



Research paper

Bio-behavioural changes in treatment-resistant socially isolated FSL rats show variable or improved response to combined fluoxetine-olanzapine versus olanzapine treatment

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ABSTRACT

Background: Exposure of Flinders Sensitive Line (FSL) rats to post-weaning social isolation rearing (SIR) causes depressive- and social anxiety-like symptoms resistant to, or worsened by, fluoxetine. SIR typically presents with psychotic-like symptoms, while the paradoxical response to fluoxetine suggests unaddressed psychotic-like manifestations. Psychotic depression (MDpsy) is invariably treatment resistant. To further explore the mood-psychosis continuum in fluoxetine resistant FSL-SIR rats (Mncube et al., 2021), mood-, psychotic-, anxiety-, and social-related behaviour and biomarker response to antidepressant/antipsychotic treatment was studied in FSL-SIR rats. **Methods:** Sprague Dawley (SD) and FSL pups were subjected to social rearing or SIR from postnatal day (PND) 21. Thereafter FSL-SIR rats received olanzapine (5 mg/kg x 14 days) or olanzapine+fluoxetine (OLZ+FLX; 5 mg/kg + 10 mg/kg for 14 days) from PND 63. Psychotic-like, depressive, anxiety, and social behaviour were assessed from PND 72, versus saline-treated FSL-SIR rats, using the prepulse inhibition (PPI), forced swim, open field and social interaction tests. Post-mortem cortico-hippocampal norepinephrine (NE), serotonin (5-HT), and dopamine (DA), as well as plasma corticosterone and dopamine-beta-hydroxylase levels were evaluated. **Results:** SD-SIR and FSL-SIR rats present with significant depressive-like behaviour ($p < 0.01$) as well as significantly reduced sensorimotor gating ($p < 0.01$), although exacerbation versus SIR alone was not observed. Anxiety was significant in FSL-SIR ($p < 0.01$) but not SD-SIR rats. No deficit in social behaviour was evident. Cortico-hippocampal monoamines (NE, 5-HT, DA; $p < .05$) and dopamine beta hydroxylase ($d = 1.13$) were reduced in FSL-SIR rats, less so in SD-SIR rats. Except for dopamine-beta-hydroxylase, these deficits were reversed by both olanzapine and OLZ+FLX ($p < 0.01$). OLZ+FLX was superior to reverse hippocampal NE and DA changes ($p < 0.01$). However, OLZ ($p < .05$) and OLZ+FLX ($p < .01$) worsened depressive-like behaviour and failed to reverse PPI deficits in FSL-SIR rats.

Conclusion: SIR-exposed FSL rats display worsened anxiety, as well as depressive and psychotic-like symptoms, variably responsive to olanzapine or OLZ+FLX. Depleted monoamines are reversed by OLZ+FLX, less so by olanzapine. FSL-SIR rats show promising face and construct but limited predictive validity for MDpsy, perhaps more relevant for bipolar disorder.

1. Introduction

Major depression (MD) has an estimated lifetime prevalence of 17% with up to a third of patients resistant to first-line antidepressants (Rush

et al., 2004; Cusin and Peyda, 2019). Underlying psychosis, otherwise known as psychotic depression (MDpsy), further contributes to treatment-resistance (Nestler et al., 2002; Fava, 2003; Schatzberg, 2003). MDpsy may have a similar or higher point prevalence as

Abbreviations: SD, Sprague-Dawley; FSL, Flinders Sensitive Line; SIR, social isolation rearing; SOC, socially-reared; SAL, saline; OLZ+FLX, olanzapine plus fluoxetine co-therapy; PPI, prepulse inhibition; NE, norepinephrine; 5-HT, serotonin; DA, dopamine.

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schizophrenia (SCZ) (Heslin and Young, 2018). MDpsy is diagnosed on the occurrence of two or more major depressive episodes separated by an interval of at least two months (MD symptom free) and the occurrence of delusions and/or hallucinations that are either mood-congruent (guilt or sadness) or mood-incongruent (persecutory or paranoid) during MD episodes (Tonna et al., 2012; APA., 2013). These patients typically suffer more intense depression, cognitive disturbances, and psychomotor agitation or retardation, with a poorer clinical diagnosis than non-psychotically depressed patients (Schatzberg, 2003; Keller et al., 2007; Cherian et al., 2019). Other features include psychosocial difficulties, increased suicide and a family history of mental disorder, especially bipolar disorder (Heslin and Young, 2018; Keller et al., 2007). MD and MDpsy have been proposed to be distinct disorders (Keller et al., 2007; Rothschild, 2013), with MDpsy presenting with depressive symptoms similar to those of schizophrenia and psychotic bipolar disorder (Keller et al., 2007; Jääskeläinen et al., 2018). Compared to psychotic bipolar disorder, MDpsy is characterised by more severe negative symptoms, albeit similar with regard to rehospitalisation and functional outcome (Jääskeläinen et al., 2018).

Combined antipsychotic-antidepressant treatment of MDpsy is recommended over monotherapy (Farahani and Correll, 2012). Olanzapine and fluoxetine co-therapy (OLZ+FLX) is approved by the Food and Drug Administration for treatment resistant depression (TRD) (Caldarone et al., 2015) and has proven useful in treating MDpsy (Rothschild, 2013). Pre-clinically, OLZ+FLX improves synaptic efficacy and cognitive performance (Zhang et al., 2000; Horowitz et al., 2003), related mechanistically to increased dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in the prefrontal cortex (Zhang et al., 2000). These actions mediate greater benefit versus either agent alone. MDpsy is associated with cerebrospinal fluid and plasma hyperdopaminergia, low dopamine- β -hydroxylase, and hypersecretion of cortisol (Schatzberg et al., 1985, 2014). Dopamine-beta-hydroxylase catalyses the conversion of DA to NE and informs on central and peripheral DA activity (Hamner and Gold, 1998). Reduced dopamine-beta-hydroxylase is also associated with antidepressant treatment resistance (Caldarone et al., 2015). Neuro-anatomically, the frontal cortex and hippocampus are implicated in depressive (Andrews et al., 2015) and psychotic (Uys et al., 2017) symptoms, with the former mediating cognitive function (Ott and Nieder, 2019) and mood regulation (Pandya et al., 2012) and the hippocampus more involved in emotion, neuroendocrine stress hormone regulation and declarative memory (Nakahara et al., 2018). Here, elevated NE (plasma) and 5-HT (platelets) are associated with MDpsy and may differentiate it from MD (Healy et al., 1986; Goekoop et al., 2012).

Effective pharmacological management of MDpsy is challenging (Nestler et al., 2002; Fava, 2003; Schatzberg, 2003). That said, part of the dilemma lies in the dearth of validated animal models of MDpsy to enable drug discovery initiatives. Some animal models emulate comorbid depression in schizophrenia (Samsom and Wong, 2015), while the ouabain model addresses the co-occurrence of MD (manifested as hypo-activity) and mania (demonstrated by hyperactivity) (El-Mallakh et al., 1995). The difficulty in reproducing the cyclical occurrence of psychotic and depressive symptoms of MDpsy, especially in bipolar disorder, and the lack of predictive validity of these models, is problematic. Post-weaning social isolation rearing (SIR) engenders late-life bio-behavioural manifestations akin to psychosis (schizophrenia) (Moller et al., 2015), anxiety (Rau et al., 2015), and depression (Arndt et al., 2015), including social deficits, aggression, and reduced sensorimotor gating (Forrest et al., 2014). Importantly, early childhood adversity (emulated by the SIR model) has been linked to the development of MDpsy or bipolar disorder (Jääskeläinen et al., 2018; Post et al., 2012). Indeed, like MDpsy initial NE activation and increased dopamine-beta-hydroxylase activity followed by chronic dopamine-beta-hydroxylase suppression, may represent common aetiologies (Hamner and Gold, 1998).

The Flinders Sensitive Line (FSL) rat is a genetic rodent model that

displays broad face and construct validity for MD, including response to various classes of antidepressants (Overstreet and Wegener, 2013). However, exposing FSL rats to a traumatic environmental stressor induces characteristics of TRD (Brand and Harvey, 2017a, 2017b). Given the mood-psychosis continuum of MDpsy, a gene-environment model combining FSL and early-life SIR holds promise. Although studies have explored later-life SIR in FSL rats (Bjornebekk et al., 2007; Fischer et al., 2012), they were not designed to consider schizophrenia or MDpsy. More recently Mncube et al., 2021 (Mncube et al., 2021) showed that FSL rats subjected to post-weaning SIR display depressive- and social anxiety-like symptoms that are resistant to, or worsened by, fluoxetine. Importantly, worsening of depression and agitation (anxiety) is a typical adverse response to an antidepressant, linking psychosis-like manifestation to MD (Gournellis et al., 2018) or a bipolar diathesis (Perugi et al., 2019).

OLZ+FLX is a recognized treatment for TRD, especially MDpsy. Using fluoxetine-resistant FSL-SIR rats (Mncube et al., 2021) we elaborate further on the predictive validity of the FSL-SIR rat. We hypothesise that fluoxetine-resistant FSL-SIR rats will present with underlying mood and psychotic-like bio-behavioural manifestations with limited response to olanzapine alone but improved response to OLZ+FLX. By addressing mood, anxiety, social and psychosis-like behaviours together with associated biological markers following chronic olanzapine or OLZ+FLX treatment, we hope to demonstrate that FSL-SIR rats are a useful preparation to model TRD, in particular MDpsy.

2. Methods

2.1. Animals

This study was approved by the AnimCare animal research committee (NHREC reg. no. AREC-130913-015) of the North West University (NWU) (Ethics approval number: NWU-00150-18-S5). The animals used were bred, supplied and housed at the Vivarium (SAVC reg. number FR15/13458; SANAS GLP compliance number G0019) of the Pre-Clinical Drug Development Platform (PCDDP) at the NWU.

Male SD and FSL rats were used in this study. Flinders Sensitive Line rats are derived from SD rats (Overstreet and Wegener, 2013), with SD rats used as a healthy control for both FSL and SD rats reared in social isolation. Before commencement of the study, it is important to determine the behavioural validity of SD rats reared in social isolation with regard to psychotic-like symptoms. In modelling MDpsy and investigating the effects of olanzapine and OLZ+FLX, it was necessary to establish the occurrence of abnormal behaviour in these animals as compared to a healthy control animal (socially reared SD) and against a psychotic model (SD plus SIR). Comparing the isolated SD and FSL rats to SDs gives an indication of “how close to healthy” each cohort is reverted following olanzapine (and OLZ+FLX treatment in the case of the FSL rats). The original colonies of FSL rats were obtained from Dr David H Overstreet, University of North Carolina, USA. The effects of SIR on increased anxiety and hyperactivity are not consistently observed in female rats (Weiss et al., 2004; Walker et al., 2019) and because this is a requirement for the proposed model, female rats were not used in the study. All animals were exposed to the same olfactory, visual, and auditory cues, although FSL-SIR rats were deprived of social contact with peer rats during this period. All rats were allowed free access to standard laboratory chow and water and housed in identical transparent cages (380 mm × 380 mm × 230 mm) in an environmentally-controlled room: constant temperature (22 ± 4 °C), humidity (50 ± 20%), and a 12:12 h light-dark cycle (lights on 06:00 and off at 18:00) with no to minimal noise. The dark cycle was induced under red light following in-house protocol (Regenass et al., 2018).

2.2. Study design

The present study sought to validate a fluoxetine-resistant FSL plus

post-weaning SIR animal model of TRD described in an earlier parallel study (Mncube et al., 2021). The aforementioned paper considered TRD and therefore used fluoxetine response as predictive validation. Given the focus on MDpsy, this paper will specifically evaluate the response to olanzapine and OLZ+FLX; FLX alone will be excluded. Since the FSL rat represents a model of MD and not psychosis, and to also limit unnecessary use of animals, a comparative group of FSL rats are excluded while the same SAL-treated control animals (SD and FSL-SIR) were used in this study and the earlier co-run TRD study (Mncube et al., 2021). Both studies were conducted at the same time so that animal behaviour varying over time is not a confounding factor.

The study design is presented in Fig. 1. Animals were weaned on post-natal day (PND) 21. SD rats were assigned to social-rearing (SD, 3 rats/cage) or social isolation rearing (SIR, 1 rat/cage) while the FSL rats were reared in social isolation. Rearing conditions were maintained for a period of 8 weeks (Moller et al., 2013; Uys et al., 2016). At PND 63, while remaining in their assigned rearing condition, SD-SIR and FSL-SIR animals were assigned to a treatment group: saline-treated (SAL) or olanzapine-treated. Socially-reared SD rats received only SAL. Thus, the resultant cohorts were as follows: SD-SOC-SAL, SD-SIR-SAL and FSL-SIR-SAL as validation groups, with the latter carried over to the drug treatment study, comprising FSL-SIR-SAL as reference versus FSL-SIR-olanzapine and FSL-SIR-OLZ+FLX. Each cohort comprised 12 rats, with a total of 72 animals used in the study. The animals were first weighed on the day of weaning and then each morning from the beginning of the treatment protocol (PND 63) until the last day of the study (PND 77), with weights used to calculate the volume of drug to be administered. The treatment regimen commenced from PND 63 and continued until PND 76. Behavioural testing commenced on PND 72 beginning with the open field test, followed by the social interaction test (SIT) on PND 74, the forced swim test (FST) on PND 75, and the prepulse inhibition test (PPI) on PND 76. All behavioural tests were performed during the dark cycle (18:30–02:30). The animals were euthanised by decapitation without prior administration of an anaesthetic. Trunk blood and brain tissue were collected for bioanalysis. For behavioural and monoamine analysis, all animals were included in the data. For ELISA analysis, plasma samples ($n = 10$ per cohort) were randomly selected from the 12 animals per cohort. This was to allow for more samples to be assayed per plate while maintaining statistical power. Dopamine-beta-hydroxylase and corticosterone were quantified in plasma rather than the brain to correlate to clinical findings (Schatzberg et al., 1985; Liu et al., 2010; Miller et al., 2011) which are mainly based on fluid sample readouts.

2.3. Drug preparation and treatment protocol

Fluoxetine hydrochloride (Pubchem CID 62857; Jade Pharmaceuticals, South Africa) was first dissolved in approximately 500 μ L distilled

water and then made up to 10 mg/mL in physiological saline. Olanzapine (Pubchem CID 135398745; DB Fine Chemicals (Pty) Ltd (Johannesburg, South Africa) was dissolved in approximately 200 μ L 0.1 N acetic acid (Pubchem CID 176) and then in saline to make a 5 mg/mL solution. All treatments were administered subcutaneously (s.c) (Zhang et al., 2000) according to the literature at the following doses: fluoxetine (10 mg/kg) (Detke et al., 1995), olanzapine (5 mg/kg) (Heidbreder et al., 2001) and OLZ+FLX (olanzapine 5 mg/kg + fluoxetine 10 mg/kg) for a period of 14 days. Control rats received SAL s.c. All treatments were administered during the light cycle between 08:00 and 10:00, with fresh solutions prepared daily.

2.4. Behavioural assessments

The behavioural experiments were performed from least to most stressful, as described by Mokoena et al., 2015 (Mokoena et al., 2015), to ensure that behaviour in subsequent tests would not be negatively affected by prior tests. All behavioural tests were performed during the dark cycle (18:30–02:30).

2.4.1. Psychosis: hyperlocomotion – open field test

Spontaneous hyperactivity in rats in response to novel environments is useful for assessing psychomotor agitation (a symptom of SCZ and MDpsy) (Moller et al., 2015; Schatzberg and Rothschild, 1992). The method of Sherif and Oreland, 1995 (Sherif and Oreland, 1995) was used to determine the total distance travelled (cm) in the open field test. Rats were placed individually into the centre of a square arena (100 \times 100 \times 50 cm). The test was conducted in a dimly lit room illuminated with red light (40 W). Animal behaviour was recorded for 5 min using a ceiling-mounted digital camera. The video files were analysed using Noldus Ethovision XT software (Noldus® Information Technology, Wageningen, The Netherlands).

2.4.2. Anxiety: thigmotaxis – open field test

Thigmotaxis is an important indicator of anxiety in rodents and is sensitive to treatment with anxiolytics and sedatives (Belovicova et al., 2017). The ratio of time (s) spent in the centre of the arena versus the time (s) spent along the walls of the arena (presented as a percentage) was used to determine relative anxiety levels in the test subjects, with behaviour recorded for 10 min, with the first 5 min scored and analysed to provide better insight into anxious behaviour (Gould et al., 2009). Video files of the behaviour in the open field test were analysed using Noldus Ethovision XT software (Noldus® Information Technology, Wageningen, The Netherlands).

2.4.3. Social interaction test

Social deficits, anxiety as well as aggression are recognised symptoms of MDpsy (Keller et al., 2007; Tyrka et al., 2006). The social interaction test was performed in the same arena and under the same lighting conditions as the open field test and as previously described (Moller et al., 2011) to assess anxiety-related social withdrawal and antisocial behaviour in rodents (File and Seth, 2003; Kaidanovich-Beilin et al., 2011). All behaviours in the social interaction test were scored manually with a stopwatch from the video recordings of the animal interactions. Pair scores were used and are expressed as percentage (%) time spent by the rat pair in a particular behaviour. The behaviours assessed are described in Table 1. For brevity, they are presented in the data as “Social (amicable)”, “Asocial (anxiety-like),” and “Aggressive (antisocial)” behaviour.

2.4.4. Despair – forced swim test

Despair is a manifest symptom of MD (Post et al., 2012). Immobility (despair), swimming (survival, coping) and climbing (escape-driven behaviour) behaviours were scored as previously described (Mncube et al., 2021). Individual rats were placed in transparent, cylindrical swim tanks containing water at 25 °C and allowed to swim for 7 min. At

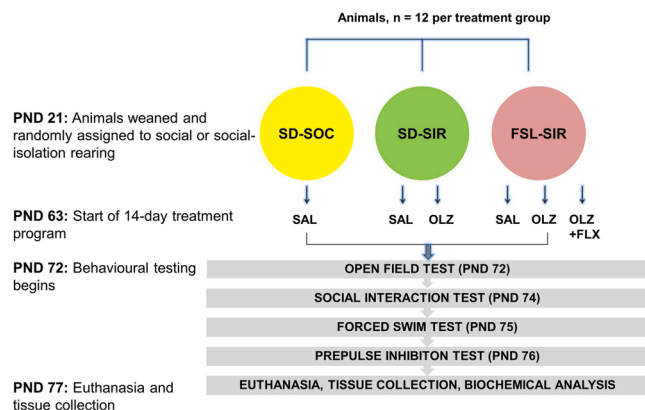


Fig. 1. Study design.

Table 1

Behaviour scored in the social interaction test (Adapted from Barnett (1958), Blanchard and Blanchard (1977), and Brain et al. (1989) (Barnett, 1958; Blanchard and Blanchard, 1977; Brain et al., 1989).

Category	Behaviour	Description
Social	Sniffing	Sniffing the head, snout, anogenital area, or body of the partner
Asocial (anxiety)	Exploring	Walking or running around the arena, not obviously directed toward the partner, supported or unsupported rearing unrelated to partner
Antisocial (aggression)	Nose-off	Defensive or offensive strategy in which partners stand immobile facing each other; may escalate to boxing
	Boxing	Two rats stand on their hind legs facing each other and push and paw at each other using front paws

the end of the swim period, the rats were removed from the tanks, dried and returned to their home cages. Immobility, swimming and climbing behaviours (timed in seconds) were scored manually from the video recordings of the animal behaviour in the cylinders by a researcher blinded to treatment. For reasons noted earlier (Oberholzer et al., 2018), the first and last minutes of the rat behaviour in the water were excluded in the score and analysis. This test was performed on PND 75 during the dark cycle.

2.4.5. Psychosis: Sensorimotor gating – prepulse inhibition (PPI) test

PPI is used to determine sensorimotor gating performance in humans and rodents (Shoji and Miyakawa, 2018), deficits of which correlate with clinical symptoms of disordered thoughts and distractibility (Forrest et al., 2014) evident in psychosis (APA., 2013). Prepulse inhibition was assessed in two ventilated and illuminated, sound-attenuating startle chambers (SR-LAB, San Diego Instruments, San Diego, USA), as described previously (Moller et al., 2011).

Per cent PPI (%PPI) for each prepulse + pulse trial was calculated using the following formula: %PPI = [100 – (startle response for PRE-PULSE+PULSE trial) / (startle response for PULSE ALONE trial) × 100] (Swanepoel et al., 2018). Average %PPI values across the four prepulse intensities were calculated and used as described previously (Uys et al., 2016).

2.4.6. Bioanalysis

2.4.6.1. Preparation of plasma and brain tissue. The animals were sacrificed by decapitation without the prior use of an anaesthetic agent the morning (12–14 h) after the final behavioural test as previously described (Mokoena et al., 2015; Möller et al., 2013). The frontal cortex and hippocampus were excised on an ice-cooled glass slab immediately after decapitation. Trunk blood was collected in pre-chilled, 4 mL vacutainer tubes (Vacuette®) containing K₃EDTA solution as anti-coagulant. The blood was centrifuged at 1000g at 4 °C for 15 min. Brain tissue and plasma were fixed in liquid nitrogen and stored at – 80 °C until the day of analysis.

2.4.6.2. Monoamine quantification. NE, 5-HT, and DA were quantified in the selected brain regions using a high-performance liquid chromatography (HPLC) system with electrochemical detection (HPLC-EC), as previously described (Viljoen et al., 2018). The brain tissue was prepared as described by Viljoen et al., 2018 (Viljoen et al., 2018). An Agilent 1200 series HPLC (Agilent Technologies Inc., Santa Clara, CA USA), equipped with an isocratic pump and autosampler, coupled to an ESA Coulochem III Electrochemical detector with a coulometric flow cell (Model 5011 A High Analytical Cell and Guard cell 5020) and Chromleon® Chromatography Management System version 6.8 (obtained from Thermo Fisher Scientific, Waltham, MA USA), was used for this analysis.

Monoamine concentrations in the tissue samples were determined by

comparing the area under the peak of each marker with that of the internal standard 5-hydroxy-N ω -methyltryptamine oxalate (5-HMT) with a final concentration of 1500 ng/mL. Calibration curves were prepared for each analyte (range 10–200 ng/mL; $r^2 > 0.99$). Monoamine concentrations were expressed as ng/g wet weight of tissue (mean \pm SEM).

2.4.6.3. Plasma biochemistry: Corticosterone and dopamine beta hydroxylase. Dopamine beta hydroxylase (Abbexa, Cambridge, UK) and corticosterone (Elabscience, Wuhan, China) were measured in the plasma by sandwich ELISA kits according to the manufacturer's protocol. Briefly, 100 μ L of plasma sample for the dopamine-beta-hydroxylase assay and 50 μ L for the corticosterone assay were incubated in monoclonal antibody-coated wells in a 96-well plate. The liquid from the wells were removed and a biotinylated detection antibody was added to each well. After a series of washes, avidin-conjugated horseradish peroxidase was added. Absorbance was measured using a Spectronic 20 (Bausch and Lomb) spectrophotometer. For each ELISA kit, a total of 10 plasma samples from each cohort was analysed in duplicate.

2.4.7. Statistical analysis

Statistical analysis was performed under the supervision of the Statistical Consultation Service of the NWU. GraphPad Prism® version 8 (GraphPad Software, San Diego California USA, www.graphpad.com) was used for statistical analysis and graphical presentations. Data are graphically presented as mean \pm SEM. Normality of data was determined using the Shapiro-Wilk test. Comparison of the bio-behavioural manifestations in SAL-treated SD-SOC, SD-SIR and FSL-SIR rats were made using one-way ANOVA with Bonferroni post-hoc multiple comparisons. Where the criteria of equality of variances for ANOVA was not met, the Kruskal-Wallis ANOVA with Dunn's post-hoc multiple comparisons was used. A 5% confidence limit for error was taken as statistically significant ($p < .05$). Given the risk of the p-value being confounded by the sample size, practical significance was calculated to decrease the risk of a type II statistical error (false negative), according to Cohen, 1988 (Cohen, 1988) and Rosnow and Rosenthal, 1996 (Rosnow and Rosenthal, 1996). Cohen's d-value (effect size) was calculated to indicate the effect size and practical significance of results demonstrating statistical significance on a 5% ($p < .05$) and 10% ($p \leq .1$) significance level. An effect size less than 0.2 is considered a small effect, 0.3–0.7 is a medium effect indicating a trend for practical significance, and effect sizes of 0.8 and greater is considered large and practically significant (Cohen, 1977).

3. Results

To confirm the translational validity of the FSL-SIR rat model for MDpsy, bio-behavioural comparisons between socialised SAL-treated SD rats and SAL-treated SD-SIR and FSL-SIR animals were first carried out. Once validity was confirmed, SAL-treated FSL-SIR rats, as a putative MDpsy model, were assessed with respect to reversal of bio-behavioural changes by olanzapine alone and OLZ+FLX. Fluoxetine alone was not considered in this study, for reasons noted earlier.

3.1. Behaviour

3.1.1. Open field test

3.1.1.1. Model validation. Locomotor activity (Fig. 2a): Distance travelled by the rats in the open field test, a measure of locomotor activity. One-way ANOVA of the data indicated no significant strain effect on distance travelled across the groups [$F(2, 33) = 0.8458, p = .4383$]. Hence no post-hoc analysis was performed.

Thigmotaxis (Fig. 2c): Kruskal-Wallis test indicated a significant strain effect on thigmotactic behaviour (Kruskal-Wallis statistic = 12.38, $p = .0020$), a measure of anxious behaviour. FSL-SIR-SAL rats were

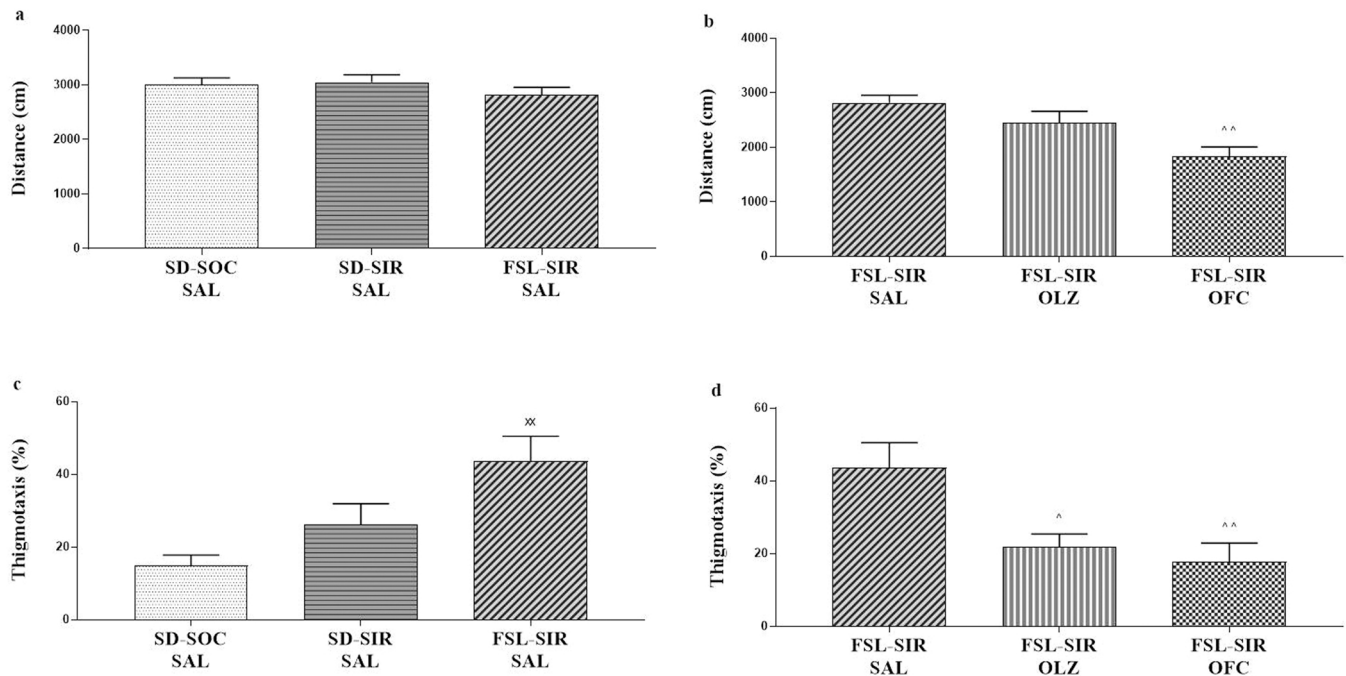


Fig. 2. Locomotor activity measured as distance travelled in the open field test ($n = 12/\text{group}$) in (a) SAL-treated SD-SOC, SD-SIR and FSL-SIR rats or in (b) FSL-SIR rats treated with drug treatment as indicated. $\sim p < .01$ vs. FSL-SIR. Thigmotaxis indicating anxiety in (c) SAL-treated SD-SOC, SD-SIR and FSL-SIR animals as determined in the open field test ($n = 12/\text{group}$). $^{**}p < .01$ vs. SD-SOC. SD-SOC or in (d) FSL-SIR rats treated with drug treatment as indicated. $\sim p < .01$, $^*p < .05$ vs. FSL-SIR-SAL. SD-SOC, socially reared Sprague-Dawley; SD-SIR, Sprague-Dawley exposed to social isolation rearing; FSL, Flinders Sensitive Line rats exposed to social isolation rearing; OFC=OLZ+FLX co-therapy.

significantly more thigmotactic (anxious) than socialised SD-SOC animals ($p = .0014$, Cohen's $d = 1.65$). SD-SIR-SAL animals did not exhibit significantly different thigmotactic behaviour at the 5% or 10% level versus SD-SOC-SAL ($p = .4717$) or FSL-SIR ($p = .1118$) animals.

3.1.1.2. Treatment response. Locomotor activity (Fig. 2b): One-way ANOVA indicated a significant effect of treatment on locomotor activity [$F(2, 33) = 7825, p = .0017$]. Post-hoc analysis showed that OLZ+FLX significantly decreased locomotor activity in FSL-SIR rats compared to those receiving SAL-treatment ($p = .0013$, Cohen's $d = 1.87$) and a similar, albeit insignificant, trend compared to those receiving olanzapine ($p = .0582$, Cohen's $d = 0.96$).

Thigmotaxis (Fig. 2d): Kruskal-Wallis ANOVA indicated a significant effect of treatment across the groups (Kruskal-Wallis statistic = 10.23, $p = .0060$). Dunn's test indicated that both olanzapine ($p = .05$, Cohen's $d = 1.21$) and OLZ+FLX-treatment ($p = .0083$, Cohen's $d = 1.29$) decreased thigmotactic behaviour (anxiety) versus SAL treated FSL-SIR rats.

3.1.2. Social interaction test

3.1.2.1. Model validation. Social (amicable) behaviour (Fig. 3a): Kruskal-Wallis test did not show a significant strain effect on social behaviour between the various groups (Kruskal-Wallis statistic = 5.135, $p = .0725$), although this was significant at the 10% level.

Asocial (socially anxious-like) behaviour (Fig. 3c): Kruskal-Wallis did not indicate a significant strain effect on asocial behaviour between various groups (Kruskal-Wallis statistic = 0.9688, $p = .6375$).

Antisocial (aggressive) behaviour (Fig. 3e): Kruskal-Wallis showed no significant strain effect between the various groups (Kruskal-Wallis statistic = 1.403, $p = .4960$).

3.1.2.2. Treatment response. Social (amicable) behaviour (Fig. 3b): One-way ANOVA did not show a significant effect of treatment across the

respective groups [$F(2, 15) = 1.556, p = .2431$].

Asocial (social anxious-like) behaviour (Fig. 3d): Kruskal-Wallis revealed a significant effect of treatment across the treatment groups (Kruskal-Wallis statistic = 7.906, $p = .0127$). Dunn's test showed a significant increase in asocial behaviour in FSL-SIR rats following OLZ+FLX-treatment versus SAL-treatment ($p = .0386$, Cohen's $d = 2.15$) and a similar, albeit insignificant, trend compared to those receiving olanzapine ($p = .0520, d = 2.65$).

Anti-social (aggressive-like) behaviour (Fig. 3f): Kruskal-Wallis indicated a significant treatment effect on aggressive behaviour across the various groups (Kruskal-Wallis statistic = 7.356, $p = .0253$). Dunn's test showed a significant decrease in aggressive behaviour in FSL-SIR rats only following OLZ+FLX-treatment versus SAL treatment ($p = .0206$, Cohen's $d = 0.95$).

3.1.3. Forced swim test

3.1.3.1. Model validation. Immobility (Fig. 4a): One-way ANOVA indicated a significant strain effect on immobility across the various groups [$F(2, 33) = 9.601, p = .0005$]. Post-hoc analysis indicated significantly increased immobility in SD-SIR rats ($p = .0008$, Cohen's $d = 1.47$) and FSL-SIR rats ($p = .0052$, Cohen's $d = 1.30$) compared to socialised SD-SOC treated rats.

Swimming (Fig. 4c): One-way ANOVA indicated a significant strain effect on swimming across the various groups [$F(2, 33) = 13.00, p < .0001$]. Post-hoc analysis revealed that only SD-SIR-SAL rats exhibited significantly less swimming behaviour than SD-SOC-SAL treated rats ($p < .0001$, Cohen's $d = 1.84$) and FSL-SIR-SAL rats ($p = .0421$, Cohen's $d = 1.81$). The latter showed a similar trend but failed to reach significance versus SD-SOC-SAL treated animals.

Climbing behaviour (Fig. 4e): One-way ANOVA indicated a significant strain effect on climbing across the groups [$F(2, 33) = 3.736, p = .0345$]. Post-hoc analysis indicated significantly increased climbing behaviour in SAL-treated FSL-SIR rats versus socialised SD-SOC rats ($p = .0348$, Cohen's $d = 1.18$).

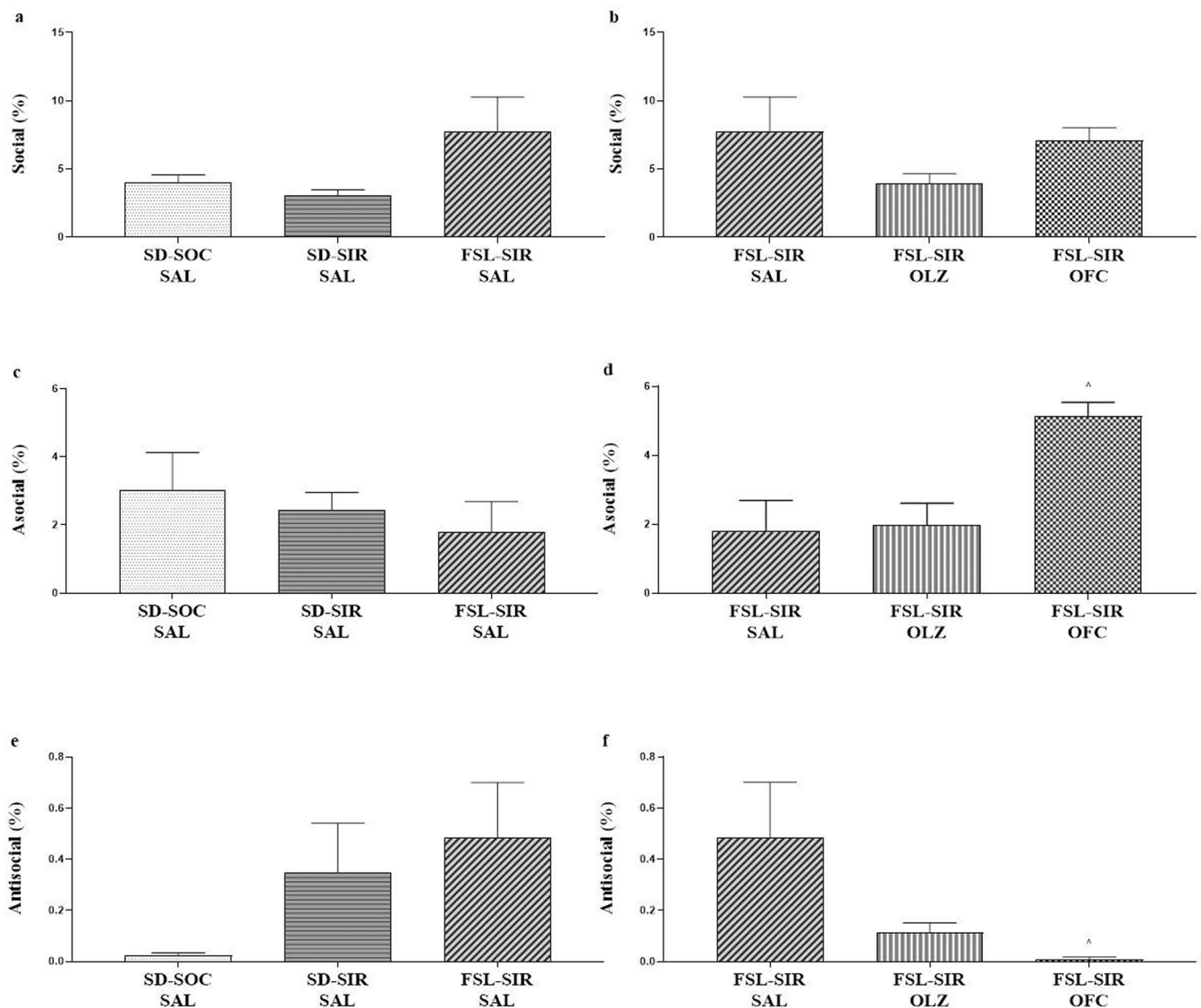


Fig. 3. Behaviour as measured in the social interaction test ($n = 12/\text{group}$). Social/amicable in (a) SAL-treated SD-SOC, SD-SIR and FSL-SIR rats or in (b) FSL-SIR rats treated with drug treatment as indicated. Asocial/socially anxious-like behaviour in (c) SAL-treated SD-SOC, SD-SIR and FSL-SIR rats or in (d) FSL-SIR rats treated with drug treatment as indicated. Antisocial/aggressive behaviour in (e) SAL-treated SD-SOC, SD-SIR and FSL-SIR rats or in (f) FSL-SIR rats treated with drug treatment as indicated. $p < .05$ vs. FSL-SIR-SAL. SD-SOC, socially reared Sprague-Dawley; SD-SIR, Sprague-Dawley exposed to social isolation rearing; FSL, Flinders Sensitive Line rats exposed to social isolation rearing; OFC=OLZ+FLX co-therapy.

3.1.3.2. Treatment response. Immobility (Fig. 4b): One-way ANOVA showed a significant effect of treatment on immobility across the various treatment groups [$F(2, 33) = 6.536$, $p = .0041$]. Both olanzapine ($p = .0268$, $d = 1.22$) and OLZ+FLX ($p = .0054$, $d = 1.70$) increased immobility in FSL-SIR rats versus those treated with SAL.

Swimming (Fig. 4d): One-way ANOVA indicated a significant effect of treatment on swimming across the various groups [$F(2, 33) = 22.54$, $p < .0001$]. Both olanzapine and OLZ+FLX significantly decreased swimming behaviour in FSL-SIR rats versus those treated with SAL (both $p < .0001$).

Climbing (Fig. 4f): One-way ANOVA indicated a significant effect of treatment on climbing across the various groups [$F(2, 33) = 3.303$, $p = .0493$]. Only OLZ+FLX treatment significantly decreased climbing behaviour in FSL-SIR rats compared to SAL-treated FSL-SIR controls ($p = .0447$, $d = 1.05$).

3.1.4. Sensorimotor gating

3.1.4.1. Model validation. %PPI (Fig. 5a): One-way ANOVA indicated a significant strain effect on PPI across the groups [$F(2, 33) = 8.045$, $p = 0.0014$]. Post-hoc analysis showed that both SD-SIR ($p = 0.0037$, Cohen's $d = 1.48$) and FSL-SIR ($p = 0.0053$, Cohen's $d = 0.05$) significantly decreased PPI compared to SD-SOC rats receiving SAL.

3.1.4.2. Treatment response. %PPI (Fig. 5b): One-way ANOVA did not indicate a significant effect of treatment on PPI across the various groups [$F(2, 33) = 0.7890$, $p = 0.4627$]. Hence a post-hoc analysis was not pursued.

3.2. Biological parameters

3.2.1. Monoamines

3.2.1.1. Model validation. Frontocortical NE (Fig. 6a): One-way ANOVA

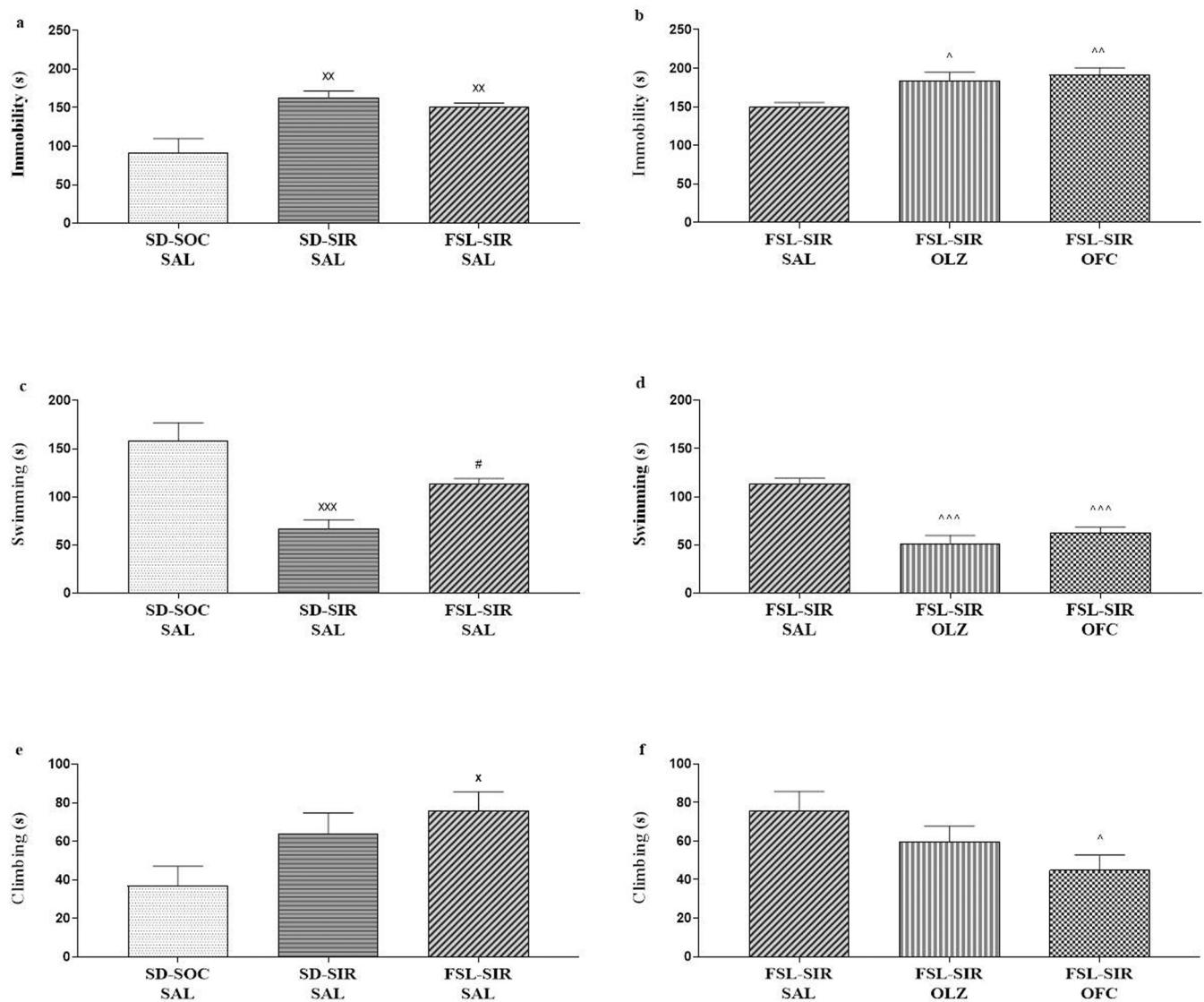


Fig. 4. Immobility, swimming and climbing behaviour of SAL-treated SD, SD-SIR and FSL-SIR rats in the forced swim test ($n = 12/\text{group}$). **Fig. 4a, b:** Immobility. $^{xx}p < .01$ vs. SD-SOC. $^{\sim}p < .01$, $^{\wedge}p < .05$ vs. FSL-SIR-SAL. **Fig. 4c, d:** Swimming. $^{xxx}p < .0001$ vs. SD-SOC, $^{\#}p < .05$ vs. SD-SIR. $^{xxx}p < .0001$ vs. FSL-SIR-SAL. **Fig. 4e, f:** Climbing. $^{\sim}p < .05$ vs. SD-SOC. $^{\wedge}p < .05$ vs. FSL-SIR-SAL, socially reared Sprague-Dawley; SD-SIR, Sprague-Dawley exposed to social isolation rearing; FSL, Flinders Sensitive Line rats exposed to social isolation rearing; OFC=OLZ+FLX co-therapy.

showed a significant strain effect across the various groups [$F(2, 33) = 144.9$, $p < .0001$]. SD-SIR-SAL rats showed significantly diminished frontocortical NE compared to SAL-treated SD-SOC rats ($p < .0001$, Cohen's $d = 0.95$). NE levels were also significantly reduced in FSL-SIR-SAL rats compared to SD-SOC-SAL ($p = .0298$, Cohen's $d = 6.46$) and versus SD-SIR-SAL rats ($p < .0001$, Cohen's $d = 8.91$).

Hippocampal NE (Fig. 6c): One-way ANOVA showed a significant strain effect across the various groups [$F(2, 32) = 135.3$, $p < .0001$]. Both SD-SIR ($p = .0306$, Cohen's $d = 0.97$) and FSL-SIR ($p < 0.0001$, Cohen's $d = 6.26$) presented with significantly lower hippocampal NE levels compared to SD-SOC rats, all treated with SAL. FSL-SIR-SAL animals also had significantly lower NE levels compared to SD-SIR-SAL rats ($p < .0001$, Cohen's $d = 10.36$).

Frontocortical 5-HT (Fig. 7a): One-way ANOVA indicated a significant strain effect across all the groups in question [$F(2, 33) = 85.31$, $p < .0001$]. Post-hoc analysis showed significantly elevated frontocortical 5-HT levels in SD-SIR-SAL rats compared to SD-SOC-SAL controls ($p = .0082$, Cohen's $d = 1.13$). FSL-SIR rats however presented with significantly diminished 5-HT levels compared to SD-SOC ($p < .0001$, Cohen's $d = 4.41$) and versus SD-SIR-SAL rats ($p < .0001$, Cohen's $d =$

6.44).

Hippocampal 5-HT (Fig. 7c): One-way ANOVA showed a significant effect of strain across the groups in question [$F(2, 32) = 66.04$, $p < .0001$]. Post-hoc tests show significantly diminished 5-HT levels in FSL-SIR rats compared to SD-SOC ($p < .0001$, Cohen's $d = 3.46$) and versus SD-SIR rats ($p < .0001$, Cohen's $d = 9.31$). Although elevated hippocampal 5-HT levels in SD-SIR rats missed significance compared to SD-SOC rats at the 5% level, it was statistically significant at the 10% level ($p = .0752$) with a large Cohen's effect size ($d = 0.83$).

Frontocortical DA (Fig. 8a): Kruskal-Wallis showed a significant strain effect across the groups in question (Kruskal-Wallis statistic = 22.52, $p < .0001$). Post-hoc analysis showed significantly diminished DA levels in SAL-treated FSL-SIR rats compared to SAL-treated SD-SOC ($p = .0058$, Cohen's $d = 1.89$) and SD-SIR rats ($p < .0001$, Cohen's $d = 2.74$).

Hippocampal DA (Fig. 8c): Kruskal-Wallis indicated significant strain effects across the groups in question (Kruskal-Wallis statistic = 21.78, $p < .0001$). Post-hoc analysis indicated significantly diminished DA levels in SAL-treated FSL-SIR rats compared to SAL-treated SD-SOC ($p = .0008$, Cohen's $d = 1.76$) and SD-SIR rats ($p < .0001$, Cohen's $d =$

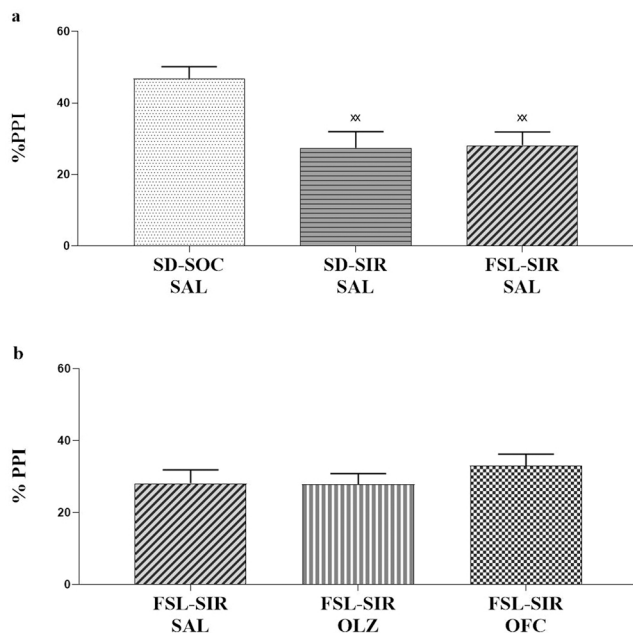


Fig. 5. Average %PPI in SAL-treated SD-SOC, SD-SIR and FSL-SIR rats (a) and in drug-treated FSL-SIR rats as indicated (n = 12/group). **p < .01 vs. SD-SOC. SD-SOC, socially reared Sprague-Dawley; SD-SIR, Sprague-Dawley exposed to social isolation rearing; FSL, Flinders Sensitive Line rats exposed to social isolation rearing; OFC=OLZ+FLX co-therapy.

2.92).

3.2.1.2. Treatment response. Frontocortical NE (Fig. 6b): One-way ANOVA in drug-treated FSL-SIR rats (Fig. 6b) demonstrated a significant effect of treatment [F(2, 33) = 25.15, p < .0001]. Both olanzapine

and OLZ+FLX-treatment significantly increased (reversed lowered) NE (both p < .0001) compared to SAL-treated FSL-SIR rats.

Hippocampal NE (Fig. 6d): One-way ANOVA in drug-treated FSL-SIR rats indicated a significant effect of treatment on [F(2, 32) = 13.16, p < .0001]. p < .0001]. SD-SIR-SAL Olanzapine (p = .0005, d = 3.00) and OLZ+FLX (p = .0002, d = 1.91) significantly raised (reversed lowered) NE compared to SAL-treated FSL-SIR rats.

Frontocortical 5-HT (Fig. 7b): One-way ANOVA in drug-treated FSL-SIR rats revealed a significant effect of treatment [F(2, 32) = 29.30, p < .0001]. Both olanzapine- and OLZ+FLX-treated FSL-SIR presented with significantly increased (reversed lowered) frontocortical 5-HT levels (both p < .0001) compared to SAL-treated FSL-SIR rats.

Hippocampal 5-HT (Fig. 7d): A one-way ANOVA in drug-treated FSL-SIR rats indicated a significant effect of treatment [F(2, 32) = 12.67, p < .0001]. OLZ+FLX-treatment significantly increased 5-HT levels compared to SAL-treatment (p < .0001, d = 1.94), and versus olanzapine-treatment (p = .0364, d = 0.93). The olanzapine-induced elevation of 5-HT in this region was significant at the 10% level (p = .0630) along with a large Cohen’s effect size (d = 2.22).

Frontocortical DA (Fig. 8b): A one-way ANOVA indicated a significant effect of treatment across the groups [F(2, 27) = 10.53, p = .0004]. Olanzapine- (p = .0062, d = 1.96) and OLZ+FLX-treatment (p = .0005, d = 2.26) significantly increased (reversed reduced) DA in FSL-SIR rats compared to SAL-treated FSL-SIR rats.

Hippocampal DA (Fig. 8d): Kruskal-Wallis ANOVA indicated a significant effect of treatment across the groups (Kruskal-Wallis statistic = 22.05, p < .0001). Olanzapine-treatment (p < .0001, d = 2.02) and OLZ+FLX-treatment (p = .0018, d = 1.59) significantly increased (reversed reduced) DA in FSL-SIR rats compared to SAL-treated FSL-SIR controls.

3.2.2. Plasma biochemistry

3.2.2.1. Model validation. Plasma dopamine-beta-hydroxylase (Fig. 9a):

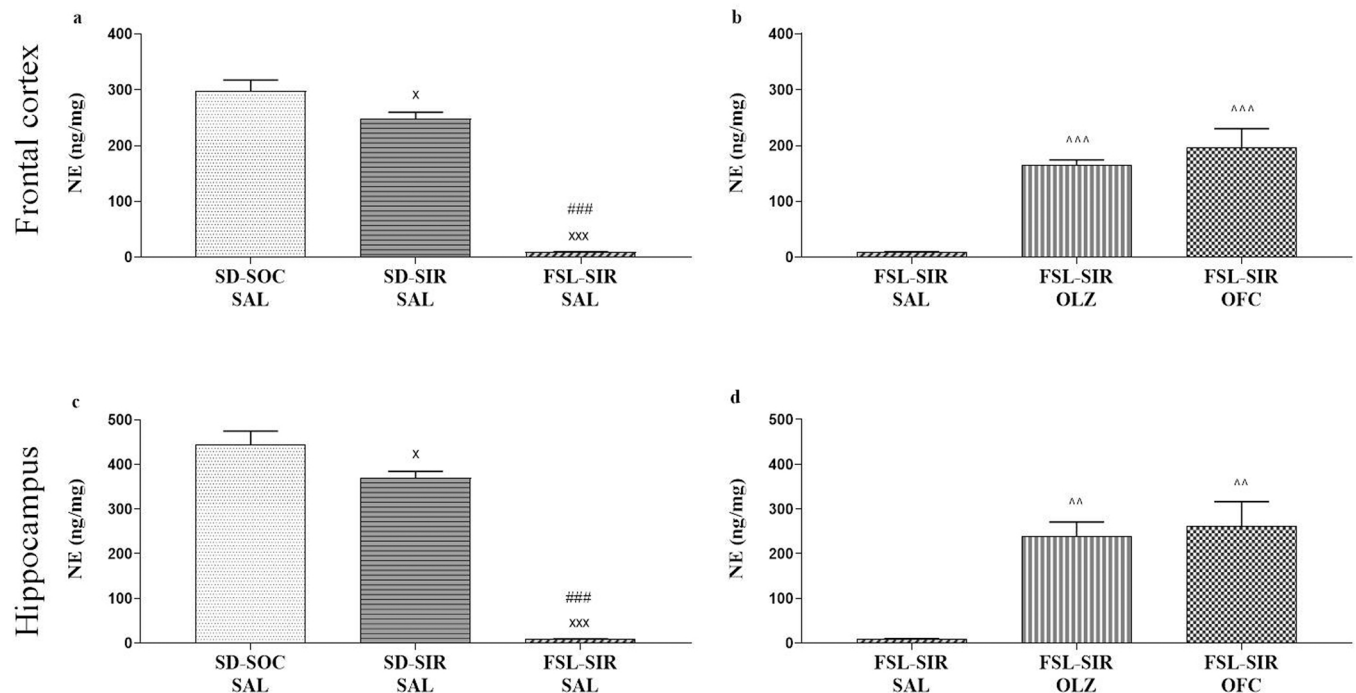


Fig. 6. Norepinephrine (NE) levels in the frontal cortex (a, b) and the hippocampus (c, d) of SAL-treated SD, SD-SIR and FSL-SIR rats. Fig. 6a: NE (frontal cortex, SAL-treated). xxxp < .0001, xp < .01 vs. SD-SOC, ###p < .0001 vs. SD-SIR (n = 12/group). Fig. 6b: NE (frontal cortex, drug-treated). ^^^p < .0001 vs. FSL-SIR-SAL (n = 12/group). Fig. 6c: NE (hippocampus, SAL-treated). xxxp < .0001, xp < .05 vs. SD-SOC, ###p < .0001 vs. SD-SIR-SAL (n = 11–12/group). Fig. 6d: NE (hippocampus, drug-treated). ^^p < .01 vs. FSL-SIR-SAL (n = 11–12/group). SD-SOC, socially reared Sprague-Dawley; SD-SIR, Sprague-Dawley exposed to social isolation rearing; FSL, Flinders Sensitive Line rats exposed to social isolation rearing; OFC=OLZ+FLX co-therapy.

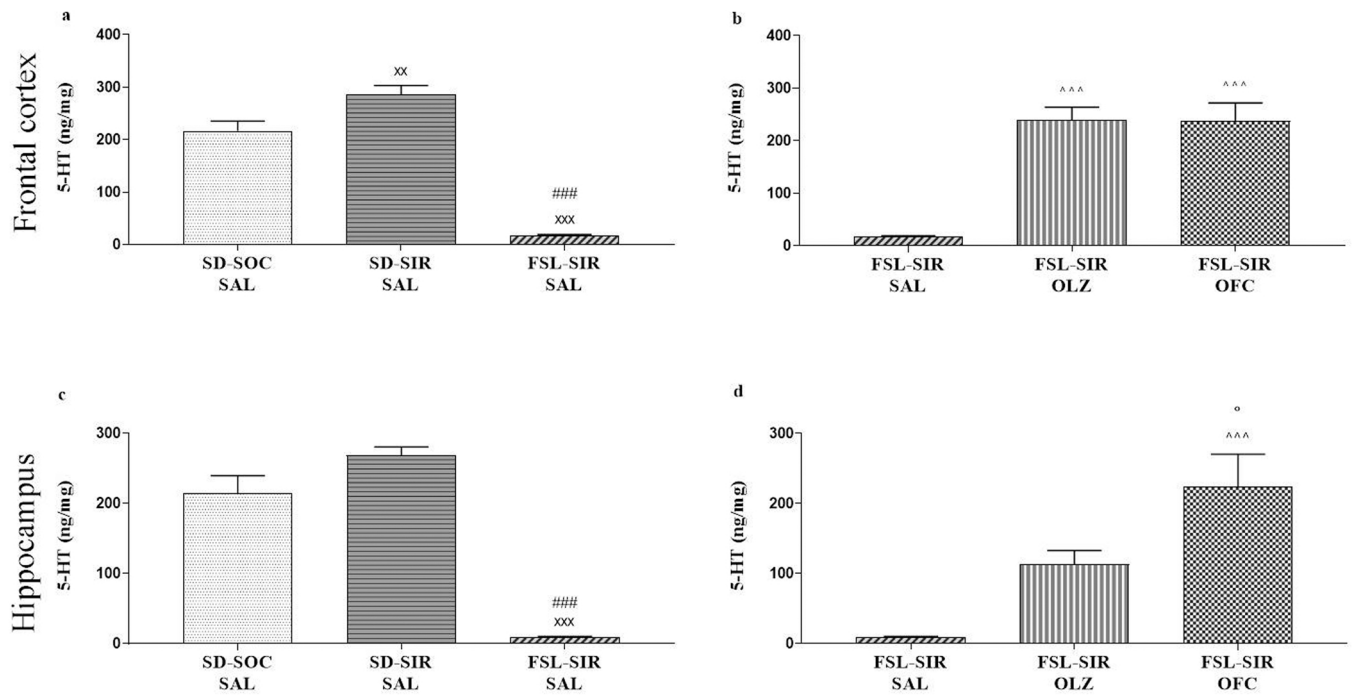


Fig. 7. Serotonin (5-HT) levels in the frontal cortex (a, b) and the hippocampus (c, d) of SAL-treated SD, SD-SIR and FSL-SIR rats. **Fig. 7a:** 5-HT (frontal cortex, SAL-treated). $xxx p < .0001$, $xx p < .01$ vs. SD-SOC, $### p < .0001$ vs. SD-SIR-SAL ($n = 12$ /group). **Fig. 7b:** 5-HT (frontal cortex, drug-treated). $^{\sim} p < .0001$ vs. FSL-SIR-SAL ($n = 11$ – 12 /group). **Fig. 7c:** 5-HT (hippocampus, SAL-treated). $xxx p < .0001$ vs. SD-SOC, $### p < .0001$ vs. SD-SIR-SAL ($n = 11$ – 12 /group). **Fig. 7d:** 5-HT (hippocampus, drug-treated). $^{\sim} p < .0001$ vs. FSL-SIR-SAL, $^{\circ} p < .05$ vs. FSL-SIR-OLZ (11–12/group). SD-SOC, socially reared Sprague-Dawley; SD-SIR, Sprague-Dawley exposed to social isolation rearing; FSL, Flinders Sensitive Line rats exposed to social isolation rearing; OFC=OLZ+FLX co-therapy.

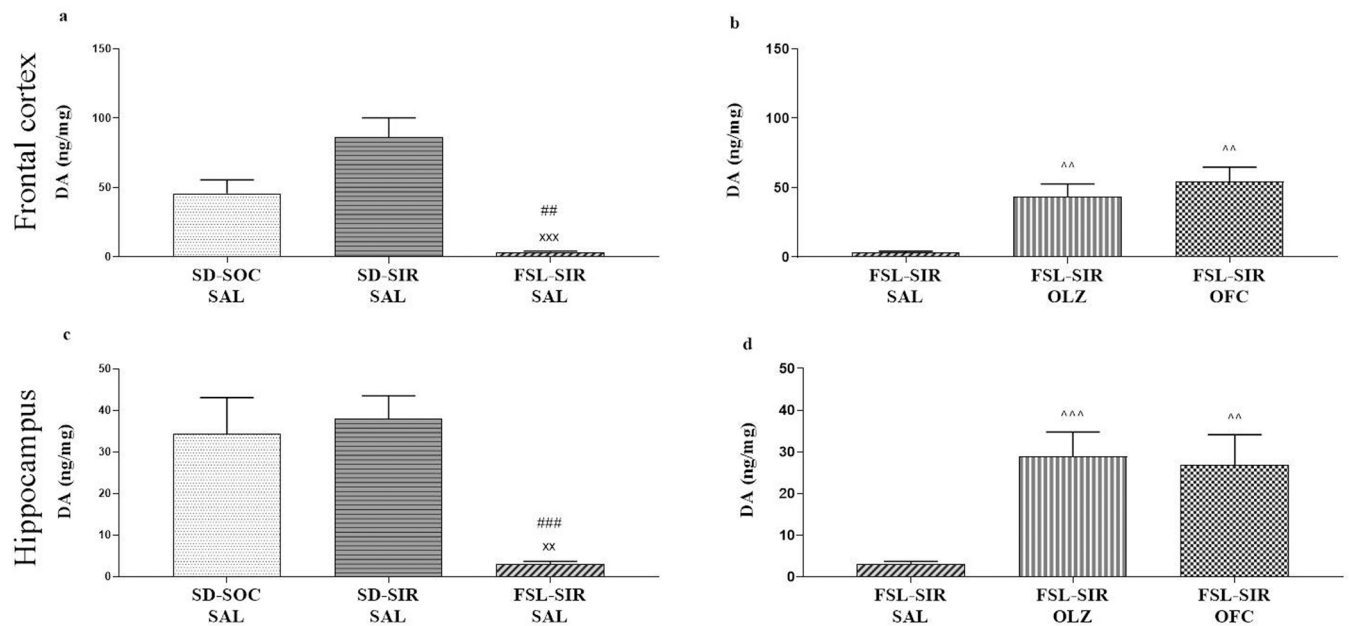


Fig. 8. Dopamine levels in the frontal cortex (a, b) and the hippocampus (c, d) of SAL-treated SD, SD-SIR and FSL-SIR rats. **Fig. 8a:** DA (frontal cortex, SAL-treated). $xxx p < .0001$ vs. SD-SOC, $## p < .01$ vs. SD-SIR ($n = 10$ – 11 /group). **Fig. 8b:** DA (frontal cortex, drug-treated). $^{\sim} p < .01$ vs. FSL-SIR-SAL ($n = 10$ /group). **Fig. 8c:** DA (hippocampus, SAL-treated). $xx p < .01$ vs. SD-SOC, $### p < .0001$ vs. FSL-SIR ($n = 10$ – 12 /group). **Fig. 8d:** DA (hippocampus, drug-treated). $^{\sim} p < .0001$, $^{\sim} p < .01$ vs. FSL-SIR ($n = 10$ – 12 /group). SD-SOC, socially reared Sprague-Dawley; SD-SIR, Sprague-Dawley exposed to social isolation rearing; FSL, Flinders Sensitive Line rats exposed to social isolation rearing; OFC=OLZ+FLX co-therapy.

Kruskal-Wallis ANOVA indicated a significant strain effect across all the groups (Kruskal-Wallis statistic = 9.557, $p = 0.0084$). *Post-hoc* analysis indicated significantly suppressed dopamine-beta-hydroxylase activity in FSL-SIR rats receiving SAL compared to SAL-treated SD-SIR rats

($p = 0.0107$, Cohen's $d = 1.79$). Dopamine-beta-hydroxylase suppression in FSL-SIR-SAL rats was significant at the 10% level ($p = 0.0558$) along with a large effect size (Cohen's $d = 1.13$) compared to SD-SOC-SAL rats.

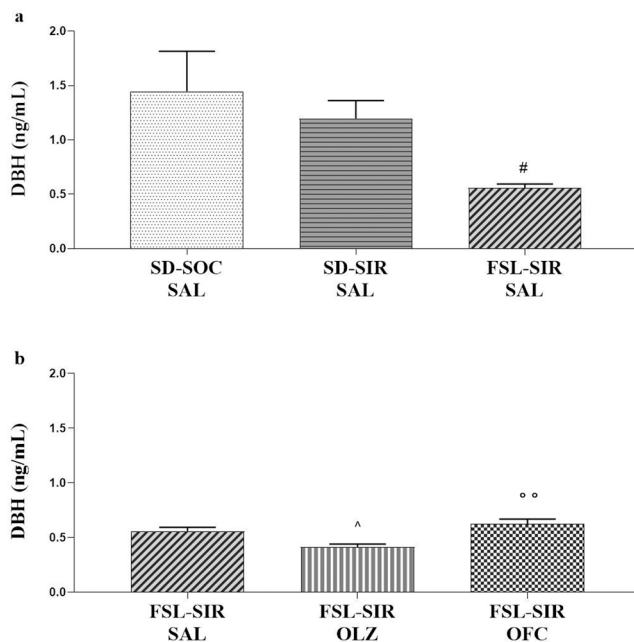


Fig. 9. Plasma dopamine beta hydroxylase measured in (a) SAL-treated SD-SOC, SD-SIR and FSL-SIR rats and in (b) drug-treated FSL-SIR rats as indicated. # $p < .05$ vs. SD-SIR, $\hat{p} < .05$ vs. FSL-SIR-SAL, $^{**}p < .05$ vs. FSL-SIR-OLZ ($n = 9-10$). SD-SOC, socially reared Sprague-Dawley; SD-SIR, Sprague-Dawley exposed to social isolation rearing; FSL, Flinders Sensitive Line rats exposed to social isolation rearing; OFC=OLZ+FLX co-therapy.

Plasma corticosterone (Fig. 10a): Kruskal-Wallis ANOVA indicated a significant strain effect across all groups (Kruskal-Wallis statistic = 6.165, $p = 0.0458$). *Post-hoc* analysis indicated a significant elevation in corticosterone in SD-SIR-SAL compared to SD-SOC-SAL rats ($p = 0.0412$, Cohen's $d = 1.07$).

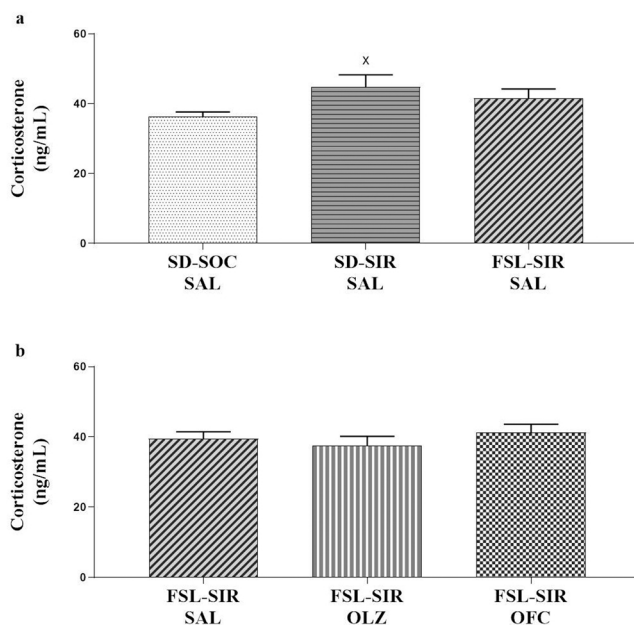


Fig. 10. Plasma corticosterone measured in (a) SAL-treated SD-SOC, SD-SIR and FSL-SIR rats and in (b) drug-treated FSL-SIR rats as indicated. CORT. $\hat{p} < .05$ vs. SD-SOC. SD-SOC, socially reared Sprague-Dawley; SD-SIR, Sprague-Dawley exposed to social isolation rearing; FSL, Flinders Sensitive Line rats exposed to social isolation rearing; OFC=OLZ+FLX co-therapy.

3.2.2.2. Treatment response. Plasma dopamine-beta-hydroxylase (Fig. 9b): Kruskal-Wallis ANOVA indicated a significant effect of treatment (Kruskal-Wallis statistic = 11.73, $p = .0028$). Olanzapine significantly (and further) reduced plasma dopamine-beta-hydroxylase in FSL-SIR rats compared to SAL-treatment ($p = .0328$, $d = 1.57$) and compared to OLZ+FLX-treatment ($p = .0032$, $d = 2.09$).

Plasma corticosterone (Fig. 10b): Kruskal-Wallis ANOVA did not note a significant effect of treatment across the groups in question (Kruskal-Wallis statistic = 1.048, $p = 0.5922$). Hence further post-hoc analysis was not done.

4. Discussion

Neither SD-SIR-SAL nor FSL-SIR-SAL rats displayed marked changes in locomotor activity, although FSL-SIR-SAL displayed significant anxiety versus SD-SOC-SAL rats (thigmotaxis) (Fig. 2a, c). While neither SD-SIR-SAL nor FSL-SIR-SAL displayed any social impairments (Fig. 3a, c, e) versus SD-SOC-SAL, both displayed significant depressive-like manifestations (Fig. 4a, c), as well as sensorimotor gating deficits (Fig. 5a). FSL-SIR-SAL rats displayed significantly increased climbing (Fig. 4e), not immediately associated with MD. SD-SIR-SAL and FSL-SIR-SAL rats had significantly reduced cortico-hippocampal NE (Fig. 6a, c), with 5-HT increased in SD-SIR-SAL but reduced in FSL-SIR-SAL rats (Fig. 7a, c), all versus SD-SOC-SAL. Only FSL-SIR-SAL rats displayed significantly reduced cortico-hippocampal DA levels (Fig. 8a, c), as well as reduced plasma dopamine-beta-hydroxylase (Fig. 9a). SD-SIR-SAL rats displayed a small increase in plasma corticosterone (Fig. 10a). These bio-behavioural data suggest an animal model with noteworthy parallels with MDpsy, which should now show predictive response to drug treatment.

Only OLZ+FLX significantly reduced locomotor activity in FSL-SIR rats versus SAL treatment (Fig. 2b), although both olanzapine and OLZ+FLX significantly reduced elevated anxiety in FSL-SIR rats (Fig. 2d). OLZ+FLX significantly reduced anti-social (aggressive) behaviour versus FSL-SIR-SAL (Fig. 3 f). As noted earlier with fluoxetine alone (Fischer et al., 2012), both treatments significantly worsened depressive-like behaviour (immobility, swimming) versus FSL-SIR-SAL, while OLZ+FLX significantly reduced climbing (Fig. 4b, d, f). Neither treatment reversed PPI deficits in FSL-SIR rats (Fig. 5b). Olanzapine and OLZ+FLX significantly reversed frontocortical NE (Figs. 6b) and 5-HT (Fig. 7b) deficits, while OLZ+FLX reversed hippocampal 5-HT deficits (Fig. 7d). Both treatments significantly reversed cortico-hippocampal DA deficits versus FSL-SIR-SAL rats (Fig. 8b, d), with olanzapine decreasing plasma dopamine-beta-hydroxylase (Fig. 9b). Neither treatment affected plasma corticosterone levels (Fig. 10b). Importantly OLZ+FLX reversed monoamine changes in FSL-SIR rats, worsened depression and failed to reverse PPI deficits. Treatment response is partially supportive of MDpsy, with a nod towards a bipolar diathesis. Deeper discussion follows.

Post-weaning SIR engenders psychosis- (Moller et al., 2011), depressive- (Fone and Porkess, 2008) and anxiety-like (Regenass et al., 2018) behaviours. That FSL-SIR rats presented with significant depressive (Fig. 4a, c) and psychosis-like (Fig. 5a) behaviour, as well as anxiety (Fig. 2c), is suggestive of an animal model with broad-ranging mood and psychosis-like manifestations. Childhood adversity (viz. SIR) as with MDpsy, may present with initial aberrations in NE and dopamine beta hydroxylase activity (Hamner and Gold, 1998), also described in FSL-SIR-SAL rats (Fig. 6a, d; Fig. 9a). Moreover, early life adversity is linked to late-life risk of developing bipolar disorder (Jääskeläinen et al., 2018; Post et al., 2012). We see that while SD-SIR-SAL rats have elevated cortical 5-HT, FSL-SIR-SAL rats show reduced cortico-hippocampal 5-HT (Fig. 7a, c), highlighting differences engendered by a combined gene-X-environment model. To this end, juvenile adversity reduces the density and function of post-synaptic 5-HT_{1A} receptors (Muchimapura et al., 2003; Kuramochi and Nakamura, 2009; Matsuzaki et al., 2011) and reduces presynaptic serotonergic function (Muchimapura et al.,

2003), ultimately affecting bio-behavioural responses (Andrews et al., 2015). This would explain why SD-SIR-SAL and FSL-SIR-SAL rats displayed reduced cortico-hippocampal DA (Fig. 8a, b) and abrogated sensory motor gating (Fig. 9a), congruent with schizophrenia or psychosis.

SIR increases (Moller et al., 2011), decreases or doesn't affect (Walker et al., 2019) locomotor activity in rats. SD-SIR and FSL-SIR rats did not exhibit hyperlocomotion (Fig. 2a). While olanzapine was ineffective, OLZ+FLX significantly reduced locomotor activity in FSL-SIR rats (Fig. 2b), perhaps anxiety related. Anxiety is a feature of schizophrenia, psychosis and MDpsy (Tandon et al., 2009; Ajob and Lacerda, 2018), and is also manifest in SIR rats (Walker et al., 2019). Importantly, FSL-SIR-SAL but not SD-SIR-SAL rats displayed significant anxiety (thigmotactic) behaviour (Fig. 2c), which again attests to a combined gene-X-environmental effect. This was fully reversed by olanzapine and OLZ+FLX (Fig. 2d), consistent with the anxiolytic properties of both olanzapine and fluoxetine (Sun et al., 2010; Willner and Belzung, 2015). FSL-SIR-SAL rats also display significantly increased climbing in the forced swim test (Fig. 4e), a noradrenaline-mediated escape behaviour akin to anxiety (Anyan and Amir, 2018) (see below). In fact, significantly reduced locomotor activity by OLZ+FLX (Fig. 2b) correlates with its reversal of increased climbing behaviour (Fig. 4 f), further supporting its anxiolytic effects (Sun et al., 2010).

SIR is linked to deficits in social interaction, social withdrawal, aggression and hyper-reactivity to novel environments (Rau et al., 2015; Forrest et al., 2014; Fone and Porkess, 2008). Here neither SD-SIR-SAL nor FSL-SIR-SAL rats exhibited notable changes (Fig. 3a, c, e). That said, a trend towards aggression was evident in FSL-SIR-SAL rats (Fig. 3e), congruent with SIR literature (Jones et al., 2011; Zamberletti et al., 2012) and that observed in psychosis, bipolar disorder and MDpsy (Østergaard et al., 2012; Baldessarini et al., 2019; Scaini et al., 2020). While olanzapine did not alter social anxiety in FSL-SIR rats, FLX+OLZ was anxiogenic (Fig. 2c). That chronic fluoxetine treatment is generally anxiolytic (Dulawa et al., 2004; Farhan and Haleem, 2016) may allude to an underlying neurobiological change in FSL-SIR rats. Translationally, psychosocial dysfunction may persist in MDpsy after mood/psychotic symptoms have resolved with treatment (Tyrka et al., 2006). Indeed, this finding correlates with 5-HT reuptake inhibitor induced anxiety, jitteriness and worsening depression in bipolar or depressed patients who have suffered early-life adversity (Coplan et al., 2014). Finally, where FSL-SIR rats tended towards greater aggression (Fig. 3e), OLX+FLX but not olanzapine significantly reduced said aggression in FSL-SIR rats (Fig. 3 f).

MDpsy presents with depressive symptoms similar to those of schizophrenia (Keller et al., 2007; Jääskeläinen et al., 2018). Congruent with literature (Hong et al., 2012), SD-SIR-SAL rats displayed significantly increased immobility (Fig. 4a) and reduced swimming (Fig. 4c), a serotonergic behaviour, with a trend to elevated climbing, a noradrenergic behaviour (Fig. 4e), compared to SD-SOC-SAL rats. The FSL-SIR-SAL configuration similarly engendered significant depressive-like behaviour but without an additive effect (Fig. 4a, c). Importantly though, FSL-SIR-SAL rats present with significantly increased climbing (Fig. 4e), suggesting bolstered noradrenergic responses, perhaps anxiety. This is of interest from a bipolar diathesis, as will be noted below.

Second-generation antipsychotics like olanzapine have antidepressant effects (Miura et al., 2020). Olanzapine and OLZ+FLX did not affect locomotor activity (Fig. 2a), thus not adversely affecting interpretation of swimming/climbing and mobility data in the forced swim test. However, olanzapine and OLZ+FLX significantly worsened depression (immobility) and coping (swimming) like behaviours in FSL-SIR versus FSL-SIR-SAL rats (Fig. 4b, d). Although OLZ+FLX is effective in MDpsy (Luan et al., 2017), our findings in FSL-SIR rats suggest a paradoxical depressogenic action. We have earlier shown that FSL-SIR rats are non-responsive to fluoxetine (Mncube et al., 2021), while the worsening of depressive-like symptoms following olanzapine treatment parallels

MDpsy (Rothschild, 1996). Also, FSL-SIR rats exhibited increased climbing reversed by OLZ+FLX, implying the presence of overt noradrenergic activity that is abrogated by standard treatment for MDpsy, i.e. OLZ+FLX. These findings emphasize a possible mood-psychosis continuum, or a bipolar diathesis, in the FSL-SIR model.

SD-SIR-SAL and FSL-SIR-SAL rats present with significant deficits in sensorimotor gating (%PPI; Fig. 5a), congruent with earlier studies (Moller et al., 2011; Veragten et al., 2020). However, where the FSL-SIR configuration exacerbated changes in anxiety, NE and 5-HT (Figs. 2c, 6a, c, 7a,c), PPI deficits did not apparently worsen. Guerrin and colleagues (Guerrin et al., 2021) show that first insults can prime/protect against later life adversity. FSL-SIR rats don't necessarily subscribe to a "dual hit" but rather a gene-X-environment model. Considering the stress sensitive nature of the FSL rat, post-weaning SIR may have tempered later behavioural responses. That said, although olanzapine (Bakshi et al., 1998) and fluoxetine (Yang et al., 2020) are able to reverse PPI deficits in rodent neurodevelopmental models, neither olanzapine nor OLZ+FLX reversed this deficit in FSL-SIR rats (Fig. 5b), emphasizing an intractable state of treatment resistance. However, non-response may be dose and duration of treatment dependent, warranting further study.

Cortico-hippocampal NE was significantly reduced in SD-SIR-SAL versus SD-SOC-SAL rats (Fig. 6a, c), congruent with earlier work (Trabace et al., 2012), and further reduced in FSL-SIR-SAL rats (Fig. 6a, c) versus SD-SOC-SAL and SD-SIR-SAL rats, suggesting an additive effect in FSL+SIR rats. Interestingly, olanzapine and OLZ+FLX significantly raised cortico-hippocampal NE in FSL-SIR rats (Fig. 6b, d), highlighting its therapeutic capabilities in MDpsy, consistent with literature (Zhang et al., 2000). An associated reduction in excessive climbing (noradrenergic) behaviour in the forced swim test in OLZ+FLX-treated FSL-SIR rats (Fig. 4 f) equal to that of SD-SOC-SAL concurs with this, while also hinting at its application in treating bipolar disorder.

Cortical 5-HT levels were significantly elevated in SD-SIR-SAL rats versus SD-SOC-SAL, typical of SIR (Han et al., 2011), but markedly lower in both regions in FSL-SIR-SAL versus SD-SOC-SAL and SD-SIR-SAL rats (Fig. 7a), again attesting to a gene-X-environment exacerbation. An increase in serotonergic behaviour (swimming) (Fig. 4c) despite decreases in cortico-hippocampal 5-HT (Fig. 7a,c) is noteworthy, although aversive stimuli (forced swimming) can attenuate 5-HT release in SIR rats (Walker et al., 2019). Deficits in cortical-hippocampal 5-HT in FSL-SIR-SAL rats (Fig. 7a,c) also correlates with anxiety (Leussis and Bolivar, 2006), i.e. increased thigmotaxis (Fig. 2c), and is consistent with the biogenic amine theory of MD and anxiety (Smolders et al., 2008). In line with earlier findings (Zhang et al., 2000), olanzapine and OLZ+FLX significantly reversed reduced cortical 5-HT in FSL-SIR rats (Fig. 7b), with OLZ+FLX significantly more effective than olanzapine in correcting hippocampal 5-HT deficits (Fig. 7d).

Cortico-hippocampal DA levels remained unchanged in SD-SIR-SAL versus SD-SOC-SAL rats (Fig. 8a, c), congruent with literature (Walker et al., 2019), but were markedly reduced in FSL-SIR-SAL versus both SD-SIR-SAL and SD-SOC-SAL rats (Fig. 8a, c). This not only concurs with fronto-cortical hypo-dopaminergia in schizophrenia (Weinstein et al., 2017) as well as the biogenic amine theory of MD (Villas Boas et al., 2019), but reaffirms the additive effect of genetic susceptibility plus early life adversity. The gene-X-environment effect is also evident in the reduction of plasma dopamine-beta-hydroxylase levels (Fig. 9a). This confirmation of DA deficiency in FSL-SIR rats is consistent with clinical findings in schizophrenia (Sternberg et al., 1982). With reduced plasma dopamine-beta-hydroxylase described in MDpsy (Schatzberg et al., 1985; Meyers et al., 1999), and the evident hypodopaminergia, these findings provide sound support for construct validity of the FSL-SIR model for both TRD and MDpsy. Importantly, olanzapine and OLZ+FLX significantly reversed DA deficits in both brain regions (Fig. 8b, d), in agreement with earlier work (Zhang et al., 2000), thus asserting the model's predictive validity. Moreover, reversing frontal cortical DA and NE dysfunction reinforces the venerated frontocortical actions of this treatment (Moller et al., 2015), confirming construct

validity. That said, olanzapine reducing dopamine-beta-hydroxylase in FSL-SIR rats and a lack of effect for OLZ+FLX (Fig. 9b) is counterintuitive, albeit concordant with literature (Meyers et al., 1999).

Finally, both schizophrenia (Cherian et al., 2019) and SIR (Mumtaz et al., 2018) present with elevated plasma cortisol/corticosterone. Indeed, SD-SIR-SAL rats demonstrated significantly increased corticosterone versus SD-SOC-SAL rats, an effect seemingly abrogated in FSL-SIR-SAL rats (Fig. 10a). This is incongruent with an over-stimulated

HPA-axis in MDpsy (Heslin and Young, 2018; Schatzberg et al., 1985; Tyrka et al., 2006). Nevertheless, this emphasises again how post weaning SIR may temper both behavioural and biological stress responses later in life. Finally, neither olanzapine nor OLZ+FLX had any effects on corticosterone levels in FSL-SIR rats (Fig. 10b), although may parallel the clinical differences between MDpsy and SCZ (Jeste et al., 1996).

With the exception of social behaviours, FSL-SIR rats show promising

Table 2

Summary of face, construct and predictive validity of the FSL-SIR model with respect to psychotic depression and psychotic bipolar disorder.

	Criteria	FSL-SIR	Congruent for MDpsy	FSL-SIR	Congruent for psychotic bipolar disorder
Face validity	Psychomotor agitation		♠ ■		♠ ■
	Anxiety		♠ ♦		♠ ♦
	Depression		♠ ♦		♠ ♦
	Social withdrawal		♠ ■		♠ ■
	Social anxiety		♠ ■		♠ ■
	Aggression		♠ ■		♠ ■
	Psychosis		♠ ♦		♠ ♦
Construct validity	NE		Elevated ■		Low in MDE ♦
	5-HT		Elevated ■		Low in MDE ♦
	DA		Elevated ■		Low in MDE ♦
	Dopamine beta-hydroxylase		Low ♦		Elevated ■
	CORT		Elevated ■		Elevated ♦
Predictive validity	Olanzapine (psychosis)		Poor response ♦		<i>NCE</i>
	OLZ+FLX (MD / psychosis)		Responsive ■		Sometimes ♦

Key: ♠ – present in the clinical disorder; ♦ – Congruent with the disorder; ■ - Incongruent with the disorder; – poor/weak validity; – moderate validity; – high/strong validity; *NCE* - no clinical evidence; MDE – major depressive episode. Indications of significance/trends, references, comparators are detailed in the text.

face, construct and predictive validity for MDpsy. The *apparent* lack of predictive validity regarding sensorimotor gating and depressive-like behaviours may be indicative of a need for more prolonged treatment, not unlike in MDpsy (Rothschild, 1996). Clinically, MDpsy presents with a diagnostic switch to bipolar disorder, with some patients developing bipolar disorder/psychotic bipolar disorder within years of diagnosis while others develop schizophrenia within a decade of MDpsy diagnosis (Jääskeläinen et al., 2018; Tohen et al., 2012). This complicates replication in an animal model. That said, worsening of depressive-like behaviour in FSL-SIR rats with olanzapine and OLZ+FLX provides an association with MD (Gournellis et al., 2018) or a bipolar diathesis (Perugi et al., 2019). Reduced plasma dopamine-beta-hydroxylase is observed in psychotic bipolar disorder, while the baseline monoaminergic profile of FSL-SIR rats mirrors the depressive phase of this disorder (Sigitova et al., 2017). The behavioural anomalies, especially worsening of mood congruent symptoms, the monoamine profile akin to bipolar disorder, and the generally good response to OLZ+FLX across most biological and behavioural parameters, suggests potential value as a preclinical model of bipolar disorder/psychotic bipolar disorder. Future work should explore approved treatments for bipolar disorder, viz. quetiapine, lurasidone, lithium, but also varying doses of olanzapine with fluoxetine. The face, predictive, and construct validity of the FSL-SIR model with respect to psychotic depression (MDpsy) and psychotic bipolar disorder are summarised in Table 2.

The practical implications and importance of this study lie in the further validation of the fluoxetine-resistant FSL-SIR rat, and that this study presents a useful *in vivo* preparation with which to undertake exploratory studies to better understand the biological basis and treatment of TRD and MDpsy. Limitations are that this study formed part of a larger more comprehensive study that could not be contained in a single manuscript due to it being too bulky and unwieldy. Hence two separate papers were prepared, one focusing on TRD (Mncube et al., 2021) and the current paper on MDpsy. To conserve animals, socially reared or socially isolated FSL rats were not assessed here, with the bio-behavioural sequelae of SIR in FSL rats and response to fluoxetine addressed in the earlier paper (Mncube et al., 2021). Although both studies were conducted at the same time, re-use of saline-treated SD and FSL-SIR control animals also needs to be mentioned. Nevertheless, together the two manuscripts provide new and important information regarding the validity of this animal model for TRD and MDpsy.

In conclusion, FSL-SIR rats present with depression and sensorimotor gating deficits, and reduced cortico-hippocampal NE, 5-HT, DA and dopamine-beta-hydroxylase. Anxiety, NE and 5-HT deficits are exacerbated in FSL-SIR rats. Except for dopamine-beta-hydroxylase, these were reversed by olanzapine and OLZ+FLX, with OLZ+FLX superior with regard to hippocampal NE and DA changes. However, while FSL-SIR rats show promising face and construct validity, that OLZ and OLZ+FLX worsened depressive-like behaviour and failed to reverse PPI deficits invites further study to confirm predictive validity for MDpsy.

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CRediT authorship contribution statement

Khulekani Mncube: Methodology, Validation, Formal analysis, Data collection, Investigation, Writing – original draft, Visualisation. **Brian Harvey:** Student supervision, Conceptualization, Resources, Data

curation, Writing – review & editing, Final submission and corresponding author, Visualisation, Project administration, Funding acquisition.

Conflict of Interest

With respect to this work, the authors declare that over the past three years, BHH has participated in advisory boards and received honoraria from Servier and Lundbeck, and has received research funding from Servier, Lundbeck, and HG&H Pharma. The authors declare that, except for income from the primary employer and research funding to BHH from the below mentioned organizations and agencies, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional services, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. KM has no conflicts of interest to declare.

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