dopaminergic pathways are still developing, so that drugs that modulate serotonergic neurotransmission are more likely to be effective than those modulating other systems [5]. That said, current treatment options for childhood or adolescent depression are limited to fluoxetine and escitalopram, both selective serotonin reuptake inhibitors (SSRIs). These two agents are approved by the United States' Food and Drug Administration (FDA) for use in children older than 8 and 12 years, respectively [6–8]. Other antidepressants, such as the tricyclic antidepressants and other SSRIs, have been shown to be ineffective (or inconclusive) in children [5,9]. In addition, a black box warning was issued by the FDA in September 2004 highlighting an increased risk of suicidal ideation in juveniles treated with SSRIs [10]. Lastly, the potential long-term consequences of early-life treatment in the developing brain have become a great concern in recent years. This has rendered paediatric prescribing a daunting task, where prescribers have to weigh the risks and benefits of early-life treatment against the unknown risks of neurodevelopmental impact and consequent outcome later in life.

A few studies focused on the potential long-term consequences of early-life exposure to centrally acting stimulants and antidepressants, in an attempt to shed some light on how such stimuli during the complex process of brain development could alter the brain's functional integrity in adulthood [11]. Interventions during neurodevelopment, such as drug use, presents a window to induce permanent neurodevelopmental alterations [12]. Some preclinical data suggest negative outcomes of early-life treatment with SSRIs, such as arrested development of spine density [13], decreased density of the serotonin transporters [14], reduced bodyweight, reduced sexual functioning and/or increased anxiety [15], increased depressive-like behaviour [16] and decreased locomotor activity [17]. However, it has been suggested that pharmacotherapy during an appropriate developmental period could alter the course of development to exert lasting beneficial effects [12]. This idea is not farfetched and it has been demonstrated that fluoxetine treatment of rodents during puberty may produce significant antidepressant-like effects and positively influence responsiveness to rewarding and aversive stimuli in adulthood [18].

Importantly, several methodological and other differences exist between studies, which may explain contradictory research findings. For example, most animal studies employ healthy rodents, without any genetic predisposition to display depressive-like behaviour, consequently limiting the translational value of the findings. Also, although striking similarities between the age-related neurodevelopment in human and other mammals have been well-documented, these are not absolute. As such, key aspects of pre-pubertal rodent neurodevelopment can be translated to that of a human child (4–12 years), the last half of which antidepressant treatment is often indicated [1]. Importantly, this would represent a vulnerable time in neurodevelopment to investigate the effects of potential interventions in early life.

Even when antidepressants may exert beneficial therapeutic effects, antidepressant treatment is associated with bothersome side-effects, such as a delayed onset of action [18], low remission rates and a high rate of relapse following discontinuation [19], highlighting the need for new treatment modalities. Such interventions typically include psychotherapy, support groups and life-style adjustments. Exercise is one such treatment option and although the efficacy of exercise has been demonstrated in adults [20–22], children [23] and rodents [24], the data in children remain limited.

Exercise has also been proposed as an augmentative strategy to antidepressant treatment due to the putative synergistic effects with antidepressants as well as the advantage of being a relatively safe and low cost intervention. The antidepressant effects of exercise have been suggested to result from increased levels of monoamines, neurotrophins, anti-inflammatory markers and antioxidants [22,25,26], whereas depression is widely regarded as an inflammatory and pro-oxidative state [27]. Immediate effects of exercise seem to be dependent upon age as well as exercise intensity [11,28]. One study reported low and high intensity exercise to induce different neurochemical effects during different developmental stages. In particular, high, but not low, intensity exercise during pre-pubertal development of Wistar rats was associated with increased pro-inflammatory cytokine levels, whereas low, but not high, intensity exercise in the same age-period increased cell proliferation rate in the dentate gyrus [28]. However high, but not low, intensity exercise in pubertal rats significantly increased proliferative cell density and anti-inflammatory cytokine concentrations [28]. This report lends further support to the idea that treatment could have differential effects depending on the time of exposure during neurodevelopment. Nevertheless, few studies have explored the potential lasting effects of exercise as a treatment option for depression.

The current study initially investigated whether the maximum oxygen consumption capacity (VO_{2max}) of the pre-pubertal Flinders Sensitive Line (FSL) rat increases with age, using an approved indirect approach. Thereafter it was determined whether pre-pubertal low or moderate exercise intensity, and low or high dose of fluoxetine treatment yields antidepressant-like effects immediately following intervention or treatment. Finally, immediate antidepressant-like behavioural effects of the combination of low intensity exercise and low dose fluoxetine was investigated. In the subsequent long-term study, we investigated the lasting effects of pre-pubertal low dose fluoxetine, low intensity exercise, or the combination of fluoxetine and exercise into early-adulthood, i.e. following a washout period of 26 days. The studies aimed to determine whether pre-puberty presents a window of opportunity, specifically in genetically susceptible rats, to exert lasting beneficial effects on behavioural and tissue biomarkers of depression (BDNF) and oxidative stress (lipid peroxidation, superoxide dismutase activity). In this regard, no Flinders Resistant Line (FRL) control animals were included in the current study, since the study did not aim to investigate the role of genetic susceptibility, but rather to investigate the role of the various interventions in an approved genetic animal model of depression. We also explored whether the combination of fluoxetine and exercise will yield an augmentative long-lasting anti-depressant-like effect in adulthood.

2. Materials and methods

2.1. Animals

Male Flinders Sensitive Line (FSL; n = 179) and Flinders Resistant Line (FRL; n = 12) rats were bred, supplied and housed at the Vivarium (SAVC reg. no. FR15/13458; SANAS GLP compliance no. G0019) of the Pre-Clinical Drug Development Platform (PCDDP), North-West University. The original rat colonies were obtained from Dr David H Overstreet, University of North Carolina, Chapel Hill, North Carolina, USA. The FSL rat is a validated genetic animal model of depression, displaying face, construct and predictive validity, whereas the FRL rat serves as a model control [29,30]. All experiments were approved by the AnimCare animal research ethics committee (NHREC reg. no. AREC-130913-015) of the North-West University (ethics approval no. **NWU-00148-14-A5**), and all animals were maintained and all procedures performed in studies involving animals were in accordance to the code of ethics in research, training and testing of drugs in South Africa and complied with national legislation.

The study aimed to employ 16 rats per test group. However, smaller numbers were sometimes employed due to a few non-