Acta Neuropsychiatrica

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The long-term bio-behavioural effects of juvenile sildenafil treatment in Sprague-Dawley versus flinders sensitive line rats

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Short Communication

Cite this article: Saayman JLB, Steyn SF, and Brink CB. (2021) The long-term bio-behavioural effects of juvenile sildenafil treatment in Sprague-Dawley versus flinders sensitive line rats. *Acta Neuropsychiatrica* **33**:200–205. doi: 10.1017/neu.2021.4

Received: 10 September 2020
Revised: 28 January 2021
Accepted: 29 January 2021
First published online: 17 February 2021

Key words:
Flinders Sensitive Line rat; long-term antidepressant-like effects; paediatric major depressive disorder; phosphodiesterase type 5 inhibitor; sildenafil

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Abstract

Objective: To investigate the long-term effects of juvenile sub-chronic sildenafil (SIL) treatment on the depressive-like behaviour and hippocampal brain-derived neurotrophic factor (BDNF) levels of adult Sprague-Dawley (SD) versus Flinders Sensitive Line (FSL) rats. *Methods:* SD and FSL rats were divided into pre-pubertal and pubertal groups, whereafter 14-day saline or SIL treatment was initiated. Pre-pubertal and pubertal rats were treated from postnatal day 21 (PND21) and PND35, respectively. The open field and forced swim tests (FST) were performed on PND60, followed by hippocampal BDNF level analysis 1 day later. *Results:* FSL rats displayed greater immobility in the FST compared to SD rats ($p < 0.0001$), which was reduced by SIL ($p < 0.0001$), regardless of treatment period. Hippocampal BDNF levels were unaltered by SIL in all treatment groups ($p > 0.05$). *Conclusion:* Juvenile sub-chronic SIL treatment reduces the risk of depressive-like behaviour manifesting during young adulthood in genetically susceptible rats.

Significant outcomes

- Juvenile sub-chronic sildenafil treatment induced long-term antidepressant-like behavioural effects in young adult Flinders Sensitive Line but not Sprague-Dawley rats.
- Juvenile sub-chronic sildenafil treatment did not have any long-term effect on the hippocampal brain-derived neurotrophic factor levels of either rat strain during young adulthood.

Limitations

- Bio-behavioural analyses, immediately following juvenile sub-chronic sildenafil treatment, were not performed.
- The FST alone was used to evaluate depressive-like behaviour. Other behavioural tests of depressive-like behaviour, such as the sucrose preference test, would be of value in prospective studies.
- Monoaminergic levels were not measured, which would confirm the conclusions drawn from the behavioural data. Similarly, prospective studies may investigate the role of the nitric oxide/cyclic guanosine 3′,5′-monophosphate/protein kinase G signalling pathway as a novel antidepressant target.

Introduction

Major depressive disorder (MDD) is a serious mood disorder (NIMH, 2018) that globally affects an estimated 322 million people (WHO, 2017), making it the leading cause of global disability (Friedrich, 2017). A recent study reported that 1.7% of children and 6.1% of adolescents suffer from MDD (Ghandour *et al.*, 2019), highlighting the high prevalence in the paediatric population. Paediatric MDD results in social dysfunction and poor academic performance (Hazell & Mirzaie, 2013), with most patients having recurrences during young adulthood (Melvin *et al.*, 2013; Kovacs *et al.*, 2016), thereby accentuating the chronicity of MDD (Monroe & Harkness, 2012).

Conventional antidepressants have major shortcomings, including a delayed onset of action, troublesome side-effect profiles and marked ineffectiveness in the treatment of treatment-resistant depression (Rosenzweig-Lipson *et al.*, 2007). Importantly, the United States' Food and Drug Administration (FDA) has only approved fluoxetine for the treatment of childhood MDD and

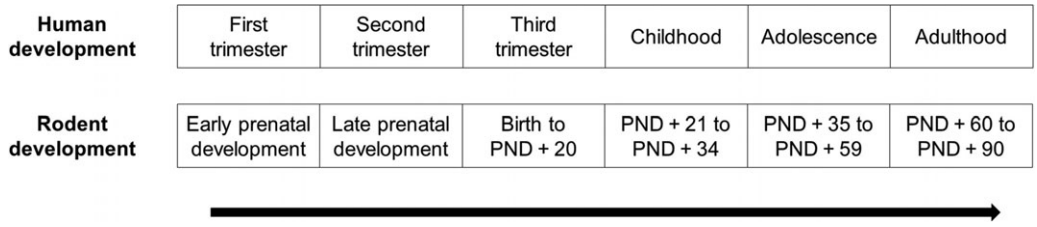

Fig. 1. A summarised comparison of rodent and human neurodevelopmental phases.
Adapted from (Steyn, 2018). PND: postnatal day.

escitalopram as additional option in adolescent MDD (FDA, 2014). Still, antidepressants are issued with a 'black-box' warning of early increased risk of suicidal ideation and behaviour in paediatric patients (FDA, 2014). This is of note, considering adolescent MDD itself is a major risk factor for suicide (Zubrick *et al.*, 2017) – the second leading cause of death in this age group (Mullen, 2018). Antidepressant-use in paediatrics has increased (Bachmann *et al.*, 2016), highlighting the difficult position prescribers are placed in – weighing the severe, sometimes fatal consequences of untreated MDD against the uncertain long-term effects of approved therapy.

Neurodevelopment displays surprising similarities and alignment of age-related brain development between humans and other mammals (Eiland & Romeo, 2013) (Fig. 1). Importantly, the serotonergic system in rats reaches maturity at the onset of the pre-pubertal period (*viz.* childhood in humans) on postnatal day 21 (PND21), whereas the noradrenergic system continues to develop throughout pre-pubertal development and only reaches maturity at the onset of puberty (*viz.* early adolescence in humans) on PND35 (Murrin *et al.*, 2007; Eiland & Romeo, 2013). These different maturity rates may explain why only serotonergic, and not adrenergic-targeting antidepressants are effective in paediatric MDD. Nevertheless, stimuli experienced by an individual during early-life development that affect these neurodevelopmental processes may either benefit or harm the functional integrity of the adult brain (Andersen, 2003; Gomes da Silva *et al.*, 2012), suggesting early-life to be a unique 'window of opportunity' to induce long-term beneficial effects via novel antidepressant treatment targets.

Preclinical studies from our laboratories were the first to demonstrate that enhanced central nitric oxide/cyclic guanosine 3′,5′-monophosphate/protein kinase G (NO/cGMP/PKG) signalling, either through selective phosphodiesterase type 5 (PDE5) inhibition (Brink *et al.*, 2008; Liebenberg *et al.*, 2010a) or intracerebroventricular infusion of a cGMP analogue (Liebenberg *et al.*, 2010b), induces antidepressant-like effects, partly by activating the cyclic adenosine monophosphate response element-binding protein/brain-derived neurotrophic factor (CREB/BDNF) downstream signalling pathway (Wang *et al.*, 2014). Indeed, MDD is associated with reduced BDNF levels (a marker of neuroplasticity) that is restored with antidepressant treatment (Pittenger & Duman, 2008; Lee & Kim, 2010).

A disordered NO/cGMP/PKG signalling cascade has been reported in the Flinders Sensitive Line (FSL) rat (Wegener *et al.*, 2010), which is a widely described and validated genetic animal model of MDD (Overstreet *et al.*, 2005). Regarding the hippocampal BDNF levels of FSL rats, findings between studies have been inconsistent. As such, a couple of studies have demonstrated that the hippocampal BDNF levels of FSL rats are comparable to those of controls (Angelucci *et al.*, 2000; Angelucci *et al.*, 2003), whereas another study indicated reduced hippocampal BDNF levels in FSL rats (Elfving *et al.*, 2010). Therefore, the FSL rat is a suitable animal model to investigate the long-term effects of juvenile sub-chronic sildenafil (SIL) treatment on depressive-like bio-behaviour during young adulthood. Conversely, Sprague-Dawley (SD) rats represent appropriate non-depressive-like comparisons (Magara *et al.*, 2015).

Taken together, PDE5 inhibitors (such as SIL) hold potential as novel candidates for the treatment of paediatric MDD, with potentially beneficial outcomes later in life. Specifically, the use of SIL in the treatment of paediatric pulmonary hypertension (Huddleston *et al.*, 2009) makes its safety profile better understood, rendering its potential use in depressed children and adolescents especially plausible.

Aims of the study

To investigate the long-term antidepressant-like effects of pre-pubertal versus pubertal sub-chronic sildenafil treatment in young adult Sprague-Dawley versus Flinders Sensitive Line rats.

Materials and methods

Figure 2 illustrates the study layout where all pups (SD and FSL) were weaned on PND21 and randomly divided into pre-pubertal (treated from PND21-34) and pubertal (treated from PND35-48) intervention groups. Hereafter, rats were again randomly divided into saline (SAL) and SIL treatment cohorts. Following 14 days of treatment, rats were housed under standard laboratory conditions, for a 'wash-out' period, until PND60 (young adulthood), when behavioural tests were performed. Finally, rats were euthanized by decapitation on PND61 to measure hippocampal BDNF levels.

Animals

Male SD ($n = 48$) and FSL ($n = 48$) rats were bred, supplied, and housed at the Vivarium of the Pre-Clinical Drug Development Platform (PCDDP) of the NWU, RSA [(SAVC reg. no.: FR15/13458), AAALAC accredited (international file #1717), GLP compliant (SANAS GLP compliance no.: G0019)]. All rats were group-housed (2–3 rats/cage) in polysulphone individually ventilated cages, under constant Vivarium conditions. Food and tap water were provided *ad libitum*.

Drug treatment

Rats were either treated with SAL or SIL citrate (3 mg/kg/day; purchased from Sigma Aldrich) (Liebenberg *et al.*, 2010a) dissolved in SAL, via daily subcutaneous (sc) injection, from PND21 (pre-pubertal intervention groups) or PND35 (pubertal intervention groups) for 14 days (Liebenberg *et al.*, 2010a; Schoeman *et al.*, 2017).

Behavioural tests

Behavioural tests were performed during the dark cycle, with the forced swim test (FST) performed 1 h after the open field test (OFT). Behaviour was recorded with a video camera, situated above (OFT) and in front of the test arenas (FST).

https://doi.org/10.1017/neu.2021.4 Published online by Cambridge University Press

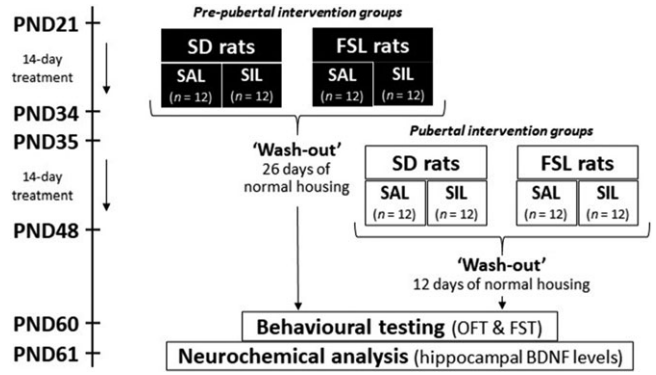

Fig. 2. A schematic illustration of the study layout. BDNF: brain-derived neurotrophic factor. FSL: Flinders Sensitive Line. FST: forced swim test. OFT: open field test. PND: postnatal day. SAL: saline. SD: Sprague-Dawley. SIL: sildenafil.

Open field test

The OFT was performed as previously described for our laboratories (Schoeman *et al.*, 2017), with each rat placed in the centre of the arena and allowed to explore for 5 min. Total distance moved was analysed with Ethovision XT 14 software (Noldus Information Technology BV, Wageningen, NLD).

Forced swim test

The FST consisted of four Perspex® cylindrical tanks, each filled to a depth of 30 cm with ambient water. The FST was performed as previously described for our laboratories (Schoeman *et al.*, 2017), with rats placed into the water-filled tanks and allowed to swim for 7 min. Behaviour was manually scored by an investigator blind to the treatment groups using a manual continuous timer software (FST Scoreboard 2.0 software; Academic Support Services: Information Technology in Education, NWU, RSA), and scoring only the mid-5 min of each trial (Badenhorst *et al.*, 2017; Schoeman *et al.*, 2017). Scored behaviour included immobility, struggling and swimming (Cryan *et al.*, 2005).

Because the FSL rat presents with inherent increased immobility in the FST, it does not require a pre-conditioning swim trial 24 h before the testing swim trial (Overstreet *et al.*, 2005). Consequently, neither SD nor FSL rats had a pre-conditioning swim trial to directly compare the behaviour of the two rat strains.

Neurochemical analysis

After euthanasia by decapitation on PND61, the whole brain was immediately extracted and placed in ice-cold SAL. The hippocampi were dissected out promptly on an ice-cooled dissection slab and individually placed into Eppendorf® tubes, which were immediately placed into liquid nitrogen before being stored at −80 °C (Harvey *et al.*, 2006).

Hippocampal BDNF level analysis

Analysis of hippocampal BDNF levels was performed using rat BDNF enzyme-linked immunosorbent assay (ELISA) kits (catalog no.: **E-EL-R1235**) that were purchased from Elabscience Biotechnology Incorporated and according to the instructions of the manufacturer. Importantly, each sample contained the same wet weight of tissue, homogenised in phosphate-buffered saline (PBS) in a 1:9 ratio (hippocampal tissue (g):PBS (ml) = 1:9). Therefore, the differences between the samples in terms of BDNF protein yield of the extraction process were negligible and the measurement of total protein levels was not required. Results were obtained from a standard curve plotted and expressed as pg/g wet weight of the hippocampus.

Statistical analysis

The minimum number of rats needed for statistically significant results were used in this study, as estimated by an evidence-based estimation (Liebenberg *et al.*, 2010a). A 5% confidence limit for error was taken as statistically significant ($p \leq 0.05$). A three-way analysis of variance (ANOVA) was performed on all data sets, followed by analyses of two-way interactions, simple main effects, and Bonferroni pairwise comparisons. The unbiased Cohen's *d* (d_{unb}) was also calculated and reported ± 95% confidence interval (CI) of the effect magnitude (Cumming, 2013). IBM® SPSS® Statistics (version 25.0. Armonk, NY: IBM Corp), together with Laerd Statistics® (https://statistics.laerd.com) were used for statistical analyses, whereas figures were created in GraphPad Prism® (version 7.0, San Diego California, USA). Effect magnitude indicators were calculated in Exploratory Software for CIs (Cumming, 2013).

Results

Data are presented according to significant two-way interactions, as identified by ANOVA analyses. Accordingly, Figure 3 reflects only the two independent variables that were identified to have significant effects on inter-group differences. Still, a complete description of the statistical analyses is described in the text.

Forced swim test

Although no significant three-way interactions for immobility ($F_{1,88} = 1.31$, $p = 0.26$) or time spent struggling ($F_{1,88} = 0.01$, $p = 0.94$) in the FST existed, significant rat strain*drug interactions were identified ($F_{1,88} = 6.80$, $p = 0.01$) (Fig. 3(A)) and ($F_{1,88} = 19.40$, $p \leq 0.0005$) (Fig. 3(B)). Compared to SAL + FSL animals, SAL + SD ($d_{unb} = 1.4$ [0.8; 2.1]) and SIL + FSL ($d_{unb} = 1.4$ [0.8; 2.0]) groups spent less time immobile in the FST, regardless of treatment period ($p \leq 0.0005$). For struggling, rat strain differences were only identified between SAL-treated animals ($p \leq 0.0005$), with SD spending more time struggling compared to FSL rats, regardless of treatment period ($d_{unb} = 2.3$ [1.6; 3.1]). Further, SIL treatment, regardless of treatment period, decreased struggling behaviour in SD rats ($p = 0.007$; $d_{unb} = 0.9$ [0.3; 1.5]) and increased it in FSL rats ($p = 0.001$; $d_{unb} = 0.8$ [0.2; 1.4]). Again, no significant three-way interaction ($F_{1,88} = 1.79$, $p = 0.18$) existed for swimming behaviour (Fig. 3(C)); however, a significant treatment period*drug interaction was identified ($F_{1,88} = 4.74$, $p = 0.03$). Pre-pubertal SIL treatment, regardless of rat strain, increased swimming behaviour relative to SAL-treated age-matched controls ($p \leq 0.0005$, $d_{unb} = 1.3$ [0.7; 1.9]) and compared to pubertal SIL-treated animals ($p = 0.02$, $d_{unb} = 0.8$ [0.3; 1.4]).

Open field test and hippocampal brain-derived neurotrophic factor levels

There were no significant three-way ($F_{1,88} = 2.08$, $p = 0.15$ and $F_{1,88} = 0.01$, $p = 0.94$) or two-way interactions for distance moved in the OFT or hippocampal BDNF levels (Table 1).

https://doi.org/10.1017/neu.2021.4 Published online by Cambridge University Press

Table 1. Total distance moved in the OFT and hippocampal BDNF levels of young adult SD and FSL rats, following sub-chronic pre-pubertal or pubertal treatment with either SAL or SIL

	Pre-pubertal treatment period				Pubertal treatment period			
	SD		FSL		SD		FSL	
	SAL	SIL	SAL	SIL	SAL	SIL	SAL	SIL
Distance moved (cm)	2431.1 ± 253.7	2936.9 ± 178.5	2204.7 ± 203.8	2030.0 ± 223.6	3249.1 ± 299.0	3022.4 ± 178.7	1985.3 ± 256.2	2000.8 ± 188.7
BDNF levels (pg/g wet weight of the hippocampus)	882.4 ± 49.6	841.2 ± 53.7	980.0 ± 70.8	935.9 ± 73.3	988.2 ± 73.0	1117.6 ± 98.8	1058.1 ± 47.0	1202.4 ± 137.8

BDNF: brain-derived neurotrophic factor. FSL: flinders sensitive line. OFT, open field test. SAL: saline. SD: Sprague-Dawley. SIL: sildenafil.
Distance moved (cm) on PND60 and hippocampal BDNF levels (pg/g wet weight of the hippocampus) on PND61. All groups are equal ($n = 12$). Data points represent the mean ± standard error of the mean.

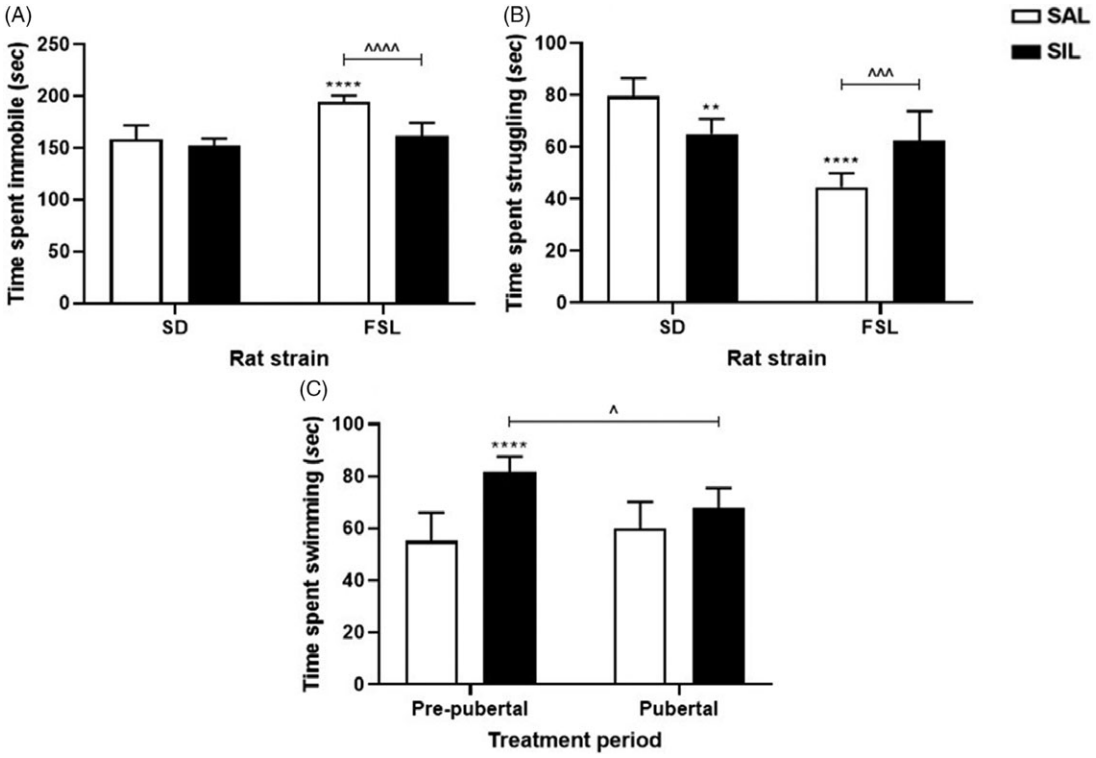

Fig. 3. FST data of SD and FSL rats on PND60, following sub-chronic pre-pubertal or pubertal treatment with either SAL or SIL. Time spent immobile (A) and struggling (B), regardless of treatment period, and time spent swimming (C), regardless of rat strain, according to ANOVA-identified independent variables. All group sizes are equal ($n = 24$). Data points represent the mean ± 95% confidence interval of the mean. Statistical analyses are reported in the text with ** $p \leq 0.01$, **** $p \leq 0.0005$ versus SAL + SD in **A** and **B** and SAL + Pre-pubertal in **C**; ^^ $p \leq 0.01$, ^^^ $p \leq 0.001$, ^^^^ $p \leq 0.0005$ versus indicated test group. FSL: Flinders Sensitive Line. SAL: saline. SD: Sprague-Dawley. SIL: sildenafil.

Discussion

On PND60 (representing young adulthood), SAL-treated FSL rats, regardless of treatment period, displayed increased depressive-like behaviour (Fig. 3(A)) and reduced struggling behaviour (putatively associated with impaired noradrenergic neurotransmission) in the FST (Fig. 3(B)) compared to SAL-treated SD rats, thereby confirming the depressive-like phenotype of the FSL rat (Overstreet *et al.*, 2005). Importantly, because general locomotor activity was comparable across all treatment groups (Table 1), FST group differences could be ascribed to treatment-induced changes or inter-strain variation in psychomotor (and not locomotor) activity.

Perhaps the most important finding is that early-life sub-chronic SIL treatment induced antidepressant-like effects in young adult FSL rats, regardless of treatment period (Fig. 3(A)). This is promising, as neither escitalopram (Steyn *et al.*, 2018) nor venlafaxine (Steyn *et al.*, 2020) were able to induce any robust long-term antidepressant-like bio-behavioural effects in FSL rats in previous studies. However, the antidepressant-like effects of early-life SIL treatment was not observed in young adult SD rats, suggesting that a genetic predisposition that makes individuals more susceptible to the development of MDD (i.e. FSL rats) may play an important role in the long-term antidepressant-like effects of SIL.

https://doi.org/10.1017/neu.2021.4 Published online by Cambridge University Press

Interestingly, early-life sub-chronic SIL treatment, irrespective of treatment period, appears to elevate noradrenergic neurotransmission (mechanism to be confirmed) in young adult FSL rats (increased struggling behaviour in the FST) and reduce it in young adult SD rats (healthy controls) (Fig. 3(B)). Therefore, it cannot be ruled out that sub-chronic SIL treatment in paediatric patients may have potentially detrimental effects in normal healthy individuals later in life in terms of hampering coping responses and therefore requires further investigation. Still, these findings suggest that having a genetic susceptibility to develop MDD (or the lack thereof) dictates the type of effects that early-life sub-chronic SIL treatment might have on noradrenergic neurotransmission during young adulthood and warrants further investigation.

Our data further suggest that putative enhancement of serotonergic-associated behaviour (mechanism to be confirmed) later in life by early-life sub-chronic SIL treatment is age-dependent, since increased swimming behaviour in the FST was only observed in pre-pubertal- and not pubertal-treated animals, irrespective of rat strain (Fig. 3(C)). Genetic susceptibility does not appear to play a role in the long-term serotonergic-associated effects of SIL, since both adult SD and FSL rats were similarly affected in this study, which is in line with the current approval of only serotonergic-targeting antidepressants in paediatric MDD patients.

Increases in BDNF levels have been associated with antidepressant action (Pittenger & Duman, 2008; Lee & Kim, 2010). In this study, early-life sub-chronic SIL treatment had no long-term or lasting effect on the hippocampal BDNF levels of either adult SD or FSL rats (Table 1). This may be due to the transient enhancement of neuroplasticity observed immediately following early-life antidepressant treatment. As such, our group demonstrated that pre-pubertal sub-chronic escitalopram treatment increased hippocampal BDNF levels on PND35 in FSL rats (immediately following treatment), however on PND60 (after a 'wash-out' period), these levels had returned to baseline (Steyn *et al.*, 2018). Prospective studies should further investigate the effect of PDE5 inhibition on neuroplasticity.

Although the biochemical mechanisms underlying the antidepressant-like effects of SIL cannot be elucidated solely based on the present findings, the pro-adrenergic effects of SIL, as delineated from the SIL-induced changes in struggling behaviour in the FST, are an intriguing possibility. In this regard, whereas it has been demonstrated that the antidepressant activity of SIL is associated with a novel mechanism of action, namely the modulation of the NO/cGMP/PKG and the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) cell signalling pathways (Liebenberg *et al.*, 2010b, 2011), we see here that SIL may induce its long-term antidepressant-like effects in FSL rats, at least in part, by ultimately normalising noradrenergic signalling. As a working hypothesis, when translating the current results to humans, it may be that individuals with a genetic susceptibility to develop MDD could benefit from early-life sub-chronic SIL treatment, putatively by modulating neurodevelopment, which results in a significantly reduced risk of developing MDD during young adulthood. Taken together, the PDE5 inhibitors (such as SIL) have potential as novel antidepressant strategies in the treatment of paediatric MDD, with beneficial behavioural outcomes later in life.

Acknowledgements. Mr. Cor Bester, Mrs. Antoinette Fick, and Mr. Kobus Venter for overseeing the welfare of the animals.

Author contributions. JLBS conducted behavioural tests, assisted with neurochemical and statistical analyses, data interpretation, wrote the first draft of the manuscript, and prepared it for submission. SFS supervised the study, assisted with statistical analyses, and data interpretation, edited and finalised the manuscript for submission. CBB: designed, planned, and funded the study, assisted with data interpretation, edited and finalized the manuscript for submission.

Financial support. This study was funded by Professor Brink (NRF – grant no. 103371IFR160118156926).

Conflict of interest. No conflict of interest to declare.

Animal welfare. The study was approved by the Animal Care, Health and Safety Research Ethics Committee (NHREC reg. no.: AREC-130913-015) of the North-West University (NWU-AnimCareREC ethics approval no.: **NWU-00277-17-A5**). All experiments adhered to the guidelines of the South African National Standards: The Care and Use of Animals for Scientific Purposes (SANS 10 386 : 2008). Animals were maintained, and all procedures performed in accordance with the code of ethics in research, training, and testing of drugs in South Africa and complied with national legislation.

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

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/neu.2021.4

References

Andersen SL (2003) Trajectories of brain development: point of vulnerability or window of opportunity? *Neuroscience & Biobehavioral Reviews* **27**, 3–18.

Angelucci F, Aloe L, Jiménez-Vasquez P and Mathé AA (2003) Lithium treatment alters brain concentrations of nerve growth factor, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor in a rat model of depression. *International Journal of Neuropsychopharmacology* **6**, 225–231.

Angelucci F, Aloe L, Vasquez PJ and Mathé AA (2000) Mapping the differences in the brain concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in an animal model of depression. *Neuroreport* **11**, 1369–1373.

Bachmann CJ, Aagaard L, Burcu M, Glaeske G, Kalverdijk LJ, Petersen I, Schuiling-Veninga CC, Wijlaars L, Zito JM and Hoffmann F (2016) Trends and patterns of antidepressant use in children and adolescents from five western countries, 2005–2012. *European Neuropsychopharmacology* **26**, 411–419.

Badenhorst NJ, Brand L, Harvey BH, Ellis SM and Brink CB (2017) Long-term effects of pre-pubertal fluoxetine on behaviour and monoaminergic stress response in stress-sensitive rats. *Acta Neuropsychiatrica* **29**, 222–235.

Brink CB, Clapton J, Eagar B and Harvey B (2008) Appearance of antidepressant-like effect by sildenafil in rats after central muscarinic receptor blockade: evidence from behavioural and neuro-receptor studies. *Journal of Neural Transmission* **115**, 117–125.

Cryan JF, Valentino RJ and Lucki I (2005) Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neuroscience & Biobehavioral Reviews* **29**, 547–569.

Cumming G (2013) The new statistics: why and how. *Psychological Science* **25**, 7–29.

Eiland L and Romeo RD (2013) Stress and the developing adolescent brain. *Neuroscience* **249**, 162–171.

Elfving B, Plougmann PH, Müller HK, Mathé AA, Rosenberg R and Wegener G (2010) Inverse correlation of brain and blood BDNF levels in a genetic rat model of depression. *International Journal of Neuropsychopharmacology* **13**, 563–572.

FDA (2014) Food and Drug Administration. Don't Leave Childhood Depression Untreated. Available at https://www.fda.gov/consumers/consumer-updates/fda-dont-leave-childhood-depression-untreated (accessed 23 November 2020).

Friedrich M (2017) Depression is the leading cause of disability around the world. *JAMA* **317**, 1517–1517.

Ghandour RM, Sherman LJ, Vladutiu CJ, Ali MM, Lynch SE, Bitsko RH and Blumberg SJ (2019) Prevalence and Treatment of Depression, Anxiety, and Conduct Problems in US Children.

Gomes Da Silva S, Unsain N, Mascó DH, Toscano-Silva M, De Amorim HA, Silva Araújo BH, Simoes PSR, Da Graça Naffah-Mazzacoratti M, Mortara RA and Scorza FA (2012) Early exercise promotes positive hippocampal plasticity and improves spatial memory in the adult life of rats. *Hippocampus*, **22**, 347–358.

Harvey BH, Brand L, Jeeva Z and Stein DJ (2006) Cortical/hippocampal monoamines, HPA-axis changes and aversive behavior following stress and restress in an animal model of post-traumatic stress disorder. *Physiology and Behavior* **87**, 881–890.

Hazell P and Mirzaie M (2013) Tricyclic drugs for depression in children and adolescents. *Cochrane Database of Systematic Reviews*.

Huddleston AJ, Knoderer CA, Morris JL and Ebenroth ES (2009) Sildenafil for the treatment of pulmonary hypertension in pediatric patients. *Pediatric Cardiology* **30**, 871–882.

Kovacs M, Obrosky S and George C (2016) The course of major depressive disorder from childhood to young adulthood: recovery and recurrence in a longitudinal observational study. *Journal of Affective Disorders*, **203**, 374–381.

Lee B-H and Kim Y-K (2010) The roles of BDNF in the pathophysiology of major depression and in antidepressant treatment. *Psychiatry Investigation* **7**, 231.

Liebenberg N, Harvey BH, Brand L and Brink CB (2010a) Antidepressant-like properties of phosphodiesterase type 5 inhibitors and cholinergic dependency in a genetic rat model of depression. *Behavioural Pharmacology* **21**, 540–547.

Liebenberg N, Müller HK, Fischer CW, Harvey BH, Brink CB, Elfving B and Wegener G (2011) An inhibitor of cAMP-dependent protein kinase induces behavioural and neurological antidepressant-like effects in rats. *Neuroscience Letters* **498**, 158–161.

Liebenberg N, Wegener G, Harvey BH and Brink CB (2010b) Investigating the role of protein kinase-G in the antidepressant-like response of sildenafil in combination with muscarinic acetylcholine receptor antagonism. *Behavioural Brain Research* **209**, 137–141.

Magara S, Holst S, Lundberg S, Roman E and Lindskog M (2015) Altered explorative strategies and reactive coping style in the FSL rat model of depression. *Frontiers in Behavioral Neuroscience* **9**, 89.

Melvin GA, Dudley AL, Gordon MS, Ford S, Taffe J and Tonge BJ (2013) What happens to depressed adolescents? A follow-up study into early adulthood. *Journal of Affective Disorders* **151**, 298–305.

Monroe S and Harkness K (2012) Is depression a chronic mental illness? *Psychological Medicine* **42**, 899–902.

Mullen S (2018) Major depressive disorder in children and adolescents. *Mental Health Clinician* **8**, 275–283.

Murrin LC, Sanders JD and Bylund DB (2007) Comparison of the maturation of the adrenergic and serotonergic neurotransmitter systems in the brain: implications for differential drug effects on juveniles and adults. *Biochemical Pharmacology* **73**, 1225–1236.

NIMH (2018) National Institute of Mental Health. Depression. Available at https://www.nimh.nih.gov/health/topics/depression/index.shtml. (accessed 23 November 2020).

Overstreet DH, Friedman E, Mathé AA and Yadid G (2005) The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. *Neuroscience & Biobehavioral Reviews* **29**, 739–759.

Pittenger C and Duman RS (2008) Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology* **33**, 88–109.

Rosenzweig-Lipson S, Beyer CE, Hughes ZA, Khawaja X, Rajarao SJ, Malberg JE, Rahman Z, Ring RH and Schechter LE (2007) Differentiating antidepressants of the future: efficacy and safety. *Pharmacology & Therapeutics* **113**, 134–153.

Schoeman JC, Steyn SF, Harvey BH and Brink CB (2017) Long-lasting effects of fluoxetine and/or exercise augmentation on bio-behavioural markers of depression in pre-pubertal stress sensitive rats. *Behavioural Brain Research* **323**, 86–99.

Steyn SF (2018) Chronic Effects of Pre-Adolescent Pharmacological and Non-Pharmacological Interventions on Depressive-Like Behaviour in Rats. North-West University.

Steyn SF, Harvey BH and Brink CB (2018) Immediate and long-term antidepressive-like effects of pre-pubertal escitalopram and omega-3 supplementation combination in young adult stress-sensitive rats. *Behavioural Brain Research* **351**, 49–62.

Steyn SF, Harvey BH and Brink CB (2020) Pre-pubertal, low-intensity exercise does not require concomitant venlafaxine to induce robust, late-life antidepressant effects in Flinders sensitive line rats. *European Journal of Neuroscience*.

Wang C, Zhang J, Lu Y, Lin P, Pan T, Zhao X, Liu A, Wang Q, Zhou W and Zhang H (2014) Antidepressant-like effects of the phosphodiesterase-4 inhibitor etazolate and phosphodiesterase-5 inhibitor sildenafil via cyclic AMP or cyclic GMP signaling in mice. *Metabolic Brain Disease* **29**, 673–682.

Wegener G, Harvey BH, Bonefeld B, Müller HK, Volke V, Overstreet DH and Elfving B (2010) Increased stress-evoked nitric oxide signalling in the Flinders sensitive line (FSL) rat: a genetic animal model of depression. *International journal of Neuropsychopharmacology* **13**, 461–473.

WHO (2017) World Health Organization. Depression and other common mental disorders: global health estimates. https://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf?sequence=1&isAllowed=y. (accessed 24 January 2021).

Zubrick SR, Hafekost J, Johnson SE, Sawyer MG, Patton G and Lawrence D (2017) The continuity and duration of depression and its relationship to non-suicidal self-harm and suicidal ideation and behavior in adolescents 12–17. *Journal of Affective Disorders* **220**, 49–56.

https://doi.org/10.1017/neu.2021.4 Published online by Cambridge University Press