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Assessment of the consequences of gestational SSRI antidepressant exposure in the rat

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A thesis submitted to the National University of Ireland Galway
for the degree of doctor of philosophy



Declaration

I hereby declare that the work presented in this thesis was carried out in accordance with the regulations of the National University of Ireland, Galway. The research is original and entirely my own with the following assistance:

- Chapter 3 oral gavage administration was assisted by Dr Hayley Doherty.
- Chapter 4 neonatal parameter recordings were assisted by Dr Silke Kleefeld, Ms Kelly McHugh, and Dr Hayley Doherty.
- Chapter 5 saccharin preference testing was assisted by Prof John Kelly.
- Chapter 6 psychopharmacological challenge dosing and behavioural testing were assisted by Prof John Kelly, Ms Kelly McHugh, Dr Hayley Doherty and Dr Zara McAleavey.
- Breeding and general husbandry activities required for such studies were generously assisted by Dr Silke Kleefeld, Ms Kelly McHugh, Dr Hayley Doherty, and Dr Zara McAleavey.

The thesis or any part thereof has not been submitted to the National University of Ireland, Galway, or any other institution in connection with any other academic award. Any views expressed herein are those of the author.

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Abstract

Although the most common psychotropic drugs prescribed in pregnancy are selective serotonin reuptake inhibitor (SSRI) antidepressants, their health impact on the resultant offspring is largely unknown due to the ethical challenges of assessment clinically. Fluoxetine, paroxetine, sertraline, and citalopram are members of the SSRI class, which alter serotonin, a key neurotransmitter dysregulated in many psychiatric disorders and a fundamental guide for embryogenesis and the formations of brain structure and function. As all SSRIs cross the placental barrier, there is the potential for them to affect the developing foetus. The incidence of perinatal depression has increased, and pharmacological intervention is deemed necessary to reduce the risks of miscarriage and harm to the pregnant woman that may manifest if the depression is untreated. Current preclinical models are lacking as many are focused on fluoxetine, however, recent clinical birth-outcome data suggests that different teratogenic risks are dependent on the particular SSRI exposed. The current work aims to individually evaluate gestational exposure in Sprague-Dawley rats from gestational day (GD) 7-21 via oral gavage to a range of doses (1.25, 2.5, 5, or 10 mg/kg depending on the SSRI) of the four aforementioned SSRIs in a longitudinal study; examining parameters of maternal wellbeing as well as male and female progeny measurements of neonatal development, behaviour in adolescence and adulthood, and behavioural response to anxiolytic and acute antidepressant drug treatment in adulthood. Fluoxetine, paroxetine, and sertraline caused profound effects on maternal wellbeing and neonatal mortality. Neonatal advances, in regards to somatic and behavioural development, were induced by fluoxetine and sertraline exposure, with fluoxetine increasing male ano-genital distance and sertraline promoting successful forelimb grip behaviours in males when compared to respective controls. Most notably, citalopram induced no effects on the parameters measured; further, no enduring consequences were found in adulthood on the specific parameters assessed after any of the SSRI exposures. Additionally, paroxetine treatment accentuated existing differences in sex by increasing male and decreasing female ambulation during adolescence. Furthermore, this model confirms a novel automated approach to assessing behavioural despair after antidepressant treatment and highlights that traditional behavioural models developed for male rodents may not be adequate in measuring female behaviour. Overall, this research highlights factors of offspring sex and age and more importantly emphasizes

the differences in dose-dependent findings attributed to distinct SSRIs. Interestingly, while citalopram had no significant effects on maternal wellbeing or offspring development, this drug was also tolerated at a higher dose range than fluoxetine or paroxetine. Moreover, the work also provides a model for assessing perinatal exposure to psychotropic drugs which acknowledges important maternal and offspring parameters in a manner that is transferable to the clinical scenario. Thus, the current results applied to the clinical scenario could inform physicians in prescribing SSRIs to treatment-naïve pregnant women which commonly face depression during pregnancy, presenting citalopram as the best treatment option of the four SSRIs for both the mothers and progeny, based on the parameters assessed here. Lastly, this longitudinal study has the potential to inform physicians of critical periods of risk for the individuals exposed to the SSRIs, thus anticipating that additional care may be required during these early life periods, while also concluding that many offspring behaviours are normalized by adulthood.

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“Science shines forth in all its value as a good capable of motivating our existence, as a great experience of freedom for truth, as a fundamental work of service. Through research each scientist grows as a human being and helps others to do likewise.”

St. John Paul II

List of Publications and Conference Proceedings

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Table of Contents

Declaration	i
Abstract	ii
Acknowledgements	iv
List of Publications and Conference Proceedings	vi
Table of Contents	ix
List of Figures	xiii
List of Tables	xv
List of Abbreviations	xviii
1 Introduction	1
1.1 Serotonin	2
1.1.1 The role of serotonin in the developing brain	3
1.1.2 Serotonergic dysfunction and resulting disorders and treatments.....	7
1.1.3 Altering brain development in rats and humans	8
1.2 Depression	11
1.2.1 Manifestation of depression in pregnant women	12
1.2.2 Impact of depression on pregnancy.....	16
1.3 Selective serotonin reuptake inhibitors	19
1.3.1 Investigations of depression and antipsychotics leading to SSRI discovery	19
1.3.2 Mechanism of action of SSRIs.....	21
1.3.3 Praise and criticism of SSRIs.....	24
1.4 Antidepressant exposure in pregnancy- a treatment dilemma	27
1.5 SSRI antidepressant profiles	31
1.5.1 Fluoxetine.....	31
1.5.2 Paroxetine.....	31
1.5.3 Sertraline	32
1.5.4 Citalopram.....	33
1.6 Clinical outcomes of gestational SSRI exposure	35
1.6.1 Limitations of current clinical studies.....	36
1.7 Preclinical outcomes of gestational SSRI exposure	39
1.7.1 Limitations of current preclinical studies.....	57
1.8 Rat model for gestational psychotropic drug exposure	59
1.8.1 Dose selection	60
1.8.2 Route of administration.....	60

1.8.3	Duration	62
1.8.4	Clinical defects modelled in animal corollaries.....	62
1.9	Research objectives	71
1.9.1	Hypothesis and aims of the present research	71
2	Materials and Methods.....	73
2.1	Materials	74
2.1.1	Animal husbandry.....	74
2.1.2	Treatments and administration.....	74
2.1.3	Behavioural equipment	75
2.1.4	Computer software.....	75
2.2	Methods.....	76
2.2.1	Animals.....	76
2.2.2	Maternal behaviour observations	81
2.2.3	Pup somatic development observations	82
2.2.4	Pup behavioural development observations.....	84
2.2.5	Behavioural testing	88
2.2.6	Data Analysis	99
3	Consequences of gestational exposure to the SSRIs fluoxetine, paroxetine, sertraline, or citalopram in the rat: maternal wellbeing and littering characteristics.	100
3.1	Introduction	101
3.1.1	Hypothesis and aims	104
3.2	Experimental methods and design.....	105
3.3	Maternal and littering results	107
3.3.1	Maternal wellbeing: Gestational body weight, food and water consumption.....	107
3.3.2	Maternal wellbeing: Postpartum body weight, food and water consumption.....	112
3.3.3	Maternal litter characteristics: Litter size and gestational length	113
3.3.4	Maternal litter characteristics: Maternal caregiving behaviours.....	114
3.3.5	Pup litter characteristics: Sex ratio, still born and total dead.....	117
3.3.6	Pup litter characteristics: Pup birth weights.....	118
3.4	Discussion.....	120
3.4.1	Aim 1: Maternal wellbeing	120
3.4.2	Aim 2: Maternal litter characteristics	122

3.4.3	Aim 3: Pup litter characteristics	125
3.4.4	Conclusion	128
4	Somatic and behavioural neonatal development of rat progeny exposed to SSRI antidepressants <i>in utero</i>.	130
4.1	Introduction	131
4.1.1	Hypothesis and aims	133
4.2	Experimental methods and design	134
4.3	Postnatal development results	137
4.3.1	Somatic development: Fur appearance and pinna unfolding	137
4.3.2	Somatic development: Eye opening, ano-genital distance, length.....	141
4.3.3	Somatic development: Daily body weight, total body weight gain ...	149
4.3.4	Behavioural development: Surface righting, negative geotaxis, forelimb grip	153
4.4	Discussion	161
4.4.1	Aim 1: Somatic development.....	161
4.4.2	Aim 2: Behavioural development	165
4.4.3	Conclusion	169
5	The effects of gestational exposure to SSRI antidepressants on anxiety, ambulatory, cognitive, and anhedonia behaviours in resultant adolescent and adult rat offspring.	171
5.1	Introduction	172
5.1.1	Hypothesis and aims	175
5.2	Experimental methods and design	176
5.3	Adolescence and adulthood results	178
5.3.1	Anxiety and motor assessment in the elevated plus maze and open field	178
5.3.2	Cognitive assessment in the novel object recognition test.....	202
5.3.3	Anhedonia assessment in the saccharin preference test.....	205
5.4	Discussion	207
5.4.1	Aim 1: Anxiety and ambulatory assessment.....	207
5.4.2	Aim 2: Cognitive assessment.....	212
5.4.3	Aim 3: Anhedonia assessment	216
5.4.4	Conclusion	218

6	Does gestational exposure to SSRIs in rats have an effect on anxiolytic and antidepressant behavioural responses in the resultant offspring?	219
6.1	Introduction	220
6.1.1	Hypothesis and aims	221
6.2	Experimental methods and design.....	223
6.3	Pharmacological challenge results.....	225
6.3.1	Pharmacological assessment of diazepam in the elevated plus maze	225
6.3.2	Pharmacological assessment of desipramine in the forced swim test	233
6.4	Discussion.....	249
6.4.1	Aim 1: Anxiolytic response	249
6.4.2	Aim 2: Antidepressant response	253
6.4.3	Conclusion	257
7	Discussion	258
7.1	Discussion.....	259
7.1.1	Conclusions.....	268
7.2	Limitations and future recommendations.....	269
7.2.1	Neonatal mortality and maternal caregiving.....	269
7.2.2	Ano-genital distance, sexual and aggressive behaviours.....	270
7.2.3	Additional behavioural tasks.....	271
7.2.4	Challenges to the psychopharmacological assessment	271
7.2.5	Selection of SSRIs and dose	272
7.2.6	Perinatal exposure and pharmacological profile.....	273
8	Bibliography	274
9	Appendices.....	295
9.1	Preliminary fluoxetine findings.....	296
9.2	Preliminary paroxetine findings	297

List of Figures

Figure 1.1 Serotonin synthesis, action, and receptors at synapse.	3
Figure 1.2 Development of 5-HT neurons in the rodent brain.....	5
Figure 1.3 Rat and human brain development timeline comparison.	9
Figure 1.4 Comparison between females and males for years lived with disability..	13
Figure 1.5 Edinburgh Postnatal Depression Scale.	15
Figure 1.6 Antidepressant prescriptions for Ireland, 2013.....	21
Figure 1.7 SSRI mechanism of action.....	23
Figure 1.8 SSRI exposure and clinical outcomes.....	36
Figure 1.9 Allometric scale dose conversion formula.	60
Figure 2.1 Oral gavage administration.....	78
Figure 2.2 PND 1 pup and tattooed pup paw.	80
Figure 2.3 Maternal caregiving behaviour.	82
Figure 2.4 Ano-genital distance and body length measurements.	84
Figure 2.5 Surface righting.	85
Figure 2.6 Negative geotaxis.....	87
Figure 2.7 Forelimb grip.	88
Figure 2.8 Elevated plus maze.	91
Figure 2.9 Open field.	93
Figure 2.10 Novel object recognition phases.....	95
Figure 2.11 Forced swim apparatus.	97
Figure 2.12 Saccharin preference test.	99
Figure 3.1 Maternal and littering measurements experimental timeline.....	105
Figure 3.2 Fluoxetine gestational weights.	108
Figure 3.3 Paroxetine gestational weights.	109
Figure 3.4 Sertraline gestational weights.	110
Figure 3.5 Citalopram gestational weights.....	111
Figure 4.1 Neonatal development experimental timeline.	135
Figure 4.2 Fluoxetine somatic development.	142
Figure 4.3 Paroxetine somatic development.	144
Figure 4.4 Sertraline somatic development.....	146
Figure 4.5 Citalopram somatic development.	148
Figure 4.6 Fluoxetine neonatal body weights.	149

Figure 4.7 Paroxetine neonatal body weights.	150
Figure 4.8 Sertraline neonatal body weights.	151
Figure 4.9 Citalopram neonatal body weights.	152
Figure 4.10 Fluoxetine behavioural development.	154
Figure 4.11 Paroxetine behavioural development.	156
Figure 4.12 Sertraline behavioural development.	158
Figure 4.13 Citalopram behavioural development.	160
Figure 5.1 Adolescence and adulthood behaviour experimental timeline.	177
Figure 5.2 NOR after gestational SSRI exposure.	203
Figure 5.3 NOR discrimination ratio.	204
Figure 5.4 SPT after gestational SSRI exposure.	206
Figure 6.1 Psychopharmacological challenge experimental timeline.	224
Figure 6.2 Fluoxetine males in the FST with SAL or DMI.	234
Figure 6.3 Fluoxetine females in the FST with SAL or DMI.	236
Figure 6.4 Paroxetine males in the FST with SAL or DMI.	238
Figure 6.5 Paroxetine females in the FST with SAL or DMI.	240
Figure 6.6 Sertraline males in the FST with SAL or DMI.	242
Figure 6.7 Sertraline females in the FST with SAL or DMI.	244
Figure 6.8 Citalopram males in the FST with SAL or DMI.	246
Figure 6.9 Citalopram females in the FST with SAL or DMI.	248

List of Tables

Table 1.1 Development 5-HT neurons in the human brain.....	4
Table 1.2 Serotonin receptor binding and activations associated with depression.	8
Table 1.3 DSM-V major depressive episode diagnostic criteria.....	12
Table 1.4 Comparison of the various antidepressant classes.	25
Table 1.5 USA FDA drug use in pregnancy ratings prior to 2015.	28
Table 1.6 Summary table comparing SSRI pharmacological profiles.	34
Table 1.7 Teratogenic effects of gestational exposure to fluoxetine.	44
Table 1.8 Teratogenic effects of gestational and lactational exposure to fluoxetine.	45
Table 1.9 Adolescent effects of gestational exposure to fluoxetine.....	46
Table 1.10 Adolescent effects of gestational and lactational exposure to fluoxetine.....	47
Table 1.11 Adult effects of gestational exposure to fluoxetine.	48
Table 1.12 Adult effects of gestational and lactational exposure to fluoxetine.	50
Table 1.13 Teratogenic effect of gestational exposure to paroxetine, sertraline, or citalopram.....	52
Table 1.14 Teratogenic effects of gestational and lactational exposure to paroxetine, sertraline, or citalopram.	53
Table 1.15 Adolescent effects of gestational exposure to paroxetine, sertraline, or citalopram.....	54
Table 1.16 Adolescent effects of gestational and lactational exposure to paroxetine, sertraline, or citalopram.	54
Table 1.17 Adult effects of gestational exposure to paroxetine, sertraline, or citalopram.....	55
Table 1.18 Adult effects of gestational and lactational exposure to paroxetine, sertraline or citalopram.	56
Table 1.19 Translation of human to animal doses using allometric scale.	60
Table 1.20 Clinical defects and animal corollaries.	63
Table 2.1 Pinnae unfolding and eye opening.	83
Table 3.1 Statistical analysis of maternal and littering results.....	106
Table 3.2 Postpartum body weight, food and water consumption.....	112
Table 3.3 Litter size and gestational length.	113
Table 3.4 Fluoxetine maternal caregiving behaviour.....	115
Table 3.5 Paroxetine maternal caregiving behaviour.....	115

Table 3.6 Sertraline maternal caregiving behaviour.	116
Table 3.7 Citalopram maternal caregiving behaviour.....	116
Table 3.8 Sex ratio, still born, and total dead.	117
Table 3.9 Pup birth weights	119
Table 4.1 Statistical analysis of neonatal development.	136
Table 4.2 Fluoxetine fur appearance and pinna unfolding.	137
Table 4.3 Paroxetine fur appearance and pinna unfolding.	138
Table 4.4 Sertraline fur appearance and pinna unfolding.	139
Table 4.5 Citalopram fur appearance and pinna unfolding.....	140
Table 5.1 Statistical analysis of adolescent and adulthood behaviour.....	177
Table 5.2 Fluoxetine EPM PND 28.	178
Table 5.3 Fluoxetine EPM PND 56.	179
Table 5.4 Fluoxetine EPM PND 84.	180
Table 5.5 Paroxetine EPM PND 28.	181
Table 5.6 Paroxetine EPM PND 56.	182
Table 5.7 Paroxetine EPM PND 84.	183
Table 5.8 Sertraline EPM PND 28.....	184
Table 5.9 Sertraline EPM PND 56.....	185
Table 5.10 Sertraline EPM PND 84.....	186
Table 5.11 Citalopram EPM PND 28.	187
Table 5.12 Citalopram EPM PND 56.	188
Table 5.13 Citalopram EPM PND 84.	189
Table 5.14 Fluoxetine OF PND 28.	190
Table 5.15 Fluoxetine OF PND 56.	191
Table 5.16 Fluoxetine OF PND 84.	192
Table 5.17 Paroxetine OF PND 28.	193
Table 5.18 Paroxetine OF PND 56.	194
Table 5.19 Paroxetine OF PND 84.	195
Table 5.20 Sertraline OF PND 28.....	196
Table 5.21 Sertraline OF PND 56.....	197
Table 5.22 Sertraline OF PND 84.....	198
Table 5.23 Citalopram OF PND 28.	199
Table 5.24 Citalopram OF PND 56.	200
Table 5.25 Citalopram OF PND 84.	201

Table 6.1 Statistical analysis of psychopharmacological challenge.	224
Table 6.2 Fluoxetine males in the EPM with SAL or DZP.	225
Table 6.3 Fluoxetine females in the EPM with SAL or DZP.	226
Table 6.4 Paroxetine males in the EPM with SAL or DZP.	227
Table 6.5 Paroxetine females in the EPM with SAL or DZP.	228
Table 6.6 Sertraline males in the EPM with SAL or DZP.	229
Table 6.7 Sertraline females in the EPM with SAL or DZP.	230
Table 6.8 Citalopram males in the EPM with SAL or DZP.	231
Table 6.9 Citalopram females in the EPM with SAL or DZP.	232
Table 7.1 Summary table of SSRI-induced effects.	262
Table 7.2 Max dose employed resulting in no deleterious effect.	268

List of Abbreviations

5-HT; serotonin

ACOG; American College of Obstetrician and Gynecologists

ACREC; Animal Care and Research Ethics Committee

ANOVA; analysis of variance

BZE; between zone entries

CIT; citalopram

CNS; central nervous system

d; day

dH₂O; distilled water

DMI; desipramine

DSM; Diagnostic and Statistical Manual of Mental Disorders

DVR; digital video recorder

DZP; diazepam

E; embryonic

EPDS; Edinburgh Perinatal Depression Scale

EPM; elevated plus maze

F; female

FDA; United States Food and Drug Administration

FLX; fluoxetine

FST; forced swim test

GD; gestation day

h; hour

HCl; hydrochloride

HPG; hypothalamus pituitary gonadal

HSE; Health Services Executive

IG; intragastric

IP; intraperitoneal

IQ; intelligence quotient

IZT; inner zone time

M; male

MAOI; Monoamine oxidase inhibitor

MDD; Major Depressive Disorder
NAMI; National Alliance on Mental Illness
NICE; United Kingdom National Institute for Health and Care Excellence
NOR; novel object recognition
OAE; open arm entry
OAT; open arm time
OF; open field
OGTT; oral glucose tolerance test
OMP; osmotic minipump
PND; postnatal day
PO; per os
P-pg; Phosphoglycoprotein
PPHN; persistent pulmonary hypertension of the new born
PRX; paroxetine
s; second
SAL; saline
SC; subcutaneous
SD rats; Sprague-Dawley rats
SD; standard deviation
SERT; sertraline
SNK; Student-Newman-Keuls
SPT; saccharin preference test
SSRI; selective serotonin reuptake inhibitor
TCA; Tricyclic antidepressant
UK; United Kingdom
USA; United States of America
USPSTF; United States Preventive Services Task Force
VEH; vehicle
YLD; years lived with disability

Most abbreviations, other than commonly used expressions, are also defined at the first point of occurrence in the text.

1 Introduction

1.1 Serotonin

The neurochemical serotonin network provides the basic framework of the mammalian brain, therefore it is important to consider the impact of altered synaptic availability of serotonin during embryogenesis (Jacobs and Azmitia, 1992). Serotonin is an important neurotransmitter most commonly found in the GI tract, blood platelets, and central nervous system (CNS) (Cryan and Lucki, 2000). As illustrated in Figure 1.1, serotonin synthesis begins with tryptophan, which is converted via tryptophan hydroxylase 2 to 5-hydroxytryptophan. Then it is converted by aromatic L-amino acid decarboxylase, finally resulting in 5-hydroxytryptamine (5-HT, serotonin) (Koeppen and Stanton, 2018). It is synthesised in neurons in the raphe nuclei and stored in presynaptic vesicles (Jacobs and Azmitia, 1992). Presynaptic serotonergic neurons originate in the brainstem dorsal and median raphe nuclei, while their axons branch out in high densities to nearly all areas of the brain (Beaudet and Descarries, 1976). When an action potential occurs, serotonin is released into the synaptic cleft and binds to a serotonin receptor, listed in Table 1.2, on the postsynaptic cell and the appropriate outcome is activated (Glover and Clinton, 2016). At termination, serotonin is recycled via the serotonin reuptake transporter and either stored for rerelease or broken down by monoamine oxidase (Koeppen and Stanton, 2018). Alterations in serotonergic function have been widely studied as a key factor in developmental disorders as well as in mental illness since the 1950s (Woolley and Shaw, 1954).

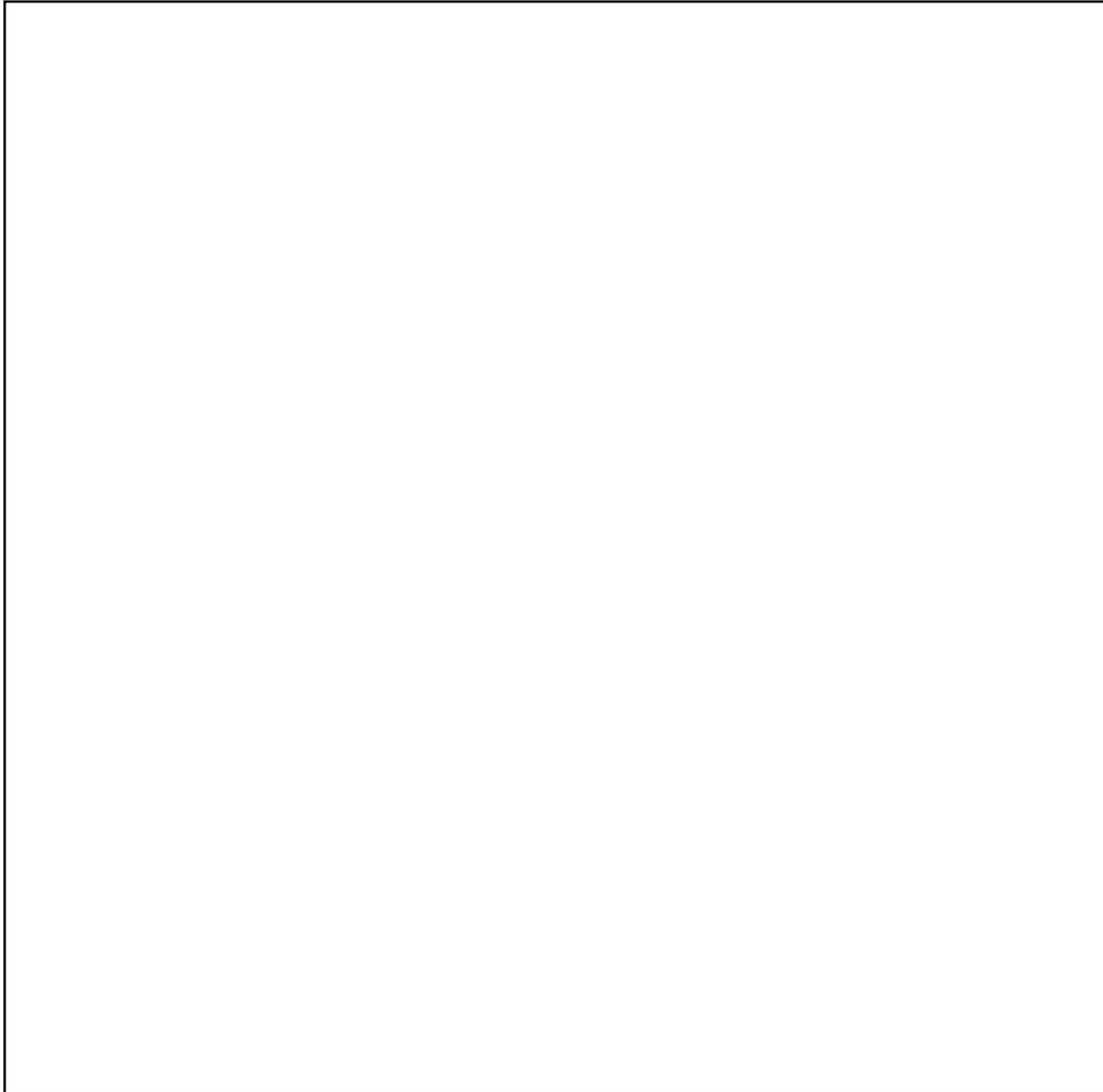


Figure 1.1 Serotonin synthesis, action, and receptors at synapse. In the presynaptic terminal, serotonin is synthesized from tryptophan and stored in vesicles before being released into the synaptic cleft via an action potential. 5-HT binds to one of its respective receptors (ligand-gated or G-protein coupled receptor (G-PCR)) to induce serotonergic activity in the postsynaptic cell or it will bind on the presynaptic cell to regulate the presynaptic activity. When serotonergic action is terminated, 5-HT is taken back up through the 5-HT reuptake transporter (5-HTRT) and is either catabolised by monoamine oxidase (MAO) or returned to the vesicles to be reused for subsequent serotonergic activation. 5-HT₃=ligand gated; 5-HT₁ & 5-HT₂=Gi/Go-PCR (↓ cyclic AMP); 5-HT₂=Gq/G11-PCR (↑ inositol trisphosphate and diacylglycerol); 5-HT₄, 5-HT₅, & 5-HT₆=Gs-PCR (↑ cyclic AMP); TPH₂=tryptophan hydroxylase 2; AADC=aromatic L-amino acid decarboxylase. Adapted from Glover and Clinton (2016).

1.1.1 The role of serotonin in the developing brain

Clinically, serotonin acts as a growth factor during embryogenesis with various cascading events that form brain structure and guide the development of other

neurotransmitter systems (Whitaker-Azmitia, 2001). During human embryogenesis, 5-HT is present as early as gestation week 5 and continues to increase throughout development until 5 years (Table 1.1.) The high turnover rate of serotonin in the immature brain suggests it is a key factor in the development process. Thus it is important to consider the possible teratogenic effects as not only do serotonergic altering agents disrupt typical activity, but exposure to such compounds disturbs the synchrony of the overall embryonic development process resulting in various delivery outcomes.

Stage	Development of serotonergic neurons
Gestation week 5	Evidence of 5-HT neurons
Gestation week 10	Rapid ↑ of 5-HT neurons
Gestation week 15	Organization of 5-HT cell bodies at raphe nuclei
2 years	Slow and steady ↑ 5-HT
5 years	5-HT ↓ through adulthood

Table 1.1 Development 5-HT neurons in the human brain. Timeline illustration of 5-HT emphasizes its importance in foetal development. Stages and developmental observations adapted from Whitaker-Azmitia (2001).

The early presence of serotonin is also witnessed preclinically in the rodent brain. Serotonergic neurons have been observed in the hindbrain (Lidov and Molliver, 1982), and serotonin increases neurite outgrowth in embryo thalamic neurons (Lotto et al., 1999), thus supporting a role in guiding early foetal brain development (Vitalis et al., 2013). The dorsal raphe is considered the main serotonergic nucleus, and in embryonic rodent brains, these axons differentiate and are projected through the forebrain as soon as embryonic day 10.5 and reach the neocortex and other rostral regions by embryonic day 14.5, highlighting the ontogenic role of 5-HT (Lidov and Molliver, 1982).

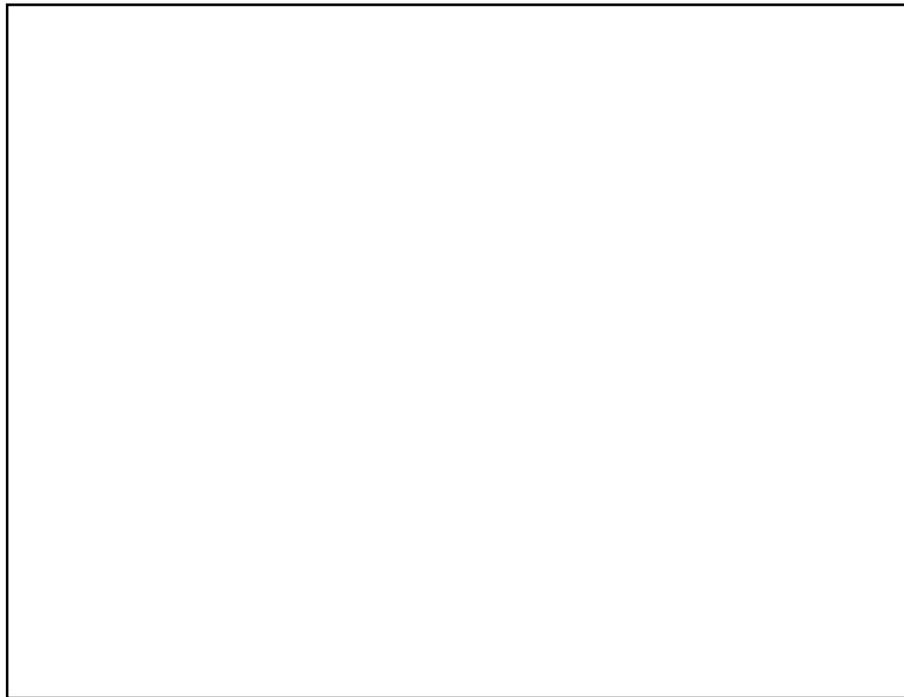


Figure 1.2 Development of 5-HT neurons in the rodent brain. Sagittal illustration of the 5-HT cell groups (B1-9) and main projections descending to the periphery (yellow) and ascending to cortical regions (purple) as early as (A) gestational day (GD) 11 and (B) GD 14 until reaching the (C) mature stage. is=isthmus; mlf=medial longitudinal fasciculus; hyp=hypothalamus; fr=fasciculus retroflexus; dt=dorsal thalamus; vt=ventral thalamus; se=septum; cx=cerebral cortex; cer=cerebellum; hip=hippocampus; th=thalamus; amg=amygdala; mfb=median forebrain bundle; st=striatum; acb=accumbens; ob=olfactory bulb. Image adapted from Vitalis et al. (2013) and Wallace and Lauder (1983).

Originally it was believed that serotonin detected in early gestation was absorbed by the placenta from the maternal blood and transferred to the foetal blood, as the serotonergic raphe neurons were not functional then (Yavarone et al., 1993a). It is now understood that the placenta is essential for producing serotonin, which is accumulated in the forebrain early in telencephalic development (Bonnin et al., 2011). This source of serotonin is vital for periods including cortical neurogenesis, migration, and initial axon targeting (Gaspar et al., 2003). The role of serotonin as a guide to axon development is further confirmed through studies illustrating that interruptions of serotonin availability can be correlated to atypical thalamocortical axon trajectories (Xu et al., 2004, Bonnin et al., 2011). Interruptions in serotonin were accomplished via the administration of the SSRI antidepressant paroxetine (Xu et al., 2004), or via knockout mice, removing the serotonin raphe transcription factor, PET1 (Bonnin et al., 2011).

As the typical serotonin system progresses, the serotonin raphe neurons are appropriated by PET1. Through this process, serotonergic neurons develop the appropriate equipment to produce and absorb serotonin (Kiyasova and Gaspar, 2011). Within the final stage of embryonic development, the foetal source of serotonin becomes independent from the placenta and the primary source of serotonin becomes the raphe neurons (Bonnin and Levitt, 2011). Moreover, altering serotonin during gestation can have persistent outcomes throughout adulthood. Depleting levels of 5-HT through the treatment of PCPA leads to an enduring reduction in dendritic maturation (Vitalis et al., 2007) and a decrease in hippocampal and cortical neurons in the resulting adult (Whitaker-Azmitia, 2001).

1.1.1.1 Pregnancy implications of altered gestational levels of serotonin

Additional effects associated with a disruption of serotonin availability throughout gestation can induce lower birth weights through the direct influence of serotonin on the uterus. The 5-HT_{2B} receptor in the uterine smooth muscle cells initiates smooth muscle contraction (Kelly and Sharif, 2006). Overall disruption of serotonin can alter uterine blood flow affecting the uterine rings which in turn can alter energy availability to the foetus resulting in lower birth weights (Wessler et al., 2007).

Cardiovascular dysfunction can also be associated with alterations in serotonergic concentrations. A reduction in peripheral serotonin synthesis in blood platelets is associated with heart failure, such findings emphasise serotonin as a key neurohormonal factor for maintaining typical cardiovascular activity (Cote et al., 2004). Moreover, serotonin receptors (5-HT_{1A}, 5-HT₃, 5-HT₇) carry out a physiological role in the regulation of the cardiovascular parasympathetic system (Ramage and Villalon, 2008). Thus, the effect of alterations of serotonin in the development of the cardiovascular system has been studied. One such study interfered with the serotonergic system by administering 12 monoamine transporter inhibitors to cultured whole rat embryos. While certain serotonergic altering compounds show a slight teratogenic effect, findings are difficult to interpret as the concentrations employed were over five times the dose expected in the clinical scenario (Sloot et al., 2009). Although, clinical databases do suggest paroxetine can be correlated to ventricular and atrial septal defects when used within the first-trimester (Cole et al.,

2007, Kallen and Olausson, 2007) thus resulting in a change of labelling as detailed in section 1.4.

1.1.2 Serotonergic dysfunction and resulting disorders and treatments

Altered levels of serotonin are associated with various psychiatric disorders (Woolley and Shaw, 1954), consequently, serotonin is a key target in the management of many mental illnesses. As serotonin regulates mood, appetite, and cognition, it is not surprising that many disorders are marked by such symptoms. A few of the illnesses or treatments which have been investigated in relation to serotonin are schizophrenia, dementia, eating disorders, anxiety and panic disorders, bipolar disorder, and depression. In the previous section, it is highlighted that altered levels of serotonin can induce many disorders, and such manipulations during the developmental period should include the assessment of such behaviours related to any of the aforementioned disorders. However, in regards to treatment, the following work mainly focuses on antidepressant interventions. Depression and antidepressants will be described in detail in the following sections; briefly, the antidepressant action to be discussed is largely influenced by the serotonergic receptors listed in Table 1.2.

Serotonin receptor	Receptor type and activation	Location	Function	Resulting effect
5-HT _{1A}	G _i /G _o -PCR ↓ cAMP	Midbrain raphe, hippocampus	↑ DA ↓ 5-HT	Secret cortisol and ACTH
5-HT _{1B/D}	G _i /G _o -PCR ↓ cAMP	Basal ganglia	↓ 5-HT	Vasoconstriction
5-HT _{2A}	G _i /G ₁₁ -PCR ↑ inositol triphosphate, diacylglycerol	Neocortex, platelets	↓ DA	Platelet aggregation
5-HT _{2C}	G _i /G ₁₁ -PCR ↑ inositol triphosphate, diacylglycerol	Cortex, brainstem	x DA x NA	Regulate anxiety
5-HT ₃	Ligand-gated cation channel	Brainstem	x DA x NA x 5-HT	Nausea and vomiting
5-HT ₆	G _s -PCR ↑ cAMP	Cortex, striatum	x ACh	Memory and cognition
5-HT ₇	G _s -PCR ↑ cAMP	Thalamus, hypothalamus	↓ 5-HT	Mood, memory and sleep

Table 1.2 Serotonin receptor binding and activations associated with depression. Serotonin receptors and subtypes including location, function and resulting effects of those associated with depression. DA, dopamine; NA, noradrenaline; ACh, acetylcholine; ACTH, adrenocorticotrophic hormone; ↑, increase; ↓, decrease; x, block. Adapted from Kohler et al. (2016) and Glover and Clinton (2016).

1.1.3 Altering brain development in rats and humans

As mentioned at the beginning of this chapter, studies altering serotonergic activity are often conducted in rats, and results are transferred to the clinical scenario. There are obvious differences between human and rats; however, they are a good alternative as clinical teratogenic testing is complicated due to ethical and logistical factors highlighted later in this chapter which ultimately make it difficult to complete well-controlled clinical studies. The information expressed in Figure 1.3 serves as a guide

when applying rat studies to the clinical context. While overcoming the clinical obstacles, rats are also easy to breed and produce large litters allowing for the assessment of multiple parameters. Furthermore, as emphasized by the timelines below, rat studies facilitate long-term and continuous assessment findings while only imposing a relatively short period of time, compared to the clinical scenario.

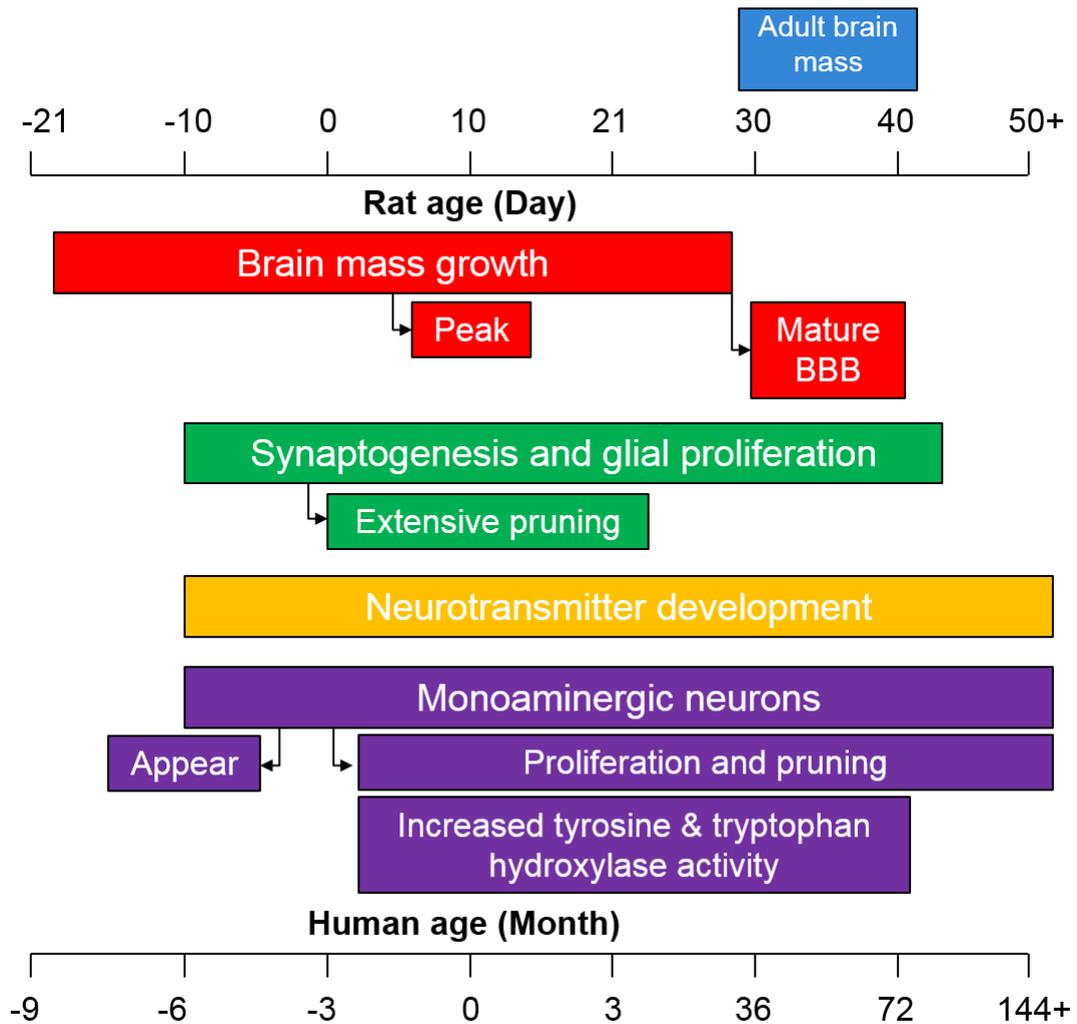


Figure 1.3 Rat and human brain development timeline comparison. Timelines informed by O'Brien and Kelly (2013) and Maciag et al. (2006).

In general, this section emphasises the important roles serotonin plays in the developing human and rodent brain, especially during embryogenesis. Dysregulation of serotonin during pregnancy can change the environment of the uterus and also have teratogenic effects. Furthermore, various 5-HT receptors that are associated with

different cascading functions altering other neurotransmitters throughout the brain. The regulatory action of serotonin is also important throughout the life-span, as altered levels of serotonin can affect many factors such as mood and cognition; moreover, many illnesses or therapies target serotonin. Overall, the human and the rodent brain both rely on serotonin for various functions and therefore information can be translated from rodent models to predict delivery outcomes for the clinical scenario when serotonin is altered during gestation.

1.2 Depression

According to the latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), Major Depressive Disorder (MDD) is a psychiatric disorder characterized by a period exceeding two weeks marked by a loss in mood from an individual's baseline and withdrawal from previously enjoyed activities (American Psychiatric Association., 2013). This global condition has a socioeconomic impact, as it is the leading cause of disability (Ferrari et al., 2013, Smith, 2011). In addition to emotional symptoms, depression also exhibits biological features including a significant change in weight, sleep, energy and ability to concentration or relax (Hamilton, 1960). Individuals are at a greater risk to develop MDD if they have a history of substance abuse, an additional medical illness or psychiatric disorder, or during periods of increased stress (American Psychiatric Association., 2013). In addition, there are endogenous features of depression as there is a strong genetic component and it has been associated with particular brain abnormalities as well as atypical monoamine and acetylcholine levels. The DSM-V classifies the disorder as moderate or severe with various symptoms (Table 1.3), such cognitive and psychotic symptoms may result in reduced performance at work or school, isolation from family and friends, irritated and aggressive moods, reduced communication and injury to others or self-inflicted harm, as well as thoughts of self-harm (American Psychiatric Association., 2013). Furthermore, these episodes of low mood manifest in both the personal and professional life, can be experienced continually for a year and often recur throughout one's lifetime (American Psychiatric Association., 2013). Overall, appropriate diagnosis and treatment of psychiatric disorders are important, as depression affects the individual as well as their children, family and the global community.

A.	Five or more of the following symptoms nearly every day in a two week period:
1	Depressed mood; feeling sad, empty, hopeless
2	Diminished interest or pleasure
3	Significant changes in weight
4	Insomnia or hypersomnia
5	Psychomotor agitation or retardation
6	Fatigue, loss of energy
7	Feeling worthless, excessive or inappropriate guilt
8	Diminished ability to think or concentrate, indecisiveness
9	Recurrent thoughts of death, suicidal ideation with/without a plan, attempted suicide
B.	Symptoms cause distress/impairment in social and occupational functioning
C.	Symptoms are not caused by a psychiatric substance or other medical condition
<i>A-C indicate a major depressive episode</i>	
D.	Symptoms are not better qualified for another psychiatric disorder
E.	No occurrences of manic or hypomanic episodes

Table 1.3 DSM-V major depressive episode diagnostic criteria. Symptoms are required to recur consistently within a two week period to confirm a diagnosis. Symptoms persisting two years indicates dysthymia, persistent depressive disorder. Adapted from DSM-V (American Psychiatric Association., 2013).

1.2.1 Manifestation of depression in pregnant women

The prevalence of depression has increased in recent years, especially in women as it is the primary source of disease-related disability for females (Kessler, 2003). While the global annual prevalence in men is 3.2%, the incidence in women is 5.5% (Albert, 2015). A study investigating the global burden of disease highlights the years lived with disability (YLD) for females and males (Figure 1.4), consistently, global rates of depression in females were higher compared to males regardless of age (Ferrari et al., 2013). Additionally, in Europe, the peak age prevalence for men is 30-50 while in women it is 18-30. Thus depression is usually diagnosed in women in early adulthood during childbearing years. This difference in sex occurs because females are at a higher risk for onset of depression from puberty (Kessler, 2003), moreover the risk of depressive episodes may increase with pregnancy and new motherhood (Burke et al., 1991). Consequently, women are at high risk of developing depression during

pregnancy, with depression manifesting in 10-20% of pregnant women (Charlton et al., 2014, Dayan et al., 2002). Additionally, females diagnosed with moderate to severe premenstrual syndrome/premenstrual dysphoric disorder are almost twice as likely to develop postpartum depression (Buttner et al., 2013).



Figure 1.4 Comparison between females and males for years lived with disability. Globally, females consistently showed higher rates of YLD compared to males in regards to depressive disorders in 2010 (Ferrari et al., 2013).

1.2.1.1 Diagnosis of perinatal depression

Perinatal depression can occur throughout the pregnancy or within 12 months post-birth. Earlier studies have found that perinatal depression becomes disguised by similar symptoms that occur during pregnancy and the puerperium period. It was found that when symptoms considered pregnancy related, such as increased appetite

and fatigue, were included for the scoring of major depression, the rate of depression rose from 1.2 to 2.8% (Klein and Essex, 1994). Dietz et al. (2007) illustrate current trends from 2007 showing that women are more likely to be treated for depression within the 39 weeks prior to conception and within the 39 weeks post-birth, rather than during the gestational period. Such results may indicate that depression occurs more in pre and postpartum women, alternatively these results may suggest that clinicians' face challenges with diagnosing depression during pregnancy when using typical depression scales due to an overlap of depression and pregnancy symptoms. In addition, a major concern for clinicians and patients are the perceived adverse effect profile of antidepressants, thus also contributing to the lower rates of treatment during pregnancy. In the UK, the high prevalence of depression manifesting in pregnancy has contributed to the 4% of women aged 20-35 who commit suicide in the perinatal period, half of which have a primary diagnosis of depression (Kim and Silver, 2016).

Routine screenings for perinatal depression are suggested due to the high prevalence of depression manifesting in pregnancy and the difficulties in separating depressive symptoms from pregnancy symptoms such as changes in appetite and fatigue (NICE, 2014). The American College of Obstetricians and Gynecologists (ACOG) even suggests that women be screened during each trimester of pregnancy, as depression can manifest at any time (ACOG Committee on Health Care for Underserved Women, 2006). The most common screening technique to assess perinatal depression is the Edinburgh Perinatal Depression Scale (EPDS) (ACOG, 2018). As illustrated in Figure 1.5, it was originally developed in 1987 for post-partum depression screening (Cox et al., 1987), however, it is now used for depression screening throughout the perinatal period. The main advantage of using the EPDS is that it is tailored to individuals experiencing pregnancy symptoms as well as episodes of depression (ACOG, 2018). Thus, it is superior to depression scales such as Patient Health Questionnaire 9, the Beck Inventory, and the Center for Epidemiological Studies Depression Scale. Inferior scales have reduced specificity as they are used to assess depression in general/non-pregnant populations. In contrast, the EPDS omits nonspecific symptoms such as changes in sleep patterns as they are expected throughout the perinatal period. However, EPDS includes symptoms of anxiety, as they are prominent in perinatal mood disorders. Other scales take longer to complete as they can have over 20 questions, whereas the EPDS typically takes less than 5 minutes to complete and is

made up of 10 self-reported questions. Furthermore, the scale is health literacy appropriate and has been translated into 50 different languages (ACOG, 2018). In conclusion, the specificity of symptoms assessed, simplicity of use, and its appropriateness over a wide scope of patients contribute to the popularity of the EPDS.

Edinburgh Postnatal Depression Scale (EPDS)

The Edinburgh Postnatal Depression Scale (EPDS) has been developed to assist primary care health professionals to detect mothers suffering from postnatal depression; a distressing disorder more prolonged than the 'blues' (which occur in the first week after delivery) but less severe than puerperal psychosis.

Previous studies have shown that postnatal depression affects at least 10% of women and that many depressed mothers remain untreated. These mothers may cope with their baby and with household tasks, but their enjoyment of life is seriously affected and it is possible that there are long-term effects on the family.

The EPDS was developed at health centres in Livingston and Edinburgh. It consists of ten short statements. The mother underlines which of the four possible responses is closest to how she has been feeling during the past week. Most mothers complete the scale without difficulty in less than 5 minutes.

The validation study showed that mothers who scored above a threshold 12/13 were likely to be suffering from a depressive illness of varying severity. Nevertheless the EPDS score should *not* override clinical judgement. A careful clinical assessment should be carried out to confirm the diagnosis. The scale indicates how the mother has felt *during the previous week*, and in doubtful cases it may be usefully repeated after 2 weeks. The scale will not detect mothers with anxiety neuroses, phobias or personality disorders.

Instructions for users

1. The mother is asked to underline the response which comes closest to how she has been feeling in the previous 7 days.
2. All ten items must be completed.
3. Care should be taken to avoid the possibility of the mother discussing her answers with others.
4. The mother should complete the scale herself, unless she has limited English or has difficulty with reading.
5. The EPDS may be used at 6–8 weeks to screen postnatal women. The child health clinic, postnatal check-up or a home visit may provide suitable opportunities for its completion.

EDINBURGH POSTNATAL DEPRESSION SCALE (EPDS)

J. L. Cox, J. M. Holden, R. Sagovsky

Department of Psychiatry, University of Edinburgh

Name:

Address:

Baby's age:

As you have recently had a baby, we would like to know how you are feeling. Please UNDERLINE the answer which comes closest to how you have felt IN THE PAST 7 DAYS, not just how you feel today.

Here is an example, already completed.

I have felt happy:
 Yes, all the time
Yes, most of the time
 No, not very often
 No, not at all

This would mean: "I have felt happy most of the time" during the past week. Please complete the other questions in the same way.

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In the past 7 days:

1. I have been able to laugh and see the funny side of things
 As much as I always could
 Not quite so much now
 Definitely not so much now
 Not at all
2. I have looked forward with enjoyment to things
 As much as I ever did
 Rather less than I used to
 Definitely less than I used to
 Hardly at all
- * 3. I have blamed myself unnecessarily when things went wrong
 Yes, most of the time
 Yes, some of the time
 Not very often
 No, never
4. I have been anxious or worried for no good reason
 No, not at all
 Hardly ever
 Yes, sometimes
 Yes, very often
- * 5. I have felt scared or panicky for no very good reason
 Yes, quite a lot
 Yes, sometimes
 No, not much
 No, not at all
- * 6. Things have been getting on top of me
 Yes, most of the time I haven't been able to cope at all
 Yes, sometimes I haven't been coping as well as usual
 No, most of the time I have coped quite well
 No, I have been coping as well as ever
- * 7. I have been so unhappy that I have had difficulty sleeping
 Yes, most of the time
 Yes, sometimes
 Not very often
 No, not at all
- * 8. I have felt sad or miserable
 Yes, most of the time
 Yes, quite often
 Not very often
 No, not at all
- * 9. I have been so unhappy that I have been crying
 Yes, most of the time
 Yes, quite often
 Only occasionally
 No, never
- *10. The thought of harming myself has occurred to me
 Yes, quite often
 Sometimes
 Hardly ever
 Never

Response categories are scored 0, 1, 2, and 3 according to increased severity of the symptom.

Items marked with an asterisk are reverse scored (i.e. 3, 2, 1 and 0). The total score is calculated by adding together the scores for each of the ten items. Users may reproduce the scale without further permission providing they respect copyright (which remains with the *British Journal of Psychiatry*) by quoting the names of the authors, the title and the source of the paper in all reproduced copies.

Figure 1.5 Edinburgh Postnatal Depression Scale. EPDS is a 10-item questionnaire, originally used for postnatal depression screening, but is now consistently employed in perinatal (pre- and/or postnatal) depression screenings (Cox et al., 1987).

1.2.1.2 Risk factors for developing perinatal depression

The most prominent risk for experiencing depression during pregnancy is a history of depression, as 90% of affected individuals will experience multiple episodes (Dietz et al., 2007, Vos et al., 2004). A recent study carried out in Nigeria concluded that additional risk factors include unemployed spouses, lower income and abuse. Moreover, 21% of women who experience verbal abuse and 45% of women who experience physical/sexual abuse developed depression during pregnancy (Busari, 2018). A family history of depression or bipolar disorder may also increase the risk for perinatal depression, as well as single motherhood and mothers under twenty years of age (Dietz et al., 2007, Stewart, 2011). Clinical studies have begun to focus on determining risks specific for antepartum depression, rather than combining risks for pre and postpartum depression. Factors such as pregnancy intention and social support may change after the birth of the baby, thus altering the risk. A multivariate analysis of English language articles published between 1980 through 2008 assessing risks for antepartum depression found that life stress, lack of social support and domestic violence consistently correlated to an increased risk for antepartum depression (Lancaster et al., 2010). The bivariate analysis concluded risks for antepartum related to maternal anxiety, life stress, history of depression, lack of social support, domestic violence, unintended pregnancy, public insurance, lower income, lower education, smoking, single status, and poor relationship quality (Lancaster et al., 2010). Such findings coincide with the risks noted previously, and emphasise the importance of having proper antenatal care that evaluates both physical and physiological health. Overall, understanding the risks associated with developing depression during pregnancy and using specialised methods of screening perinatal depression assists clinicians in the appropriate diagnosis of and thus treatment of perinatal depression.

1.2.2 Impact of depression on pregnancy

When depression is left untreated it can further complicate the pregnancy. In addition to having lasting effects on the mother's health, it can also impact the health of the offspring. Untreated depression can cause reduced foetal growth, lower birth weights and intelligence quotient (IQ), increased risk for developmental delays, and depression and anxiety in childhood (Reis and Kallen, 2010). Furthermore, the high level of stress induced by depression can lead to spontaneous abortion (Schetter and Tanner, 2012).

Thus depression should be appropriately managed within a pregnancy to avoid damaging effects. The adverse outcomes of depression exposure in pregnancy are difficult to measure clinically as most studies are poorly controlled for and thus are not consistent among data sets. Outcomes such as miscarriage are challenging to measure, as most miscarriages typically occur prior to pregnancy recognition. Consequently, it is unlikely that pregnancy-appropriate depression screenings, such as the EPDS, have occurred. Thus, current findings of correlation are limited to small sample sizes. Interestingly, recent studies have found a positive correlation with depression amongst post-miscarriage women (Mutiso et al., 2018). Furthermore, there is evidence of post-miscarriage women having increased positive results for depression in the subsequent pregnancy (McCarthy et al., 2015). Overall, further investigations on the relationship between miscarriage and depression are necessary to determine if depression induces a miscarriage or rather miscarriage induces perinatal depression.

Preterm delivery and foetal growth effects associated with lower birth weights or small for gestational age have been researched in offspring exposed to depression throughout gestation. A study carried out in British Columbia showed that infants exposed to depressive symptoms during pregnancy resulted in shorter gestations and preterm births as well as birth weights 10% lower than expected for gestational age (Oberlander et al., 2006). However, these findings were refuted by a study published in the following year which required a structured diagnosis for MDD (Suri et al., 2007). Furthermore a recent systematic review reported that preterm delivery and foetal growth effects were associated with approximately half of the studies assessing perinatal depression in pregnancy (Accortt et al., 2015), thus leaving correlations inconclusive and suggesting future high quality studies with valid screening tools, defined exposure periods, and medical histories of patients preconception.

Perinatal depression has been researched to observe potential neonatal and early childhood effects on offspring. The behaviour of neonates exposed to depression mimicked their depressed mothers in that during assessments they showed more irritability and less activity and endurance (Abrams et al., 1995). A subsequent study concluded that offspring were less expressive, as exposed infants exhibited less attentiveness during evaluations and fewer facial responses to happy and surprise modelled facial expressions (Lundy et al., 1996). In addition, the physiological

profiles of the offspring also mirror depressed mothers. Infants born to depressed mothers are shown to have elevated levels of cortisol and diminished levels of peripheral serotonin and dopamine, compared to infants born to mothers without depression (Field et al., 2004). Similarly, depression-exposed infants showed lower vagal tone and greater relative right frontal EEG activation. Similar patterns are observed in individuals with depression and emotional dysregulation (Roose et al., 1989, Henriques and Davidson, 1990). Poor emotion regulation can be associated with developmental disorders such as attention-deficit/hyperactivity disorder and autism spectrum disorder and is characterized by a longer period to return to baseline after emotional stimuli (American Psychiatric Association., 2013). Limited research exists on the effects of gestational depression exposure to early childhood development. Current research collected at 18 and 32 weeks gestation indicates that antenatal exposure relates to greater developmental delays in the resulting children followed up with at 18 months (Deave et al., 2008). Interestingly, the large cohort study found no effect for postnatal depression exposure on developmental delays however the evidence reported is weak as it relied on maternal reports of child performance. Overall the current understanding of the results incurred by perinatal depression exposure is limited and additional well-controlled investigations are required to comprehend the risks imposed by untreated perinatal depression.

In general, this section describes the burden depression can impose on a pregnancy for the mother and the progeny. Despite its prevalence, treatments are often avoided during pregnancy due to multiple factors such as an improper diagnosis or for fear of the offspring implications. However, maternal depression can also effect the pregnancy by increasing the risk of spontaneous abortion or preterm birth, and the progeny are likely to experience developmental delays as well as mood and anxiety disorders in childhood. Therefore, proper therapeutic management of depression during pregnancy is necessary for both the health of the mother and her resultant child.

1.3 Selective serotonin reuptake inhibitors

1.3.1 Investigations of depression and antipsychotics leading to SSRI discovery

As the name may suggest, the SSRI class developed by a decision to selectively target serotonin. The theory to target serotonin in the treatment of depression resulted from the further investigation of the pathophysiology of depression and the closer examination of the action of previous antidepressant classes including monoamine oxidase inhibitors (MAOI) and tricyclic antidepressants (TCA) (Hill and Rang, 2012, Freemantle and Mason, 2000). The neurochemical theory of depression was first referenced in 1965, as the monoamine theory (Schildkraut, 1965). It suggests that depression is caused by a reduction in monoaminergic neurotransmission as a result of a shortage of noradrenaline and serotonin (Nair and Sharma, 1989). This theory was proposed due to evidence that use of the antihypertensive, reserpine, caused depression in patients due to its monoamine-depleting agents (Harris, 1957). Interestingly, iproniazid was developed to treat tuberculosis and inhibits the metabolism of monoamines (Nair and Sharma, 1989). In 1952, iproniazid was recognized for having antidepressant properties after reports of increasing mood (Kuhn, 1957), thus further supporting the monoamine theory of depression and leading to the development of the first class of antidepressants, MAOIs in the early 1950s (Lopez-Munoz and Alamo, 2009). Peaking in popularity from 1957-1970, MAOIs are prescribed to alleviate symptoms of depression by inhibiting the breakdown of monoamines. However toxic reports of MAOIs dangerous interactions with foods containing tyramine and sympathomimetic drugs, caused their popularity to diminish (Lopez-Munoz and Alamo, 2009). Also in the 1950s, the first TCA imipramine was developed though originally as an antipsychotic, as an analogue of the successful antipsychotic chlorpromazine (Shorter, 2005). Imipramine became recognized as an antidepressant in 1957 after reports of patients experiencing severe depressive episodes with comorbid psychosis showed a reduction in depression symptoms (Ban, 2006). TCAs target the noradrenaline and serotonin reuptake transporters, relieving the symptoms of depression by blocking the reuptake and consequently increasing the concentrations of noradrenaline and serotonin (Elhwuegi, 2004). Unfortunately, MAOIs and TCAs have a delay between onset of treatment and therapeutic effect, however this obstacle is still present in newer antidepressant classes. Other major inadequacies of MAOIs and TCAs are their rates of efficacy and safety complications.

It was proposed that these inadequacies were a result of the manipulation of the different monoamines and other off-target effects. Therefore a therapy with a different mechanism of action from the previous antidepressants was required (Table 1.4). Thus it was hypothesized that selectively targeting serotonin would allow for effective antidepressant therapy without the unwanted downstream effects (Lopez-Munoz and Alamo, 2009).

While others were developed previously, fluoxetine (FLX) was one of the first successfully marketed SSRIs. Developed in 1974, fluoxetine entered the market in 1987 (Wong et al., 1995). Entering the market prior to fluoxetine in 1982 were indalpine and zimeldine; however, they were only available for a brief period as they were removed from the market after reported risks of hematologic toxicity and Guillain-Barré syndrome, respectively (Galbaud du Fort, 1988, Fagius et al., 1985). The exploration of targeting serotonin was propelled by the discovery of reduced concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid in the cerebral spinal fluid of the untreated depressed individuals as well as in the post-mortem brain tissue of depressed and/or suicidal patients (Asberg et al., 1976, Roy et al., 1989). More recently, a prospective study of bipolar patients discovered a negative correlation between the reduction in serotonin metabolites 5-hydroxyindoleacetic acid, homovanillic acid, and 3-methoxy-4-hydroxyphenylglycol and the lethality of suicide (Sher et al., 2006). With the success of early SSRIs and additional studies pointing to the serotonergic system, further investigation of SSRI action led to the discovery of various other derivatives. Before fluoxetine, many SSRI compounds were developed but never marketed, and some SSRIs such as indalpine and zimelidine were discontinued due to adverse effects. However, modifications to these previous compounds prompted the development of some SSRIs that have been successfully marketed. While fluoxetine is still widely prescribed today, other currently marketed SSRI antidepressants include fluvoxamine, paroxetine (PRX), sertraline (SERT), citalopram (CIT), and escitalopram (Wong et al., 1995). However, the following work is focused on four of these SSRIs, namely fluoxetine, paroxetine, sertraline, and citalopram. Interestingly, SSRIs accounted for over half of the antidepressant prescriptions for Ireland in 2013 (Figure 1.6). Also contributing to their popularity, SSRI use is not restricted to MDD, as they can be prescribed for a variety of anxiety disorders as noted within their clinical profiles in section 1.5. SSRIs are also common

in certain populations as they are the most commonly prescribed psychotropic drug in pregnancy (Alwan et al., 2016). Like other antidepressants, SSRIs still face criticisms due to their delay in onset of therapeutic effects. Although SSRIs have comparative efficacy to other antidepressant classes, they are usually prescribed over other classes due to their higher tolerability and anxiolytic properties (Cryan and Lucki, 2000). The noted SSRI safety profile is largely attributed to their unique mechanism of action.

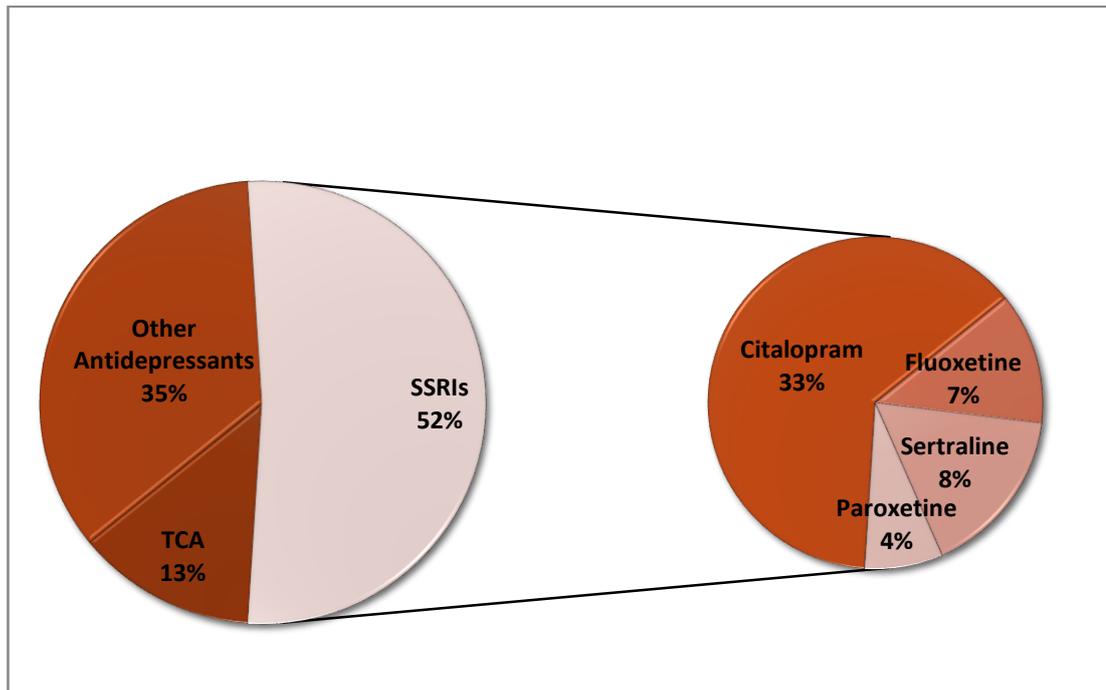


Figure 1.6 Antidepressant prescriptions for Ireland, 2013. SSRIs make up over half of the antidepressant prescriptions in Ireland. This data was jointly collected for citalopram and escitalopram, therefore the above illustration reflects a combined prescription percentage of the two SSRIs. Data provided by Health Service Executive (HSE) (2013).

1.3.2 Mechanism of action of SSRIs

SSRIs produce less severe side effects as compared to other antidepressants because of their unique mechanism of action. As explained in the previous section, this refers to other antidepressants regulating various monoamines, which result in adverse side effects and consequently lower tolerability. SSRIs exert therapeutic effects by selectively increasing the neurotransmission of serotonin in limbic regions of the depressed brain (Goodnick and Goldstein, 1998). Serotonin modulates mood, appetite, sleep, and cognition. Interestingly, many symptoms of depression (Table 1.3) align with these features which are modulated by serotonin including low mood, change in appetite, fatigue, insomnia, and difficulty concentrating (American Psychiatric Association., 2013). Furthermore, studies have concluded that individuals affected by

MDD or suicide have lower levels of serotonin in the cerebrospinal fluid and platelets (Arango et al., 2002). Overall, SSRIs work by binding to the presynaptic cell at the serotonin reuptake transporter, therefore inhibiting the reuptake of 5-HT. By blocking reabsorption, the synaptic concentration of serotonin increases, thereby resulting in increased serotonergic activity at the receptor sites on the postsynaptic neuron (Figure 1.7) (Glover and Clinton, 2016). When depression is left untreated it can further complicate the pregnancy. In addition to having lasting effects on the mother's health, it can also impact the health of the offspring. Untreated depression can cause reduced foetal growth, lower birth weights and intelligence quotient (IQ), increased risk for developmental delays, and depression and anxiety in childhood (Reis and Kallen, 2010). Furthermore, the high level of stress induced by depression can lead to spontaneous abortion (Schetter and Tanner, 2012). Thus depression should be appropriately managed within a pregnancy to avoid damaging effects. The adverse outcomes of depression exposure in pregnancy are difficult to measure clinically as most studies are poorly controlled for and thus are not consistent among data sets. Outcomes such as miscarriage are challenging to measure, as most miscarriages typically occur prior to pregnancy recognition. Consequently, it is unlikely that pregnancy-appropriate depression screenings, such as the EPDS, have occurred. Thus, current findings of correlation are limited to small sample sizes. Interestingly, recent studies have found a positive correlation with depression amongst post-miscarriage women (Mutiso et al., 2018). Furthermore, there is evidence of post-miscarriage women having increased positive results for depression in the subsequent pregnancy (McCarthy et al., 2015). Overall, further investigations on the relationship between miscarriage and depression are necessary to determine if depression induces a miscarriage or rather miscarriage induces perinatal depression.

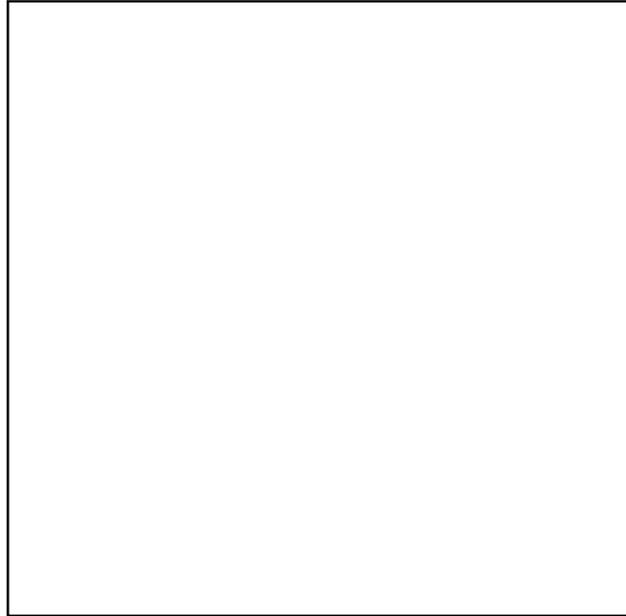


Figure 1.7 SSRI mechanism of action. SSRIs bind to the reuptake transporter thereby increasing the concentration of serotonin in the synapse. Image taken from Lattimore et al. (2005).

While there are fourteen serotonin receptor subtypes, studies have shown this SSRI action is largely mediated by the 5-HT_{1A} and 5-HT_{2A} subtypes (Cryan and Leonard, 2000, Celada et al., 2004, Artigas, 2013). It is anticipated that SSRI action occurs at the 5-HT_{1A} receptor as chronic administration of SSRIs results in the desensitisation of 5-HT_{1A} autoreceptors (Jolas et al., 1994). Additionally, mood disorders have been associated with 5-HT_{1A} autoreceptor abnormalities in genetic studies (Neff et al., 2009). This association is further supplemented via neuroimaging reports amongst postmortem subjects with MDD which demonstrate a reduction in 5-HT_{1A} density compared to healthy controls (Stockmeier et al., 1998, Boldrini et al., 2008). The 5-HT_{1A} and 5-HT_{2A} receptors can be linked in their association with antidepressant action and depression. Under a similar condition of chronic antidepressant exposure, 5-HT_{2A} receptors are down-regulated (Gray and Roth, 2001). In the neocortex, 5-HT_{1A} is commonly expressed with 5-HT_{2A}, and blocking 5-HT_{2A} increases 5-HT_{1A} in cortical and limbic areas presumably resulting in SSRI efficacy (Amargos-Bosch et al., 2004). Furthermore, both 5-HT receptors can be tied anxiety, a common comorbidity or feature of depression. Partial agonists of the 5-HT_{1A} receptor produce an anxiolytic effect (Schreiber and De Vry, 1993). Meanwhile, antagonists of the 5-HT_{2A} receptor will also produce an anxiolytic effect. Overall, 5-HT_{1A} and 5-HT_{2A} receptors are the targets of numerous psychiatric therapeutics (Artigas, 2013).

1.3.3 Praise and criticism of SSRIs

While SSRIs are not without side effects current data suggests that they are the lesser of evils. The difference between SSRIs and other antidepressants are largely attributed to their different mechanisms of action (Table 1.4). A meta-analysis, investigating SSRI and TCA reports that were well controlled and double-blinded, has suggested that the side effects of TCAs tend to cause more severe adverse reactions, compared to SSRIs (Trindade et al., 1998). The safety of SSRIs is further emphasized in another systematic review and network meta-analysis which showed that when 21 antidepressants went head to head, the SSRIs citalopram, fluoxetine, sertraline, and escitalopram, along with other antidepressants agomelatine and vortioxetine had significantly better odds ratios of acceptability (Cipriani et al., 2018). Furthermore, SSRIs have a higher toxic dose, thus reducing the risk of poisoning and fatal overdose (Goldstein and Goodnick, 1998, Barbey and Roose, 1998).

SSRIs are also frequently prescribed to treat symptoms of a variety of anxiety disorders such as obsessive-compulsive disorder and panic disorder (Drapier et al., 2007). The anxiolytic effect of SSRIs is advantageous for the treatment of depression, as the DSM-V describes anxiety as a common feature and/or comorbidity of depression (American Psychiatric Association., 2013). Several preclinical studies have indicated an anxiolytic effect after chronic treatment (Borsini et al., 2002, Zhang et al., 2000, Fish et al., 2004). While reviews of the clinical prescribing patterns would agree with preclinical data indicating that SSRIs are commonly prescribed to treat anxiety disorders (Nutt, 2005, Montgomery et al., 2007), clinical reviews of serotonergic compounds have also suggested that acute treatment may have an anxiogenic effect (Gordon and Hen, 2004). Thus suggesting a biphasic anxiolytic feature of SSRIs.

Though it is clear that SSRIs have a superior safety profile compared to MAOIs and TCAs, their efficacy is similar. While the previously mentioned review did indicate that all 21 antidepressants showed significantly better efficacy compared to placebo groups, the direct comparison between antidepressants for efficacy identified seven antidepressants with superior odds ratio, only two of which were the SSRIs paroxetine and escitalopram (Cipriani et al., 2018). Another major criticism of the SSRI class and other antidepressant classes alike is they incur a lag period between onset of treatment and the resulting therapeutic effect. While paroxetine seems to show a modest

improvement of physical symptoms within 1-2 weeks, SSRIs generally require a 4-8 week dosing period before full relief of psychological symptoms. Physiologically, after the initial 2 weeks of treatment, there is an increase in the concentration of serotonin in the synaptic cleft, yet it is after chronic treatment that antidepressant effects are observed due to a down-regulation of presynaptic serotonin receptors and an increase in the rate of neuronal firing (Blier, 2003). However, it has been observed that using a 5-HT_{1A} antagonist in combination with an SSRI can reduce the delay of therapeutic onset (Artigas et al., 1996) overall suggesting that the introduction of another drug during the initial period may be necessary to optimize SSRI efficacy. The major criticism in relation to this work is psychotropic drugs, such as SSRIs, also cross the placental barrier when used during pregnancy. Consequently, SSRIs alter the serotonergic activity in the developing brain, causing major concerns as serotonin plays a critical role in brain development, as outlined in section 1.1.1.

Class	Target	Action	Effect
MAOI	MAO-A & MAO-B	Inhibit monoamine oxidase, blocking reuptake of MAO-A & MAO-B	↑ MAO-A (serotonin, melatonin, epinephrine, norepinephrine & tyramine) ↑ MAO-B (phenethylamine trace amines, dopamine)
TCA	NA & 5-HT reuptake transporters	Block reuptake of NA & 5-HT	↑ noradrenaline & ↑ serotonin
SSRI	5-HT reuptake transporters	Selectively block reuptake of 5-HT	↑ serotonin
SNRI	5-HT & NA	Inhibit the reuptake of 5-HT & NA	↑ serotonin & ↑ noradrenaline
NDRI	NA & DA	Block action of NA & DA transporters	↑ noradrenaline & ↑ dopamine
NASSA	H ₁ , α ₂ , & 5-HT	Block negative feedback of NA & 5-HT	↑ noradrenaline & ↑ serotonin

Table 1.4 Comparison of the various antidepressant classes. MAOI, monoamine oxidase inhibitor; TCA, tricyclic antidepressant; SSRI, selective serotonin reuptake inhibitor; SNRI, selective noradrenaline reuptake inhibitor; NDRI, noradrenaline dopamine reuptake inhibitors; NASSA, noradrenergic and specific serotonergic antidepressant; MAO, monoamine oxidase; NA, noradrenaline; 5-HT, serotonin; DA, dopamine; H, histamine.

Largely, this section provides a general background on SSRI antidepressants, which are the compounds of interest for this thesis. There were many serendipitous findings in depression and psychotropic drug research which eventually led to the SSRI class. While their efficacy and therapeutic lag is equal to other antidepressants, they have a higher tolerability which is often attributed to their selectivity of serotonin, whereas other antidepressants regulate various monoamines. The 5-HT_{1A} and 5-HT_{2A} subtypes predominantly mediate the action of SSRI antidepressants and are often linked to depression and anxiety. As depression and anxiety are often comorbid, SSRIs are advantageous therapies because in addition to their antidepressant effects they are also noted to have anxiolytic effects. Although SSRI use is not confined to depression, they are not superior to other antidepressants in terms of efficacy or onset of therapeutic effect. Nonetheless, SSRIs are frequently prescribed in the clinical scenario, and although many have been developed this thesis will highlight fluoxetine, paroxetine, sertraline, and citalopram.

1.4 Antidepressant exposure in pregnancy- a treatment dilemma

Depression manifests in many pregnancies and can pose threats to the pregnancy for both the mother and the developing child. To avoid these risks in the UK, the National Institute of Health and Care Excellence (NICE) guidelines lists SSRIs as the first-line drug therapy for managing depression during pregnancy, especially in cases of antidepressant-naïve pregnant women (NICE, 2014). NICE guidelines also recommend SSRIs in pregnancy over other anti-anxiety medications such as benzodiazepines. However, limited literature could be found which definitively recommends a particular SSRI over others, although most sources imply that efficacious compounds and doses should be used on a case by case basis (McAllister-Williams et al., 2017). Maudsley Guidelines recommend that moderate or severe maternal depression should be treated with antidepressants; in particular, it is noted that the majority of experience informing this recommendation is based on the TCAs amitriptyline and imipramine, and the SSRI fluoxetine. Therefore, SSRI treatments commence in pregnancy, even though the drugs cross the placental barrier and most are listed by the US Food and Drug Administration (FDA) as category C. This results in exposing the progeny to pharmacologically active drugs that impact synaptic availability of 5-HT, an important neurotransmitter in brain development. Until recently, the FDA used a drug categorising system developed in 1979 to convey the risks associated with drug use during pregnancy (Table 1.5). SSRIs were listed as category C except for paroxetine which was listed as category D, to reflect the prevalence of cardiac malformation (O'Connor et al., 2016). Category C indicates that animal studies suggest a risk however appropriate human studies have not been completed, whereas category D indicates well-controlled human studies have been carried out and they do indicate risk. Both category C and D include that drug use during pregnancy may be appropriate as the potential benefits exceed the potential risks. The labelling of drugs for use in pregnancy changed in 2015, in an effort to better inform the patients and clinicians on the risks associated with drug use (Food and Drug Administration, 2014). Shifting from the letter category system, now risks of drug use are broken down into three subsections. The new subsections listed on labels are “Pregnancy”, which includes labour and delivery, “Lactation,” and “Females and Males of Reproductive Potential.” This new system describes the risk for each detailed subsection, allowing patients and clinicians to make informed

decisions about appropriate treatment in relation to the specified reproductive periods (Food and Drug Administration, 2014).

Category	Interpretation
A	Well-controlled studies on pregnant woman throughout pregnancy suggesting no risk to mother or child
B	Clinical related animal reproductive studies indicate there are no adverse effects. Or animal studies do suggest risks, however, well-controlled clinical studies contradict this evidence.
C	Animal studies do show an adverse effect and there are no subsets of human pregnancy studies. Potential benefits may exceed potential risks.
D	Well-controlled studies on pregnant woman indicate risks to the unborn baby and mother. Potential benefits may exceed potential risks.
X	Well controlled human and animal studies indicate positive harm to the foetus. Risks incurred exceed the potential benefit of the drug.

Table 1.5 USA FDA drug use in pregnancy ratings prior to 2015. The highlighted SSRIs of this thesis are categorized as C (fluoxetine, sertraline, and citalopram) or D (paroxetine).

Changes made to the labelling system are especially important for health care providers. They can now make meaningful decisions on drug use in pregnancy based on the specific risks, rather than the oversimplified 5 letter system which simply indicated that potential benefits outweighed potential risks. Due to a lack of understanding of the risks and for fear from the Thalidomide crisis, some clinicians avoid treatment completely and thereby may cause greater disease induced risks. Overall, this presents a treatment dilemma, as depression its self can cause harm to the progeny, and SSRIs alter serotonin—inhibiting typical development. Therefore, despite the unknown outcomes, women continue to receive treatment as physicians are advised to treat women on a case by case basis, acknowledging that maternal health and stability is key in a successful pregnancy (McAllister-Williams et al., 2017).

Preclinical studies have indicated that prenatal stress can induce “anxiety-like” and “depressive-like” maternal behaviours resulting in affective symptoms in male offspring, however when prenatal maternal depression is treated with an SSRI such as fluoxetine, the treatment reverses the “anxiety- and depressive-like” symptoms in male offspring (Salari et al., 2016). Thus emphasizing that SSRI treatment should not

be ruled out, however, more information on the safety of SSRI use in pregnancy is required. Case studies have also been conducted listing the delivery outcomes after an SSRI exposure. However these studies can be misleading as although one SSRI is featured, conclusions are assumed to apply to all SSRIs. Recently, clinical databases of antidepressant gestational exposure have been created. Using national perinatal/birth registry databases the prevalence of use and the potential teratogenicity can be listed for specific populations such as British Columbia (Oberlander et al., 2006, Oberlander et al., 2008), Sweden (Reis and Kallen, 2010) Quebec (Berard et al., 2015), The Netherlands (Zomerdijk et al., 2015), Finland (Malm et al., 2015), and Denmark (Liu et al., 2017). This has enabled large scale studies to be conducted observing various delivery outcomes in the offspring. The Swedish study explicitly examines the differences between delivery outcomes after specific SSRIs are exposed. Interestingly, although all SSRIs have the same mechanism of action, their implications in pregnancy range (Reis and Kallen, 2010). This could be due to the fact that although all SSRIs are selective for serotonin, they are so with different affinities, potentially having different consequences in the offspring. When controlling for maternal illness and medication dose a study from British Columbia found that the duration of exposure rather than the period of exposure may also contribute to the different delivery outcomes such as an increased risk for respiratory distress, lower birth weight, and reduced gestational age (Oberlander et al., 2008). Another study completed on the Quebec population specifically observed exposure during the first trimester and also isolated the effects of a specific SSRI, sertraline. In the study, sertraline was compared to other groups of gestational exposure such as depression, non-sertraline SSRIs, and non-SSRI antidepressants. Sertraline exposure in the first trimester was found to increase the risk of ASD/VSD and craniosynostosis, while non-sertraline SSRI exposure increased the risk of craniosynostosis and musculoskeletal defects (Berard et al., 2015). Thus stressing that the effects of gestational SSRI exposure are not universal amongst different SSRIs or exposure periods, further emphasizing the importance of conducting research which considers each SSRI individually with regards to the period of exposure to adequately account for the risks incurred by prescribing SSRIs during pregnancy.

Building on the information provided in the previous sections of the thesis which highlighted (1.1) the role of serotonin in development and various disorders, (1.2) the prevalence and burden of perinatal depression, and finally (1.3) an introduction to the antidepressant class of choice- SSRIs, section 1.4 emphasizes the treatment dilemma mothers and clinicians face with regards to the decision to use SSRIs during pregnancy, as they cross the placental barrier altering typical development. While the benefits often exceed the risks in the proposed treatment dilemma, there are limited well-controlled studies assessing delivery outcomes and even fewer which make conclusions based on the individual SSRIs. However, retrospective assessments isolating individual SSRIs are beginning to surface, and findings do in fact suggest that different SSRIs incur different birth outcomes. The following sections will continue to build off of this information, providing clinical profiles of the particular SSRIs in question, a general consensus on the clinical and preclinical outcomes as well as their limitations, the model used in this work to help answer this dilemma, and finally provide specific research objectives examined throughout this thesis.

1.5 SSRI antidepressant profiles

1.5.1 Fluoxetine

Fluoxetine is prescribed clinically as an SSRI antidepressant. As the first SSRI successfully marketed, fluoxetine was discovered in 1974, entered the market in 1987 and went off patent in 2001 (Wong et al., 1995). It is commonly prescribed for the management of depression and several anxiety disorders. Being an SSRI, fluoxetine increases serotonergic transmission in the synaptic cleft, by selectively binding to the serotonin reuptake transporter (Goodnick and Goldstein, 1998). While an initial dose may begin as low as 10 mg a day before being titrated up, in a typical dosing regimen, fluoxetine is administered orally at a 20-40 mg dose per day, with 60 mg a day for the usual maximum dose to treat depression, or 80 mg a day in the treatment of OCD. With a reasonably high bioavailability, peak plasma concentrations are attained within 6-8 hours (Magni et al., 2013). Fluoxetine is absorbed by the gastrointestinal tract and metabolized in the liver by the cytochrome P450 isoenzyme CYP2D6. It is eliminated through the kidneys, notably slower than other antidepressants, as fluoxetine and its metabolite (norfluoxetine) inhibit its own metabolism. Thus resulting in a 1-3 day single dose half-life and a 4-6 day chronic dose half-life (Magni et al., 2013). After a 4-6 week dosing period, fluoxetine will increase mood and decrease anxiety compared to placebo. Side effects may include nausea, headaches, dizziness, drowsiness, insomnia, fatigue, anxiety, reduced libido, sexual dysfunction or changes in weight. In 1994 fluoxetine surpassed its antidepressant uses and was approved for the treatment of obsessive-compulsive disorder, panic disorder and bulimia (Wong et al., 1995, Etain and Bonnet-Perrin, 2001, Walsh et al., 2000). There are several unlabelled uses for fluoxetine, including the management of anxiety, obesity and binge eating.

1.5.2 Paroxetine

Paroxetine is prescribed clinically as an SSRI antidepressant. Paroxetine first entered the market in 1992 by GlaxoSmithKline and went off patent in 2003. It is commonly prescribed for the management of depression and several anxiety disorders. Being the most potent SSRI, paroxetine increases serotonergic transmission in the synaptic cleft, by selectively binding to the serotonin reuptake transporter (Goodnick and Goldstein, 1998). In a typical dosing regimen for the treatment of depression, paroxetine is administered orally at a 10-40 mg dose per day, with a maximum dose of 50 mg a day.

Anxiety disorders such as obsessive-compulsive disorder may require doses at a maximum of 60 mg a day. With a reasonably high bioavailability, peak plasma concentrations are attained within 2-4 hours after administration (Bayer et al., 1989). Paroxetine is absorbed orally with 50% of the dose entering general circulation. It is then metabolized in the liver by the cytochrome P4502D6 isoenzyme, elimination occurs slowly through the kidneys, as the compound inhibits the activity of the enzyme (Heydorn, 1999). Thus resulting in an 18-24 hour half-life. According to the National Alliance on Mental Illness (NAMI), after a 1-2 week dosing period, physical symptoms such as sleep, energy, and appetite may improve, however, a 6-8 week dosing period is typically required for depressed mood and lack of interest symptoms to fully improve. Side effects may include nausea, headaches, drowsiness, reduced libido and sexual dysfunction. Paroxetine is also noted to have a worse withdrawal reaction compared to other SSRIs. In addition to depression, paroxetine also has a range of off label uses including generalized anxiety disorder, premature ejaculation, diabetic neuropathy, neurocardiogenic syncope, obsessive-compulsive disorder, panic disorder, and social anxiety disorder (Stone et al., 2003, Kroenke et al., 2001, Rocca et al., 1997, Waldinger et al., 1998, Sindrup et al., 1990). The labelling of paroxetine was updated from Category C to D in 2005 when meta-analysis showed that infants exposed within the first-trimester had increase risks for ventricular and atrial septal cardiovascular malformations (Cole et al., 2007, Kallen and Olausson, 2007).

1.5.3 Sertraline

Sertraline is prescribed clinically as an SSRI antidepressant. The development of sertraline began in the early 1970s, chemists working with compounds based on the structure of the neuroleptic chlorprothixene developed a compound called tametraline. Then from tametraline, they derived sertraline in 1977 (Welch, 1995). Sertraline made it to market in the UK in 1990 and the US in 1991, the patent expired in 2006 (Couzin, 2005). It is commonly prescribed for the management of depression and several anxiety disorders. Being the second most potent and selective SSRI, sertraline increases serotonergic transmission in the synaptic cleft, by selectively binding to the serotonin reuptake transporter (Goodnick and Goldstein, 1998). Though it has mild clinical relevance, sertraline is the only SSRI to bind to dopamine transporters, occasionally being described as a serotonin-dopamine reuptake inhibitor (Richelson,

1994). Sertraline is typically administered orally. An initial daily dose of 50 mg is required for most disorders with sertraline. A daily dose of 100 mg can be more effective than the initial dose in clinical practice and some cases may require a dose of 200 mg per day. For the treatment of some anxiety disorders, a dose of 25 mg per day is used; in the case of eating disorders or panic disorder, in the second week of treatment, the dose is often increased to 50 mg a day. With a bioavailability of 44%, sertraline is absorbed slowly through the gastrointestinal tract, reaching maximum plasma concentration after 6-8 hours (Hiemke and Hartter, 2000). Little is known about its metabolism, however, 0.2% is excreted unchanged in the urine and 50% in the faeces (Murdoch and McTavish, 1992). Thus resulting in a 24-36 hour half-life, with males clearing the compound faster than females (Ronfeld et al., 1997). After a 1-2 week dosing period, physical symptoms such as sleep, energy, and appetite may improve, however, a 6-8 week dosing period is typically required for depressed mood and lack of interest symptoms to fully improve. Side effects may include nausea, headaches, dizziness, drowsiness, insomnia, fatigue, anxiety, reduced libido, sexual dysfunction or changes in weight. Compared to other SSRIs, sertraline has higher rates of psychiatric side effects and diarrhoea. In addition to antidepressant effects, unlabelled uses include the treatment of obsessive-compulsive disorder, panic disorder, social phobia, generalized anxiety disorder, premenstrual dysphoric disorder, premature ejaculation, and neurocardiogenic syncope (Waldinger et al., 1998, Grubb et al., 1994, Stone et al., 2003, Hansen et al., 2008, Marjoribanks et al., 2013)

1.5.4 Citalopram

Citalopram is prescribed clinically as an SSRI antidepressant. Citalopram was first discovered by Lundbeck in 1972, entering the market in Denmark in 1989 and the US in 1998, and patent protection ended in 2003. It is commonly prescribed for the management of depression and several anxiety disorders. Being the SSRI with the highest selectivity for serotonin reuptake, citalopram increases serotonergic transmission in the synaptic cleft, by selectively binding to the serotonin reuptake transporter (Owens et al., 1997). In a typical dosing regimen, citalopram is administered orally at a 20-40 mg dose per day. Doses of 60 mg a day have also demonstrated efficacy (Montgomery et al., 2001), however mostly in the case of obsessive-compulsive disorder or severe depression, if lower doses are insufficient.

Furthermore, in the aftermath of a controversial 2011 FDA issued warning of prolonged QTc risks after treatment with citalopram, it has been recommended that patients with pre-existing cardiac risk factors and should be monitored with an electrocardiogram for doses above 40 mg (McCarrell et al., 2019). Unlike other SSRIs, citalopram undergoes minor first-pass metabolism. Further, it has a bioavailability of 80%, allowing for the peak to be reached after 4 hours. Citalopram is metabolized in the liver by the isoenzymes CYP3A4 and CYP2C19 and is eliminated through the kidneys, with a 24-36 hour half-life (Sindrup et al., 1993, Hill and Rang, 2012). After a 4-6 week dosing period, citalopram will increase mood and decrease anxiety compared to placebo. Side effects may include drowsiness, insomnia, nausea, fatigue, reduced libido, sexual dysfunction, or changes in weight. While citalopram is typically prescribed for the management of depression, it also has efficacy in treating fibromyalgia, panic disorder, and generalized anxiety disorder (Anderberg et al., 2000, Perna et al., 2001, Varia and Rauscher, 2002).

	Fluoxetine	Paroxetine	Sertraline	Citalopram
Dose range (mg)	20-40	10-40	25-50	20-40
Max dose (mg)	80	50-60	200	40-60
Bioavailability	60-80%	50%	44%	80%
Peak plasma	6-8 h	2-4 h	6-8 h	4 h
Metabolism	CYP2D6	CYP2D6	CYP2B6	CYP2C19 CYP3A4
Active metabolite	norfluoxetine	none	N-desmethyl-sertraline	dimethyl-citalopram
Half-life	Acute: 24-72 h Chronic: 96-144 h	18-24 h	24-36 h	24-36 h
Elimination	Kidney	Kidney	Renal (0.2%) Faecal (50%)	Kidney

Table 1.6 Summary table comparing SSRI pharmacological profiles. Information in table is based on the findings cited throughout the preceding text in section 1.5. See Ewing et al. (2015) for further information regarding inconsistent patterns of placental antidepressant transfer.

1.6 Clinical outcomes of gestational SSRI exposure

As SSRIs pass the placental barrier, they alter the availability of serotonin, an important growth factor in embryogenesis and critical in brain development (Lauder et al., 2000). Clinically, gestational exposure is associated with increased risks for delivery outcomes including lower birth weights, smaller size in relation to gestational age, preterm birth, and developmental delays (Velasquez et al., 2013). In addition, cardiovascular malformations have been linked to gestational exposure to fluoxetine (Diav-Citrin et al., 2008), paroxetine (Kallen and Otterblad Olausson, 2006), sertraline (Louik et al., 2007), and citalopram (Pedersen et al., 2009). These findings are largely supported by various recent observational case studies. As the highlighted SSRIs only entered the market from 1987-1992, and their usage during pregnancy has only become common in the last 20 years, there is restricted data showing the effects of gestational exposure clinically. The existing data is limited as it is currently unethical to carry out drug studies on pregnant women. Though it is unethical to use pregnant women for a drug trial study, pregnant women are still using SSRIs. For example, Denmark reported that since 1997 a 16-fold increase of antidepressant exposure occurred, with 2010 exposures reaching 3.2% of pregnancies (Kjaersgaard et al., 2013). In the US, it was found that 3.4% of women filled an antidepressant prescription during late pregnancy, with 2.7% being filled for an SSRI (Huybrechts et al., 2015). Therefore case studies have begun tracking the pregnancy and delivery outcomes of gestational SSRI exposure. This research is helpful in aiding clinicians to understand and anticipate many complications caused by the exposure. Studies as those referenced in Figure 1.8, highlight that the period of exposure is critical in predicting the associated risks. For example exposure during the first twelve weeks (trimester) increases risks of congenital cardiovascular malformations. As noted previously in Table 1.1, the first evidence of serotonergic neurons is during the first trimester, with 5-HT present as early as gestational week five, which coincides with the development of the heart at week six. This is important when considering cases where depression manifests within a pregnancy, especially with 5-HT neurons rapidly increasing in the developing brain from the end of the first trimester through to five years of age. However, as they take SSRIs as a whole, they assist clinicians little in determining the best SSRI option for treatment-naïve women. Overall, most studies conclude that SSRIs are a better option compared to other antidepressants or to

depression, but there is a deficit in research which could suggest a particular SSRI. A recent study carried out using Swedish data centres does propose different progeny malformations being associated with individual SSRIs (Reis and Kallen, 2010). However, there is still a lack of data describing the behavioural effects of each of the four SSRIs, featured throughout this thesis, which could impose consequences from gestation throughout adulthood. Furthermore, discrepancies exist between the actual impact of an SSRI, independent from the impact of maternal depression.

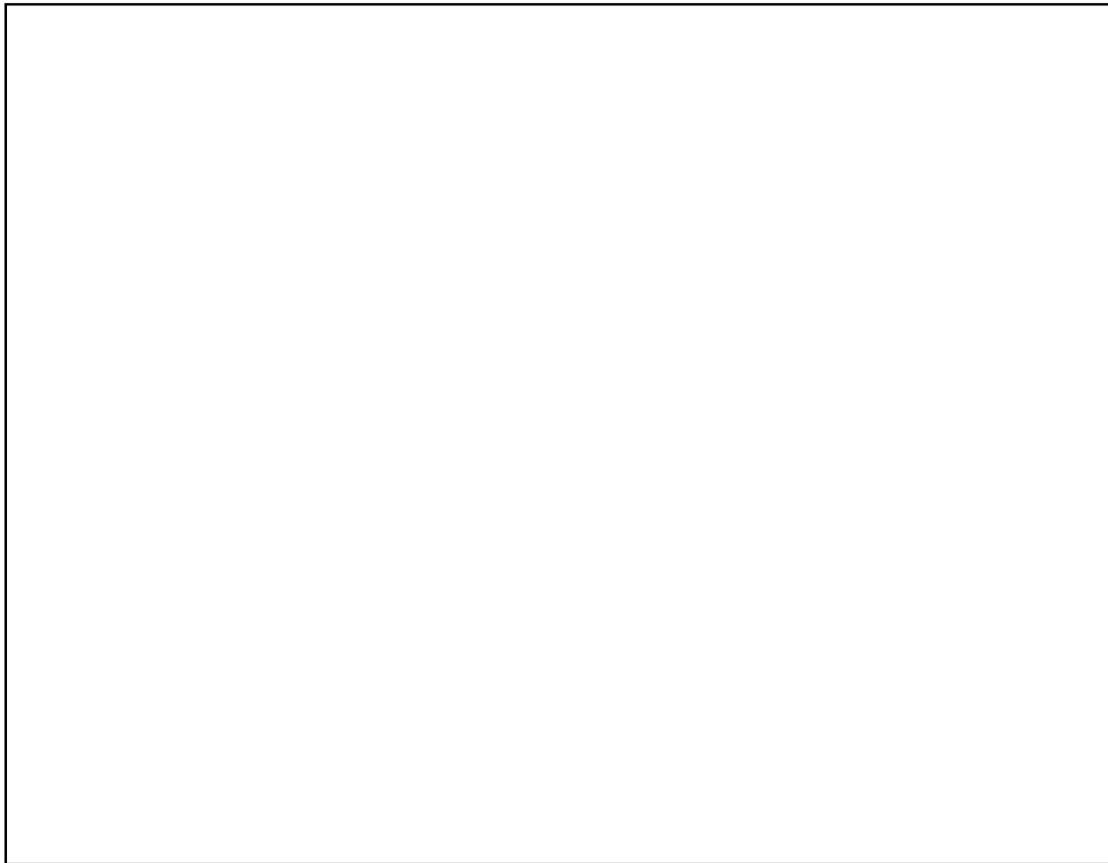


Figure 1.8 SSRI exposure and clinical outcomes. This image was taken from Velasquez et al. (2013) and highlights various clinical outcomes associated with gestational SSRI exposure based on trimester of exposure. PPHN, persistent pulmonary hypertension of the neonate.

1.6.1 Limitations of current clinical studies

A major limitation incurred by clinical studies are the ethical limitations to carry out clinical trials in pregnant women. While it has long been the case to exclude pregnant women from clinical trials, some bioethicists such as Anne Lyerly, Ruth Faden and Maggie Little, believe that this has led to longstanding scientific negligence, depriving

pregnant women and fetuses; as described earlier, often an untreated disease during pregnancy has a greater magnitude than the implications of the treatment. Lyerly, Faden, and Little have founded the 'Second Wave Initiative' project, aimed at developing a legal framework to allow for responsible inclusion of pregnant women in biomedical research (Lyerly et al., 2008). Extraordinarily, Lyerly et al. (2008) have pointed out that while the Thalidomide disaster is often cited as a primary reason not to include pregnant women in clinical trials, ironically, the case is not the result of a clinical trial in pregnant women. Furthermore, had there been a clinical trial in pregnant women for the drug that was prescribed to treat morning sickness, the same tragic birth defects would have occurred, albeit on a much smaller scale. Thus, it is argued that the Thalidomide case should encourage clinical trials rather than discourage such studies (Lyerly et al., 2008). Overall, while there may be a new shift coming in clinical trials, funding for such research is difficult to find as there are few incentives for pharmaceutical companies to carry these studies out, and in fact, the 'ignorance is bliss' approach is in the best interest of the company. Essentially, if a medication has not been approved in pregnancy and it does induce a teratogenic deficit, then the company can hide behind that fact that the use of the medication during pregnancy was 'off label'. Conversely, if the manufacturer does fund drug exposure in pregnancy studies and no birth defects are identified, but after releasing the compound a teratogenic effect is later observed, then they are open to a lawsuit. Therefore the consequences of this accepted, perhaps unethical-ethical standard, to exclude pregnant women from clinical trials, result in continued uncertainty for physicians and patients of SSRI antidepressant use during pregnancy. Aside from this new movement in clinical trials, current data that exists on antidepressant prescribing during pregnancy is limited.

As time goes on, such studies are building up and clinicians are able to more appropriately prescribe SSRIs to treatment-naïve pregnant women and predict labour outcomes. However, despite efforts, there are still many uncontrollable variables in the clinical situation. A primary variable is the pathophysiology of depression and how depression may alter a woman's behaviour, thus causing further complications to pregnancy. Depression is a wide-ranging disorder and the symptoms incurred can differ, thus affecting a pregnancy differently. Consequently, it is difficult to decipher if the delivery outcomes are specific to the depression or the SSRI. For example, in

the large Swedish study mentioned previously, it was concluded that TCA exposure did not significantly differ compared to SSRIs and other antidepressants for many parameters including preterm birth, low birth weight, hypoglycaemia, respiratory diagnosis, low Apgar score, jaundice and cardiac malformations (Reis and Kallen, 2010). However, these findings may be confounded, as the nature of the mother and their particular symptoms of depression are individual, thus requiring a specific antidepressant treatment. Overall, the conclusions for the direct adverse results of particular antidepressants remain uncertain.

Additionally, although treatment may be prescribed, it is common among individuals prescribed antidepressants to be at risk for low compliance behaviour as 50% of both psychiatric and primary care patients discontinue antidepressant use prematurely (Sansone and Sansone, 2012). Noncompliance to antidepressants can be attributed to many features of antidepressants such as side effects and a delay in therapeutic onset (Fortney et al., 2011, Blier, 2003, Kennedy et al., 2008). Similarly, non-adherence can also be attributed to symptoms of depression or the individual's behaviour, such as forgetfulness, low motivation, a misunderstanding of the dosing regimen, their concern for reliance/addiction, and a concern for changes in personality (Bulloch and Patten, 2010, Masand, 2003, Chakraborty et al., 2009). Considering the symptoms of both depression and pregnancy in a clinical setting, it cannot be assumed that self-administration of antidepressant treatment is carried out perfectly. Therefore, clinical evidence of adverse birth effects of antidepressants is confounded by unreliable compliance and consequently unreliable gestational exposure.

In addition to the literature compiling women with a range of depression symptoms, studies will also group women who are prescribed a range of dosages. Thus adding to the inconsistencies of self-administration and the concentration exposed. Overall, it is unclear if the current observations made within the clinical scenario are altered by SSRI, dosage, or the pathophysiology of depression. Further, it is unknown if these outcomes will persist through adulthood. Thus a study investigating the direct effects of each SSRI at a range of dosages and observing various parameters from gestation through adulthood would allow for a better illustration of the implications of each SSRI on a pregnancy and the resulting progeny.

1.7 Preclinical outcomes of gestational SSRI exposure

To account for the confounding variables witnessed in the clinical scenario, preclinical tests are carried out within several rodent models detailed in Table 1.7-Table 1.18. Preclinical studies overcome limitations of the clinical scenario by permitting specified drugs and dosages, as well as confirmation of exposure and avoidance of maternal depressive behaviours. Various studies have been advantageous in uncovering the role of 5-HT in development. From such studies, it is evident that 5-HT directs neuronal connections during foetal and neonatal development (Azmitia, 2001, Bonnin et al., 2007). Additional evidence has shown that SSRIs cross the placental barrier in rats, making them a good model for preclinical studies investigating alterations in serotonin levels during critical periods of development. Furthermore, many regulatory studies have been carried out in animal models to indicate toxic effects of gestational SSRI exposure. Current findings in the animal model note a wide range of findings including cardiac malformations (Yavarone et al., 1993b, Haskell et al., 2017), persistent pulmonary hypotension in the neonate (Hooper et al., 2016, Fornaro et al., 2007), more frequent skin hematomas at birth (Stanford and Patton, 1993), altered thermal threshold (Vartazarmian et al., 2005, Lisboa et al., 2007, Knaepen et al., 2013, Lee, 2009), and nest quality (Svirsky et al., 2016).

In addition to an SSRIs' direct effect on offspring development, exposure *in utero* may also impact how other drugs are processed. This is important to consider as in the clinical context it is not unusual for depressed individuals to be filling multiple prescriptions. An unintended effect of SSRI exposure *in utero* may include their impact on certain regulatory proteins such as Phosphoglycoprotein (P-gp). P-gp is a protein in the placental wall that allows nutrients to be circulated between the embryo and the mother (Bhuiyan et al., 2012). It also acts as a filter, preventing xenobiotics from reaching the foetus. SSRIs inhibit the action of P-gp, and therefore it was assumed that exposure during pregnancy may reduce drug efflux for the placenta and foetal blood-brain barrier. Conversely, when gestational sertraline exposure was evaluated in FVB mice, it was found that it increased P-gp mediated efflux, thus minimizing drugs from entering through the placenta (Bhuiyan et al., 2012). However, this effect was tissue-specific as the opposite was observed in brain tissue. Gestational sertraline exposure caused a decrease in P-gp mediated efflux and thus increased the transfer of drugs in the foetal and maternal brain (Bhuiyan et al., 2012). Therefore,

when prescribing in pregnancy it is important to consider the effects of drug interactions as well as the direct effect of the SSRI.

Multiple approaches have been taken to assess exposure to drugs during pregnancy. The different methodologies used complicate making direct comparisons across four of the clinically used drugs namely, fluoxetine, paroxetine, sertraline, and citalopram. Therefore, the succeeding tables have been compiled which highlight the implications of treatment in regards to various longitudinal periods throughout development to provide a preclinical profile of the four drugs. As fluoxetine has been extensively studied, the first set of tables (Table 1.7-Table 1.12) only feature fluoxetine, whereas the limited findings of paroxetine, sertraline, and citalopram have been combined to make up the following set of tables (Table 1.13-Table 1.18) which in the subsequent text is referred to as 'other SSRIs'. Each particular period is represented in two tables, the first of which are the outcomes of studies observing strict gestational exposure and the latter observe exposure from gestation through lactation or solely the lactational period. Due to a lack of preclinical studies observing the other SSRIs, the extended exposures into lactation results were included to enrich their limited findings and to create a fuller perception of the implications imposed by early-life exposure to SSRIs. The three consequential time periods highlighted are the gestational and neonatal period for dams and pups (FLX Table 1.7-Table 1.8; other SSRIs Table 1.13-Table 1.14), the adolescent period which was after progeny were weaned and before adulthood (FLX Table 1.9-Table 1.10; other SSRIs Table 1.15-Table 1.16), and the progeny adulthood period (FLX Table 1.11-Table 1.12; other SSRIs Table 1.17-Table 1.18). The overall findings portrayed in these tables are in regards to maternal wellbeing, littering characteristics, neonatal development, and offspring behaviour.

Gestational exposure to fluoxetine has induced many effects throughout the three examined periods. Table 1.7 reports reductions in maternal body weight and food consumption in rats exposed to 16 (not 8) mg/kg (IG, GD 15-20) (da-Silva et al., 1999), but not for mice dams exposed to fluoxetine (10 mg/kg, DW) during pregnancy (Bauer et al., 2010). Surprisingly, twelve of the fourteen studies represented in the table neglected to report maternal wellbeing parameters. Furthermore, only two studies measured maternal caregiving behaviours, one of which indicated an increase in contact with the pups; however this effect was only found at the higher dose employed (8 mg/kg) rather than the lower doses (2, 4 mg/kg) (Johns et al., 2005)

inferring dose-dependent findings in relation to fluoxetine. Interestingly, da-Silva et al. (1999) was the only study, of five measuring gestational lengths, which saw a significant reduction. Furthermore nearly half reported an increase in offspring mortality, implying that fluoxetine may have profound effects on neonatal mortality depending on the species as well as the period and dose of exposure. Interestingly, only one of the nine studies which recorded litter size reported a decrease after exposure to 12 mg/kg fluoxetine (GD 11-21) (Olivier et al., 2011) whereas a higher dose and a shorter exposure period (16 mg/kg, GD 15-20) in the same species and via the same route of administration had no effect on litter size (da-Silva et al., 1999), nor did the latter study report increased mortality. This particularly highlights that the period of gestational exposure can impose significant effects on foetal development; underlining the difficulties in comparing studies with different exposure periods. Almost all studies reported birth weights, approximately a third of which indicated a decrease in offspring birth weight; again with differences in the model concerning the route of administration, period of exposure, and dose, likely dictating the differences amongst study observations.

Consequences of fluoxetine exposure *in utero* for adolescent and adult progeny are listed in Table 1.9 and Table 1.11 respectively. Despite the decrease in birth weight mentioned previously, two studies reported effects on adolescent weight and a third study reported an increase in weight compared to controls (Olivier et al., 2011). The discrepancy in weight amongst the three studies features the different influences of species, route, exposure period, and dose. However, when comparing to the birth weights, another contradiction is exposed as birth weights were reported to show a decrease or no treatment-induced differences, whereas the adolescent weights are either consistent or increased. Jumping ahead to the following period, one adulthood study shows no differences in weight and another shows a decrease, reflecting the birth weights more than the adolescent body weights. Overall this supports the importance of continuous assessment longitudinal studies which depict growth at critical developmental milestones. In regards to behavioural measures, increased anxiety was reported in half of the adolescent and adulthood observations (Noorlander et al., 2008, Butkevich and Mikhailenko, 2018, Capello et al., 2011, Olivier et al., 2011). Bairy et al. (2007) reported reduced exploration time for male and female Wistar rats after exposure to fluoxetine (8, 12 mg/kg, IG, GD 6-20) in the adolescent period, and only

at the 12 mg/kg dose in the adulthood period. Neither study measuring acoustic startle for male and female adult progeny found a significant effect of fluoxetine treatment despite the variations amongst each of the study factors represented in the table (Vartazarmian et al., 2005, Vorhees et al., 1994). Further, one study of male and female adolescent Wistar rats indicated that fluoxetine (8, 12 mg/kg, IG, GD 6-20) significantly increased motor skill (Bairy et al., 2007), whereas Long Evans adult rats showed no significant differences in motor skill after gestational fluoxetine exposure (8, 11, 12 mg/kg, OMP, GD12-littering) (Capello et al., 2011). No significant changes in “depressive-like” behaviours and few cognitive defects were noted at either period. Mainly, in the adolescent period spatial memory was enhanced after exposure (8, 12 mg/kg, IG, GD 6-20) in Wistar (Bairy et al., 2007) but not SD rats who had a similar exposure approach (1, 5, 12 mg/kg, IG, GD 7-20) (Vorhees et al., 1994); emphasizing the known differences in strains as Wistar are reportedly less active than SD rats (Asano, 1986), thus the Wistar model could be more sensitive to subtle increases of activity. Considering the two aforementioned studies by Bairy et al. (2007) and Vorhees et al. (1994) in further cognitive parameters of the adolescent period, strain (and dose-dependent) differences are reinforced as the Wistar rat, exposed to 12 but not 8 mg/kg fluoxetine, reduced fear memory, whereas the SD study showed no effect of treatment, regardless of dose (1, 5, 12 mg/kg). Sex and age differences were noted when measuring social interaction after gestational fluoxetine exposure. For example, in adolescent offspring, Svirsky et al. (2016) report a significant increase in social interaction for female CD1 mice, but no change for males after *in utero* exposure (10 mg/kg, SC, GD 1-littering). No reports were found describing fluoxetine induced effects on social interaction for female rats, however, male Wistar rats exposed to fluoxetine (12 mg/kg, IG, GD 11-21) experienced a transient decrease in social behaviours during the adolescent period, however, behaviours were normalized in adulthood (Olivier et al., 2011). Fluoxetine studies neglected to represent exposure in females in regards to aggressive and sexual behaviours. Nevertheless, 10 mg/kg fluoxetine was found to increase male aggressive behaviours in adolescent mice (SC, GD 1-littering) and adult rats (IP, GD 13-21) (Svirsky et al., 2016, Singh et al., 1998). Furthermore, two different routes (SC, IG) and exposure periods (GD 13-20, GD 11-21) to 12 mg/kg indicated no change in sexual behaviours (Cagiano et al., 2008, Olivier et al., 2011). Overall, such findings emphasize the effects of factors such as

species, route, exposure period, dose, and sex are important features in dictating the consequences as well as the particular SSRI employed.

Species	Route	Exposure period	Dose (mg/kg)	Change	Reference
Gestational length					
Mouse (129/SvEvTac)	DW	Pregnancy	10	↔	(Bauer et al., 2010)
Rat	SC	GD13-20	5, 10	↔	(Cagiano et al., 2008)
Rat (SD)	IG	GD7-20	1, 5, 12	↔	(Vorhees et al., 1994)
Rat (Wistar)	IG	GD6-20	8, 12	↔	(Bairy et al., 2007)
Rat (Wistar)	IG	GD15-20	8, 16	↓	(da-Silva et al., 1999)
Mortality					
Mouse (C57BL/6J)	IP	GD8-18	0.3, 0.6, 0.8	↑	(Noorlander et al., 2008)
Rat (Fischer)	IG	GD6-15	2, 5, 12.5	↔	(Byrd and Markham, 1994)
Rat (SD)	IG	GD7-20	1, 5	↔	(Vorhees et al., 1994)
Rat (SD)	IG	GD7-20	12	↑	(Vorhees et al., 1994)
Rat (SD)	IG	GD11-21	10	↑	(Fornaro et al., 2007)
Rat (Wistar)	IG	GD15-20	8, 16	↔	(da-Silva et al., 1999)
Rat (Wistar)	IG	GD6-20	8, 12	↔	(Bairy et al., 2007)
Litter size					
Mouse (129/SvEvTac)	DW	Pregnancy	10	↔	(Bauer et al., 2010)
Mouse (ICR (CD1))	SC	GD1-littering	10	↔	(Svirsky et al., 2016)
Rat	SC	GD13-20	5, 10	↔	(Cagiano et al., 2008)
Rat (SD)	IG	GD7-20	1, 5, 12	↔	(Vorhees et al., 1994)
Rat (SD)	SC	GD13-20	10	↔	(Cabrera and Battaglia, 1994)
Rat (Wistar)	IG	GD6-20	12	↔	(Bairy et al., 2007)
Rat (Wistar)	IG	GD11-21	12	↓	(Olivier et al., 2011)
Rat (Wistar)	IG	GD15-20	8, 16	↔	(da-Silva et al., 1999)
Rat (Wistar)	PO	GD0-21	7	↔	(Taghizadeh et al., 2016)
Birth weight					
Mouse (C57BL/6J)	IP	GD8-18	0.3, 0.6, 0.8	↔	(Noorlander et al., 2008)
Mouse (ICR (CD1))	SC	GD1-littering	10	↔	(Svirsky et al., 2016)
Rat (Fischer)	IG	GD6-15	2, 5, 12.5	↔	(Byrd and Markham, 1994)
Rat (SD)	IG	GD7-20	1, 5	↔	(Vorhees et al., 1994)
Rat (SD)	IG	GD7-20	12	↓	(Vorhees et al., 1994)
Rat (SD)	IG	GD11-21	10	↔	(Fornaro et al., 2007)
Rat (SD)	SC	GD13-20	5, 10 / 10	↓	(Cagiano et al., 2008, Cabrera and Battaglia, 1994)
Rat (Wistar)	IG	GD15-20	8, 16	↔	(da-Silva et al., 1999)
Rat (Wistar)	IG	GD6-20	8	↔	(Bairy et al., 2007)
Rat (Wistar)	IG	GD6-20	12	↓	(Bairy et al., 2007)
Rat (Wistar)	IP	GD9-20	10	↓	(Butkevich and Mikhailenko, 2018)
Parental observations					
<i>Maternal body weight</i>					
Mouse (129/SvEvTac)	DW	Pregnancy	10	↔	(Bauer et al., 2010)
Rat (Wistar)	IG	GD15-20	8	↔	(da-Silva et al., 1999)
Rat (Wistar)	IG	GD15-20	16	↓	(da-Silva et al., 1999)
<i>Maternal food consumption</i>					
Rat (Wistar)	IG	GD15-20	8	↔	(da-Silva et al., 1999)
Rat (Wistar)	IG	GD15-20	16	↓	(da-Silva et al., 1999)
Maternal caregiving behaviours					
<i>Contact with pups</i>					
Rat (SD)	SC	GD1-20	2, 4	↔	(Johns et al., 2005)
Rat (SD)	SC	GD1-20	8	↑	(Johns et al., 2005)
<i>Grooming pups</i>					
Rat (SD)	SC	GD1-20	2, 4, 8	↔	(Johns et al., 2005)
<i>Pup retrieval</i>					
Mouse (ICR (CD1))	SC	GD1-littering	10	↔	(Svirsky et al., 2016)
<i>Self-care</i>					
Rat (SD)	SC	GD1-20	2, 4, 8	↔	(Johns et al., 2005)
Somatic development-Pinna unfolding and eye opening					
Rat (Wistar)	IG	GD6-20	8, 12	↔	(Bairy et al., 2007)
Behavioural development-Negative geotaxis (PND 8, then 10 and 12)					
Rat (Wistar)	IG	GD6-20	8, 12	↔	(Bairy et al., 2007)
Rat (Wistar)	IG	GD6-20	8, 12	↓	(Bairy et al., 2007)

Table 1.7 Teratogenic effects of gestational exposure to fluoxetine. Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	Change	Reference
<i>Gestational length</i>					
Rat (Wistar)	IG to dam	GD7-PND21	0.4, 1.7, 17	↔	(Muller et al., 2013)
<i>Mortality</i>					
Rat (Wistar)	IG to dam	GD7-PND21	0.4, 1.7, 17	↑	(Muller et al., 2013)
<i>Litter size</i>					
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	↔	(Lisboa et al., 2007, Favaro et al., 2008)
Rat (Wistar)	IG to dam	GD7-PND21	0.4, 1.7, 17	↔	(Muller et al., 2013)
Rat (Wistar)	OMP to dam	GD14-PND7	10	↔	(Forcelli and Heinrichs, 2008)
Rat (Wistar)	PO to dam	GD(-14)-PND21	10	↔	(De Long et al., 2015a)
<i>Sex ratio</i>					
Rat (Wistar)	IG to dam	GD7-PND21	0.4, 1.7, 17	↔	(Muller et al., 2013)
<i>Birth weight</i>					
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	↔	(Lisboa et al., 2007, Favaro et al., 2008)
Rat (SD)	SC to pup	GD6-PND20	5	↓	(Sprowles et al., 2017)
Rat (Wistar)	IG to dam	GD7-PND21	0.4, 1.7, 17	↓	(Muller et al., 2013)
Rat (Wistar)	OMP to dam	GD14-PND7	10	↔	(Forcelli and Heinrichs, 2008)
Rat (Wistar)	PO to dam	GD0-PND21	5	↔	(Francis-Oliveira et al., 2013, Dos Santos et al., 2016)
Rat (Wistar)	PO to dam	GD(-14)-PND21	10	↔	(De Long et al., 2015a)
<i>Parental observations</i>					
<i>Maternal body weight</i>					
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	↔	(Favaro et al., 2008)
Rat (Wistar)	IG to dam	GD7-PND21	0.4, 1.7	↔	(Muller et al., 2013)
Rat (Wistar)	IG to dam	GD7-PND21	17	↓	(Muller et al., 2013)
<i>Maternal behaviour</i>					
<i>Nursing-Active</i>					
Rat (SD)	OMP	PND1-PND28	5	↑	(Pawluski et al., 2012)
<i>Nursing-Passive</i>					
Rat (SD)	OMP	PND1-PND28	5	↔	(Pawluski et al., 2012)
<i>Nursing-Total</i>					
Rat (SD)	PO via food wafer	GD10-PND21	5	↑	(Gemmel et al., 2018a)
Rat (SD)	OMP	PND1-PND28	5	↑	(Pawluski et al., 2012)
<i>Grooming pups</i>					
Rat (SD)	OMP	PND1-PND28	5	↔	(Pawluski et al., 2012)
<i>Somatic development</i>					
<i>External genitalia</i>					
Rat (SD)	OMP	PND1-21	5	↔	F (Rayen et al., 2013)
Rat (SD)	OMP	PND1-21	5	↓	M (Rayen et al., 2013)
<i>Behavioural development</i>					
Mouse (C57BL/6J)	PO with saccharin	GD0-PND14	16	↓	(Maloney et al., 2018)

Table 1.8 Teratogenic effects of gestational and lactational exposure to fluoxetine.

Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	Sex	Change	Reference
Body weight						
Mouse (C57BL/6J)	IP	GD8-18	0.3, 0.6, 0.8	M,F	↔	(Noorlander et al., 2008)
Rat (SD)	SC	GD13-20	10	M,F	↔	(Cabrera and Battaglia, 1994)
Rat (Wistar)	IG	GD11-21	12	M,F	↑	(Olivier et al., 2011)
Anxiety						
Mouse (C57BL/6J)	IP	GD8-18	0.3, 0.6, 0.8	M,F	↔	(Noorlander et al., 2008)
Mouse (C57BL/6J)	IP	GD8-18	0.8	M,F	↑	(Noorlander et al., 2008)
Rat (Wistar)	IG	GD6-20	8, 12	M,F	↔	(Bairy et al., 2007)
Rat (Wistar)	IP	GD9-20	10	F	↑	(Butkevich and Mikhailenko, 2018)
Exploration						
Mouse (C57BL/6J)	IP	GD8-18	0.3, 0.6, 0.8	M,F	↔	(Noorlander et al., 2008)
Mouse (ICR (CD1))	SC	GD1-littering	10	M,F	↔	(Svirsky et al., 2016)
Rat	SC	GD13-20	5, 10	M,F	↔	(Cagiano et al., 2008)
Rat (SD)	IG	GD7-20	1, 5	M,F	↔	(Vorhees et al., 1994)
Rat (Wistar)	IG	GD6-20	8, 12	M,F	↓	(Bairy et al., 2007)
Depressive behaviour						
Rat (Wistar)	IP	GD9-20	10	F	↔	(Butkevich and Mikhailenko, 2018)
Cognition						
<i>Novel object recognition</i>						
Mouse (ICR (CD1))	SC	GD1-littering	10	M,F	↔	(Svirsky et al., 2016)
<i>Spatial memory</i>						
Rat (SD)	IG	GD7-20	1, 5, 12	M,F	↔	(Vorhees et al., 1994)
Rat (Wistar)	IG	GD6-20	8, 12	M,F	↑	(Bairy et al., 2007)
Rat (Wistar)	IP	GD9-20	10	F	↔	(Butkevich and Mikhailenko, 2018)
<i>Fear memory</i>						
Rat (SD)	IG	GD7-20	1, 5, 12	M,F	↔	(Vorhees et al., 1994)
Rat (Wistar)	IG	GD6-20	8	M,F	↔	
Rat (Wistar)	IG	GD6-20	12	M,F	↓	(Bairy et al., 2007)
Motor skill						
Rat (Wistar)	IG	GD6-20	8, 12	M,F	↑	(Bairy et al., 2007)
Social interaction						
Mouse (ICR (CD1))	SC	GD1-littering	10	M	↔	(Svirsky et al., 2016)
Mouse (ICR (CD1))	SC	GD1-littering	10	F	↑	(Svirsky et al., 2016)
Rat (Wistar)	IG	GD11-21	12	M	↓	(Olivier et al., 2011)
Aggressive behaviour						
Mouse (ICR (CD1))	SC	GD1-littering	10	M	↑	(Svirsky et al., 2016)

Table 1.9 Adolescent effects of gestational exposure to fluoxetine. Behavioural effects occurring after weaning (PND 21) and before adulthood (PND 56). Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	Sex	Change	Reference
Body weight						
Mouse (C5BL/6J)	IP to pup	PND4-21	10	M,F	↓	(Karpova et al., 2009)
Rat (SD)	PO to dam	GD0-PND21	5	M	↔	(Gemmel et al., 2018b)
Rat (SD)	PO to dam	GD0-PND21	5	F	↓	(Gemmel et al., 2018b)
Rat (SD)	SC to pup	GD6-PND20	5	M,F	↓	(Sprowles et al., 2017)
Rat (Wistar)	PO to dam	G13-PND21	10, 20	M	↓	(de Oliveira et al., 2013)
Anxiety						
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	M,F	↔	(Lisboa et al., 2007)
Mouse (Swiss)	OG	GD0-PND21	7.5	M,F	↔	(Favaro et al., 2008)
Rat (Wistar)	IG to dam	GD0-PND21	5	M,F	↓	(Francis-Oliveira et al., 2013)
Rat (Wistar)	OG	GD0-PND21	5	M	↔	(Toffoli et al., 2014)
Rat (Wistar)	PO to dam	Pregnancy & lactation	5	M,F	↔	(Matsumoto et al., 2016)
Rat (Wistar)	SC to pup	PND0-4	20	M	↔	(Lee and Lee, 2012)
Exploration						
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	F	↔	(Lisboa et al., 2007)
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	M	↓	(Lisboa et al., 2007)
Rat (SD)	OMP	PND1-21	5	M,F	↔	(Rayen et al., 2011)
Rat (Long Evans)	SC to pup	PND8-21	5	F	↔	(Rodriguez-Porcel et al., 2011)
Rat (Long Evans)	SC to pup	PND8-21	5	M	↓	(Rodriguez-Porcel et al., 2011)
Rat (Wistar)	SC to pup	PND0-4	20	M	↔	(Lee and Lee, 2012)
Rat (Wistar)	SC to pup	PND0-4	10 / 20	M,F /M	↓	(Lee, 2009, Lee and Lee, 2012)
Rat Wistar	IG to dam	GD0-PND21	7.5	M	↓	(Vieira et al., 2013)
Depressive behaviour						
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	M	↔	(Lisboa et al., 2007)
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	F	↑	(Lisboa et al., 2007)
Rat (SD)	OMP	PND1-21	5	M,F	↔	(Rayen et al., 2011)
Rat (Wistar)	IG to dam	GD0-PND21	5	M/F	↔	(Francis-Oliveira et al., 2013)
Cognition						
<i>Novel object recognition</i>						
Rat (Wistar)	OG to dam	GD11-PND7	12	M	↔	(Kroeze et al., 2016)
Motor skill						
Rat (Wistar)	SC to pup	PND0-4	10 / 20	M,F /M	↓	(Lee, 2009, Lee and Lee, 2012)
Social interaction						
Rat (Long Evans)	SC to pup	PND8-21	5	F	↔	(Rodriguez-Porcel et al., 2011)
Rat (Long Evans)	SC to pup	PND8-21	5	M	↓	(Rodriguez-Porcel et al., 2011)
Rat (SD)	PO to dam	GD0-PND21	5, 10	M,F	↔	(Gemmel et al., 2017)
Aggressive behaviour						
Rat (SD)	PO to dam	GD0-PND21	5, 10	M,F	↔	(Gemmel et al., 2017)
Reproductive development						
Rat (Wistar)	PO to dam	GD1-PND21	5	F	↓	(Dos Santos et al., 2016)
Rat (Wistar)	PO to dam	GD13-PND21	5, 10, 20	M	↓	(de Oliveira et al., 2013)
Thermal threshold						
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	M,F	↔	(Lisboa et al., 2007)
Rat (SD)	PO to dam	GD21-PND21	10	M	↔	(Knaepen et al., 2013)
Rat (Wistar)	SC to pup	PND0-4	10	M,F	↑	(Lee, 2009)

Table 1.10 Adolescent effects of gestational and lactational exposure to fluoxetine. Behavioural effects occurring after weaning (PND 21) and before adulthood (PND 56). Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	Sex	Change	Reference
Body weight						
Mouse (C57BL/6J)	IP	GD8-18	0.3, 0.6, 0.8	M,F	↔	(Noorlander et al., 2008)
Rat	SC	GD13-20	10	M/F	↓	(Cagiano et al., 2008)
Anxiety						
<i>Elevated plus maze</i>						
Mouse (C57BL/6J)	IP	GD8-18	0.3, 0.6	M/F	↔	(Noorlander et al., 2008)
Mouse (C57BL/6J)	IP	GD8-18	0.8	M/F	↑	(Noorlander et al., 2008)
Rat (Wistar)	IG	GD11-21	12	M	↔	(Olivier et al., 2011)
<i>Open field</i>						
Mouse (C57BL/6J)	IP	GD8-18	0.3, 0.6, 0.8	M,F	↑	(Noorlander et al., 2008)
<i>Novelty-suppressed feeding/drinking</i>						
Rat (Long Evans)	OMP	GD12-littering	8, 11, 12	M,F	↑	(Capello et al., 2011)
Rat (Wistar)	IG	GD11-21	12	M	↑	(Olivier et al., 2011)
<i>Defensive withdrawal test / Marble burying</i>						
Rat (Long Evans)	OMP	GD12-littering	8, 11, 12	M,F	↔	(Capello et al., 2011)
Exploration						
Mouse (C57BL/6J)	IP	GD8-18	0.3, 0.6, 0.8	M,F	↔	(Noorlander et al., 2008)
Rat	SC	GD13-20	10	M	↔	(Cagiano et al., 2008)
Rat (SD)	IG	GD7-20	1, 5, 12	M,F	↔	(Vorhees et al., 1994)
Rat (Wistar)	IG	GD6-20	8	M,F	↔	(Bairy et al., 2007)
Rat (Wistar)	IG	GD6-20	12	M,F	↓	(Bairy et al., 2007)
Rat (Wistar)	IG	GD11-21	12	M	↔	(Olivier et al., 2011)
Depressive behaviour						
Rat (Wistar)	IG	GD11-21	12	M	↔	(Olivier et al., 2011)
Acoustic startle						
Guinea pig (Hartley)	OMP	pregnancy	5	M/F	↔	(Vartazarmian et al., 2005)
Rat (SD)	IG	GD7-20	1, 5, 12	M/F	↔	(Vorhees et al., 1994)
Cognition						
<i>Reward, Fear memory</i>						
Rat	SC	GD13-20	10	M	↔	(Cagiano et al., 2008)
Motor skill						
Rat (Long Evans)	OMP	GD12-littering	8, 11, 12	M/F	↔	(Capello et al., 2011)
Social interaction						
Rat (Wistar)	IG	GD11-21	12	M	↔	(Olivier et al., 2011)
Aggressive behaviour						
Rat	IP	GD13-GD21	10	M	↑	(Singh et al., 1998)
Sexual behaviour						
Rat	SC	GD13-20	12	M	↔	(Cagiano et al., 2008)
Rat (Wistar)	IG	GD11-21	12	M	↔	(Olivier et al., 2011)

Table 1.11 Adult effects of gestational exposure to fluoxetine. Behavioural effects occurring in adulthood (after PND 56). Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	Sex	Change	Reference
Body weight						
Mouse (C5BL/6)	DW to dam	GD15-PND12	25	F	↓	(McAllister et al., 2012)
Mouse (C5BL/6J)	IP to pup	PND4-21	10	M/F	↓	(Karpova et al., 2009)
Rat (SD)	PO to dam	GD0-PND21	5	M,F	↔	(Gemmel et al., 2018b)
Rat (SD)	SC to pup	GD6-PND20	5	M,F	↓	(Sprowles et al., 2017)
Rat (Wistar)	PO to dam	GD(-14)-PND21	10	M	↔	(De Long et al., 2015a)
Rat (Wistar)	PO to dam	GD(-14)-PND21	10	F	↑	(De Long et al., 2015a)
Rat (Wistar)	PO to dam	GD1-PND21	5	F	↔	(Dos Santos et al., 2016)
Anxiety						
<i>Elevated plus maze</i>						
Mouse	IP to pup	PND4-21	10	M,F	↔	(Ansorge et al., 2004, Ansorge et al., 2008)
Mouse (C5BL/6)	DW to dam	GD15-PND12	25	F	↓	(McAllister et al., 2012)
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	M,F	↔	(Lisboa et al., 2007)
Rat (Wistar)	OMP to dam	GD14-PND7	10	M,F	↔	(Forcelli and Heinrichs, 2008)
Rat (Wistar)	PO to dam	GD0-PND21	5	M	↔	(Silva et al., 2018)
Rat (Wistar)	SC	PND0-4	20	M	↑	(Ko et al., 2014)
<i>Open field</i>						
Mouse (C5BL/6)	DW to dam	GD15-PND12	25	F	↔	(McAllister et al., 2012)
Mouse (C5BL/6J)	IP to pup	PND4-21	10	M,F	↔	(Ansorge et al., 2008, Karpova et al., 2009, Zheng et al., 2011)
<i>Light-dark box</i>						
Mouse (C5BL/6J)	IP to pup	PND4-21	10	M	↔	(Karpova et al., 2009)
Rat (SD)	DW to dam	PND2-21	17.2	M	↔	(Nagano et al., 2012)
<i>Novelty-suppressed feeding/drinking</i>						
Rat (Wistar)	IG to dam	GD0-PND21	5	M/F	↔	(Francis-Oliveira et al., 2013)
Rat (Wistar)	SC to pup	PND0-4	20	M	↑	(Lee and Lee, 2012)
<i>Defensive withdrawal test / Marble burying</i>						
Rat (SD)	SC to pup	GD6-PND20	5	M,F	↑	(Sprowles et al., 2017)
Exploration						
Mouse (C5BL/6J)	DW to dam	GD15-PND12	25	F	↔	(McAllister et al., 2012)
Mouse (C5BL/6J)	IP to pup	PND4-21	10	M,F	↓	(Ansorge et al., 2004, Ansorge et al., 2008, Karpova et al., 2009, Zheng et al., 2011)
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	M,F	↔	(Lisboa et al., 2007)
Rat (SD)	DW to dam	PND2-21	17.2	M	↔	(Nagano et al., 2012)
Rat (SD)	PO to dam	GD21-PND21	10	M	↔	(Knaepen et al., 2013)
Rat (Wistar)	OMP to dam	GD14-PND7	10	M,F	↓	(Forcelli and Heinrichs, 2008)
Depressive behaviour						
Mouse (C5BL/6)	DW to dam	GD15-PND12	25	F	↓	(McAllister et al., 2012)
Mouse (C5BL/6J)	IP to pup	PND4-21	10	M	↓	(Karpova et al., 2009)
Mouse (NMRI)	DW to dam	GD10-PND20	8	M	↔	(Salari et al., 2016)
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	M	↔	(Lisboa et al., 2007)
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	F	↑	(Lisboa et al., 2007)
Rat (Wistar)	IG to dam	GD0-PND21	5	M/F	↔	(Francis-Oliveira et al., 2013)
Rat (Wistar)	SC	PND0-4	20	M	↑	(Ko et al., 2014)
Acoustic startle						
Mouse (C5BL/6)	DW to dam	GD15-PND12	25	F	↔	(McAllister et al., 2012)
Rat (SD)	SC to pup	GD6-PND20	5	M/F	↑	(Sprowles et al., 2017)
Rat (Wistar)	SC	PND0-4	20	M	↓	(Ko et al., 2014)
Cognition						
<i>Novel object recognition</i>						
Rat (Wistar)	OG to dam	GD11-PND7	12	M	↔	(Kroeze et al., 2016)
<i>Spatial memory</i>						
Mouse (C5BL/6)	DW to dam	GD15-PND12	25	F	↔	(McAllister et al., 2012)
Rat (SD)	SC to pup	GD6-PND20	5	M/F	↑	(Sprowles et al., 2017)
<i>Reward, Fear memory</i>						
Mouse	IP to pup	PND4-21	10	M,F	↓	(Ansorge et al., 2004, Ansorge et al., 2008)
<i>Reward, Reinforcement by cocaine</i>						
Rat (Wistar)	OMP to dam	GD14-PND7	10	M,F	↑	(Forcelli and Heinrichs, 2008)

Motor skill						
Mouse (C5BL/6)	DW to dam	GD15-PND12	25	F	↔	(McAllister et al., 2012)
Rat (SD)	SC to pup	GD6-21 & PND1-20	5	M/F	↑	(Sprowles et al., 2017)
Rat (Wistar)	SC	PND0-4	20	M	↓	(Ko et al., 2014)
Social interaction						
Rat (Long Evans)	SC to pup	PND8-21	5	M,F	↓	(Rodriguez-Porcel et al., 2011)
Rat (SD)	PO to dam	GD10-PND21	10	M,F	↑	(Gemmel et al., 2019)
Rat (Wistar)	PO to dam	GD0-PND21	5	M	↓	(Silva et al., 2018)
Rat (Wistar)	SC	PND0-4	20	M	↑	(Ko et al., 2014)
Aggressive behaviour						
Mouse (Swiss)	IG to dam	GD0-PND21	7.5	M	↔	(Lisboa et al., 2007)
Sexual behaviour						
Mouse (Swiss)	IG	GD0-PND21	7.5	M	↓	(Gouvea et al., 2008)
Rat (Long Evans)	SC to pup	PND8-21	5	M	↓	(Rodriguez-Porcel et al., 2011)
Rat (SD)	OMP to dam	PND1-21	5	F	↑	(Rayen et al., 2014)
Rat (SD)	OMP to dam	PND1-21	5	M	↓	(Rayen et al., 2013)
Rat (Wistar)	IG to dam	GD1-PND21	7.5	M	↔	(Vieira et al., 2013)
Rat (Wistar)	PO to dam	GD1-PND21	5	F	↔	(Dos Santos et al., 2016)
Reproductive development						
Rat (Wistar)	PO to dam	GD1-PND21	5	F	↔	(Dos Santos et al., 2016)
Rat (Wistar)	PO to dam	G13-PND21	5, 10	M	↔	(Monteiro Filho et al., 2014)
Rat (Wistar)	PO to dam	G13-PND21	20	M	↓	(Monteiro Filho et al., 2014)
Circadian behaviour						
<i>Phase-advance to light</i>						
Mouse (C57BL/6)	DW to dam	GD15-PND12	25	M	↑	(Kiryanova et al., 2013)
<i>Free-running period</i>						
Mouse (C57BL/6)	DW to dam	GD15-PND12	25	M	↓	(Kiryanova et al., 2013)

Table 1.12 Adult effects of gestational and lactational exposure to fluoxetine. Behavioural effects occurring in adulthood (after PND 56). Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	SSRI	Change	Reference
Gestational length						
Mouse (CD1-1)	PO	GD(-14)-GD16	30	PRX	↔	(Rayburn et al., 2000)
Rat (Fischer)	PO	GD15-21	10	PRX	↓	(Van den Hove et al., 2008)
Rat (Wistar)	SC	GD0-littering	10	SERT	↔	(De Long et al., 2015)
Mortality						
Mouse (C57BL/6)	SC	3rd trimester	5	CIT	↔	(Hsiao et al., 2005)
Rat (Fischer)	PO	GD15-21	10	PRX	↑	(Van den Hove et al., 2008)
Mortality before PND 4						
Rat (SD)	PO	GD(-64)/GD(-14) until GD14sac/littering	10	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	GD(-64)/GD(-14) until GD14sac/littering	40, 80	SERT	↑	(Davies and Klowe, 1998)
Rat (SD)	PO	GD(-71)/GD(-15) until GD20sac/littering	10	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	GD(-71)/GD(-15) until GD20sac/littering	20, 80	SERT	↑	(Davies and Klowe, 1998)
Rat (SD)	PO	GD0-5 or 10 or 15	80	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	GD0-littering	80	SERT	↓	(Davies and Klowe, 1998)
Mortality after PND 4						
Rat (SD)	PO	GD(-64)/GD(-14) until GD14sac/littering	10, 40, 80	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	GD(-71)/GD(-15) until GD20sac/littering	10, 20, 80	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	GD0-5 or 10 or 15 or littering	80	SERT	↔	(Davies and Klowe, 1998)
Still born						
Rat (Wistar)	SC	GD0-littering	10	SERT	↔	(De Long et al., 2015b)
Embryo mortality						
Mouse (ICR whole embryo)	culture	GD9-12	20uM	SERT	↑	(Shuey et al., 1992)
Maternal toxicity						
Rabbit (NZ White)	PO	organogenesis-c-section	40	SERT	↑	(Davies and Klowe, 1998)
Pregnancy success rate						
Rat (SD)	PO	GD(-64)/GD(-14) until GD14sac/littering	10, 40	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	GD(-64)/GD(-14) until GD14sac/littering	80	SERT	↓	(Davies and Klowe, 1998)
Rat (SD)	PO	GD(-71)/GD(-15) until GD20sac/littering	10, 20, 80	SERT	↔	(Davies and Klowe, 1998)
Rat (Wistar)	SC	GD0-littering	10	SERT	↔	(De Long et al., 2015)
Ossification of foetuses						
Rabbit (NZ White)	PO	organogenesis-c-section	40	SERT	↓	(Davies and Klowe, 1998)
Rat (SD)	PO	organogenesis-c-section	10, 20, 80	SERT	↓	(Davies and Klowe, 1998)
Embryo toxicity						
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↔	(Rayburn et al., 2000)
Mouse (ICR whole embryo)	culture	GD9-12	5, 10, 20 uM	SERT	↔	(Shuey et al., 1992)
Rabbit (NZ White)	PO	organogenesis-c-section	5, 20, 40	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	organogenesis-c-section	10, 20, 80	SERT	↔	(Davies and Klowe, 1998)
Teratogenicity						
Rabbit (NZ White)	PO	organogenesis-c-section	5, 20, 40	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	GD(-71)/GD(-15) until GD20sac/littering	10, 20, 80	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	organogenesis-c-section	10, 20, 80	SERT	↔	(Davies and Klowe, 1998)
Litter size						
Mouse (C57BL/6)	SC	3 rd trimester	5	CIT	↔	(Hsiao et al., 2005)
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↔	(Rayburn et al., 2000)
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↔	(Coleman et al., 1999)
Mouse (Wild-derived outbred house mice)	PO (dam & males)	Breeding-littering	22.5	PRX	↔	(Gaukler et al, 2015)
Rat (Wistar)	SC	GD0-littering	10	SERT	↑	(De Long et al., 2015)
Sex ratio						
Mouse (C57BL/6)	SC	3 rd trimester	5	CIT	↔	(Hsiao et al., 2005)
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↔	(Coleman et al., 1999)

<i>Pup weights</i>						
<i>Birth weight</i>						
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↓	(Rayburn et al., 2000)
Rat (Fischer)	PO	GD15-21	10	PRX	↓	(Van den Hove et al., 2008)
Rat (SD)	PO	GD(-64)/GD(-14) until GD14sac/littering	80	SERT	↓	(Davies and Klowe, 1998)
Rat (SD)	PO	GD(-71)/GD(-15) until GD20sac/littering	20, 80	SERT	↓	(Davies and Klowe, 1998)
Rat (Wistar)	SC	GD0-littering	10	SERT	↔	(De Long et al., 2015)
<i>Small for gestational age</i>						
Rat (Wistar)	SC	GD0-littering	10	SERT	↑	(De Long et al., 2015)
<i>Birth-PND 4 weight</i>						
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↓	(Coleman et al., 1999)
Rat (SD)	PO	GD(-64)/GD(-14) until GD14sac/littering	10, 40, 80	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	GD0-5 or 10 or 15	80	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO	GD0-littering	80	SERT	↓	(Davies and Klowe, 1998)
<i>PND 4-PND 21 weight</i>						
Rat (SD)	PO	GD(-71)/GD(-15) until GD20sac/littering	10, 20, 80	SERT	↔	(Davies and Klowe, 1998)
<i>Parental observations</i>						
<i>Maternal body weight</i>						
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↔	(Rayburn et al., 2000)
Rat (SD)	PO	organogenesis-c-section	20, 80	SERT	↓	(Davies and Klowe, 1998)
<i>Paternal body weight</i>						
Mouse (Wild-derived outbred house mice)	PO (dam & males)	Breeding-littering	22.5	PRX	↓	(Gaukler et al., 2015)
<i>Paternal reproductive performance</i>						
Mouse (Wild-derived outbred house mice)	PO (dam & males)	Breeding-littering	22.5	PRX	↓	(Gaukler et al., 2015)
Rat (SD)	PO	GD(-71)/GD(-15) until GD20sac/littering	10, 20, 80	SERT	↔	(Davies and Klowe, 1998)
<i>Maternal caregiving behaviours</i>						
<i>Nursing-Active</i>						
Rat (SD)	OG	GD(-16)-littering	20	SERT	↔	(Kott et al., 2018)
<i>Nursing-Passive</i>						
Rat (SD)	OG	GD(-16)-littering	20	SERT	↔	(Kott et al., 2018)
<i>Nursing-Total</i>						
Rat (SD)	OG	GD(-16)-littering	20	SERT	↔	(Kott et al., 2018)
<i>Out of nest</i>						
Rat (SD)	OG	GD(-16)-littering	20	SERT	↔	(Kott et al., 2018)
<i>Somatic development</i>						
<i>External genitalia</i>						
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↔	(Rayburn et al., 2000)
<i>Eye opening</i>						
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↔	(Rayburn et al., 2000)
<i>Head width</i>						
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↓	(Rayburn et al., 2000)
<i>Incisor eruption</i>						
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↔	(Rayburn et al., 2000)
<i>Separation vocalization</i>						
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↑	(Coleman et al., 1999)
<i>Geotaxis</i>						
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	↔	(Coleman et al., 1999)

Table 1.13 Teratogenic effect of gestational exposure to paroxetine, sertraline, or citalopram. Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	SSRI	Change	Reference
Gestational length						
<i>Labour duration</i>						
Rat (SD)	IP to dam	GD12-PND2	10	SERT	↑	(Craft et al., 2010)
Mortality						
<i>Mortality before PND 4</i>						
Rat (SD)	PO to dam	GD15-PND20	80	SERT	↑	(Davies and Klowe, 1998)
<i>Mortality after PND 4</i>						
Rat (SD)	PO to dam	GD15-PND20	80	SERT	↑	(Davies and Klowe, 1998)
Litter size						
Rat (SD)	IP to dam	GD12-PND2	10	SERT	↓	(Craft et al., 2010)
Pup weights						
<i>Birth weight</i>						
Rat (SD)	PO to dam	GD15-PND20	20, 80	SERT	↓	(Davies and Klowe, 1998)
<i>PND 4-PND 21 weight</i>						
Mouse (C57BL/6)	IP to dam, IP to pup	GD0-littering, PND1-14	5, 1.5	SERT	↑	(Gemmel et al., 2018a)
Rat (Long Evans)	SC to pup	PND8-21	10, 20	CIT	↓ (M)	(Harris et al., 2012)
Rat (SD)	PO to dam	GD15-PND20	80	SERT	↓	(Davies and Klowe, 1998)
Rat (SD)	PO to dam	GD15-PND20	20, 80	SERT	↓	(Davies and Klowe, 1998)
Rat (SD)	SC to pup	GD6-21 & PND1-20	5	CIT	↓	(Sprowles et al., 2017)
Rat (SD)	SC to pup	PND11-20	5, 7.5	CIT	↓ (M)	(Schaefer et al., 2013)
Parental observations						
<i>Maternal body weight</i>						
Rat (SD)	PO to dam	GD15-PND20	20, 80	SERT	↓	(Davies and Klowe, 1998)
Rat (SD)	PO to dam	GD15-PND20	80	SERT	↓	(Davies and Klowe, 1998)
Rat (SD)	SC to pup	GD6-21 & PND1-20	10	CIT	↔	(Sprowles et al., 2016)
<i>Maternal food and water consumption</i>						
Rat (SD)	PO to dam	GD15-PND20	20, 80	SERT	↓	(Davies and Klowe, 1998)
<i>Dam activity</i>						
Rat (SD)	PO to dam	GD15-PND20	20, 80	SERT	↑	(Davies and Klowe, 1998)
Somatic development						
<i>External or visceral anomalies</i>						
Rat (SD)	PO to dam	GD15-PND20	10, 20, 80	SERT	↔	(Davies and Klowe, 1998)
Rat (SD)	PO to dam	GD15-PND20	80	SERT	↔	(Davies and Klowe, 1998)

Table 1.14 Teratogenic effects of gestational and lactational exposure to paroxetine, sertraline, or citalopram. Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	SSRI	Sex	Change	Reference
Anxiety							
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	M,F	↔	(Coleman et al., 1999)
Mouse (CD-1)	PO	GD(-14)-littering	30	PRX	M,F	↔	(Christensen et al, 2000)
Exploration							
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	M,F	↔	(Coleman et al., 1999)
Mouse (CD-1)	PO	GD(-14)-littering	30	PRX	M,F	↔	(Christensen et al, 2000)
Social interaction							
Mouse (CD-1)	PO	GD(-14)-GD16	30	PRX	M,F	↔	(Coleman et al., 1999)

Table 1.15 Adolescent effects of gestational exposure to paroxetine, sertraline, or citalopram. Behavioural effects occurring after weaning (PND 21) and before adulthood (PND 56). Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	SSRI	Sex	Change	Reference
Body weight							
Mouse (C57BL/6)	IP to dam, IP to pup	GD0-littering, PND1-14	5 dam, 1.5 pup	SERT	M,F	↔	(Meyer et al., 2018)
Rat (Long Evans)	SC to pup	PND8-21	5, 10, 20	CIT	M	↔	(Harris et al., 2012)
Rat (SD)	PO to dam	GD15-PND20	10, 20, 80	SERT	M,F	↔	(Davies and Klowe, 1998)
Rat (SD)	SC to pup	GD6-PND20	5 / 10	CIT	M,F	↓	(Sprowles et al., 2016, Sprowles et al., 2017)
Rat (SD)	SC to pup	PND11-20	5, 7.5	CIT	M	↓	(Schaefer et al., 2013)
Exploration							
<i>Novel object approach</i>							
Rat (Long Evans)	SC to pup	PND8-21	10	CIT	M	↓	(Rodriguez-Porcel et al., 2011)
Rat (Long Evans)	SC to pup	PND8-21	10	CIT	F	↔	(Rodriguez-Porcel et al., 2011)
Motor skill							
Rat (Long Evans)	SC to pup	PND8-21	10	CIT	M,F	↓	(Rodriguez-Porcel et al., 2011)
Social interaction							
Rat (Long Evans)	SC to pup	PND8-21	10	CIT	M,F	↓	(Rodriguez-Porcel et al., 2011)

Table 1.16 Adolescent effects of gestational and lactational exposure to paroxetine, sertraline, or citalopram. Behavioural effects occurring after weaning (PND 21) and before adulthood (PND 56). Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	SSRI	Sex	Change	Reference
Anxiety							
<i>Elevated plus maze</i>							
Mouse (C57BL/6)	IP to dam	GD13-21	20	CIT	M,F	↑	(Zahra et al., 2018)
Mouse (C57BL/6)	SC to dam	3 rd trimester	5	CIT	M,F	↔	(Hsiao et al., 2005)
Mouse (CD-1)	PO to dam	GD(-14)-GD16	30	PRX	M,F	↔	(Coleman et al., 1999)
Rat (SD)	OG	GD(-16)-littering	20	SERT	M,F	↔	(Kott et al., 2019)
<i>Defensive withdrawal test</i>							
Rat (Long Evans)	OMP	GD12-littering	3, 8	PRX	M,F	↔	(Capello et al., 2011)
Rat (Long Evans)	OMP	GD12-littering	7, 10	SERT	M,F	↔	(Capello et al., 2011)
Exploration							
Mouse (C57BL/6)	IP to dam	GD13-21	20	CIT	M,F	↓	(Zahra et al., 2018)
Mouse (CD-1)	PO to dam	GD(-14)-GD16	30	PRX	M,F	↔	(Coleman et al., 1999)
Depressive behaviour							
Mouse (CD-1)	PO to dam	GD(-14)-GD16	30	PRX	M,F	↔	(Coleman et al., 1999)
Rat (SD)	OG	GD(-16)-littering	20	SERT	M,F	↔	(Kott et al., 2019)
Cognition							
<i>Novel object recognition</i>							
Rat (SD)	OG	GD(-16)-littering	20	SERT	M,F	↔	(Kott et al., 2019)
<i>Spatial memory</i>							
Mouse (CD-1)	PO to dam	GD(-14)-littering	30	PRX	M,F	↔	(Christensen et al., 2000)
<i>Fear memory</i>							
Mouse (CD-1)	PO to dam	GD(-14)-littering	30	PRX	M,F	↔	(Christensen et al., 2000)
<i>Reward, Reinforcement by cocaine</i>							
Mouse (C57BL/6)	SC to dam	3 RD trimester	5	CIT	M,F	↔	(Hsiao et al., 2005)
Motor skill							
Mouse (C57BL/6)	SC to dam	3 rd trimester	5	CIT	M,F	↔	(Hsiao et al., 2005)
Rat (Long Evans)	OMP	GD12-littering	3, 8	PRX	M,F	↔	(Capello et al., 2011)
Rat (Long Evans)	OMP	GD12-littering	7, 10	SERT	M,F	↔	(Capello et al., 2011)
Social interaction							
Mouse (C57BL/6)	IP to dam	GD13-21	20	CIT	M,F	↓	(Zahra et al., 2018)
Aggressive behaviour							
Mouse (CD-1)	PO to dam	GD(-14)-GD16	30	PRX	M,F	↑	(Coleman et al., 1999)
Reproductive success							
Mouse (CD-1)	PO to dam	GD(-14)-GD16	30	PRX	M,F	↔	(Rayburn et al., 2000)/(Coleman et al., 1999)
Rat (SD)	PO to dam	GD(-64)/GD(-14)-GD14sac/littering	10, 40, 80	SERT	M,F	↔	(Davies and Klowe, 1998)
Rat (SD)	PO to dam	GD(-71)/GD(-15)-GD20sac/littering	10, 20, 80	SERT	M,F	↔	(Davies and Klowe, 1998)

Table 1.17 Adult effects of gestational exposure to paroxetine, sertraline, or citalopram. Behavioural effects occurring in adulthood (after PND 56). Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

Species	Route	Exposure period	Dose (mg/kg)	SSRI	Sex	Change	Reference
Body weight							
Mouse (C57BL/6)	IP to dam, IP to pup	GD0-littering, PND1-14	5 dam, 1.5 pup	SERT	M,F	↔	(Meyer et al., 2018)
Rat (Long Evans)	SC to pup	PND8-21	10	CIT	M	↓	(Harris et al., 2012)
Rat (SD)	SC to pup	GD6-21, PND1-20	5 /10	CIT	M,F/M	↓	(Sprowles et al., 2017) / (Sprowles et al., 2016)
Rat (SD)	SC to pup	GD6-21, PND1-20	10	CIT	F	↔	(Sprowles et al., 2016)
Rat (SD)	SC to pup	PND11-20	5, 7.5	CIT	M	↓	(Schaefer et al., 2013)
Anxiety							
<i>Elevated plus maze</i>							
Mouse (C57BL/6)	IP to dam, IP to pup	GD0-littering, PND1-14	5 dam, 1.5 pup	SERT	M,F	↔	(Meyer et al., 2018)
Rat (Long Evans)	SC to pup	PND8-21	5, 10, 20	CIT	M	↔	(Harris et al., 2012)
Rat (Wistar)	DW to dam	GD7-PND21	10	CIT	M	↑	(Zohar et al., 2016)
Rat (Wistar)	DW to dam	GD7-PND21	10	CIT	F	↔	(Zohar et al., 2016)
<i>Open field</i>							
Rat (SD)	SC to pup	GD6-21, PND1-20	10	CIT	M,F	↑	(Sprowles et al., 2016)
<i>Marble burying</i>							
Rat (SD)	SC to pup	GD6-21, PND1-20	5,10	CIT	M,F	↑	(Sprowles et al., 2017) / (Sprowles et al., 2016)
Exploration							
Mouse (C57BL/6)	IP to dam, IP to pup	GD0-littering, PND1-14	5 dam, 1.5 pup	SERT	M,F	↔	(Meyer et al., 2018)
Depressive behaviour							
Rat (SD)	SC to pup	GD6-21, PND1-20	10	CIT	M,F	↑	(Sprowles et al., 2016)
Rat (Wistar)	DW to dam	GD7-PND21	10	CIT	M,F	↑	(Zohar et al., 2016)
Acoustic startle							
Rat (SD)	SC to pup	GD6-21, PND1-20	5/10	CIT	M,F	↑	(Sprowles et al., 2017) / (Sprowles et al., 2016)
Cognition							
<i>Spatial memory</i>							
Mouse (C57BL/6)	IP to dam, IP to pup	GD0-littering, PND1-14	5 dam, 1.5 pup	SERT	M,F	↔	(Meyer et al., 2018)
Rat (SD)	SC to pup	GD6-21, PND1-20	5/10	CIT	M,F	↓	(Sprowles et al., 2017) / (Sprowles et al., 2016)
Rat (SD)	SC to pup	PND11-20	5, 7.5	CIT	M	↓	(Schaefer et al., 2013)
<i>Egocentric learning</i>							
Rat (SD)	SC to pup	GD6-21, PND1-20	10	CIT	M,F	↔	(Sprowles et al., 2016)
Rat (SD)	SC to pup	PND11-20	5, 7.5	CIT	M	↓	(Schaefer et al., 2013)
Motor skill							
Rat (Long Evans)	SC to pup	PND8-21	5	CIT	M	↑	(Maciag et al., 2006)
Rat (SD)	SC to pup	GD6-21, PND1-20	5	CIT	M,F	↑	(Sprowles et al., 2017)
Social interaction							
Mouse (C57BL/6)	IP to dam & IP to pup	GD0-littering, PND1-14	5 dam, 1.5 pup	SERT	M,F	↔	(Meyer et al., 2018)
Rat (Long Evans)	SC to pup	PND8-21	10	CIT	M,F	↓	(Rodriguez-Porcel et al., 2011)
Rat (SD)	SC to pup	GD6-21, PND1-20	10	CIT	M,F	↔	(Sprowles et al., 2016)
Sexual behaviour							
Rat (Long Evans)	SC to pup	PND8-21	10, 20 / 5 / 10	CIT	M	↓	(Harris et al., 2012)/(Maciag et al., 2006)/(Rodriguez-Porcel et al., 2011)

Table 1.18 Adult effects of gestational and lactational exposure to paroxetine, sertraline or citalopram.

Behavioural effects occurring in adulthood (after PND 56). Observational change indicated by arrows; ↔, no change; ↑, increase; ↓, decrease.

1.7.1 Limitations of current preclinical studies

Although regulatory studies have illustrated risks of gestational SSRI exposure, they have done so without always mimicking the clinical experience as much as possible. As such studies often use toxic doses, it is difficult to translate littering outcomes of therapeutic doses. Additionally, many regulatory studies record gross malformations upon birth, rather than enduring consequences that occur after neonatal development. Likewise, often various methods of drug exposure are used, rather than the clinically relevant oral exposure. Furthermore, as fluoxetine is one of the oldest successfully marketed SSRIs, the majority of preclinical research has been developed solely using fluoxetine as a predictor of all SSRIs. Further, this over-representation of fluoxetine also strays from the clinical situations, as paroxetine, sertraline and citalopram are also commonly prescribed SSRIs and clinical results are beginning to indicate that these four SSRIs will affect pregnancy differently (Reis and Kallen, 2010). This can be highlighted by the number of studies featured in the fluoxetine tables (Table 1.7-Table 1.12), compared to the combined paroxetine, sertraline, and citalopram tables (Table 1.13-Table 1.18). Although continuous assessment has been completed in some fluoxetine studies, current research scarcely considers the longitudinal effects of the other frequently prescribed SSRIs. Furthermore, it is very difficult to compare the outcomes of studies as various species, routes, and exposure periods have been employed in most studies, along with an over-representation of fluoxetine. In some cases, there is also a deficit in studies assessing both male and female offspring. Overall rodent studies closely modelling the clinical experience and comparing all four featured SSRIs under consistent study designs would be advantageous in understanding the individual longitudinal consequences experienced by subjects from the gestational through the adulthood period.

Of note, many studies within Table 1.7-Table 1.18, regardless of exposure period or dose, seemed to assess anxiety parameters over others, and many also observed depressive behaviours. In addition to the fluoxetine studies, citalopram also frequently exhibited increased “anxiety-” and “depressive-like” behaviours in adulthood. This is interesting to note, as the clinical scenario often suggests that untreated maternal depression can produce similar behaviours in the progeny as well (Oberlander et al., 2006). Therefore, if progeny are pre-exposed to SSRI antidepressants *in utero* that also have an anxiolytic feature, it could suggest an altered response to anxiolytic and

antidepressants later in adulthood. Furthermore altered responses to psychotropic drugs such as diazepam and desipramine in adulthood when the brain is fully developed, implies altered GABAergic or noradrenergic and serotonergic neurotransmissions, respectively. Interestingly, of the four SSRIs studied, only one other study could be found which assessed the progeny in regards to anxiolytic response to diazepam after fluoxetine exposure (see Favaro et al. (2008)). Therefore additional studies in this area would be beneficial to better inform clinicians treating psychiatric disorders in adults exposed to particular SSRI antidepressants *in utero*.

1.8 Rat model for gestational psychotropic drug exposure

Preclinical evaluation of drugs is an essential aspect of drug discovery and development, as well as teratogenicity studies. Animal models are commonly employed to measure pharmacological and toxicological propitiates of novel drugs. Such studies can also be carried out to the *in utero* experience, to model drug exposure throughout the gestational period. Although FDA ratings characterise most SSRIs as schedule C, suggesting that they can impact pregnancy, lactation and reproductive potential, in many cases exposure during gestation is necessary. Therefore preclinical studies are carried out observing the toxicological effects. However current studies fail to mimic the clinical experience, with major limitations including dose selection, route of administration, duration, and clinical defects evaluated. Therefore the concept of safety pharmacology has emerged on the idea that differences in organ function should be assessed, similar to how organ structure is assessed in toxicity studies, and resulting undesirable outcomes should be examined after a therapeutic dose range and above, exposure (Bass et al., 2004). Safety pharmacology measures are important in assessing the CNS as a function of behaviour, locomotor activity/coordination, sensorimotor reflexes and pain perception (Hamdam et al., 2013). In particular, severe CNS adverse effects account for approximately 10% of drugs taken off the market worldwide (Fung, 2001), therefore companies are encouraged to use such safety pharmacology assessments earlier in the drug discovery process as it is beneficial both financially and time-wise (Pugsley et al., 2008). Overall, the following study is designed to assess pharmacological doses to produce translatable work to the clinical scenario, rather than toxic exposure *in utero*. To calculate pharmacological doses an allometric scale can be used to determine appropriate doses. The body surface area (BSA) normalization method is suggested. Across various mammalian species, the BSA accounts for several biological factors including oxygen utilization, caloric expenditure, basal metabolism, blood volume, circulating, plasma proteins, and renal function. Thus, the allometric scale is an important tool when attempting to extrapolate data from the clinical to the preclinical scenario and *vice versa*; it accounts for the body weight and surface area (Km) of the human or animal to determine the appropriate dose (Reagan-Shaw et al., 2008). A relevant dose extrapolation is important when considering SSIR use as there are various interspecies differences in regards to CYP-mediated drug metabolism. This is particularly relevant for isoforms

CYP2C, -2D, and 3A, which as noted in Table 1.6, are required for the metabolism of SSRIs (Martignoni et al., 2006).

$$\text{Human dose (mg/kg)} = \text{Animal dose (mg/kg)} \times \frac{\text{Animal Km}}{\text{Human Km}}$$

Figure 1.9 Allometric scale dose conversion formula. Animal Km=6, Human Km=37.

Human Dose (mg)	Rat Dose (mg)
20	2.06
50	5.14
75	7.71
160	16.44
200	20.56

Table 1.19 Translation of human to animal doses using allometric scale. Conversions are calculated using above formula reported by Reagan-Shaw et al. (2008). Human dose (mg) based on 60 kg human.

1.8.1 Dose selection

Selecting appropriate doses is an important aspect of developing a model which mimics the clinical experience. As noted previously, many studies employ only toxic doses, which does not hold true to the clinical experience. Across the four SSRIs under investigation, clinical prescribing begins with an initial dose of 20-25 mg per day, and max dose will range depending on the SSRI in question and the therapeutic treatment required (60-200 mg per day). The intention of the model was to use a range of doses within the therapeutic range, generating a dose response. Parameters used to determine the highest doses include a reduction in body weight gain and a noticeable increase in embryo-foetal mortality. Such doses would not typically be used in the clinical setting. Thus in attempts to mimic the clinical scenario, doses were chosen that were below this range.

1.8.2 Route of administration

Though many preclinical studies use SC routes of administration, clinical administration of SSRIs, exposing progeny *in utero*, occurs through oral administration. Therefore an oral route is necessary to best mimic the clinical situation.

Current studies feature different routes of oral administration including via gavage, intragastric, or with the dose incorporated in the food, a wafer or the drinking water. The gavage has been criticised, as it requires the animals to be restrained. The stress of dosing during the gestational period could result in early termination, litter rejection, or behavioural deficits in the pups. However proper training and administration, as well as a proper vehicle group, can ameliorate such criticism. Furthermore, studies in this lab have reported that oral gavage administration had no effects on maternal wellbeing or litter characteristics such as dam body weight gain, gestational length, litter size, or neonatal mortality (McDonnell-Dowling et al., 2017). These findings are not previously observed as habituation to the oral gavage occurs due to the chronic exposure regime. Furthermore, handling the rats daily the week prior to gavage administration provides habituation to the handling and thus enables an easier transition into gavage dosing (Turner et al., 2012). Meanwhile, supplying the drug through the drinking water does not allow for the precise weight based dosing achieved in the gavage method. Furthermore, a sweetener is sometimes necessary to ensure the solution is palatable and will be consumed. This is problematic as an originally intended features of this study observe diabetic components of SSRI exposure *in utero*, and the introduction of the sweet solution creates risk for gestational diabetes. Furthermore, integration of the dose into food or wafers requires surveillance of animals which would not be possible due to the size of the study. Moreover, it does not portray the clinical scenario, and whether the drug was administered through the drinking water or the food, it is difficult to confirm that the full dose is consumed at the specified time imposed by the dosing schedule. Logistically speaking, it is difficult to ensure the total dose is consumed through the drinking water or food when spills in the cage are common and the drug may also impact water consumption or food consumption. Thus the gavage method was used, with daily handling, for both the SSRI and the vehicle-treated mothers. This method allows for a strict dosing regimen where animals can be dosed the complete and appropriate amount each day. Furthermore, for the two weeks leading up to dosing, females were handled each day to acclimate the rats to the researcher.

1.8.3 Duration

While the length of gestation in rats is approximately 21-22 days, different critical periods can be extrapolated to the 40 week human experience. Gestational day (GD) 0-10 parallels the first trimester of human pregnancy and GD 11-littering corresponds to the second trimester of human pregnancy. Subsequently, postnatal day (PND) 0-10, coincides with the third trimester clinically. There are various clinical exposure scenarios that can be modelled for gestational exposure to SSRIs. Such circumstances and their relevant exposure periods can include: (1) a woman taking an SSRI becomes pregnant resulting in a continuation of treatment; (2) a pregnant woman becomes depressed requiring the commencement of an SSRI during gestation; (3) a postpartum mother becomes depressed, imposing SSRI-treatment after birth. While all these scenarios are relevant this study features the second scenario, depression manifesting in pregnancy and consequently SSRI-treatment manifesting in pregnancy. The first scenario, of a depressed woman becomes pregnant, was not chosen to be modelled as it was mentioned in previous sections that it is not recommended for women who are stable on antidepressants to change their medication as the withdrawal rates are very high. In the case of post-partum depression, exposure is limited to lactation if the mother chooses to nurse and even so a smaller portion of the drug is actually transferred. Therefore it is important to especially consider the second scenario, as the mother is treatment-naïve and the foetus is undergoing critical brain development. Henceforth, rats were administered SSRI antidepressants at a range of pharmacological doses from GD 7-21.

1.8.4 Clinical defects modelled in animal corollaries

There are various progeny outcomes witnessed clinically that can be modelled in preclinical studies. Such clinical consequences could include changes in typical maternal parameters or typical neonatal development, difficulties with anxiety and motor function at different developmental periods, challenges with learning and memory, risk of developing depression, and impaired drug-induced behavioural response. The rodent corollaries of these clinical symptoms are illustrated in Table 1.20. Specifics of the rodent corollaries are detailed in Chapter 2, description of their individual justification and clinical relevance to this model of SSRI drug exposure *in utero* are further explained in the following subsections (1.1.1.1-1.8.4.4).

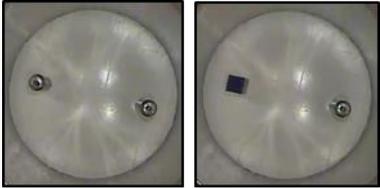
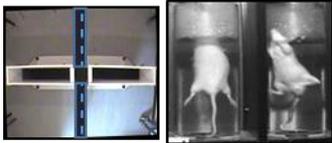
Clinical defect	Rodent corollary	Corollary illustration
Maternal parameters and birth outcomes	Body weight, food and water consumption. Caregiving behaviour, gestational length, litter size, sex ratio, mortality, and birth weight.	
Neonatal development	Fur appearance, pinna unfolding, eye opening, ano-genital distance, body length, weight gain, surface righting, negative geotaxis, and forelimb grip.	
Anxiety and ambulation	Elevated plus maze and open field.	
Cognition	Novel object recognition.	
Depression-anhedonia	Saccharin preference test.	
Psychotropic responses	Elevated plus maze with diazepam. Forced swim test with desipramine.	

Table 1.20 Clinical defects and animal corollaries. Rodent models used in the work to observe various parameters at different critical developmental milestones. Rats present in areas highlighted in blue indicate less “anxiety-like” behaviour.

1.8.4.1 Maternal parameters and birth outcomes

Multiple parameters were used to assess maternal wellbeing such as maternal body weight, food and water consumption. These were monitored throughout the entire perinatal period. Monitoring the whole period is critical to determine possible harmful effects of SSRIs on the dams at both initiation and withdrawal of the treatment. Similar to the clinical experience, dams are expected to gain a considerable amount of weight throughout their pregnancy. Therefore an inhibition or reduction in weight gain from treatment implies a toxic effect of the dose on maternal wellbeing. Secondly, the dams' ability to support a typical pregnancy is considered by observing the effects of treatment on the maternal litter characteristics of gestational length and litter size. Furthermore, lasting implications of SSRI use and cessation on the dam are also examined by surveying the maternal behaviour postpartum to ensure the usual profile of caregiving behaviours are exhibited. Moreover, pup litter characteristics such as sex ratio, mortality, and birth weights are observed after *in utero* SSRI exposure, within the first week postnatal. Lastly, an effect of sex was considered, by recording the sex ratio and pup body weights of males and females at birth.

1.8.4.2 Neonatal development in the rat

Somatic development monitoring is critical for recognizing possible teratogenic effects on the physical development of the SSRI exposed offspring, especially as clinical reports suggest an association between *in utero* exposure to SSRIs and developmental delays (Pedersen et al., 2010). Observations of somatic development are explained in detail in Chapter 2. Concisely, note is taken regarding fur appearance, pinna unfolding, eye opening, ano-genital distance, body length, and body weight at various critical time points. The alteration of such features offers a window into the physical development of the pups. For example, the first feature considered is fur appearance, as it is typically apparent within the first 2-3 days following littering. Alopecia in rats can be associated with endocrine diseases and is often an indication of stress (Botchkarev, 2003, Peters et al., 2006). Furthermore, a hormonal influence has been noted to affect fur growth and appearance (Paul et al., 2007). In the rat pup, a delay in fur growth may also indicate a maternal insufficiency, possibly signifying inadequate lactational nutrition or deprivation of nursing altogether as previous reports have shown such delays in pups of malnourished dams compared to those born of dams receiving a normal protein diet (Khanna et al., 1991). Additionally, eye opening

in rodents produces an illustration of neocortical development in response to sensory stimulation. The maturation of the visual cortex hastens after the eyes open, triggering synaptic potentiation, transmission, plasticity, and refinement, thus a delay or advance in eye opening could imply significant effects on visual cortex development (Lu and Constantine-Paton, 2004, Guan et al., 2017). Ano-genital distance is often recorded in rodent models, as male ano-genital distance is nearly twice the size of females, similar to the sexual dimorphism observed clinically (Salazar-Martinez et al., 2004). It is also important to consider ano-genital distance, as alterations in ano-genital distance have been a marker of adult reproductive disorders, fertility difficulties, altered testosterone levels, and endocrine disruption clinically and in rodent models (Pereira et al., 2006, Welsh et al., 2008). Consequently, reduced ano-genital distance has been associated with infertility in males whereas longer ano-genital distance has been linked to normal reproductive potential (Eisenberg et al., 2011). Altered measurements in new-borns have also been correlated with body weight and length (Sathyanarayana et al., 2010). In rats, reduced ano-genital distance is associated with defects in the reproductive tract such as hypospadias, altered testicular descent, and a smaller testicular volume (Wolf et al., 1999). Furthermore, exposure to androgens during the perinatal period can alter ano-genital distance, which may lead to adverse reproductive consequences (Gray et al., 2001, Mylchreest et al., 1998). Collectively, somatic measurements enable the investigation of the physical maturation and development of the offspring, as well as the ability to predict consequences in adulthood.

Further, this study considers the behaviour of the pups as well to uncover possible lasting implications on sensorimotor coordination, vestibular function, or righting and gripping reflexes in pups exposed to the SSRIs *in utero*. Administration of the behavioural tests is described in previous chapters, briefly, behavioural tests include surface righting, negative geotaxis, and forelimb grip. The first behavioural test observed following littering is the surface righting reflex, which is carried out to measure the progression of the pups righting mechanisms and the development of vestibular function (Khan et al., 2004, Mesquita et al., 2007). Surface righting is similar to the labyrinthine righting reactions observed clinically which measure the development of posture and movement by observing the alignment and relationship of the head and torso of the infant. This reflex also relies on the vestibular system to process the sensory information, triggering movement and ultimately a change in

position. Later, negative geotaxis is measured, which is employed to measure sensorimotor progression (Mesquita et al., 2007). Lastly, the sensorimotor and vestibular function is further observed via the forelimb grip, which indicates the pups gripping reflex and forelimb strength (Sousa et al., 2006). Taking all of these behavioural measurements together enables the examination of sensorimotor maturation and the development of vestibular function or reflexes of the resulting exposed progeny, in addition to anticipating complications throughout the life span.

1.8.4.3 Adolescent and adulthood behaviours in the rat

1.8.4.3.1 Anxiety and ambulation

Anxiety and motor function can be assessed at critical time points throughout development, translating to adolescence, early and late adulthood periods. Monitoring these three periods allows for the interpretation of the progression or regression of induced gestational exposure effects. The elevated plus maze (EPM) and the open field (OF) are the models used to measure anxiety and ambulatory behaviour. Briefly, the EPM and OF are based on the exploitation of the behaviour of the rat, conflicted between their natural tendency for exploration versus thigmotaxis, the phenomenon by which the rat remains close to the wall within each arena. The EPM is a common preclinical model used to detect the anxiolytic properties of drug treatments. Raised off the floor, with two open arms and two closed (walled) arms, it relies on the tendency of the animal to avoid the brighter open arms and explore the darker closed arms. This test has been validated in rodent models across labs as a measure of anxiety (Pellow et al., 1985). More specifically, the EPM has also been assessed in Sprague-Dawley albino rats for sensitivity to serotonin-altering drugs (McDermott and Kelly, 2008). Prior to the development of the EPM, the OF was developed in 1932 to illustrate emotionality of rodents, based on the inclination and ability of the rat to explore an open space (Hall and Ballachey, 1932). Originally developed as a square field, a circular arena is commonly used to assess anxiolytic effects. The bright field with reflective walls stimulates anxiety in rats, and thus the time spent in the outer ring signifies an anxiogenic effect. In addition to measuring anxiety, the OF can also be used to assess induced motor deficits after drug exposure, by monitoring overall distance moved within the arena and the number of entries between the zones. Ultimately, the aversion to exploring the bright open spaces translates to the clinical experience of anxiety.

1.8.4.3.2 Cognition

Cognition is measured in adulthood by evaluating learning and memory of the exposed offspring. As previously mentioned, serotonin has an important role in cognitive function, thus this is an important parameter to consider. The novel object recognition (NOR) test is used to assess object recognition. The task was first described as a measure of recognition memory (Ennaceur and Delacour, 1988) and relies on the natural tendency of rats to spend more exploration time with the novel rather than the familiar object (Berlyne, 1950). Equal time spent between the two objects would suggest a deficit in recognition memory. Such a technique mirrors the visual object recognition tests carried out clinically which measures the capacity to recognize the physical properties of an object, such as shape, colour, size, orientation or texture. One example is method M of the Benton Visual Retention Test, which assesses short-term memory by allowing subjects to view a geometric shape or design for 10 seconds, then the image is removed and the subject must choose the correct match from four possible answer choices, a correct answer would imply adequate short-term visual retention (Benton, 1983, Benton, 1994). Furthermore, inadequate recognition processes can also suggest improperly developed or cooperating visual and prefrontal cortices. Overall, such tests offer a window into the brain, illuminating the lasting implications of treatment exposure on brain structure and function in adulthood.

1.8.4.3.3 Anhedonia

Anhedonia is a symptom of depression, required for a clinical diagnosis of MDD (American Psychiatric Association., 2013). It was first termed in 1896 and refers to a defect in hedonic function and results in an inability to feel pleasure in pleasurable activities (Rizvi et al., 2016). To measure anhedonia in rats, the saccharin preference test (SPT) is employed, which measures the tendency of the rat to seek out the pleasurable saccharin solution versus plain water, as a percentage of total fluid consumption. This model depends on the natural hedonic tendency of the rat to favour sweet solutions compared to plain solutions (Willner, 2005). If a “depressive-like” anhedonia effect is present, a reduction in the percentage of saccharin preference is observed compared to the control animals. As mentioned previously, clinical evaluations of depression are often questionnaires, and such questions often refer to desire or motivation (Rizvi et al., 2016). An example of a question referring to anhedonia from the previously described EPDS scale would include “I have looked

forward with enjoyment to things” (Cox et al., 1987). Overall, anhedonia is a consistent symptom of depression, which can be measured in preclinical and clinical scenarios.

1.8.4.4 Psychotropic drug response

1.8.4.4.1 Anxiolytic response

To assess the efficacy of diazepam, an anxiolytic compound, in SSRI exposed progeny, the EPM was used again. In addition to measuring baseline anxiety behaviours, the EPM is a standard preclinical model proved to detect the anxiolytic properties of drug treatments across labs (Pellow et al., 1985, Pellow and File, 1986, Lister, 1987). Diazepam, a benzodiazepine first marketed in 1963, is used clinically for its therapeutic anxiolytic features; which are accomplished by acting as a positive allosteric modulator of the GABA_A receptor, binding to the benzodiazepine site of the GABA receptor, thereby increasing GABA affinity and consequently GABAergic effects (Julien et al., 2011). Therefore, it is expected to reduce baseline “anxiety-like” behaviours in rats, which are often manifest as thigmotaxis. While an inadequate response would suggest compromised efficacy, it also proposes that GABAergic neurotransmission was altered by SSRI exposure *in utero*, and this deficit was not overcome in the fully developed adult brain.

1.8.4.4.2 Antidepressant response

To assess the efficacy of the antidepressant desipramine, the forced swim test (FST) was employed as it is the most common preclinical technique for the assessment of antidepressant drug activity (Lucki, 1997). Guided by the behavioural despair principle, the animal is exposed to the threat of drowning and their behavioural reaction to the situation, or absence of activity, is correlated to negative mood. Developed in 1977, the model has been validated for use with various drugs and rodents (Porsolt et al., 1978b). Reliably, the model illustrates that when rodents are treated prior to the swim test, depressive drugs increase immobility, anxiolytic drugs do not affect immobility, and antidepressant drugs reduce immobility, compared to saline-treated rodents (Porsolt et al., 1977). This phenomenon is consistent even after tricyclic antidepressant exposure, despite drug-induced sedative effects, decrease muscle tone and motor activity (Porsolt et al., 1977). While the original model compared immobility to mobility behaviours, newer drugs have been found to induce different behaviours, therefore, the test has evolved to include not just immobility but

also climbing and swimming behaviours (Lucki, 1997, Borsini and Meli, 1988, Bogdanova et al., 2013). Furthermore, the reliability of this model had been recently criticized for the subjectivity of manually scoring behaviours, thus resulting in variable outcomes for behavioural durations (Bogdanova et al., 2013). Therefore, many labs have altered behavioural scoring to include additional parameters such as time sampling and behaviour frequency. Henceforth, the current study employs an automated activity tracking technology in order to produce unbiased and uniformed results. This measure is made possible by advances in the EthoVision[®] XT 11.5 video tracking software, which allows the activity of the animal to be quantified. This is achieved by assessing the change over time in the number of grey-scale valued pixels in the arena. Therefore a higher rate of pixel changes would indicate high activity and less behavioural despair, whereas a low rate of pixel changes infers low activity and more behavioural despair. Therefore, activity states can be assigned by selecting particular activity percentage thresholds which correlate with the corresponding behaviours of immobility and mobility.

In 1964, desipramine, a tricyclic antidepressant, was developed for its antidepressant properties. Most tricyclic antidepressants bind to the noradrenaline and serotonin reuptake transporters, thereby increasing noradrenaline and serotonergic activity by raising the synaptic concentrations of these neurotransmitters. As desipramine is a secondary amine, it binds to both of these neurotransmitters, however, it has a greater affinity to noradrenaline (Julien et al., 2011). Secondary amine tricyclic antidepressants are the result the metabolized tertiary amine tricyclic antidepressants. However, secondary amine tricyclic antidepressants are preferred as they induce less pronounced histaminic, cholinergic, α_1 -adrenergic receptor blockade, when compared to tertiary amine tricyclic antidepressants (Preskorn et al., 2010). Akin to the SSRI antidepressants described previously, desipramine also faces a pause between initiations of treatment and therapeutic effect on mood (Katz et al., 1996). Similarly, the FST also requires sub-chronic or chronic antidepressant administration to show better antidepressant effectiveness compared to acute dosing (Detke et al., 1997). Therefore, desipramine is expected to alleviate behavioural despair in rats, which is expressed in the FST as inactivity. Reduced responsiveness suggests compromised efficacy while also proposing that noradrenergic and serotonergic neurotransmission

was altered by SSRI exposure *in utero*, and this deficit was not overcome in the fully developed adult brain.

1.9 Research objectives

The primary objective of this investigation is to examine whether exposure during pregnancy in rats of four SSRI antidepressants (fluoxetine, paroxetine, sertraline, or citalopram) is associated with deleterious effects on the resulting offspring, using a range of developmental and behavioural parameters that begin at birth and extend into adulthood. These parameters have been selected to provide a comprehensive clinically relevant assessment of the potentially deleterious impact of members of the SSRI group. This project uses an animal model to increase the knowledge of the consequences of exposure to the highlighted SSRIs at a variety of doses. By using these four SSRIs, a comparative assessment can be made. Overall this research has the potential to select an SSRI of the four studied with a superior safety profile. Long-term, these findings can provide a preclinical test system for evaluating future antidepressants with regards to safety in the progeny.

1.9.1 Hypothesis and aims of the present research

It is hypothesized that *in utero* SSRI antidepressant (fluoxetine, paroxetine, sertraline, or citalopram) exposure at relevant doses that do not cause maternal toxicity, will result in sustained deleterious consequences in the rat offspring as pups, adolescents and adults in regards to somatic, behavioural, and drug-induced response measurements.

In order to explore this hypothesis comprehensively, detailed hypotheses are addressed throughout the work, each addressing the specific aims numbered below:

Chapter 3: *In utero* exposure to fluoxetine, paroxetine, sertraline, or citalopram at clinically relevant doses that are not significantly toxic to (1) maternal wellbeing in the gestational and postnatal period, will not have a significant impact on (2) dam litter characteristics and (3) pup litter characteristics.

Chapter 4: *In utero* exposure to fluoxetine, paroxetine, sertraline, or citalopram at clinically relevant doses that do not cause maternal toxicity will have enduring implications on (1) somatic and (2) behavioural neonatal development in rat offspring.

Chapter 5: *In utero* exposure to fluoxetine, paroxetine, sertraline, or citalopram at clinically relevant doses that do not cause maternal toxicity will have enduring implications on (1) “anxiety-like” and locomotor behaviours in adolescent and adult rat offspring. Additionally, deficits in (2) cognition and (3) “depressive-like” behaviours of anhedonia will be significantly present in resulting adult rat offspring.

Chapter 6: *In utero* exposure to fluoxetine, paroxetine, sertraline, or citalopram at clinically relevant doses that do not cause maternal toxicity will alter the behavioural response of resulting adult rat offspring after subsequent adulthood exposure to (1) anxiolytic and (2) antidepressant psychotropic drugs.

2 Materials and Methods

2.1 Materials

2.1.1 Animal husbandry

Male Sprague-Dawley rats (275-325g): Charles River, Margate, United Kingdom

Female Sprague-Dawley rats: in-house bred, CNS Pharmacology Laboratory, NUI, Galway

Male and female offspring Sprague-Dawley rats: in-house bred, CNS Pharmacology Laboratory, NUI, Galway

Plastic cage bottoms with metal lids (42 cm x 25.5 cm x 13 cm): North Kent Plastics, Coalville, United Kingdom

Water bottles: North Kent Plastics, Coalville, United Kingdom

3 Rs Paper bedding: Fibrecycle Ltd., North Lincolnshire, United Kingdom

Poplar-abp3 premium wood chip bedding: Eco-Pure™, Manchester, United Kingdom

20% protein rodent diet Advanced Protocol® Verified 75 IF Irradiated (5V75): LabDiet®, Brentwood, MO, USA

Nesting materials—Cotton wool nesting material, toilet paper rolls and glove boxes

Environmental enrichment—sizzle nest: LBS Biotechnology, Horley, United Kingdom

Nutritional enrichment—Coco Pops, hazelnut in shell, sunflower seeds, sesame seeds, Cheerios, dry porridge oats, muesli, raisins, Rice Krispies

Temperature/humidity monitor: Radionics Ltd., Dublin Ireland

Top pan balance: Mason Technology, Dublin, Ireland

India ink 30ml: Windsor & Newton Ink, Arts & Hobby, Galway, Ireland

2.1.2 Treatments and administration

Distilled H₂O

Saline (0.9% w/v NaCl): Sigma-Aldrich, Dublin, Ireland

Fluoxetine hydrochloride 5g (Cat # F0750): TCI, Belgium

Citalopram hydrobromide 5g (Cat # C2370): TCI, Belgium

Paroxetine hydrochloride hemihydrate 5g (Cat # P1977): TCI, Japan

Sertraline hydrochloride 5g (Cat # S0507): TCI, Belgium

Desipramine hydrochloride (Cat # D3900): Sigma-Aldrich, Dublin, Ireland

Diazepam (Diazemuls emulsion): University Late Night Pharmacy, Galway, Ireland

Saccharin sodium salt hydrate 500g (Cat # S1002): Sigma-Aldrich, Dublin, Ireland

1ml syringes: BD Microlance, Oxford, United Kingdom

Stainless steel oral gavage curved dosing cannulas, 38mm x 20ga (Cat # 34-0322):

Harvard Apparatus, United Kingdom

Needles (25G x 5/8"): BD Microlance, Oxford, United Kingdom

Insulin syringes (29G x 12.7mm): BD Microlance, Oxford, United Kingdom

2.1.3 Behavioural equipment

Black plastic cage insert for surface righting: Ambrose O'Halloran, Pharmacology and Therapeutics, NUI, Galway

Negative geotaxis: Ambrose O'Halloran, Pharmacology and Therapeutics, NUI, Galway

Forelimb grip: Ambrose O'Halloran, Pharmacology and Therapeutics, NUI, Galway

Home cage apparatus (8-place rack): Ambrose O'Halloran, Pharmacology and Therapeutics, NUI, Galway

Elevated plus maze, arms 50cm x 10cm, closed arm walls 30cm high, centre 10cm x 10cm: Ambrose O'Halloran, Pharmacology and Therapeutics, NUI, Galway

Open field, 75cm diameter x 41cm high: Ambrose O'Halloran, Pharmacology and Therapeutics, NUI, Galway

Forced swim test cylinders (45 x 20 cm): Ambrose O'Halloran, Pharmacology and Therapeutics, NUI, Galway

DVD recorders (Sony): Currys, Galway, Ireland

DVR Recorder (8 place Inspire DVR range): Tracksys, Nottingham, United Kingdom

2.1.4 Computer software

Microsoft Office 2013: Microsoft Ireland, Dublin, Ireland

IBM SPSS Statistics 24: SPSS Inc., New York, NY, USA

GraphPad Prism 8: GraphPad Software Inc., La Jolla, CA, USA

EthoVision[®] XT 11.5: Noldus, Wageningen, The Netherlands

2.2 Methods

2.2.1 Animals

All experimental procedures on animals were conducted with the approval of the Animal Care and Research Ethics Committee (ACREC) of the National University of Ireland, Galway (12/NOV/07, 15/MAR/05, 16/FEB/02), under licence from the Irish Department of Health and Children and in compliance with European Communities Council directive 86/609 guidelines. Sprague-Dawley (SD) rats were used for all of the experiments described. Mothers, as well as male and female treatment-exposed offspring, were bred in-house. Fathers used for breeding of treatment-exposed offspring were supplied by Charles River. Rats were housed in plastic-bottomed cages (42 x 25.5 x 13 cm; L x W x H), and bedding was changed every 5-7 days. Rats were maintained on a 12 h light/dark cycle (lights on at 0800 h) in a temperature controlled room (23 ± 2 °C) with relative humidity ranging at 35-60%. Rat chow pellets and water were available *ad libitum*. In studies where anhedonia was assessed, a saccharin drinking solution was also provided to the rats. In all cases, animals were randomly assigned to their treatment groups.

2.2.1.1 Breeding colonies

Male and Females were supplied by Charles River. Four days before mating, males were singly housed on paper bedding while females stayed housed in groups of three. During the two week mating period males and females were housed together at a ratio of 1:2 or 1:3. Paper bedding was changed once per week. Following the mating period, females were singly housed in cages with poplar wood chip bedding, and materials to facilitate nest building such as cotton wool and cardboard tubes or boxes. The dams were left undisturbed until littering. Upon littering the mothers and pups were left untouched until PND 2 at which point the pups were counted. From postnatal day 2 until weaning at day 21, the pups were counted daily and from PND 5 cages were changed weekly. Litter size, sex ratio, deaths and unplanned pup sacrifices were documented. On PND 21, weaning was carried out. Pups were housed in cages of four by sex with one littermate when possible. Only the number of female pups needed for the study were weaned, the remaining male pups were either allocated to another study/research group or sacrificed if surplus to requirements. At three months old the

females were weighed and re-housed in groups of three by weight for the commencement of the study.

2.2.1.2 Study breeding

The females used for the breeding part of the study were offspring of the breeding colonies described above. At approximately four months old these females were mated with males supplied by Charles River. Four days before mating, males were singly housed on paper bedding while females stayed housed in groups of three. At 16:30 h on the first day of breeding, the female groups of three were rehoused in with the single housed male. From 08:00 h the following morning a vaginal smear swab was taken from each female. The sample was viewed using a microscope to detect the presence of sperm. GD 0 was determined upon a positive sample, females who were not pregnant were placed back into the same male's cage and were checked daily from 08:00 h until producing a positive smear or the breeding period ended. On GD 0 females were singly housed on poplar wood chip bedding and daily body weight, food and water consumption monitoring began to ensure a healthy pregnancy. Cages were changed on GD 6, 13 and 19. Cotton wool and cardboard cylinders were given to dams on GD 0 and 13 to facilitate nest building. During cage changes, old nesting material was transferred to the fresh cage, and a scoop of dirty bedding from the dam was sprinkled over fresh bedding to retain the dams scent and minimize maternal stress.

2.2.1.3 Gestational exposure to SSRIs

Aside from when being weighed, the dams were left undisturbed until GD 7. On GD 7 dams were randomly allocated to a treatment group by weight. From 13:00 h daily, dams were weighed and dosed via oral gavage (OG) (Figure 2.1) at a 2 ml/kg volume from GD 7 until littering (GD 21 or 22). Treatment groups included vehicle (H₂O) or a particular SSRI prepared in H₂O. Doses administered were based on daily weights and included fluoxetine (2.5 mg/kg), paroxetine (1.25, 2.5 and 5 mg/kg), sertraline (2.5, 5 and 10 mg/kg) or citalopram (2.5, 5 and 10 mg/kg). Excluded doses from preliminary studies included fluoxetine (5, 10 and 20 mg/kg) and paroxetine (10 mg/kg). Body weights, food and water consumption were recorded during this period

to detect any effects of treatment or toxicity to the pregnancy. Doses found to have toxic effects in earlier cohorts were excluded from later cohorts.



Figure 2.1 Oral gavage administration.

2.2.1.4 Initial fluoxetine study findings and resulting dose selections

Before completing this work, studies were researched which used doses ranging from 5-20 mg/kg for *in utero* exposure to fluoxetine. As much of the research informing this study had been completed solely employing fluoxetine, a primary study began featuring fluoxetine. The doses selected were 5, 10 or 20 mg/kg at a 2 ml/kg volume of fluoxetine or H₂O. Each of the parameters and animal corollaries listed in the following sections were expected to be carried out upon littering. However, a significant decrease was found for fluoxetine at 10 and 20 mg/kg for maternal body weight gain, food consumption, water consumption and pup weights at postnatal day (PND) 1. Furthermore, a two-fold increase in stillborn pups was observed at 5, 10 and 20 mg/kg of fluoxetine compared to vehicle. The reduced weights and high rates of mortality suggest a toxic effect of the doses used and thus inappropriate when considering doses within the pharmacological range. Consequently, limited data (reported in Appendix 9.1) were collected due to the restricted number of surviving offspring.

Following studies were carried out in three cohorts, featuring all the SSRIs, to ensure the dosages employed were non-toxic. By conducting a preliminary analysis on the first cohort appropriate doses were selected for each SSRI. To moderate excess use of animals, fluoxetine was further observed only at the 2.5 mg/kg dose. The other SSRIs, paroxetine, sertraline, and citalopram, were trialled at 2.5, 5 or 10 mg/kg doses. The

paroxetine doses had to be amended after the first cohort to remove the 10 mg/kg dose (reported in Appendix 9.2). Thus, later cohorts included paroxetine at 1.25, 2.5 and 5 mg/kg. While the dose range for SSRIs prescribed in the clinical scenario does vary between compound, dose levels were selected that would not severely alter the maternal or litter parameters reported in Chapter 3 and thus alter a typical pregnancy. Therefore particular SSRIs, such as sertraline, which can be prescribed at a 200 mg dose clinically, were measured at a max dose of 10 mg/kg in the rat due to preliminary findings concerning reduced maternal weight gain during gestation and higher rates of mortality.

As the resulting SSRI exposed offspring were taken from the three cohorts, the vehicle group used to make comparison was also a combination from the three cohorts, where the percentage of vehicle offspring taken from each cohort mirrored the percentage of the certain SSRI in question. Thus resulting in four vehicle groups, each composed randomly to best suit the SSRI cohort membership.

2.2.1.5 Study littering

Upon littering dosing stopped and the mothers and pups were left untouched until the pups had been cleaned off and put in the nest and then PND 1 was designated. PND 1 was considered on the following day if litters were not cleaned off by 17:00 h. Litters were checked each day, during this the mothers were put in a spare cage with their original cage topper (with their food and water) and were slid back into their original spot on the rack. The original cage bottom with the pups was placed on a heating pad. Many steps were taken to preserve the dam's scent on the pups and to prevent rejection of the litter by the mother. Before touching the pups, the researchers ran their hands through the bedding. Every time the researcher touched a testing tool, they ran their hands through the bedding again before touching the pups. After the daily litter observation was completed pups were returned to their nest and dirty bedding was sprinkled over the litter to preserve the dams scent and dilute the researcher's scent. The mother was then placed back in the cage, on top of the nest of pups. Cages were changed once during the postnatal period on PND 10. Before changing the cage the placement of the nest was noted and a scoop of the dirty bedding was set aside. After refreshing the bedding the nest was put back in its original placement in the cage with the pups in it and the dirty bedding was sprinkled throughout the new bedding and

over the nest of pups. Additional cotton wool was placed in the cage to replenish any unintentionally lost during the cage change.

2.2.1.6 Recording of postnatal observations

On PND 1, pups were counted, sexed and the number of stillborn pups was recorded. After weighing the pups, the male and female pup with the weight closest to the average male and female weight of that litter were tattooed on the bottom of their back paw using an insulin needle and India ink (Figure 2.2). Daily somatic and behavioural tests, as well as PND 28 testing, were carried out on the tattooed litter representative when possible. Only one male and female were considered from each litter for every test to avoid litter effects.



Figure 2.2 PND 1 pup and tattooed pup paw. The average male and female pup were tattooed at PND 1 to represent the litter in all neonatal observations through PND 28.

Mothers were weighed daily, and food and water consumption were monitored to detect any withdrawal effect of the drug. During daily checks, pup deaths and unplanned pup sacrifices were documented. If litter size dropped lower than 8 pups, then the litter was removed from the study to avoid litter size effects. Likewise, if there were more than 14 pups in a litter, surplus pups were sacrificed to avoid litter size effects. On PND 18, food and water consumption monitoring of the mothers concluded, and food pellets were scattered throughout the cage bottom to prepare the pups for weaning. On PND 21, weaning was carried out. 8-10 pups were weaned from each litter and when possible 5 male and 5 female pups were weaned to control for sex effects. Surplus pups were either allocated to another study/research group if they were vehicle exposed, or sacrificed if surplus to requirements. Pups were housed with littermates on paper bedding with a cardboard cylinder and sizzle nest for environmental enrichment. Food pellets were scattered throughout the cage bottom

until PND 23 to assist the pups in the weaning transition. On PND 29, after PND 28 testing, the tattooed pups were sacrificed and tissues samples were collected. The remaining littermates were randomly rehoused by sex in cages of 4 with other non-littermate pups that were born on the same PND \pm 1. Pups were tail marked with a sharpie to indicate their litter origin and housed on paper bedding with food and sizzle nest. Cages were changed once a week until PND 56 and tail marks were refreshed each time. Nutritional enrichment was given every second cage change, but not within a week of testing. Pups were only exposed to an individual enrichment once and could include sesame seeds, sunflower seeds, hazelnuts, dry porridge oats, Cheerios, muesli, Coco Pops, Rice Krispies and raisins.

2.2.2 Maternal behaviour observations

Maternal behaviour was observed to determine if there was a lasting effect of gestational SSRI exposure on PND 10. The camera was suspended above the home cage (Figure 2.3) and recorded the dam for 50 min in the home cage. The video recording was made at the beginning of the light phase between 08:00-10:00 h. The video recording was later scored and observations were made of 5 second clips at a 5 minute interval, thus a total of 10 observations were made. There were 15 maternal behaviours scored: arched nursing, blanket nursing, passive nursing, in the nest, out of the nest, in contact with the pups, grooming the pups, carrying the pups, moving the nest or bedding, resting with eyes closed, eating, drinking, grooming, sniffing and rearing. At each interval, a score of 1 was given if a behaviour occurred within the 5 second clip.



Figure 2.3 Maternal caregiving behaviour. Time sampling observations were recorded on PND 10 for an hour.

2.2.3 Pup somatic development observations

As noted previously, a male and female pup from each litter were tattooed to represent the average pup of each litter (Figure 2.2). These pups were used to track the somatic development of the exposed offspring, from littering at PND 1, through weaning at PND 21, until reaching sexual maturity at PND 28 and tissue samples at PND 29. To ensure the physical maturation of the pup, somatic parameters were measured in both male and female offspring in comparison to the vehicle exposed pups. Observations were made from 10:00 h, during daily checks of the mothers and litters, so that mothers were only separated from their pups once a day for approximately 5 minutes. Furthermore, both mother and pups remained in the home cage room while testing, though the mother was placed in a spare cage for the duration of testing.

Somatic parameters included fur appearance, pinna (ear) unfolding, and eye opening. Fur appearance was recorded from PND 3, the first day of occurrence was when fur appeared, usually most easily observed at the back of the neck. The first day for pinna unfolding to occur was PND 3, while eye opening first occurred on PND 14 (Table 2.1). Recordings noted if no, one, or two pinnae/eyes were unfolded/opened. For analysis, the features were only considered present if both pinnae had unfolded or both eyes had opened. Recording of fur, pinna and eye observations continued until the feature was fully present for the tattooed male and female pup. In addition to these

somatic parameters, body weight, ano-genital distance, and body length were measured to track the somatic development of exposed offspring.

Time point & Present / Absent	Pinnae unfolding	Time point & Present / Absent	Eye opening
PND 3 Absent		PND 12 Absent	
PND 4 Present		PND 14 Present	
PND 5 Present		PND 16 Present	

Table 2.1 Pinnae unfolding and eye opening. Illustration of somatic development features including pinna unfolding at PND 3, 4 & 5 and eye opening at PND 12, 14, & 16. Both features (pinna/eye) must be present to meet criterion.

2.2.3.1 Body weight

The tattooed male and female pups were weighed many times throughout the postnatal period. Recordings occurred at PND 1, 2, 4, 8, 11, 15, 18, 21, and 28 for male and female offspring. Observations made were based on treatment group compared to vehicle, as well as weight gain over time.

2.2.3.2 Ano-genital distance

Ano-genital distance was measured to observe possible masculinising or feminising effects of gestational SSRI exposure. Digital callipers were used to measure the distance between the base of the genitals and the top of the anus (Figure 2.4) for each tattooed pup. Measurements were recorded in mm and performed on PND 3 and 24 for male and female offspring.

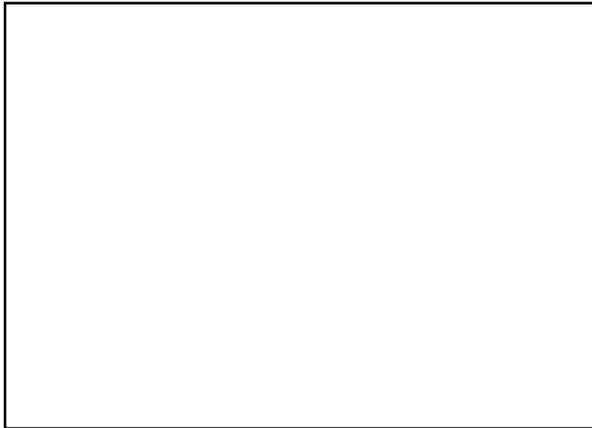


Figure 2.4 Ano-genital distance and body length measurements. Tattooed male and female pups were measured at PND 3 and 24 for ano-genital distance and PND 7 and 14 for body length. Image adapted from (Christiansen et al., 2014).

2.2.3.3 Body length

Body length was measured to observe the possible effects of gestational SSRI exposure and compare growth sizes. Digital callipers were used to measure the distance between the tip of the nose and the base of the tail for each tattooed pup (Figure 2.4). Measurements were recorded in mm and performed on PND 7 and 14 for male and female offspring.

2.2.4 Pup behavioural development observations

In addition to somatic testing, various behavioural tests were also carried out during the neonatal period to track behavioural development. As noted previously, a male and female pup from each littered were tattooed to represent the average pup of each litter. To ensure the behavioural maturation of the pup, sensorimotor and reflex tests were carried out frequently throughout the neonatal period in both male and female offspring. Observations were made from 10:00 h, during daily checks of the mothers and litters, so that mothers were only separated from their pups once a day for

approximately 5 minutes. Furthermore, both mother and pups remained in the home cage room while testing, though the mother was placed in a spare cage for the duration of testing. Such tests included surface righting, negative geotaxis and forelimb grip.

2.2.4.1 Surface righting

Sensorimotor development was observed through righting reflexes to detect the development of body righting mechanisms. The surface righting reflex was carried out by placing the tattooed pup in the supine position on a flat surface. A timer was used to measure the duration, in seconds, required for the pup to turn over and return to its normal prone position (Figure 2.5). For the task to be completed the pup must be securely up on all four paws, with no paws stuck/folded under the body. The maximum time allotted was 30 seconds and if the pup failed to right itself within that time, 30 seconds was recorded and the test ended. The surface righting task was carried out on PND 2, 3, 4 and 5 on both male and female pups to investigate and compare the effects of gestational SSRI exposure.

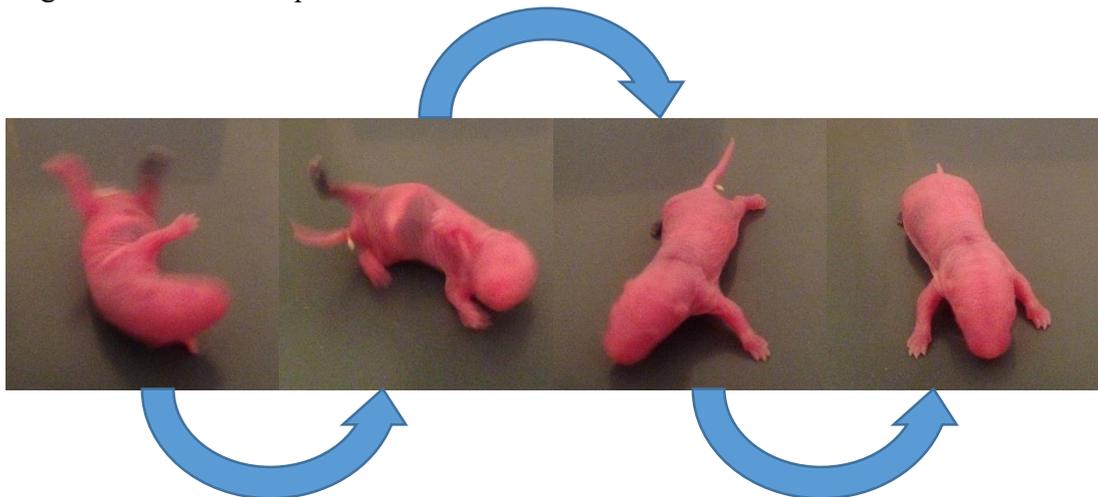


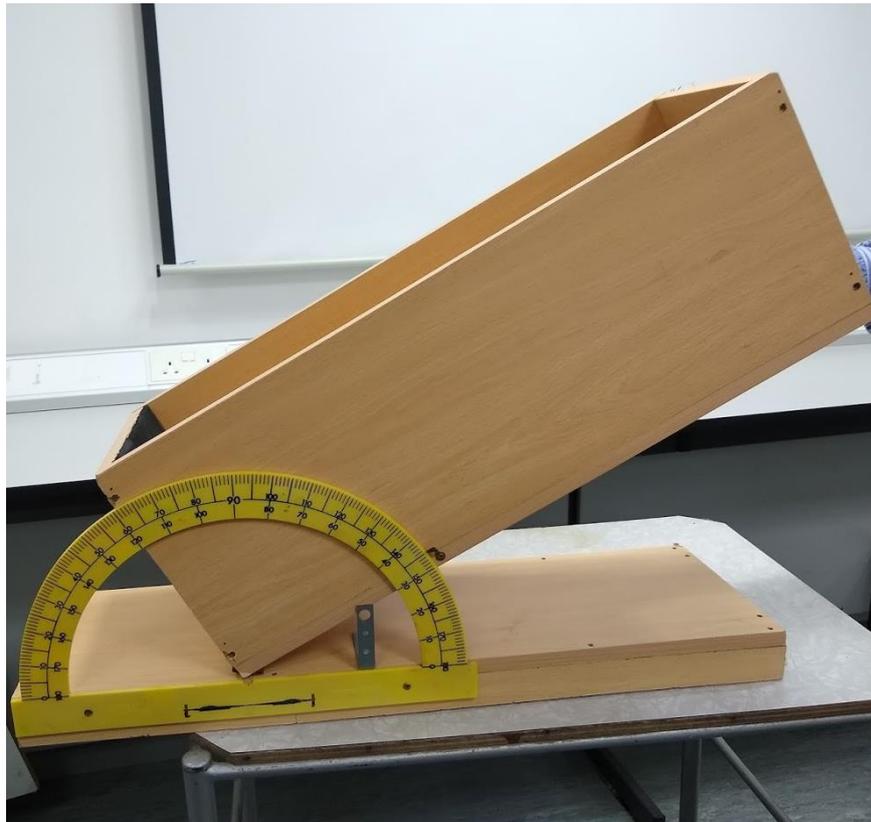
Figure 2.5 Surface righting. Depiction of tattooed male pup at PND 3 successfully completing the surface righting reflex task. Criterion was met if the pup was successful in <10 s.

2.2.4.2 Negative geotaxis

Another sensorimotor development test used was the negative geotaxis test. The negative geotaxis apparatus consists of a flat timber surface slide that is inclined at a 30° angle (Figure 2.6 A). At the top of the slide, there is a sandpaper surface (15 cm x 12 cm) for the rat pups to grip to. Tattooed pups were placed in the middle of the rough surface at the top of the slide with the tip of their nose facing downward. Then a timer was used to record the duration, in seconds, for the pup to turn 180° (Figure 2.6 B). For the task to be completed, the pup must have made the complete turn with the tip

of the nose facing upward. The maximum time allotted was 30 seconds and if the pup failed to turn 180° within that time, 30 seconds was recorded and the test ended. A soft folded towel was placed at the bottom of the slide in order to cushion any falls. If the pup fell when trying to turn, it was given one second chance. If the pup fell a second time or failed to turn 180°, 30 seconds was recorded and the pup was returned to the home cage. The negative geotaxis task was carried out on PND 9 and 11 on both male and female pups to investigate and compare the effects of gestational SSRI exposure.

A)



B)



Figure 2.6 Negative geotaxis. (A) Apparatus used set at a 30° angle and (B) PND 9 pup resting on sandpaper, successfully turning 180° from initial placement facing down to final position facing up. Criterion was met if the pup was successful in <15 s.

2.2.4.3 Forelimb grip

The development of the gripping reflex was observed via the forelimb grip test. The test indicates the strength, vestibular function and sensorimotor coordination of the pups. The forelimb grip apparatus consists of a thin steel bar 20 cm in length and 0.2 cm in diameter. The bar is suspended, by two adjustable poles, 25 cm above the base

of the platform. A soft towel is folded at the base of the platform to cushion any falls. The tattooed pups were held from the base of the tail and lowered onto the bar so that both forepaws could grasp the bar. When the pup successfully gripped the bar with both forepaws, the tail was gently lowered and released (Figure 2.7). A timer was used to measure the duration, in seconds, that the pup was able to hold onto the bar before falling. The maximum time allotted was 20 seconds. If the pup fell before 20 seconds, the time was noted and the task was terminated. If the pup managed to manoeuvre about the bar, i.e. pulling itself up to sit upon the bar on its hind legs or moving itself across the bar to cling to either of the two support polls, the test was terminated and the time noted was 20 seconds. After completing the task, the pup was returned to the home cage. The forelimb grip task was carried out on PND 14 and 17 on both male and female pups to investigate and compare the effects of gestational SSRI exposure.

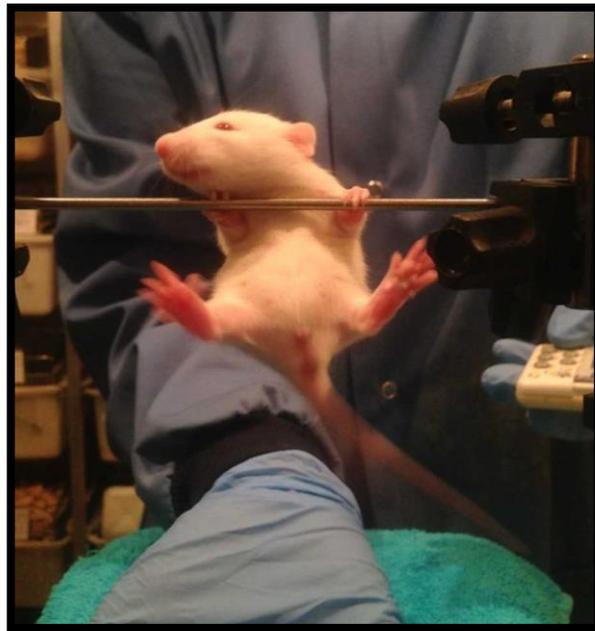


Figure 2.7 Forelimb grip. Tattooed female pup performing the forelimb grip task at PND 17. Criterion was met if the rat held on for >10 s.

2.2.5 Behavioural testing

In addition to early neonatal behavioural testing, various tasks were also carried out during the juvenile, adolescent and adult periods to track behavioural development and observe sustained deleterious consequences. When possible, both male and female pups were tested from each litter, to evaluate a sex effect. The tattooed male and

female pups representing the average pup of each litter that was used in previous neonatal testing was also used at PND 28 in the elevated plus maze (EPM) and the open field (OF).

2.2.5.1 Elevated plus maze (EPM)

The elevated plus maze was introduced in 1984 by Handley and Mithani and further developed in 1986 by Pellow and File to the test used today. The test is one of the most frequently used methods validated for evaluating “anxiety-like” behaviours in rats and mice (Pellow et al., 1985, Pellow and File, 1986, Lister, 1987). The theory behind the test is based on the conflict between the rat’s natural aversion to open areas and heights, and the rat’s natural exploratory behaviour or tendency to investigate the open arms (Montgomery, 1954). The plus shaped apparatus depicted in Figure 2.8 A, is made of black polycarbonate plastic and consists of two enclosed and two open arms (50 cm×13 cm each) and a central platform where all four arms meet (10 cm×13 cm). The closed/walled arms have 30 cm high walls and the floor of the apparatus is raised 55 cm off the floor. Two 100 W bulbs with dimmer switches were installed above each open arm to avoid shadows. Using a Lux meter the light intensity was set to 80 Lux for the open arm and 35 Lux for the closed arms. An overhead camera was fixed approximately 1.3 meters above the apparatus, focused on the central platform, and behaviours were recorded to a DVR recorder in the same room. Trials were then scored at a later date by an experimenter blinded to the treatment groups Figure 2.8 B-C. EthoVision[®] XT 11.5 video tracking software was used to score behaviours automatically (distance) and manually (duration of time in and frequency of entry to each zone (centre, open and closed arms)). For the rat to be considered in the open or closed zone all four paws had to be present in the zone. If less than four paws were in the open or closed arm, then the rat would be considered to be in the centre zone.

The morning of testing, each rat was weighed before undergoing the test. Rats were taken directly from their home cages, carried to the EPM room, and placed in the centre of the EPM facing an open arm. The test concluded after 5 minutes and the animal was transferred directly to the open field test. The EPM was cleaned between each trial, using hot water and fairy liquid, and dried with a towel before the next animal was placed in the maze. Testing occurred from 09:00-11:00 h, at various critical developmental periods for each of the exposed offspring groups (PND 28, 56,

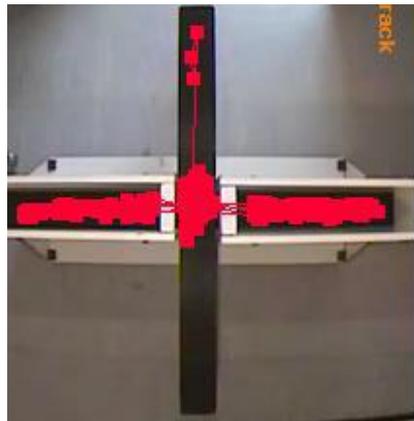
84). If the rat fell from the apparatus for either of these developmental periods, the animal was not used for analysis.

The pharmacological challenge study (Chapter 6) assessed the effect of diazepam compared to saline on exposed adult offspring groups. The test was carried out on rats between PND 92-96. All animals were weighed the morning of the test. Thirty minutes prior to testing, animals received a SC injection of diazepam (DZP) or saline (SAL) at 1 mg/kg dose with a volume of 1 ml/kg. Rats were placed in the arena as specified above. After the 5 minute test, the rat was returned to the home cage and the apparatus was cleaned as described above. During the 5 minute exposure, an experimenter watched a live stream of the trial from an adjacent room with the timer. If a rat fell from the apparatus, the timer was paused. The researcher retrieved the rat and placed it back in the centre of the EPM facing an open arm. Then the trial resumed and the fall was noted.

A)



B)



C)

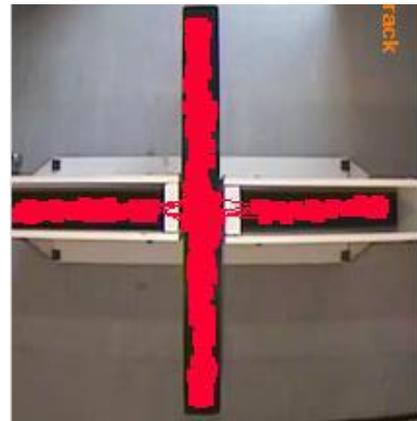


Figure 2.8 Elevated plus maze. EthoVision[®] XT images of the EPM (A) with the open arms highlighted in blue, where open arm activity was manually scored to determine if the rat was exhibiting (B) more “anxiety-like” behaviour or (C) less “anxiety-like” behaviour.

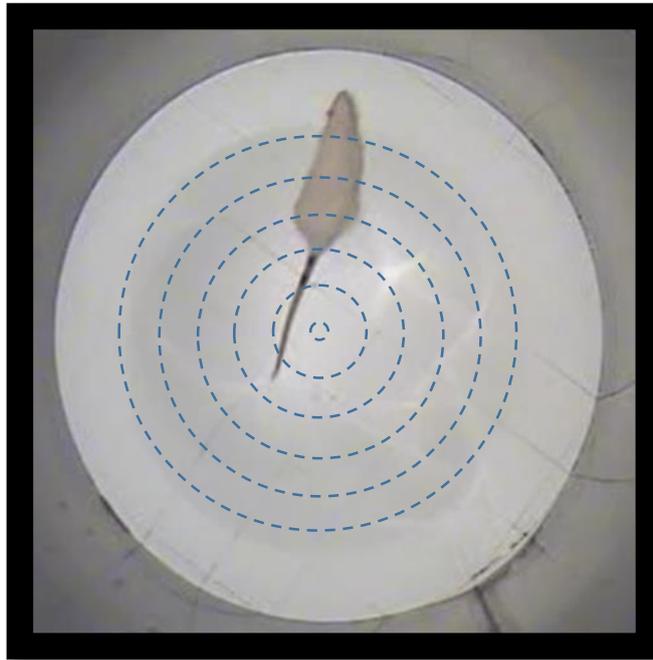
2.2.5.2 Open field (OF)

In addition to the EPM, the open field (OF) test is also used to assess “anxiety-like” rat behaviours, as well as general locomotor activity. The test relies on the theory of thigmotaxis, or the rodent’s natural tendency to remain at the border of the arena rather than the open spaces and bright lights at the centre of the arena (Sousa et al., 2006). The cylindrical apparatus has a white or grey base (75 cm diameter) surrounded by a metallic wall (43 cm high) as depicted in Figure 2.9 A. Four 100 W bulbs with dimmer

switches were installed above the arena evenly to avoid shadows. Using a Lux meter the light intensity was set to 190-210 Lux. An overhead camera was fixed approximately 1.3 meters above the apparatus, focused on the centre of the arena, and behaviours were recorded through a DVR recorder in the same room. Trials were then scored at a later date by an experimenter blinded to the treatment groups (Figure 2.9 B-C). The arena was divided into two sections the bright inner zone at the centre of the arena and the outer zone at the periphery of the arena. EthoVision[®] XT 11.5 video tracking software was used to automatically score various parameters, measuring locomotor activity (distance and number of entries between zones) and “anxiety-like” behaviour (duration of time spent in each zone (inner and outer)).

Aside from when the OF was used as the arena for the novel object recognition test, all animals entered the OF immediately after EPM testing. Rats were placed in the centre of the open field and left for 5 minutes to explore the arena. After the trial, the animal was transferred directly back to the home cage. The OF was cleaned between each trial, using hot water and fairy liquid, and dried with a towel before the next rat was placed in the maze. Testing occurred from 09:00-11:00 h, at various critical developmental periods for each of the exposed offspring groups (PND 28, 56, 84). If the rat fell from the prior test (EPM apparatus) for either of these developmental periods, the animal was not used for analysis.

A)



B)



C)



Figure 2.9 Open field. EthoVision[®] XT images of the OF (A) with the inner zone highlighted in blue, where ambulatory behaviour and inner time duration was automatically tracked to determine if the rat was exhibiting (B) more “anxiety-like” behaviour or (C) less “anxiety-like” behaviour.

2.2.5.3 Novel object recognition (NOR)

The novel object recognition test is used to assess learning and memory. By observing the ability of the animal to distinguish between a familiar and novel object, their recognition capability can be measured. Testing occurred between PND 83-86, and the apparatus used to carry out the NOR was the same as the arena used in the OF test, detailed above. The animal set used to complete the NOR test was different from the animal set which completed the OF test. Using a Lux meter the light intensity was set

to 35-45 Lux. Two identical black and green pint-sized cans were used as the ‘familiar’ objects and a blue, green and yellow Lego block tower was used as the ‘novel’ object. During both the familiarisation and the testing phases, the objects were placed at opposite sides of the arena diagonally from each other and at a distance of 16 cm from the arena walls. Therefore the two objects were approximately 43 cm from one another. The objects were wiped with IMS between each phase of the test. The OF was also cleaned between each of the three phases, using hot water and fairy liquid, and dried with a towel.

The test took place over two days, with day one being a 20 minute habituation exposure to the apparatus used for testing, the open field arena. Day two consists of 3 phases of exposure, each lasting 3 minutes. Phase 1, the habituation period, in which the animal is placed in the empty arena (Figure 2.10 A). Phase 2, the familiarisation period, in which the rat explores two identical objects in the arena (Figure 2.10 B). Phase 3, the test period, in which the one familiar object is replaced with a novel object and the amount of time the rat explores each object is measured (Figure 2.10 C). Between phase 1 and 2 there is a 7 minute delay and between phase 2 and 3 there is a 3 minute delay. During both delays, the rat is placed in a fresh cage in the testing room while the arena and objects are cleaned. Each time the rat was placed into the centre of the arena facing the same wall. Between each animal, the objects were placed in different areas around the perimeter of the circular arena floor, always opposing one another.

All trials were recorded by a video camera attached above the centre of the arena. At a later time, all trials were scored manually by an experimenter blinded to the treatment groups. EthoVision[®] XT 11.5 video tracking software was used automatically track distance moved and velocity. This software was also used to manually score the amount of time the animal spent exploring each object. The nose of the animal had to be within 2 cm of the object, for an interaction to be considered.

A)

Phase 1: Habituation



B)

Phase 2: Familiar object exposure



C)

Phase 3: Novel object exposure



Figure 2.10 Novel object recognition phases. The three phases of the novel object recognition test carried out on day two, which follow a 20 minute habituation exposure 24 hours prior.

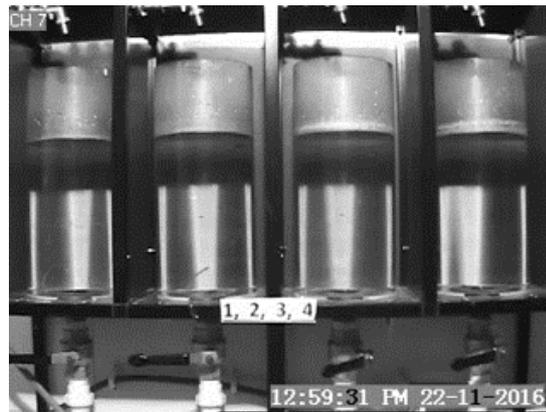
2.2.5.4 Forced swim test (FST)

The forced swim test is the most frequently used method for evaluating antidepressant efficacy of compounds in preclinical behavioural testing. It was developed on the principle of behavioural despair, where the animal is subjected to the threat of drowning and their response to the threat correlates to negative mood. The FST apparatus consists of four Pyrex cylinders (45 x 20 cm), resting 75cm above the floor (Figure 2.11). Each cylinder is isolated from the next by black polycarbonate plastic walls. The apparatus was illuminated by a 100 W bulb placed in front of the FST with the intensity of light set at 40 lux. A water spigot was mounted above each cylinder, and the cylinders were filled to 30 cm high with water (23-26 °C). All testing occurred in a separate room from the home cage room. The test occurred via two exposures, occurring 24 h from another, and was carried out on PND 98-103.

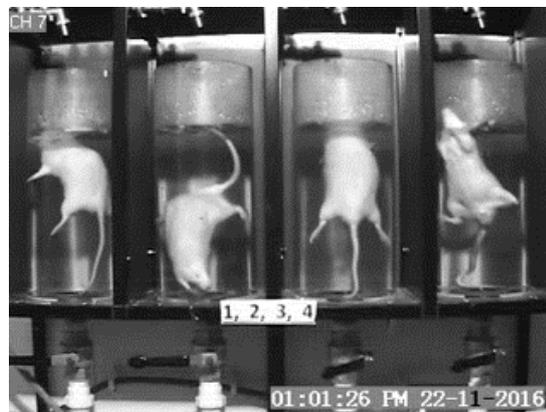
Rats were weighed and taken directly from the home cage and placed in the swimming arena twice over two days. The habituation 'pre-swim' exposure lasted 15 minutes and 24 h later the rats were reintroduced to the same cylinder and the test 'post-swim' exposure lasted 5 minutes. Between each animal, the cylinders were emptied and refilled with clean water. The FST was used as part of the drug challenge study. Therefore between the pre- and post-swim the animal received subacute SC injections of desipramine (DMI) or saline at 10 mg/kg dose with a volume of 1 ml/kg. From 15 minutes after the pre-swim, injections occurred 24 h, 5 h, and 1 h prior to the post-swim.

All trials were recorded by a DVR recorder from a camera that was placed at a certain distance from the apparatus so that all four cylinders could be viewed together and clearly. The 5 minute trials were later scored automatically. The parameters considered were activity stages. The activity thresholds considered were highly active (>10%), active (8-10%), moderately active (4-8%), and inactive (<4%). Scoring techniques were carried out using the automated activity analysis scoring setting on the EthoVision® XT 11.5 software package (Figure 2.11 C). Overall, activity thresholds were assigned to correspond to behaviours of activity and inactivity. High activity implies less behavioural despair and is exhibited by a higher rate of pixel changes. Inversely, less activity implies more behavioural despair and is measure by fewer pixel changes.

A)



B)



C)



Figure 2.11 Forced swim apparatus. (A) Vacant swim tanks, (B) occupied swim tanks, and (C) EthoVision[®] XT image of activity analysis. Images B and C depict different common behaviours observed including swimming, diving, immobile, and climbing.

2.2.5.5 Saccharin preference test (SPT)

The saccharin preference test is used to measure anhedonia which is observed clinically as a symptom of depression. It can be characterised as an inability to feel pleasure in pleasurable activities. Thus, this test measures the rat's preference for a sweet pleasurable saccharin solution. If the rat experiences "depressive-like" anhedonia, there is a reduction in saccharin preference. This test was carried on the saline group of animals which had been exposed to the FST four days prior. Thus, this test took place through 4 overnight exposures between PND 105-112.

Five days prior to and throughout the procedure, animals were singly housed in order to accurately measure food, water and saccharin consumption. The procedure was carried out through 4 overnight exposures to a choice of both 0.5% saccharin solution and water. The first three nights, animals were trained to develop a preference for saccharin, and the 4th night was the test night. An extra water bottle was placed in the rat's home cage, beside the normal water bottle position, allowing for access to both water and saccharin (Figure 2.12). To account for any preference in bottle positioning, as the rats are used to the water on the left side, the bottles were randomly placed amongst treatment groups on either the right or the left side, and each night bottles were placed in the same position as the previous evening. Each evening, saccharin was made available 2-3 h prior to the dark cycle (17:00-18:00 h) and removed approximately 1-2 h after onset of the light cycle the following morning (09:00-10:00 h). Before introducing the bottles in the evening, both the water and the saccharin bottles were weighted. Upon removal, water and saccharin bottles were weight again, along with the animal and the food.



Figure 2.12 Saccharin preference test. Image of the random placement of bottles filled with either water or saccharin for overnight exposure to saccharin. In the morning, the saccharin bottle was removed and the water bottle was returned to the original position to the left of the food.

2.2.6 Data Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences 24.0 for Windows (SPSS Inc., IBM, New York, USA). The following results chapters will describe the specific analysis used. In all requiring data sets, normality and homogeneity of variance ($p < 0.05$) was determined. Data was analysed parametrically if it passed normality and homogeneity. When possible, non-parametric data was transformed and analysed parametrically. If data could not be transformed, it was evaluated non-parametrically. In all cases $p < 0.05$ was deemed statistically significant. All figures were constructed using GraphPad Prism 8. Details on each graphical and tabular representation are outlined in each chapter. When data was deemed parametric it was represented as mean \pm SD, non-parametric data was represented as median \pm interquartile range. Bonferroni correction was applied to non-parametric data to account for multiple comparisons. Pup litter characteristics data were represented in tables and figures as ratio or percentage, however, χ^2 statistical analysis was carried out on raw values. Chapters 5 and 6 used Scheirer-Ray-Hare extension of Kruskal Wallis test followed by a Mann Whitney U *post hoc* test with appropriate Bonferroni correction. This test is analogous to a two-way ANOVA and it was used so that all SSRI data sets could be analysed using the same statistical approach.

3 Consequences of gestational exposure to the SSRIs fluoxetine, paroxetine, sertraline, or citalopram in the rat: maternal wellbeing and littering characteristics.

3.1 Introduction

From puberty, women are at a greater risk than males to develop depression. Consequently, depression is the leading cause of disease-related disability for females (Kessler, 2003). A cross-sectional study in the USA reports that of 3,705 subjects, major depression is experienced by 1 in 20 non-pregnant women of reproductive age, i.e. 20-44 years, 1 in 3 of which are prescribed antidepressants (Guo et al., 2018). Overall, antidepressant prescribing has increased in recent years, however the cause is unknown (Kosidou et al., 2010, Ma et al., 2005). Factors contributing to the rise in antidepressant use could be a combination of reasons, including a genuine increase in depression occurrences or an increase in depression diagnoses as focus has been brought to reducing the stigma and bringing a greater awareness to mental health. According to the UK Office for National Statistics surveys, this increase is most noted in women 19-24 years compared to other age groups or sexes (Collishaw et al., 2010, McManus, 2016). In the UK, a longitudinal study has shown that the rate of prenatal depression has grown over two generations (1990-1992, 2012-2016) from 17% to 25%, with current estimates suggesting 1 in 7 pregnant women are depressed (Pearson et al., 2018). Among other concerns, one resultant difficulty of perinatal mental illness is the economic impact, approximately costing the UK £8 billion (~\$10.6 billion) with an average case of perinatal depression costing society £74,000, with 31% incurred by the mother and 69% by the child (Bauer, 2014). Furthermore, mental illness places a significant burden not only on the wider economy but also all aspects of society including the individuals, their families, and the workplace. An analysis considering a UK electronic healthcare database proposes that 25% of women that stop taking antidepressants prior to pregnancy restart treatment within three months after birth (Charlton et al., 2015). It is not specified if the pause in treatment was after consultation with their doctor, however, such results suggest that women either experience a relapse postpartum or they stopped their treatment prematurely due to the pregnancy, rather than being asymptomatic. Within the same study, considering multiple European regions, such as the Netherlands and Wales, it estimates that 0.5% of women received their first SSRI prescription during pregnancy, suggesting that depression manifested during pregnancy (Charlton et al., 2015). The collective term 'perinatal depression' combines the periods of prenatal and postnatal depression, i.e. depressive episodes before or within the year after the baby is born. Taking all this

information into account, the prevalence of perinatal depression is estimated to be around 11.9% (Woody et al., 2017).

The increasing prevalence of depression during pregnancy has caused the American College of Gynaecologists (ACOG) to encourage continuous assessments of depression in each trimester of pregnancy by obstetrician-gynaecologists and other obstetric care providers as symptoms can present at any time (ACOG Committee on Health Care for Underserved Women, 2006, ACOG, 2018). The decision to regularly screen for depression at obstetrician appointments is largely based on the recommendations of the US Preventive Services Task Force (USPSTF) (Siu et al., 2016). As described previously, The Edinburgh Perinatal Depression Scale (EPDS) is the most commonly used screening technique to assess perinatal depression (ACOG, 2018). Diagnosis of depression during pregnancy is important for the health of the mother and the child. Untreated maternal depression leads to higher risks of postpartum depression and suicidality (Shi et al., 2018). Additionally, untreated maternal depression during pregnancy can be associated with increased nausea and vomiting as well as an increase in physician office visits and duration of sick leave (Andersson et al., 2004). Depressive symptoms during pregnancy have also been linked to a higher incidence of preterm delivery or altered gestational lengths (Nezvalova-Henriksen et al., 2016), as well as birth weights 10% lower than expected for gestational age (Oberlander et al., 2006).

SSRIs are the most commonly prescribed psychotropic drug during pregnancy (Alwan et al., 2016). In addition to their antidepressant properties, SSRIs are also commonly prescribed for their anxiolytic effects (Stone et al., 2003), thus increasing the prescription rates in reproductive-aged women. Indeed, the UK NICE guidelines list SSRIs as the first-line drug intervention for depression (NICE, 2009) and for treatment-naïve women experiencing perinatal depression (NICE, 2014). Furthermore, SSRIs are often prescribed to treat anxiety during pregnancy, as benzodiazepines can pose a serious risk (NICE, 2014). Benzodiazepine use can be linked to misuse/dependence, oral cleft, and reduced motor skills (Jones et al., 2010, Dolovich et al., 1998, Salisbury et al., 2016). With an increased incidence of depression occurring during pregnancy, the effects of these drugs have become a major concern for physicians and families. Despite the high incidence of maternal depression and the guidelines that advise SSRI use, studies suggest that while physicians are

willing to prescribed antidepressants prior to conception and after birth, they are reluctant to prescribe SSRIs when symptoms manifest during gestation (Dietz et al., 2007). This is due to the lack of safety information available for the use of serotonin-altering medication during pregnancy and its impact on foetal development.

SSRIs alter the synaptic availability of serotonin, an important neurotransmitter involved in the regulation of mood, appetite, sleep and cognition. Although SSRIs may have a therapeutic effect on mood, consideration must also be given to off-target effects relating to appetite, sleep, and cognition, especially in vulnerable populations. While SSRI side effects are less severe than other antidepressants (Trindade et al., 1998) they can include nausea, headaches, dizziness, drowsiness, insomnia, fatigue, anxiety, or changes in weight. During pregnancy, such SSRI side effects can be more difficult for the patient to endure, when coinciding with considerable physiological changes that are a consequence of pregnancy.

The knowledge surrounding drug use in pregnancy is limited to clinical case studies and preclinical regulatory studies. This is because clinical testing in pregnancy is impossible due to the unknown maternal and foetal implications involved, deeming them unethical. While it is typically the standard to exclude pregnant women from clinical trials, some bioethicists acknowledge that this rule leads to longstanding scientific negligence. Ultimately pregnant woman and their progeny receive a lower level of care, due to the unknown treatment effects, although in many cases the untreated illness has a greater magnitude on the pregnancy than the treatment (Lyerly et al., 2008). Therefore until a legal framework is built for the responsible inclusion of pregnant women in clinical studies, the majority of research is limited to confounded anecdotal evidence. Although many countries are beginning to build up databases for drug exposure during pregnancy, it is difficult to conduct well-controlled studies which rely on self-reports. In such reports, researchers must navigate the multiple confounding factors listed previously, i.e. the symptoms and pathophysiology of depression and pregnancy, the low compliance associated with psychoactive drugs, the balance of multiple prescriptions, and genetic and environmental factors. Animal models are therefore a valuable resource when testing drug use during pregnancy. They can be used in preclinical research to determine the therapeutic potential of pharmacological compounds. However, very few preclinical studies consider the

effects that treatments, at pharmacological doses, may have during pregnancy on the wellbeing and behaviour of the mother and the phenotypic characteristics of the litter.

As mentioned previously, it is important to use an animal model that resembles the clinical experience as much as possible in order to remain clinically relevant and translatable. Briefly, the current design depicts SSRI treatment manifesting within the pregnancy, thus drawing a parallel to the clinical scenario involving the treatment of SSRI-naïve women experiencing depression during pregnancy. In this study, SSRIs are administered orally, the usual route used clinically. Additionally, an allometric scale was used to help determine appropriate doses, which accounts for the body weight and surface area of the human or animal, so the doses can be extrapolated from the clinical to the preclinical scenario (Reagan-Shaw et al., 2008). Furthermore, the current work applies safety pharmacology which considers organ function as well as structure, by employing a range of therapeutic doses and above (Bass et al., 2004). Overall this study aims to assess pharmacological doses to produce findings reflecting safety pharmacology for SSRI exposure *in utero* rather than toxicology reports of toxicity exposure *in utero* via SSRIs.

This study employs multiple parameters of maternal wellbeing to ensure that the dose exposed does not induce maternal toxicity. Additionally, the ability to carry typical a pregnancy is measured through various litter characteristics and postpartum care is also measured to expose residual effects of the dose. Moreover, pup litter characteristics are also assessed to determine implications on neonatal survival and sex induced effects.

3.1.1 Hypothesis and aims

It is specifically hypothesized in this chapter that *in utero* exposure to fluoxetine, paroxetine, sertraline, or citalopram at clinically relevant doses that are not significantly toxic to maternal wellbeing will not have significant consequences on the litter.

This hypothesis was measured through three specific aims throughout this chapter, ultimately investigating the effects of SSRI administration from GD 7-21 on (1) maternal parameters of wellbeing in the gestational and postpartum period, as well as evaluating the effects that gestational SSRI exposure has on (2) maternal litter characteristics, and (3) pup litter characteristics.

3.2 Experimental methods and design

A comprehensive description of the breeding colonies and study breeding was detailed in Chapter 2, along with protocols for the measurement of litter characteristics and maternal caregiving assessment.

Female Sprague-Dawley (SD) rats, approximately 4 months old, were mated and singly housed following positive vaginal smear for sperm. From GD 7 until littering, dams received either vehicle, FLX (2.5 mg/kg), PRX (1.25, 2.5, or 5 mg/kg), SERT or CIT (2.5, 5 or 10 mg/kg) via oral gavage (OG) (n=9-13 dams or litters per group). Testing occurred with all four SSRIs under investigation simultaneously, however, research was collected over three cohort studies in order to successfully manage a study of this size.

During the gestational and postnatal period, maternal body weight, food and water consumption were monitored. Upon littering, litter characteristics were recorded including, gestational length, number of pups born, sex ratio, pup mortality and birth weights. Maternal caregiving behaviour was observed for an hour on PND 10, to assess nursing, litter contact, and self-care activities.

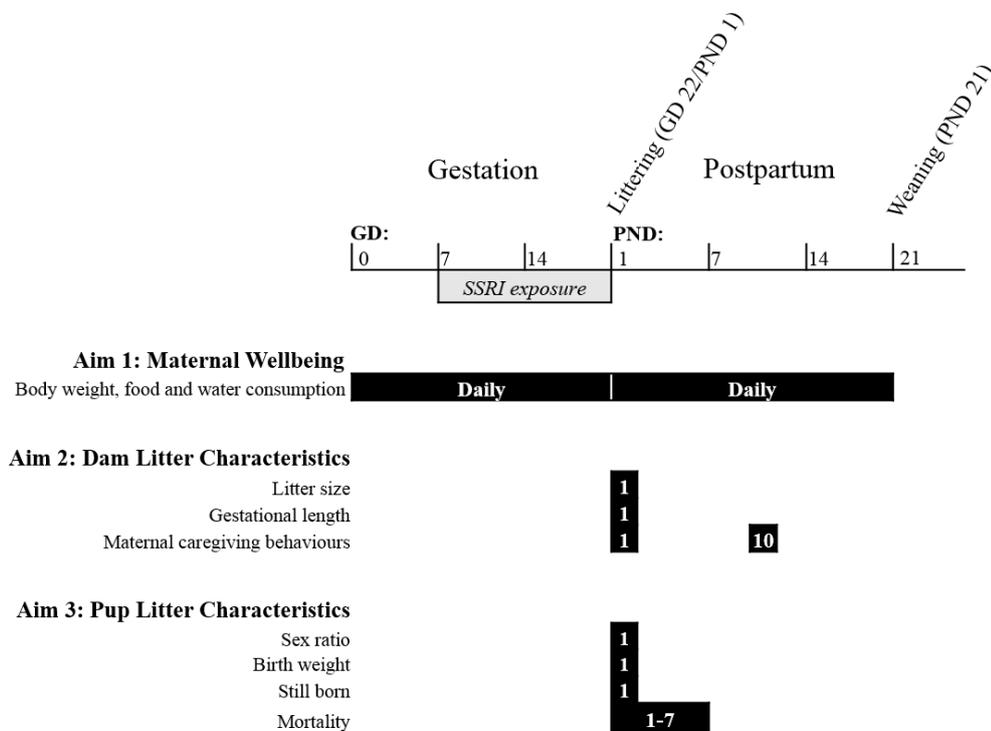


Figure 3.1 Maternal and littering measurements experimental timeline.

Statistical analysis was performed using the Statistical Package for Social Sciences 24.0 for Windows (SPSS Inc., IBM, New York, USA) on the parameters listed in Table 3.1. Primarily, normality and homogeneity of variance ($p < 0.05$) were determined to choose the appropriate analysis. Data was analysed parametrically if it passed normality and homogeneity. Parametric maternal parameters (gestational and postnatal weights) with four treatment groups were analysed using a one-way ANOVA with a factor of dose, data assessing two treatment groups (fluoxetine) required analysis via an independent sample T-test. Offspring weights were analysed using a two-way ANOVA with factors of dose, sex, and dose x sex. When appropriate ($p < 0.05$), ANOVAs were followed up by an SNK (Student-Newman-Keuls) *post hoc* test. Non-parametric data (litter size, gestational length, and maternal caregiving behaviour) with four groups were analysed via a Kruskal Wallis by dose, while data assessing two treatment groups (fluoxetine) were analysed via a Mann Whitney U test. When appropriate ($p < 0.05$), *post hoc* Mann Whitney U tests were used to determine differences amongst individual treatment groups. Pup litter characteristics (sex ratio and mortality) were assessing present vs absent features, therefore, a χ^2 statistical analysis was carried out on raw values despite being represented in tables and figures as ratio or percentage. Bonferroni correction was applied to non-parametric data to account for multiple comparisons ($*p < 0.017$).

Parameter	Shapiro-Wilk & Levene's test $p > 0.05$	Shapiro-Wilk & Levene's test $p < 0.05$
Weights, litter size, gestational length, maternal care giving behaviours	ANOVA → SNK FLX data: Independent sample T-test	Kruskal Wallis → Mann Whitney U, Bonferroni correction
Presence of: Sex, mortality		Chi Square → Bonferroni correction

Table 3.1 Statistical analysis of maternal and littering results. In the case of paroxetine, sertraline and citalopram a Bonferroni correction was employed to correct for multiple comparisons was $*p < 0.017$. SNK (Student-Newman-Keuls).

3.3 Maternal and littering results

3.3.1 Maternal wellbeing: Gestational body weight, food and water consumption

Fluoxetine (Figure 3.2)

There were no significant effects of treatment on body weight gain [$t_{(23)}=1.26$, $p=0.220$], food consumption [$t_{(23)}=0.78$, $p=0.446$], or water consumption [$t_{(23)}=-0.89$, $p=0.382$].

Paroxetine (Figure 3.3).

There were no significant effects of treatment on body weight gain [$F_{(3,44)}=0.13$, $p=0.943$], food consumption [$F_{(3,44)}=1.68$, $p=0.185$], or water consumption [$F_{(3,44)}=0.44$, $p=0.723$].

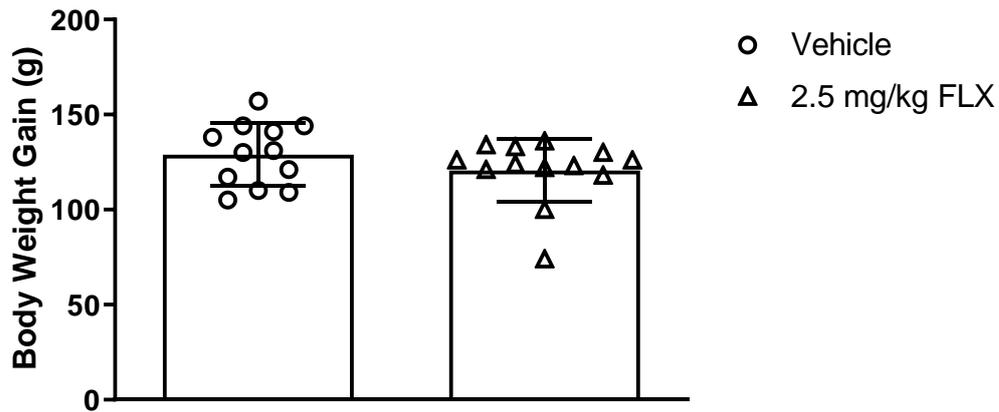
Sertraline (Figure 3.4)

There was a significant effect of treatment on body weight gain [$F_{(3,44)}=7.02$, $p=0.001$] and food consumption [$F_{(3,44)}=3.05$, $p=0.039$] but not water consumption [$F_{(3,44)}=0.17$, $p=0.915$]. A *post hoc* SNK revealed that the 10 mg/kg dose significantly reduced body weight gain and food consumption ($p<0.05$ vs vehicle).

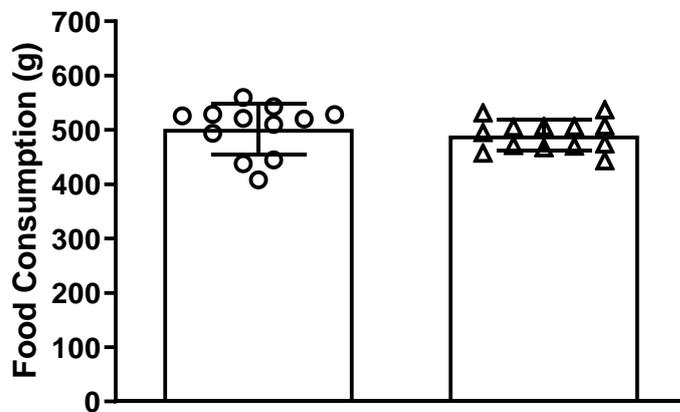
Citalopram (Figure 3.5)

There were no significant effects of treatment on body weight gain [$F_{(3,45)}=2.18$, $p=0.105$], food consumption [$F_{(3,45)}=0.58$, $p=0.630$], or water consumption [$F_{(3,45)}=0.85$, $p=0.476$].

A)



B)



C)

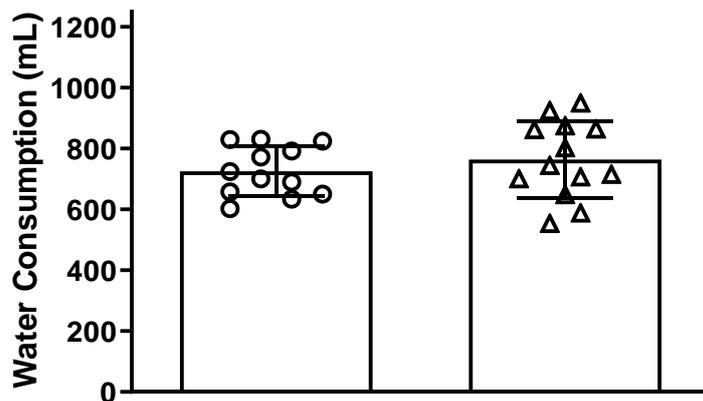
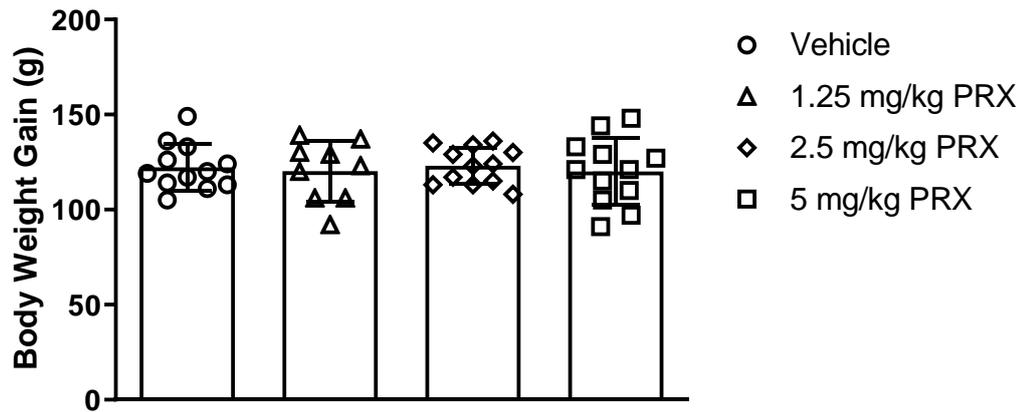
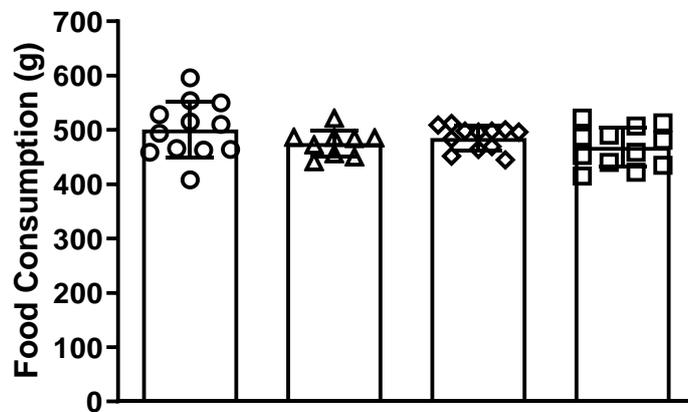


Figure 3.2 Fluoxetine gestational weights. Total (A) body weight gain, (B) food consumption, and (C) water consumption, of pregnant females treated with either vehicle or FLX (2.5 mg/kg) via OG from GD 7-21. Data are expressed as mean \pm SD, n=9-13 dams/group. No significant effect of treatment was found, see text for statistical analysis.

A)



B)



C)

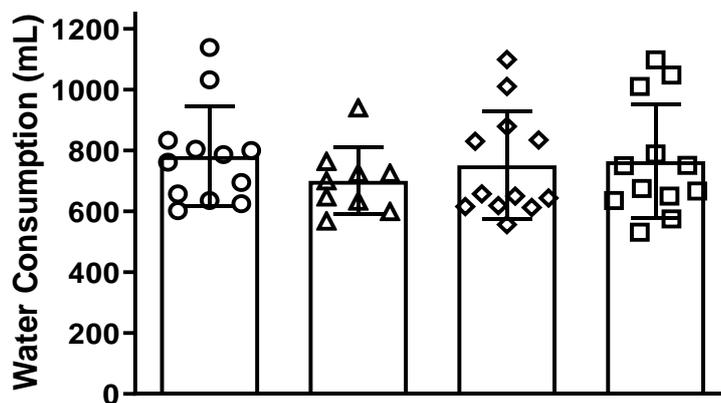
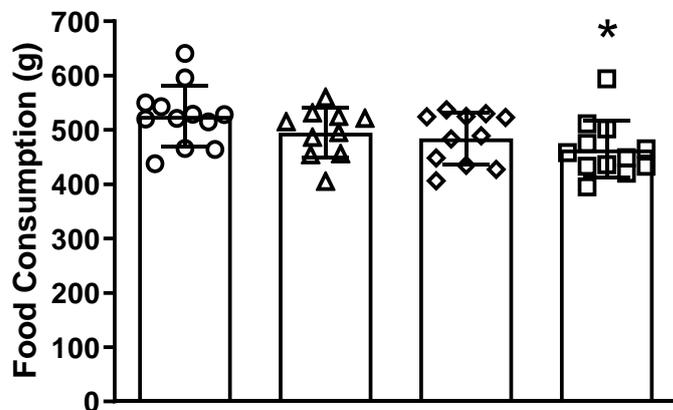


Figure 3.3 Paroxetine gestational weights. Total (A) body weight gain, (B) food consumption, and (C) water consumption, of pregnant females treated with either vehicle or PRX (1.25, 2.5 or 5 mg/kg) via OG from GD 7-21. Data are expressed as mean \pm SD, n=9-13 dams/group. No significant effect of treatment was found, see text for statistical analysis.

A)



B)



C)

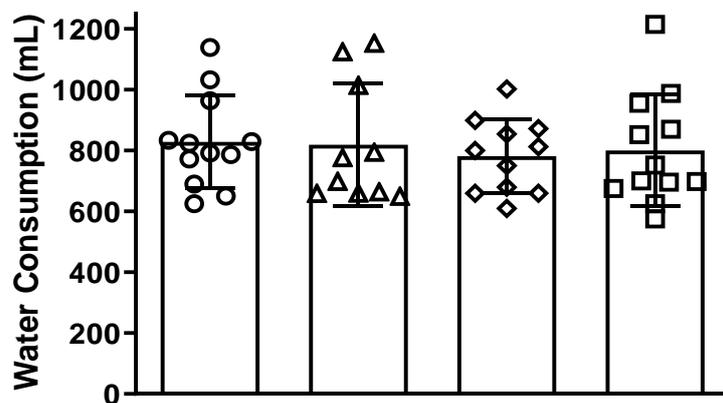
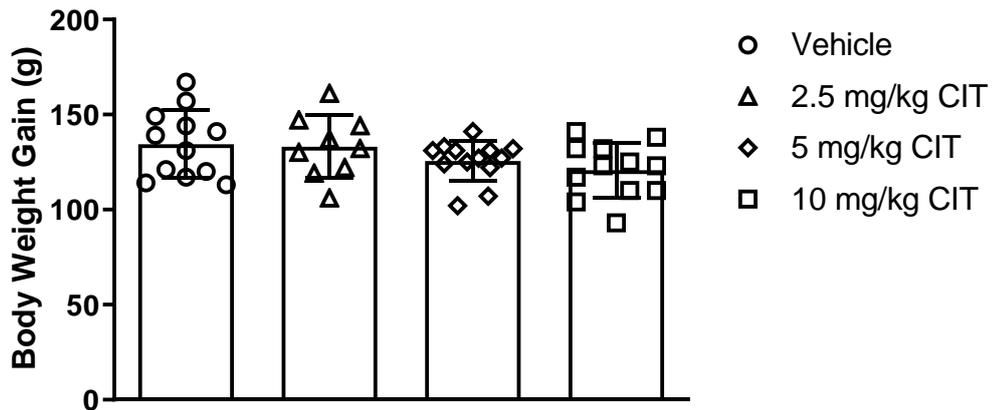
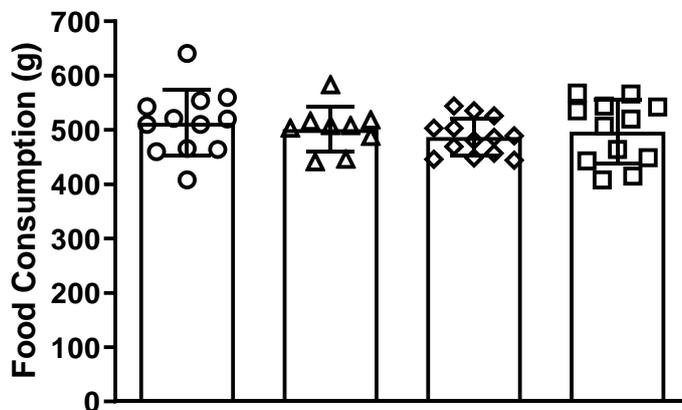


Figure 3.4 Sertraline gestational weights. Total (A) body weight gain, (B) food consumption, and (C) water consumption, of pregnant females treated with either vehicle or SERT (2.5, 5, or 10 mg/kg) via OG from GD 7-21. Data are expressed as mean \pm SD, n=9-13 dams/group, * p <0.05 vs vehicle. SERT (10 mg/kg) significantly reduced body weight gain and food consumption. See text for statistical analysis.

A)



B)



C)

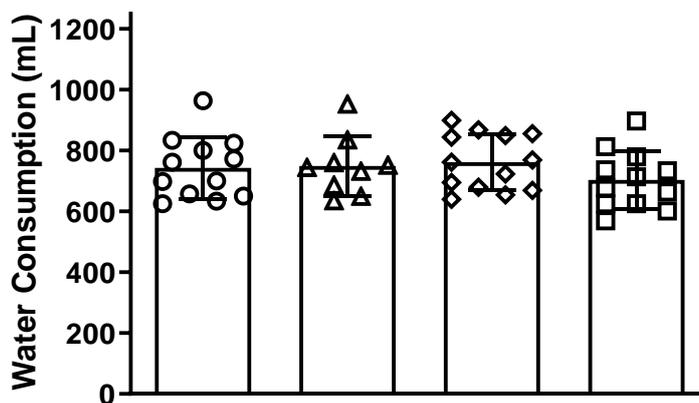


Figure 3.5 Citalopram gestational weights. Total (A) body weight gain, (B) food consumption, and (C) water consumption, of pregnant females treated with either vehicle or CIT (2.5, 5, or 10 mg/kg) via OG from GD 7-21. Data are expressed as mean \pm SD, n=9-13 dams/group. No significant effect of treatment was found, see text for statistical analysis.

3.3.2 Maternal wellbeing: Postpartum body weight, food and water consumption

As shown in Table 3.2, there were no significant effects of treatment on postnatal body weight gain, food consumption, or water consumption, regardless of treatment.

Group	n	Weight gain (g)	Food consumption (g)	Water consumption (mL)
Vehicle	12	14±15	1092±98	1517±150
2.5 mg/kg FLX	13	8±16	1035±184	1488±235
t		0.86	1.54	0.36
p		0.398	0.137	0.719
df		23	23	23
Vehicle	12	6±15	1016±99	1357±199
1.25 mg/kg PRX	9	6±17	1011±99	1286±89
2.5 mg/kg PRX	12	13±14	989±74	1397±135
5 mg/kg PRX	12	11±10	1021±113	1465±209
F		0.68	0.23	1.95
p		0.572	0.873	0.137
df		3, 42	3, 42	3, 42
Vehicle	12	7±12	1055±129	1500±214
2.5 mg/kg SERT	10	17±14	1046±92	1504±231
5 mg/kg SERT	11	8±8	1002±79	1475±173
10 mg/kg SERT	12	12±21	952±93	1376±155
F		1.12	2.51	1.10
p		0.351	0.073	0.361
df		3, 43	3, 43	3, 43
Vehicle	12	5±17	1050±113	1477±229
2.5 mg/kg CIT	9	16±11	1041±76	1468±175
5 mg/kg CIT	13	8±12	1010±105	1448±155
10 mg/kg CIT	12	2±14	972±127	1301±201
F		1.90	1.24	2.20
p		0.144	0.31	0.102
df		3, 46	3, 46	3, 46

Table 3.2 Postpartum body weight, food and water consumption. Females treated with either vehicle or SSRI via OG from GD 7-21. Data are expressed as mean±SD. No significant effects of treatment were found.

3.3.3 Maternal litter characteristics: Litter size and gestational length

Fluoxetine (Table 3.3)

There were no significant effects of treatment on litter size [$U=77.00$, $p=0.956$] or gestational length [$U=72.50$, $p=0.596$].

Paroxetine (Table 3.3).

There were no significant effects of treatment on litter size [$K_{(3)}=1.42$, $p=0.701$] or gestational length [$K_{(3)}=4.65$, $p=0.200$].

Sertraline (Table 3.3)

There were no significant effects of treatment on litter size [$K_{(3)}=0.37$, $p=0.946$] or gestational length [$K_{(3)}=2.04$, $p=0.565$].

Citalopram (Table 3.3)

There were no significant effects of treatment on litter size [$K_{(3)}=5.42$, $p=0.143$] or gestational length [$K_{(3)}=3.09$, $p=0.379$].

Group	Number of litters	Litter size (# of pups born)	Gestational length (days)
Vehicle	12	15 (12-17)	22 (22-22)
2.5 mg/kg FLX	13	14 (14-18)	22 (22-22)
Vehicle	12	14 (12-17)	22 (22-22)
1.25 mg/kg PRX	9	14 (12-15)	22 (22-23)
2.5 mg/kg PRX	12	14 (13-15)	22 (22-23)
5 mg/kg PRX	12	14 (13-15)	22 (22-22)
Vehicle	12	13 (12-17)	22 (22-22)
2.5 mg/kg SERT	10	14 (13-15)	22 (22-22)
5 mg/kg SERT	11	14 (12-15)	22 (22-22)
10 mg/kg SERT	12	14 (12-15)	22 (22-22)
Vehicle	12	15 (13-17)	22 (22-22)
2.5 mg/kg CIT	9	15 (14-16)	22 (22-22)
5 mg/kg CIT	13	14 (12-16)	22 (22-23)
10 mg/kg CIT	12	13 (11-14)	22 (21-22)

Table 3.3 Litter size and gestational length. Dams treated with either vehicle, FLX (2.5 mg/kg), PRX (1.25, 2.5, or 5 mg/kg), SERT or CIT (2.5, 5 or 10 mg/kg), via OG from GD 7-21. Data are expressed as median and interquartile range. No significant effect of treatment was found, see text for statistical analysis.

3.3.4 Maternal litter characteristics: Maternal caregiving behaviours

As shown in Table 3.4, Table 3.5, Table 3.6, and Table 3.7 no significant effect of treatment was found for maternal caregiving in regards to the number of times activities such as nursing, litter interactions, or self-care occurred.

Group	Nursing (counts)			Litter interactions (counts)				Self-care (counts)
	Active	Passive	Total	In nest	Out of nest	Contact with pups	Groom pups	Eat, drink, groom
Vehicle	4.0 (3.0-6.8)	0.0 (0.0-2.0)	5 (3.0-7.0)	7.0 (5.0-8.0)	3.0 (2.3-5.0)	2.0 (0.5-3)	2.0 (0.3-2.8)	1.0 (0.3-3.0)
2.5 mg/kg FLX	5.0 (4.5-6.0)	1.0 (0.0-2.5)	6.0 (6.0-8.0)	6.0 (6.0-8.0)	4.0 (2.0-4.5)	3.0 (2.0-4.0)	2.0 (1.0-4.0)	1.0 (0.0-3.5)
U	56.50	62.00	54.00	69.50	72.00	53.50	59.50	64.00
p	0.237	0.360	0.186	0.635	0.739	0.168	0.303	0.429

Table 3.4 Fluoxetine maternal caregiving behaviour. Nursing, litter interactions, and self-care activities on PND 10 of dams treated with either vehicle or FLX (2.5 mg/kg) via OG from GD 7-21. Data are expressed as median and interquartile range for the number of times the activities occurred, n=9-13 dams/group. No significant effect of treatment was found.

Group	Nursing (counts)			Litter interactions (counts)				Self-care (counts)
	Active	Passive	Total	In nest	Out of nest	Contact with pups	Groom pups	Eat, drink, groom
Vehicle	2.5 (0.3-4.8)	1.5 (0.0-5.0)	5.0 (2.3-9.0)	5.5 (4.3-9.8)	4.5 (0.3-6.5)	1.5 (0.3-3.0)	1.5 (0.3-2.8)	1.0 (0.3-2.8)
1.25 mg/kg PRX	5.0 (4.0-6.5)	0.0 (0.0-1.0)	6.0 (4.0-7.0)	7.0 (5.0-8.0)	4.0 (2.0-5.0)	3.0 (2.0-5.0)	3.0 (2.0-4.0)	2.0 (1.0-3.0)
2.5 mg/kg PRX	3.0 (0.0-6.0)	1.0 (0.0-1.0)	5.0 (1.0-9.0)	6.0 (2.0-9.0)	4.0 (1.0-9.0)	2.0 (0.0-2.0)	1.0 (0.0-2.0)	1.0 (0.0-3.0)
5 mg/kg PRX	5.0 (3.0-6.0)	1.0 (0.0-1.0)	5.0 (3.0-7.0)	6.0 (4.0-7.0)	4.0 (3.0-6.0)	2.0 (1.0-4.0)	1.0 (1.0-3.0)	3.0 (0.0-4.0)
K	3.98	4.01	0.12	0.38	0.24	5.68	5.70	0.99
p	0.264	0.260	0.990	0.945	0.971	0.128	0.127	0.803
df	3	3	3	3	3	3	3	3

Table 3.5 Paroxetine maternal caregiving behaviour. Nursing, litter interactions, and self-care activities on PND 10 of dams treated with either vehicle or PRX (1.25, 2.5, or 5 mg/kg) via OG from GD 7-21. Data are expressed as median and interquartile range for the number of times the activities occurred, n=9-13 dams/group. No significant effect of treatment was found.

Group	Nursing (counts)			Litter interactions (counts)				Self-care (counts)
	Active	Passive	Total	In nest	Out of nest	Contact with pups	Groom pups	Eat, drink, groom
Vehicle	4.5 (2.3-6.8)	1.0 (0.0-2.8)	6.5 (4.3-8.5)	7.5 (5.0-8.8)	3.0 (1.3-5.0)	2.0 (0.3-3.0)	2.0 (0.3-2.8)	1.0 (0.0-2.5)
2.5 mg/kg SERT	5.5 (4.0-7.0)	0.0 (0.0-1.3)	6.5 (5.0-7.0)	7.0 (7.0-8.3)	3.0 (3.0-3.250)	4.0 (1.5-5.0)	3.0 (0.8-4.0)	0.0 (0.0-1.3)
5 mg/kg SERT	5.0 (4.5-6.3)	1.0 (1.0-2.3)	7.0 (5.8-8.0)	8.0 (6.0-8.0)	2.0 (2.0-4.3)	2.0 (1.0-4.0)	2.0 (1.0-3.3)	1.0 (0.0-2.3)
10 mg/kg SERT	4.5 (3.0-5.8)	1.5 (0.0-2.0)	5.5 (4.3-8.0)	7.0 (4.3-8.8)	4.5 (1.3-5.8)	3.5 (2.0-4.0)	2.5 (1.3-3.8)	1.0 (0.0-1.8)
K	2.10	3.95	1.50	0.81	1.21	1.65	3.36	1.59
p	0.552	0.267	0.683	0.848	0.751	0.104	0.339	0.661
df	3	3	3	3	3	3	3	3

Table 3.6 Sertraline maternal caregiving behaviour. Nursing, litter interactions, and self-care activities on PND 10 of dams treated with either vehicle or SERT (2.5, 5 or 10 mg/kg) via OG from GD 7-21. Data are expressed as median and interquartile range for the number of times the activities occurred, n=9-13 dams/group. No significant effect of treatment was found.

Group	Nursing (counts)			Litter interactions (counts)				Self-care (counts)
	Active	Passive	Total	In nest	Out of nest	Contact with pups	Groom pups	Eat, drink, groom
Vehicle	4.0 (3.0-6.5)	1.5 (0.0-5.5)	6.5 (4.0-9.8)	6.5 (5.0-9.8)	3.5 (0.3-5.0)	2.0 (0.3-3.0)	2.0 (0.3-2)	3.0 (1.0-4.0)
2.5 mg/kg CIT	3.5 (2.3-5.5)	0.5 (0.0-1.5)	6.0 (2.3-7.0)	6.0 (2.3-7.0)	4.0 (2.8-7.0)	3.0 (0.8-3.5)	2.5 (0.8-3.0)	1.5 (0.8-3.0)
5 mg/kg CIT	3.0 (1.0-7.5)	1.0 (0.0-2.5)	7.0 (2.0-9.0)	7.0 (2.0-9.0)	2.0 (1.0-7.0)	3.0 (0.0-4.0)	2.0 (0.0-3.5)	0.0 (0.0-1.0)
10 mg/kg CIT	4.0 (2.0-6.0)	1.0 (0.0-2.0)	6.0 (4.0-8.0)	6.0 (4.0-8.0)	4.0 (2.0-5.0)	2.0 (1.0-4.0)	2.0 (1.0-3.0)	1.0 (0.0-3.0)
K	0.18	2.14	2.27	1.84	2.28	1.59	1.20	2.73
p	0.981	0.543	0.518	0.606	0.517	0.661	0.754	0.436
df	3	3	3	3	3	3	3	3

Table 3.7 Citalopram maternal caregiving behaviour. Nursing, litter interactions, and self-care activities on PND 10 of dams treated with either vehicle or CIT (2.5, 5 or 10 mg/kg) from GD 7-21. Data are expressed as median and interquartile range for the number of times the activities occurred, n=9-13 dams/group. No significant effect of treatment was found.

3.3.5 Pup litter characteristics: Sex ratio, still born and total dead

As shown in Table 3.8 there were no significant effects of treatment on sex ratio. Paroxetine and sertraline affected stillborn and total dead. Paroxetine (2.5 mg/kg) significantly increased the number of stillborn compared to vehicle litters. Paroxetine (2.5 and 5 mg/kg) and sertraline (10 mg/kg) significantly increased the number of total dead the week post-littering compared to vehicle litters.

Group	Number of litters	Sex ratio (males born)	Still born (%)	Total dead (%)
Vehicle	12	0.49	1	5
2.5 mg/kg FLX	13	0.56	3	11
χ^2		1.90	1.72	3.61
p		0.168	0.189	0.058

Vehicle	12	0.52	1	5
1.25 mg/kg PRX	9	0.50	1	7
2.5 mg/kg PRX	12	0.52	9*	21*
5 mg/kg PRX	12	0.53	2	15*
χ^2		0.23	24.11	25.95
p		0.972	<0.001	<0.001

Vehicle	12	0.51	1	5
2.5 mg/kg SERT	10	0.48	0	7
5 mg/kg SERT	11	0.48	1	6
10 mg/kg SERT	12	0.46	6	19*
χ^2		0.93	13.47	21.98
p		0.817	0.004	<0.001

Vehicle	12	0.50	1	9
2.5 mg/kg CIT	9	0.47	2	7
5 mg/kg CIT	13	0.56	1	11
10 mg/kg CIT	12	0.50	1	12
χ^2		3.08	0.51	3.06
p		0.380	0.918	0.382

Table 3.8 Sex ratio, still born, and total dead. Pups exposed to either vehicle, FLX (2.5 mg/kg), PRX (1.25, 2.5, or 5 mg/kg), SERT or CIT (2.5, 5 or 10 mg/kg), via OG to dam from GD 7-21. Data are expressed as ratio or percentage, * $p < 0.05$ vs vehicle, with appropriate correction due to multiple comparisons. PRX (2.5 mg/kg) increased stillborn. PRX (2.5, 5 mg/kg) and SERT (10 mg/kg) significantly increased total dead.

3.3.6 Pup litter characteristics: Pup birth weights

Fluoxetine (Table 3.9)

There was a significant effect of sex on birth weight [$F_{(1,46)}=6.90, p=0.012$]. No effects were found for dose or dose x sex. There were no significant effects of treatment on birth weight within male [$t_{(23)}=1.15, p=0.263$] or female [$t_{(23)}=1.21, p=0.239$] groups.

Paroxetine (Table 3.9)

There was a significant effect of sex on birth weight [$F_{(1,82)}=10.65, p=0.002$]. No effects were found for dose or dose x sex. There were no significant effects of treatment on birth weight within male [$F_{(3,44)}=1.23, p=0.312$] or female [$F_{(3,44)}=0.94, p=0.429$] groups.

Sertraline (Table 3.9)

There was a significant effect of sex on birth weight [$F_{(1,82)}=10.30, p=0.002$]. No effects were found for dose or dose x sex. There were no significant effects of treatment on birth weight within male [$F_{(3,44)}=1.38, p=0.264$] or female [$F_{(3,44)}=1.48, p=0.235$] groups.

Citalopram (Table 3.9)

There was a significant effect of sex on birth weight [$F_{(1,86)}=6.54, p=0.012$]. No effects were found for dose or dose x sex. There were no significant effects of treatment on birth weight within male [$F_{(3,46)}=0.41, p=0.748$] or female [$F_{(3,46)}=0.76, p=0.524$] groups.

Group	Number of litters	Male birth weight (g)	Female birth weight (g)
Vehicle	12	6.56±0.67	6.15±0.71*
2.5 mg/kg FLX	13	6.30±0.43	5.86±0.46
Vehicle	12	6.39±0.77	6.01±0.62*
1.25 mg/kg PRX	9	6.63±0.75	6.18±0.64
2.5 mg/kg PRX	12	6.15±0.44	5.81±0.43
5 mg/kg PRX	12	6.46±0.30	6.07±0.39
Vehicle	12	6.59±0.65	6.24±0.66*
2.5 mg/kg SERT	10	6.34±0.52	5.91±0.53
5 mg/kg SERT	11	6.16±0.55	5.74±0.48
10 mg/kg SERT	12	6.22±0.46	5.91±0.63
Vehicle	12	6.30±0.70	5.99±0.71*
2.5 mg/kg CIT	9	6.49±0.54	6.12±0.50
5 mg/kg CIT	13	6.56±0.62	6.11±0.54
10 mg/kg CIT	12	6.59±0.43	6.46±0.54

Table 3.9 Pup birth weights Male and female birth weights for resulting pups exposed to either vehicle, FLX (2.5 mg/kg), PRX (1.25, 2.5, or 5 mg/kg), SERT or CIT (2.5, 5 or 10 mg/kg), via OG to dam from GD 7-21. A significant effect of sex was found for all groups, no significant effect of treatment was found. Data are expressed as mean±SD, * p <0.05 vs vehicle male. See text for statistical analysis.

3.4 Discussion

Overall, this chapter has revealed that sertraline significantly reduce maternal body weight gain and food consumption during pregnancy, although no residual weight, consumption, or behavioural effects were observed in the postnatal period. Similarly, gestational exposure to paroxetine or sertraline were associated with an increase in pup mortality.

3.4.1 Aim 1: Maternal wellbeing

The first aim of this study was to examine the effects of SSRI administration from GD 7-21, via oral gavage, on maternal parameters of wellbeing in the gestational and postpartum period. Maternal wellbeing was measured by monitoring the body weight, food and water consumption of the dams from conception to weaning.

3.4.1.1 Fluoxetine

During the gestational and postnatal period, no effect was found for fluoxetine treatment on body weight, food or water consumption. Interestingly, when treatment was given during the pre and postnatal period at lower doses (0.4 or 1.7 mg/kg, IG to dam, GD 7-PND 21), no effect was found for treatment in either period; however, at a higher dose of fluoxetine (17 mg/kg), a decrease in weight gain was found during both periods (Muller et al., 2013). Similarly, no effect of fluoxetine treatment was found for maternal body weight or food consumption when rats were treated only in the last week of gestation (8 mg/kg, IG, GD 15-20); yet, a significant decrease was observed in both parameters when that dose was doubled (16 mg/kg) (da-Silva et al., 1999). In an earlier study, using the same model portrayed in this chapter, however at higher doses (5, 10, and 20 mg/kg), a significant effect of treatment was observed on gestational weight gain for all three doses. Further, the middle and high dose (10 and 20 mg/kg) caused a reduction in food consumption, while the highest dose even caused reduced water intake, compared to vehicle exposed dams (Appendix 9.1). Overall, the dose of fluoxetine employed in the current study did not impact maternal parameters of wellbeing, however, a decrease in these parameters is observed in fluoxetine at higher doses signifying that higher doses of fluoxetine can, in fact, be toxic.

3.4.1.2 Paroxetine

Throughout the gestational and postnatal period, no effect was found for paroxetine treatment on body weight, food or water consumption. Preceding literature has not been reported on these parameters in rats. However, studies completed in mice also found no effect on body weight gain throughout the gestational period, although dosing began prior to conception and stopped during pregnancy and was at higher doses than used in the current study (30 mg/kg, oral, GD (-14)-GD 16) (Rayburn et al., 2000). Therefore, the current study suggests that paroxetine at the doses employed does not alter maternal parameters of wellbeing related to weight or feeding throughout the perinatal period.

3.4.1.3 Sertraline

During the gestational period, a significant effect was found for the highest dose of sertraline used (10 mg/kg) on maternal weight gain and food consumption during the gestational period, but not water consumption. Interestingly, no effect was found on these parameters during the postnatal period, inferring an effect of treatment prenatally. Previously, studies completed in rats also found a significant decrease in body weight at even higher doses (20 or 80 mg/kg, oral, GD 15-PND 20) as well as a significant decrease in food consumption when dosing continued until weaning (Davies and Klowe, 1998). Due to reports on a decrease in food consumption, it can be inferred that higher doses of sertraline may cause a decrease in appetite and consequently an inhibition of body weight gain. A previous investigation, not modelling pregnancy, had similar findings in male SD rats food deprived for 24 hours, with sertraline treatment (10 or 18 mg/kg, IP, 30 min prior to feeding test) causing a reduction in feeding behaviour without affecting water consumption, whereas saline or lower doses (2 or 3.3 mg/kg) had no effect on such behaviours (Lucki et al., 1988). Within the same lab, free-feeding rats were administered 10 mg/kg for 7 days, causing an inhibition of body weight gain by 40% compared to vehicle-treated rats. Overall, these results taken with the previous studies, propose that sertraline at doses of 10 mg/kg and higher can have an anorexic effect and/or cause an inhibition of body weight gain, even in pregnant rats when weight gain is expected.

3.4.1.4 Citalopram

Throughout the gestational and postnatal period, no effect was found for citalopram treatment on body weight, food or water consumption. Previous literature supports

such findings on gestational weight gain, reporting no significant effect of treatment (10 mg/kg, SC to pup, GD 6-PND 20) for percent gestational weight gain in SD rats (Sprowles et al., 2016). No literature could be found observing food and water consumption or on postpartum maternal weights. Therefore, the current study proposes that the doses employed for citalopram do not modify maternal wellbeing in relation to typical weight gain, feeding, or drinking during the prenatal period, nor does it affect such parameters postpartum when treatment concludes at birth.

3.4.2 Aim 2: Maternal litter characteristics

The second aim of this study was to examine the effects of SSRI administration from GD 7-21, via oral gavage, on maternal litter characteristics. Maternal litter characteristics were measured by monitoring the litter size and gestational length at PND 1, and maternal caregiving behaviours at PND 10.

3.4.2.1 Fluoxetine

On the day of littering, no effect was found for fluoxetine treatment on litter size or gestational length. Consistent with PND 1 findings, no residual effect of treatment was observed for maternal caregiving activities at PND 10. The majority of the previous literature considering fluoxetine, noted in Table 1.7, reports no changes in litter size or gestational length, regardless of species, at doses ranging from 1-16 mg/kg with exposure periods varying up to the entire gestational period (Bauer et al., 2010, Cagiano et al., 2008, Vorhees et al., 1994, Bairy et al., 2007, Svirsky et al., 2016, Cabrera and Battaglia, 1994, Taghizadeh et al., 2016). Olivier et al., 2011 does report a difference in litter size after gestational treatment (12 mg/kg, IG, GD 11-21) however, litter size was measured at PND 7, not birth. This suggests that gestational fluoxetine exposure or withdrawal may affect neonatal survival within the first week postpartum, but does not conclude that treatment affects the number of pups the mother is able to carry to term. Unlike the current results, an earlier study reports that fluoxetine (8 or 16 mg/kg, IG, GD 15-20) causes a shortened gestational length to 21.1 or 21.2 days compared to control data 21.6 days (da-Silva et al., 1999), nevertheless this still falls within the typical gestational length of 21-22 days. Although the dosing began a week after the treatment regime described in this chapter, all animals littered on either GD 21 or 22, and there was no effect of treatment on litter size, similar to the

current results. As this study only exposed dams from GD 15-20, rather than until littering, it could be possible that the early littering shown by both fluoxetine groups could actually be a withdrawal of drug or stress-related effect; proposing that a change in treatment so close to labour may induce earlier labour. Similar to the maternal litter results at birth, no lasting effect of treatment was found for fluoxetine on maternal caregiving behaviours at PND 10. A similar study observing exposure throughout gestation (2 or 4 mg/kg, SC, GD 1-20) also concluded no effect of treatment on litter interactions or self-care albeit on PND 1 (Johns et al., 2005), thus fluoxetine treatment had no effect on maternal caregiving at birth or at PND 10. Overall, the current results agree with previous findings that *in utero* fluoxetine exposure, at a lower dose, has no effect on litter size at PND 1, nor does it alter the gestational length that is typically expected for rodents, or have residual effects on postnatal maternal caregiving behaviour.

3.4.2.2 Paroxetine

At postnatal day one, no effect was found for paroxetine treatment on litter size or gestational length. Similarly, no lasting effect of treatment was found on maternal caregiving activities at PND 10. The majority of literature published on paroxetine exposure *in utero* tends to be in mice rather than rats, however, prior to the current study, none have reported parameters of maternal caregiving activities. In keeping with these findings, no effect of paroxetine treatment was reported in mice on litter size or gestational length when exposure began prior to mating and ended before birth at a much higher dose (30 mg/kg, PO, GD (-14)-GD 16) (Coleman et al., 1999, Rayburn et al., 2000). Further, paroxetine exposure to breeding pairs (22.5 mg/kg, PO, GD (-8)-littering) had no effect on litter size in mice (Gaukler et al., 2015). Conversely, a study conducted in rats found paroxetine 10 mg/kg exposed in the last week before littering caused a significant reduction in gestational length compared to vehicle litters (Van den Hove et al., 2008); although, treated and untreated litters all fell within the expected length of rat gestation, between 21-22 days. Taken together, paroxetine (1.25, 2.5 or 5 mg/kg) has no effect on litter size or gestational length, while additional evidence may suggest higher doses could reduce the gestational length in rats, albeit within the expected gestational window. Furthermore, for the first time, it is reported that gestational paroxetine exposure does not impact postnatal dam activities of litter or self-care.

3.4.2.3 Sertraline

On the day of littering, no effect was found for sertraline treatment, from GD 7-littering, on litter size or gestational length. Likewise, maternal behaviour was not altered on PND 10. Converse to the current findings, others have reported that exposure all throughout gestation (10 mg/kg, SC, GD 0-littering) produced an increase in litter size, without affecting gestational length (De Long et al., 2015b). On the other hand, perinatal sertraline exposure (10 mg/kg, IP to dam, GD 12-PND 2) reduced litter size and increased the duration of labour in SD rats, but had no effect on the length of gestation (Craft et al., 2010). In regards to nursing behaviours, a similar study also found that pregestational and gestational sertraline exposure (20 mg/kg, OG, GD (-16)-littering) had no effect on any nursing behaviours (active, passive, or total) nor did it affect the frequency the mother was out of the nest, even when measurements were made earlier (PND 2-8) than in the present study (Kott et al., 2018). The current findings, taken with previous results, suggest that exposure to sertraline has no effect on average litter size or gestational length, however, it is important to note that a sertraline effect on litter size cannot be ruled out when considering the upper dose of exposure outside of GD 7-littering treatment periods. Furthermore, there are no residual effects of gestational sertraline exposure on maternal caregiving behaviours in the early postpartum period, when dams are exposed to a range of doses in this study (2.5, 5 or 10 mg/kg) or in previous studies (20 mg/kg).

3.4.2.4 Citalopram

At postnatal day one, no effect was found for citalopram treatment on litter size or gestational length. Neither is there a lasting effect of treatment on maternal behaviours at PND 10. Unfortunately, there is little published preclinical data observing the effects of *in utero* citalopram exposure on litter size and gestational length, and no literature in regards to maternal caregiving behaviours. One study in mice supports these findings, noting that exposure to citalopram (5 mg/kg, SC) for the third trimester had no effect on litter size (Hsiao et al., 2005), however, no information was provided in regards to effects on gestational length. Overall it is inferred that citalopram exposure at the doses studied has no effect on litter size or gestational length. In addition, novel findings present that gestational exposure does not alter postnatal maternal behaviours in regards to nursing, interactions with the litter, or self-care activities.

3.4.3 Aim 3: Pup litter characteristics

The third aim of this study was to examine the effects of SSRI administration from GD 7-21, via oral gavage, on offspring litter characteristics. Offspring litter characteristics were measured by recording the sex ratio at PND 1, mortality at PND 1 and within the first week, and male and female weights at PND 1.

3.4.3.1 Fluoxetine

Sex ratio and mortality were not affected by fluoxetine exposure in the present study. In addition, no effect was found for treatment on birth weight, however, there was a sex effect in that males weighed more than females at PND 1. Regarding pup littering characteristics, the majority of preclinical literature on gestational SSRI exposure features fluoxetine (Table 1.7 and Table 1.8). Narrowing the literature to rats administered orally and from GD 7-21±1 indicates that sex ratio is not altered by treatment at the current dose or at doses ranging lower or higher (0.4, 1.7 or 17 mg/kg) (Muller et al., 2013). Consistent with the mortality results for 2.5 mg/kg exposure, doses of fluoxetine ranging from 1-8 mg/kg also have no impact on pup mortality (Vorhees et al., 1994, Bairy et al., 2007). Interestingly, the literature suggests that higher doses, such as 12 mg/kg, do increase mortality rates in exposed offspring (Vorhees et al., 1994). Further, in the same model, albeit at higher doses (5, 10, and 20 mg/kg), a significant effect treatment effect on mortality was found with a twofold dose response increase in stillborn pups, compared to vehicle exposed litters (Appendix 9.1). Extending from these results showing an absence of treatment effect on birth weights, supplementary studies with doses reaching from 1-8 mg/kg, also found no effect of treatment on birth weight (Vorhees et al., 1994, Bairy et al., 2007). However, the same studies found that when fluoxetine treatment increased to 12 mg/kg, there was a significant decrease in pup body weight at PND 1, suggesting a possible dose-dependent effect of exposure on birth weights (Vorhees et al., 1994, Bairy et al., 2007). In the previous study, an effect of treatment was also noted for birth weights, with the highest doses significantly reducing male and female weights (Appendix 9.1). Predominantly, fluoxetine exposure at the current dose employed had no effect on pup litter characteristics, however additional preclinical literature suggests that higher doses may actually increase mortality rates and decrease birth weights.

3.4.3.2 Paroxetine

While sex ratio was not affected by paroxetine, a treatment effect was found for mortality for stillborn and total dead pups. Additionally, no effect was found for treatment on birthweight, however, there was a sex effect in that males weighed more than females at PND 1. While literature at the doses employed in this study has not been identified, there are a few studies that exist at higher doses which support the trends reported. These findings regarding exposure having no effect on sex ratio are reinforced by previous results, albeit in mice (30 mg/kg, PO, GD (-14)-GD 16) (Coleman et al., 1999). Under the same conditions, lower birth weights and a reduction in weight gain, from PND 1-4, have also been reported in mice (Rayburn et al., 2000, Coleman et al., 1999). Furthermore in rats, exposure (10 mg/kg, PO, GD 15-21) also significantly reduced birth weights (Van den Hove et al., 2008). This is interesting to note as at the lower doses which were employed here (1.25-5 mg/kg), no effect of treatment on weight was prominent at birth, however, particular doses (2.5 and 5 mg/kg) still increased mortality within the first week, and some doses (2.5 mg/kg) even increased stillborn rates. Previous studies assessing gestational paroxetine exposure and pup mortality are also limited, and to our knowledge, none present mortality data for PND 1 or for the first week postpartum. However, the aforementioned study in rats by Van den Hove et al. (2008) reports increases in pup mortality rates, perhaps a factor of their reported decreased birth weights. This supports the current results that exposure impacts pup mortality. Furthermore, a preliminary study conducted in the laboratory, also attempted a higher dose (10 mg/kg), however profound mortality effects were noted as well as reduced birth weights (Appendix 9.2). Thus the 10 mg/kg group was removed and the lowest dose (1.25 mg/kg) was installed instead. Overall, gestational paroxetine exposure, at the doses employed and reported elsewhere, does not affect sex ratio. These results taken with the previous findings suggest that while only doses over 10 mg/kg alter birth weights, doses as low as 2.5 and 5 mg/kg can still have significant effects on offspring mortality.

3.4.3.3 Sertraline

Although sex ratio and stillborn parameters were not affected by sertraline, a treatment effect was found in mortality for total pups dead within the first week. In addition, no effect was found for treatment on birth weight, however, there was a sex effect in that

males weighed more than females at PND 1. To date, previous findings have not been reported on sertraline exposure and sex ratio at littering in rats. Similar to these findings, no effect was found on stillborn rates in a study exposing dams all throughout gestational (10 mg/kg, SC, GD 0-littering) (De Long et al., 2015b). As noted in Table 1.13, Davies and Klowe (1998) have conducted many analyses of the effects of sertraline on mortality before and after PND 4, with varying exposure periods and ranging doses (10-80 mg/kg, PO). Similar to De Long et al., (2015b) and the current study, which found no effect of treatment on stillborn, Davies and Klowe (1998) also reported no effect of 10 mg/kg treatment on mortality before PND 4, rather an increase is noted at doses such as 20, 40, or 80 mg/kg sertraline with exposures from mating through littering. While the current results indicate that 10 mg/kg increases total death within the first week, the aforementioned study indicates after PND 4 there were no effects of treatment on mortality, regardless of dose (Davies and Klowe, 1998). Agreeing with the reported findings, De Long et al., (2015b) suggests that 10 mg/kg has no effect on birth weight, however, a decrease in birth weight is noted for doses of 20 mg/kg or greater (Davies and Klowe, 1998). Overall, sertraline exposure *in utero* has no effect on sex ratio or birth weights, nevertheless, the highest dose of sertraline does increase total pup dead within the week following birth.

3.4.3.4 Citalopram

Sex ratio and mortality were not affected by citalopram exposure in the present study. Additionally, no effect was found for treatment on birth weight, however, there was a sex effect in that males weighed more than females at PND 1. Regarding pup littering characteristics, limited literature exists on gestational citalopram exposure in rat offspring. However, one study completed in mice also found no effect on sex ratio or mortality when pups were exposed (5 mg/kg, SC) throughout the third trimester of pregnancy (Hsiao et al., 2005). Aside from the present study, no literature was found demonstrating the outcomes of citalopram exposure during pregnancy on birth weights. Overall, citalopram exposure had no effects on any of the pup litter characteristics, and novel findings have been contributed in regards to the absence of effect citalopram has on birth weights.

3.4.4 Conclusion

In conclusion, the results presented in this chapter suggest that the doses ultimately employed do allow for a clinically relevant model without having a toxic effect on maternal parameters of wellbeing, thus avoiding the measurement of *in utero* toxicity rather than *in utero* SSRI exposure on dam, litter and pup observations. Few effects were noted due to paroxetine and sertraline treatment, but no effects were noted due to citalopram or fluoxetine treatment at the doses employed in the current study. It is important to consider however that sertraline and citalopram were examined at a higher range of doses (2.5, 5 or 10) whereas fluoxetine assessment occurred at a single low dose (2.5 mg/kg) and paroxetine reached to a lower dose range (1.25, 2.5, 5 mg/kg) in comparison to sertraline and citalopram. This transpired because a previous study in the lab involving fluoxetine at higher doses (5, 10, 20 mg/kg) had profound effects on maternal weight gain and stillborn rates at all doses, and a significant effect on birth weights for the highest dose. Furthermore, a preliminary study with paroxetine suggested similar effects for the 10 mg/kg dose, hence the introduction of the lowest dose (1.25 mg/kg). The replacement of the higher dose with this lower dose proved valuable as although paroxetine showed no effects on maternal wellbeing or litter size, surprisingly paroxetine still had a significant impact on pup mortality at birth and within the first week following birth, even at the 2.5 mg/kg dose level, whereas all other SSRIs showed no impact on mortality at this level. The difference observed amongst the four SSRIs effect on neonatal survival emphasizes the idea that inferences cannot be drawn for the effects of a particular SSRI to the clinical context, based on preclinical research on a different SSRI. Furthermore, the paroxetine data suggest that pharmacological doses of paroxetine exposure *in utero* effects neonatal survival both immediately and longitudinally, which may have an impact on prescribing patterns clinically, especially considering treatment-naïve women. Sertraline, at the highest dose, did show a decrease in total maternal weight gain during the gestational period, this is mirrored in gestational food consumption. Consequently, as a weight gain inhibiting effect at this dose (10 mg/kg) has been noted previously in non-pregnant models, it could be suggested that this is an upper pharmacological dose, but not toxic to the pregnancy as there was no effect on litter size, gestational length, stillborn pups, or birth weights; thus, proposing that this effect did not impact the dam's ability to carry a typical pregnancy to full term. However, although the same parameters of maternal weights were not altered in the postpartum period, it is possible that the

smaller weight gain during gestation complicated the dam's ability to recover from labour and as a consequence, resulted in a significant increase in total pups dead within the first week of littering. It is suggested that the residual treatment effects contributing to mortality in the first week post-birth were not carried over to the second week post-birth, as there were no differences noted for maternal caregiving behaviours. In conclusion, no effects were observed by citalopram or fluoxetine exposed litters at the resultant doses studied; however, significant mortality effects were observed due to paroxetine treatment, while the highest doses of sertraline led to a decrease in maternal wellbeing and subsequently an increase in mortality within the first week postpartum. Extended to the clinical scenario, such results can inform physicians, particularly when prescribing to treatment-naïve women, further anticipating maternal wellbeing and delivery outcomes.

**4 Somatic and behavioural
neonatal development of rat
progeny exposed to SSRI
antidepressants *in utero*.**

4.1 Introduction

As mentioned in previous chapters, 1 in 3 women of reproductive age are prescribed antidepressants (Guo et al., 2018) and 1 in 7 pregnant women are depressed (Pearson et al., 2018). Consequently, rates of antidepressant exposure during gestation are increasing (Pearson et al., 2018), thus contributing to SSRIs being reported as the most widely prescribed psychotropic drug during pregnancy (Alwan et al., 2016).

SSRIs are suggested as the first line for drug treatment to manage depression in pregnancy (NICE, 2014). Untreated depression complicates the mother's ability to care for her own health and shows increased risks of self-medication, overall placing the health of the unborn at risk. Subsequently, untreated depression during pregnancy is associated with maternal postpartum depression and suicidality, thus further impeding the mother's ability to care for the child after birth. In addition to foetal growth restrictions and low birth weights, offspring exposed to untreated depression and stress in pregnancy can be characterized as having lower IQs, developmental delays, abnormal cognitive and emotional outcomes, and depression and anxiety in childhood (Oberlander et al., 2006, Kim et al., 2006, Leigh and Milgrom, 2008).

As previous chapters described, SSRIs alter the synaptic availability of serotonin, an important growth factor in embryogenesis which is present clinically as early as gestational week 5. Further, serotonin guides the development of brain structure and many other neurotransmitter systems (Whitaker-Azmitia, 2001). A resulting complication of altered levels of serotonin in pregnancy includes uterine dysfunction (Kelly and Sharif, 2006, Wessler et al., 2007). Serotonin also mediates typical cardiovascular activity as a neurohormonal factor (Cote et al., 2004) and regulates the cardiovascular parasympathetic system (Ramage and Villalon, 2008).

Interestingly, conclusions drawn from gestational exposure to SSRIs are often summarized to the entire SSRI class. Observations of the combined SSRI class include preterm delivery, low birth weight, postnatal adaptation syndrome, low Apgar scores, and developmental delays. When serotonin is altered by paroxetine exposure *in utero*, clinical databases suggests a correlation between exposure and congenital heart malformations (Cole et al., 2007, Kallen and Olausson, 2007) however no clear correlations have been made for other SSRIs. Thus, as mentioned previously, the FDA updated paroxetine's categorization from C (foetal risks may exist, not enough clinical

data has been collected) to D (clinical data shows a correlation for foetal risks). Fluoxetine, sertraline, and citalopram remain in category C, since clear conclusions cannot be drawn due to limited data. Therefore studies considering SSRIs individually are needed to understand the consequences of each drug in the resulting progeny.

Animal models are used to reveal the teratogenic effects of pharmacological compounds on offspring, which helps to predict clinical birth outcomes, avoiding potentially harmful effects on the baby. Clinical trials do not occur in pregnancy in order to avoid devastating effects on the progeny; however, limited information is gained through clinical case studies and databases, after accounting for multiple confounding factors. Thus, it is important that the design and methodologies of animal studies have high clinical relevance. Regulatory studies carried out do not typically mirror the clinical experience and more closely reveal overdose consequences during pregnancy, as higher doses are used than typically prescribed clinically. Therefore, the work presented here applies the concept of safety pharmacology, which considers a range of therapeutic doses and above, to examine organ function along with structure (Bass et al., 2004). Therefore, adding to the gross malformations reported in toxicological studies, safety pharmacology assess the CNS as a function of behaviour, locomotor activity/coordination, sensorimotor reflexes and pain perception (Hamdam et al., 2013). In addition, the majority of relevant preclinical literature focuses on one SSRI, namely fluoxetine. Similar to the conclusions made in clinical literature that consider all SSRIs as having the same effect, preclinical literature focuses on the effect of fluoxetine and lends fluoxetine findings to be characteristics of all SSRIs. Overall, using animal studies to mimic the typical clinical scenario, rather than the worst case scenario (i.e. therapeutic dose rather than overdose), can yield clinically relevant information regarding the somatic and behavioural maturation of offspring exposed to individual SSRIs during gestation.

This study uses the oral gavage, which is an oral route of administration to deliver the SSRIs to match the clinical scenario. Additionally, while SSRI exposure can occur at various periods during pregnancy, the current research illustrates exposure from GD 7, mimicking the manifestations of treatment often observed clinically. The somatic and behavioural maturation of the exposed pups was monitored throughout the neonatal period. In addition, consideration was given to an interaction between the SSRI effect and sex of the offspring, by recording both male and female maturation in

all parameters observed. Further, parameters are measured at several time points and they have been selected based on critical milestones when the characteristics are typically acquired to track pup growth and expose developmental delays or advances.

As clinical reports suggest an association between gestational SSRI exposure and developmental delays (Pedersen et al., 2010) monitoring somatic and behavioural development is important in identifying possible teratogenic effects on the physical development of exposed progeny, as well as implications on sensorimotor coordination, vestibular function, or righting and gripping reflexes. Taking note of alterations in features such as fur appearance, pinna unfolding, eye opening, ano-genital distance, body length, and body weight at various critical time points, offers a window into the somatic growth of the pups. Delays in somatic development also help illustrate maternal wellbeing, as maternal insufficiencies could result in inadequate lactational nutrition or deprivation of nursing altogether. As such, pups of malnourished dams exhibit significant delays compared to progeny of normal protein dams (Khanna et al., 1991). Additionally, administering behavioural tasks such as surface righting, negative geotaxis, and forelimb grip task allows for the behavioural representation of the exposed offspring, particularly in regards to motor function. Collectively, the aforementioned somatic and behavioural parameters enable the examination of the physical maturation and the characterization of sensorimotor coordination and reflex development of the exposed offspring at different critical periods throughout neonatal development. Subsequently, these features can help to predict enduring consequences in adulthood.

4.1.1 Hypothesis and aims

It is specifically hypothesized in this chapter that *in utero* exposure to fluoxetine, paroxetine, sertraline, or citalopram at clinically relevant doses that are not significantly toxic to maternal wellbeing will alter neonatal development.

This hypothesis was assessed through two specific aims throughout this chapter, ultimately investigating the initial and longitudinal effects of SSRIs administered from GD 7-21 on offspring parameters of (1) somatic and (2) behavioural development, with considerations to potential differences in sex within each of these parameters.

4.2 Experimental methods and design

A detailed description of the study breeding, gestational exposure, recording of postnatal observation, and pup somatic and behavioural development can be found in Chapter 2.

Briefly, Sprague-Dawley pups were born on PND 1, after being exposed via the dam *in utero* to either vehicle, FLX (2.5 mg/kg), PRX (1.25, 2.5, or 5 mg/kg), SERT or CIT (2.5, 5 or 10 mg/kg) via oral gavage from GD 7 until littering (n=9-13 litters per group). Testing occurred with all four SSRIs under investigation simultaneously, however, research was collected over three cohort studies in order to successfully manage a study of this size.

When possible, the same male and female litter representatives were used for tracking the somatic and behavioural maturation of the exposed pups. Only one male and one female pup were used per litter to avoid litter effects. Postnatal developmental testing occurred from PND 1–24. Somatic parameters included fur appearance, pinna unfolding, eye opening, ano-genital distance, body length, and body weight. Behavioural parameters included surface righting, negative geotaxis, and forelimb grip.

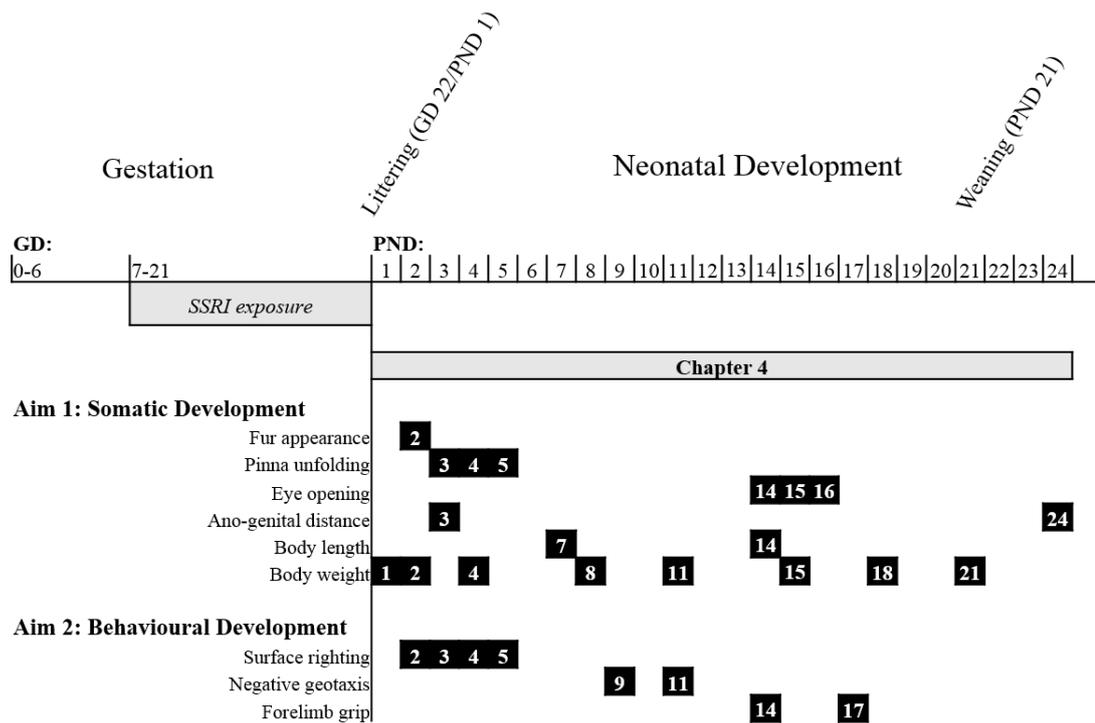


Figure 4.1 Neonatal development experimental timeline.

Statistical analysis was performed using the Statistical Package for Social Sciences 24.0 for Windows (SPSS Inc., IBM, New York, USA) on the parameters listed in Table 4.1. Primarily, normality and homogeneity of variance ($p < 0.05$) were determined to choose the appropriate analysis. Parametric somatic development parameters (ano-genital distance, length, and pup body weights) were analysed using a two-way ANOVA with factors of dose, sex, and dose x sex. When pup weights were measured at multiple time-points during neonatal development, they were analysed using a two-way ANOVA repeated measures. Sphericity could not be assumed, therefore the Greenhouse-Geisser correction was employed. When appropriate ($p < 0.05$), ANOVAs were followed up by an SNK (Student-Newman-Keuls) *post hoc* test. Non-parametric data of somatic and behavioural development (fur, pinna, eye, surface righting, negative geotaxis, and forelimb grip) were assessed as present vs absent features, therefore, a χ^2 statistical analysis was carried out on raw values, despite being represented in tables and figures as ratio or percentage. When appropriate ($p < 0.05$), additional *post hoc* χ^2 tests were used to determine differences amongst individual treatment groups. Bonferroni correction was applied to non-

parametric data to account for multiple comparisons. In the case of fluoxetine, when making three comparisons (two doses and sex) $*p < 0.025$ was used. In the cases of paroxetine, sertraline, and citalopram, when making five comparisons (four doses and sex) $*p < 0.013$ was used.

Parameter	Shapiro-Wilk & Levene's test $p > 0.05$	Shapiro-Wilk & Levene's test $p < 0.05$
Presence of: fur, pinna, eye, behavioural development		Chi Square → Bonferroni correction
Ano-genital distance, length, body weight	ANOVA → SNK	

Table 4.1 Statistical analysis of neonatal development. In the case of fluoxetine, Bonferroni correction employed to correct for multiple comparisons was $*p < 0.025$. For all other SSRIs, Bonferroni correction employed to correct for multiple comparisons was $*p < 0.013$.

4.3 Postnatal development results

4.3.1 Somatic development: Fur appearance and pinna unfolding

As shown in Table 4.2, Table 4.3, Table 4.4, and Table 4.5 there were no significant effects of sex or treatment on fur appearance or pinna unfolding, regardless of SSRI.

Group	n	Fur appearance (%)		Pinna unfolding (%)			
		PND 3	PND 4	PND 3	PND 4	PND 5	PND 6
<i>Male</i>							
Vehicle	12	83	100	17	83	92	100
2.5 mg/kg FLX	12	92	100	8	83	100	100
<i>Female</i>							
Vehicle	12	83	100	17	82	100	100
2.5 mg/kg FLX	11	91	100	18	91	100	100
Sex	χ^2	0.00	0.00	0.00	0.38	1.04	0.00
	<i>p</i>	1.000	1.000	1.000	0.537	0.307	1.000
Dose	χ^2	0.67	0.00	0.12	0.00	0.98	0.00
	<i>p</i>	0.413	1.000	0.727	0.955	0.322	1.000

Table 4.2 Fluoxetine fur appearance and pinna unfolding. Pups exposed to either vehicle or FLX (2.5 mg/kg) from GD 7-21, via oral gavage to dam. Data are expressed as the percentage of pups with fur or with both pinnae. No significant effect of treatment was found, see table for statistical analysis.

Group	n	Fur appearance (%)		Pinna unfolding (%)			
		PND 3	PND 4	PND 3	PND 4	PND 5	PND 6
<i>Male</i>							
Vehicle	12	92	100	8	67	100	100
1.25 mg/kg PRX	9	100	100	44	89	100	100
2.5 mg/kg PRX	10	100	100	50	90	100	100
5 mg/kg PRX	11	91	100	27	91	100	100
<i>Female</i>							
Vehicle	12	83	100	25	75	100	100
1.25 mg/kg PRX	9	100	100	33	89	100	100
2.5 mg/kg PRX	10	90	100	20	90	100	100
5 mg/kg PRX	11	100	100	36	100	100	100
Sex	χ^2	0.38	0.00	1.20	0.20	0.00	0.00
	<i>p</i>	0.537	0.537	1.000	0.273	1.000	1.000
Dose	χ^2	3.09	0.00	2.99	6.49	0.00	0.00
	<i>p</i>	0.378	0.378	1.000	0.393	1.000	1.000

Table 4.3 Paroxetine fur appearance and pinna unfolding. Pups exposed to either vehicle or PRX (1.25, 2.5, 5 mg/kg) from GD 7-21, via oral gavage to dam. Data are expressed as the percentage of pups with fur or with both pinnae. No significant effect of treatment was found, see table for statistical analysis.

Group	n	Fur appearance (%)		Pinna unfolding (%)			
		PND 3	PND 4	PND 3	PND 4	PND 5	PND 6
<i>Male</i>							
Vehicle	12	83	100	8	75	92	100
2.5 mg/kg SERT	10	90	100	20	80	100	100
5 mg/kg SERT	10	100	100	10	90	100	100
10 mg/kg SERT	12	83	100	25	92	100	100
<i>Female</i>							
Vehicle	12	83	100	25	83	100	100
2.5 mg/kg SERT	9	89	100	11	89	100	100
5 mg/kg SERT	9	100	100	22	100	100	100
10 mg/kg SERT	12	92	100	25	92	100	100
Sex	χ^2	0.00	0.00	1.20	0.25	1.04	0.00
	<i>p</i>	1.000	1.000	0.273	0.615	0.307	1.000
Dose	χ^2	0.10	0.00	0.91	2.94	2.61	0.00
	<i>p</i>	0.992	1.000	0.824	0.401	0.455	1.000

Table 4.4 Sertraline fur appearance and pinna unfolding. Pups exposed to either vehicle or SERT (2.5, 5, 10 mg/kg) from GD 7-21, via oral gavage to dam. Data are expressed as the percentage of pups with fur or with both pinnae. No significant effect of treatment was found, see table for statistical analysis.

Group	n	Fur appearance (%)		Pinna unfolding (%)			
		PND 3	PND 4	PND 3	PND 4	PND 5	PND 6
<i>Male</i>							
Vehicle	12	75	100	8	67	100	100
2.5 mg/kg CIT	9	67	100	11	89	100	100
5 mg/kg CIT	13	85	100	31	77	100	100
10 mg/kg CIT	12	82	100	27	73	91	100
<i>Female</i>							
Vehicle	12	75	100	17	75	100	100
2.5 mg/kg CIT	9	80	100	10	90	100	100
5 mg/kg CIT	13	69	100	31	77	100	100
10 mg/kg CIT	12	73	100	45	82	91	100
Sex	χ^2	0.00	0.00	0.38	0.20	0.00	0.00
	<i>p</i>	1.000	1.000	0.537	0.653	1.000	1.000
Dose	χ^2	0.10	0.00	6.25	2.20	6.41	6.41
	<i>p</i>	0.992	1.000	0.100	0.532	0.093	0.492

Table 4.5 Citalopram fur appearance and pinna unfolding. Pups exposed to either vehicle or CIT (2.5, 5, 10 mg/kg) from GD 7-21, via oral gavage to dam. Data are expressed as the percentage of pups with fur or with both pinnae. No significant effect of treatment was found, see table for statistical analysis.

4.3.2 Somatic development: Eye opening, ano-genital distance, length

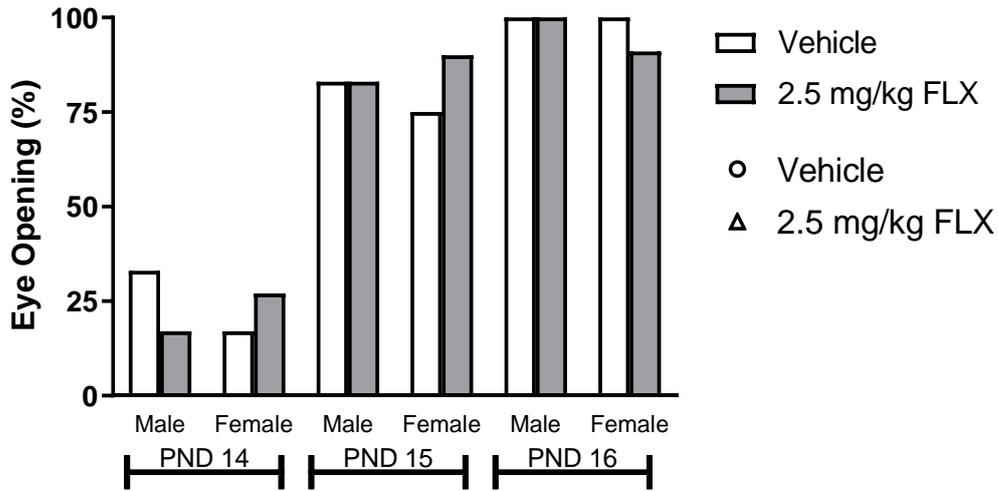
4.3.2.1 Fluoxetine (Figure 4.2)

In regards to eye opening, on PND 14, 15, 16, and 17 there was no significant effect of sex [PND 14: $\chi^2=0.89$, $p=0.346$; PND 15: $\chi^2=0.25$, $p=0.615$; PND 16: $\chi^2=0.00$, $p=1.000$; PND 17: $\chi^2=0.00$, $p=1.000$] or treatment [PND 14: $\chi^2=0.07$, $p=0.792$; PND 15: $\chi^2=0.41$, $p=0.520$; PND 16: $\chi^2=1.07$, $p=0.302$; PND 17: $\chi^2=0.00$, $p=1.000$]. By PND 16, both eyes were opened for all male pups. By PND 17, both eyes were opened for all pups.

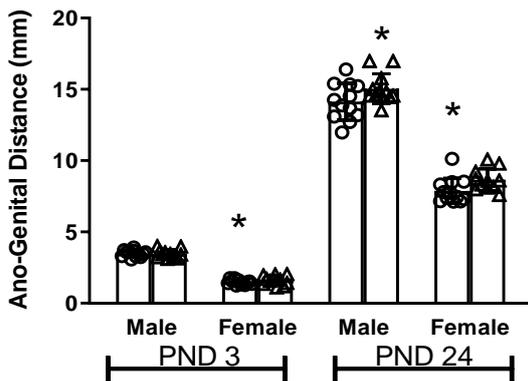
In the analysis of ano-genital distance, on PND 3 and 24, there was a significant effect of sex [PND 3: $F_{(1,43)}=630.96$, $p<0.001$; PND 24: $F_{(1,43)}=452.35$, $p<0.001$], with males having a larger ano-genital distance than females. There was no effect of treatment at PND 3 [$F_{(1,43)}=0.46$, $p=0.501$], however, an effect of treatment was found at PND 24 [$F_{(1,43)}=8.00$, $p=0.007$]. A *post hoc* analysis revealed an effect within the male groups, with treated males having a larger ano-genital distance at PND 24 than untreated males.

Body length analysis initially indicated a significant effect of sex at PND 7 [$F_{(1,43)}=5.37$, $p=0.025$], with males being bigger than females but no effect of sex was observed at PND 14 [$F_{(1,43)}=0.12$, $p=0.731$]. No significant effect of treatment was found on length for either time point [PND 7: $F_{(1,43)}=3.63$, $p=0.063$; PND14: $F_{(1,43)}=3.15$, $p=0.083$].

A)



B)



C)

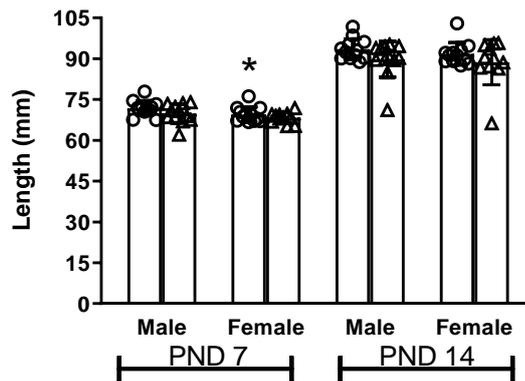


Figure 4.2 Fluoxetine somatic development. (A) Eye opening, (B) ano-genital distance, and (C) length, for resulting pups exposed to either vehicle or FLX (2.5 mg/kg) from GD 7-21. Data are expressed as the percentage of pups with both eyes opened, or data are expressed as mean \pm SD, n=11-12/group, * $p < 0.05$ vs vehicle male. An effect of sex was found for ano-genital distance and length, with males having a larger ano-genital distance and body length. A treatment effect was found within the male group, producing a significantly larger ano-genital distance. No significant deleterious effect of treatment was found, see text for statistical analysis.

4.3.2.2 Paroxetine (Figure 4.3)

In regards to eye opening, on PND 14, 15, and 16 there was no significant effect of sex [PND 14: $\chi^2=0.00$, $p=1.000$; PND 15: $\chi^2=0.18$, $p=0.673$; PND 16: $\chi^2=0.00$, $p=1.000$]. Also at PND 14 and 16, there was no significant effect of treatment [PND 14: $\chi^2=3.03$, $p=0.387$; PND 16: $\chi^2=0.00$, $p=1.000$]. At PND 15, there was a significant effect of treatment [$\chi^2=13.93$, $p=0.003$], although a *post hoc* analysis showed no effect within the male or female treatment groups. By PND 16, both eyes were opened for all pups.

In the analysis of ano-genital distance, a significant effect of sex was revealed on PND 3 and 24 [PND 3: $F_{(1,76)}=1048.04$, $p<0.001$; PND 24: $F_{(1,76)}=809.62$, $p<0.001$], with males having a larger ano-genital distance than females. No treatment effect was observed at PND 3 [$F_{(3,76)}=1.76$, $p=0.163$], and although a treatment effect was observed at PND 24 [$F_{(3,76)}=2.83$, $p=0.044$] no treatment effects were observed within male or female groups after a *post hoc* analysis.

Body length analysis indicated no significant effect of sex [PND 7: $F_{(1,76)}=3.696$, $p=0.058$; PND 14: $F_{(1,76)}=1.81$, $p=0.183$] or treatment [PND 7: $F_{(3,76)}=1.08$, $p=0.364$; PND 14: $F_{(3,76)}=0.70$, $p=0.554$] were found at either time point.

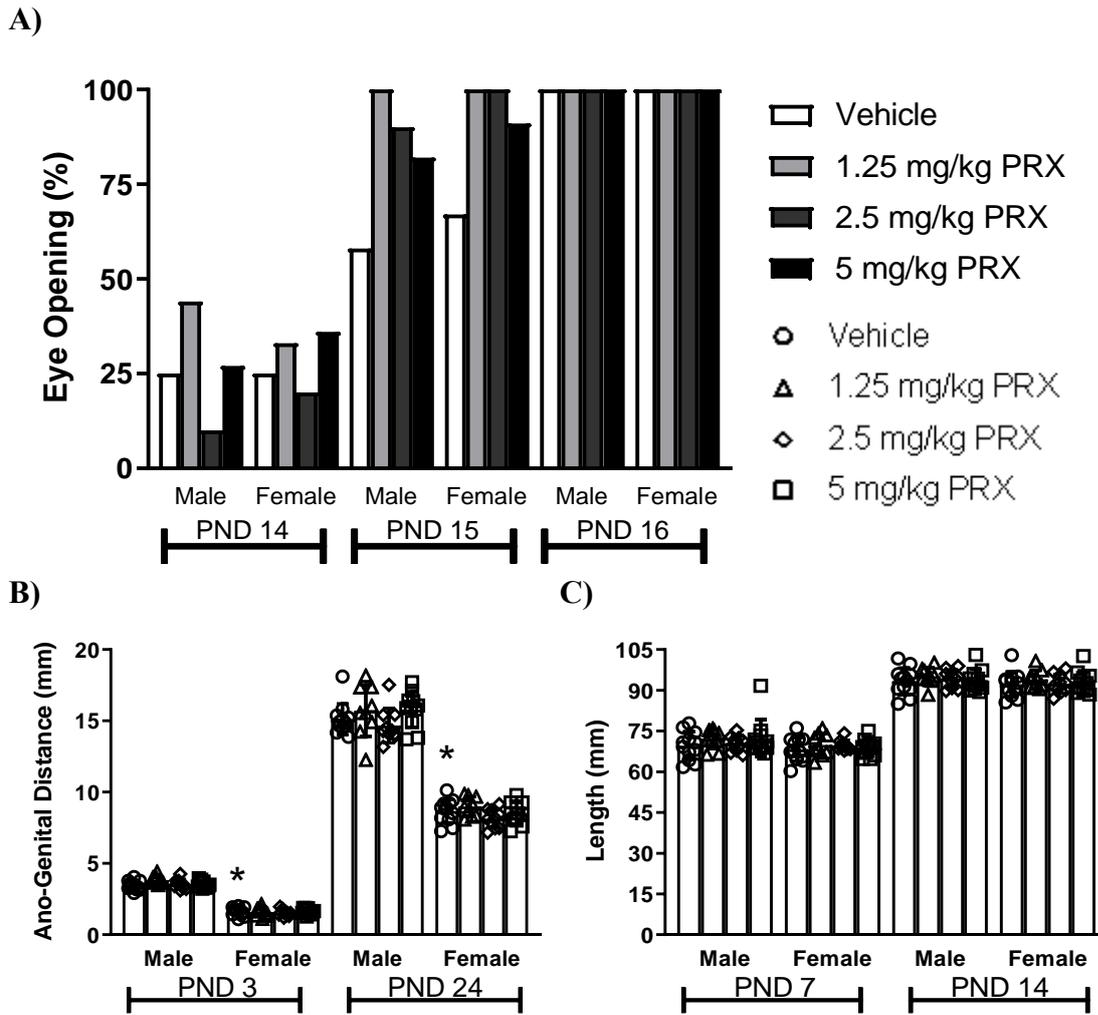


Figure 4.3 Paroxetine somatic development. (A) Eye opening, (B) ano-genital distance, and (C) length, for resulting pups exposed to either vehicle or PRX (1.25, 2.5, or 5 mg/kg) from GD 7-21. Data are expressed as the percentage of pups with both eyes opened or data are expressed as mean \pm SD, n=9-12/group, * p <0.05 vs vehicle male. An effect of sex was found for ano-genital distance, with males having a larger ano-genital distance. No significant effect of treatment was found, see text for statistical analysis.

4.3.2.3 Sertraline (Figure 4.4)

In regards to eye opening, on PND 14, 15, 16, or 17 there was no significant effect of sex [PND 14: $\chi^2=0.25$, $p=0.615$; PND 15: $\chi^2=0.00$, $p=1.000$; PND 16: $\chi^2=0.00$, $p=1.000$; PND 17: $\chi^2=0.00$, $p=1.000$] or treatment [PND 14: $\chi^2=4.77$, $p=0.190$; PND 15: $\chi^2=6.18$, $p=0.277$; PND 16: $\chi^2=3.57$, $p=0.312$; PND 17: $\chi^2=0.00$, $p=1.000$]. By PND 16, both eyes were opened for all female pups. By PND 17, both eyes were opened for all pups.

In the analysis of ano-genital distance a significant effect of sex was found on PND 3 and 24 [PND 3: $F_{(1,78)}=833.47$, $p<0.001$; PND 24: $F_{(1,78)}=659.20$, $p<0.001$], with males having a larger ano-genital distance than females. No treatment effects were found at either time point [PND 3: $F_{(3,78)}=0.26$, $p=0.851$; PND 24: $F_{(3,78)}=0.99$, $p=0.400$].

Body length analysis indicated a significant effect of sex at PND 7 and 14 [PND 7: $F_{(1,78)}=6.70$, $p=0.011$; PND 14: $F_{(1,78)}=4.75$, $p=0.032$], with males having a longer body length than females. No treatment effect was observed at PND 7 [$F_{(3,78)}=2.06$, $p=0.112$], and although a treatment effect was found at PND 14 [$F_{(3,78)}=3.04$, $p=0.034$] a *post hoc* analysis revealed no differences within male or females treatment groups.

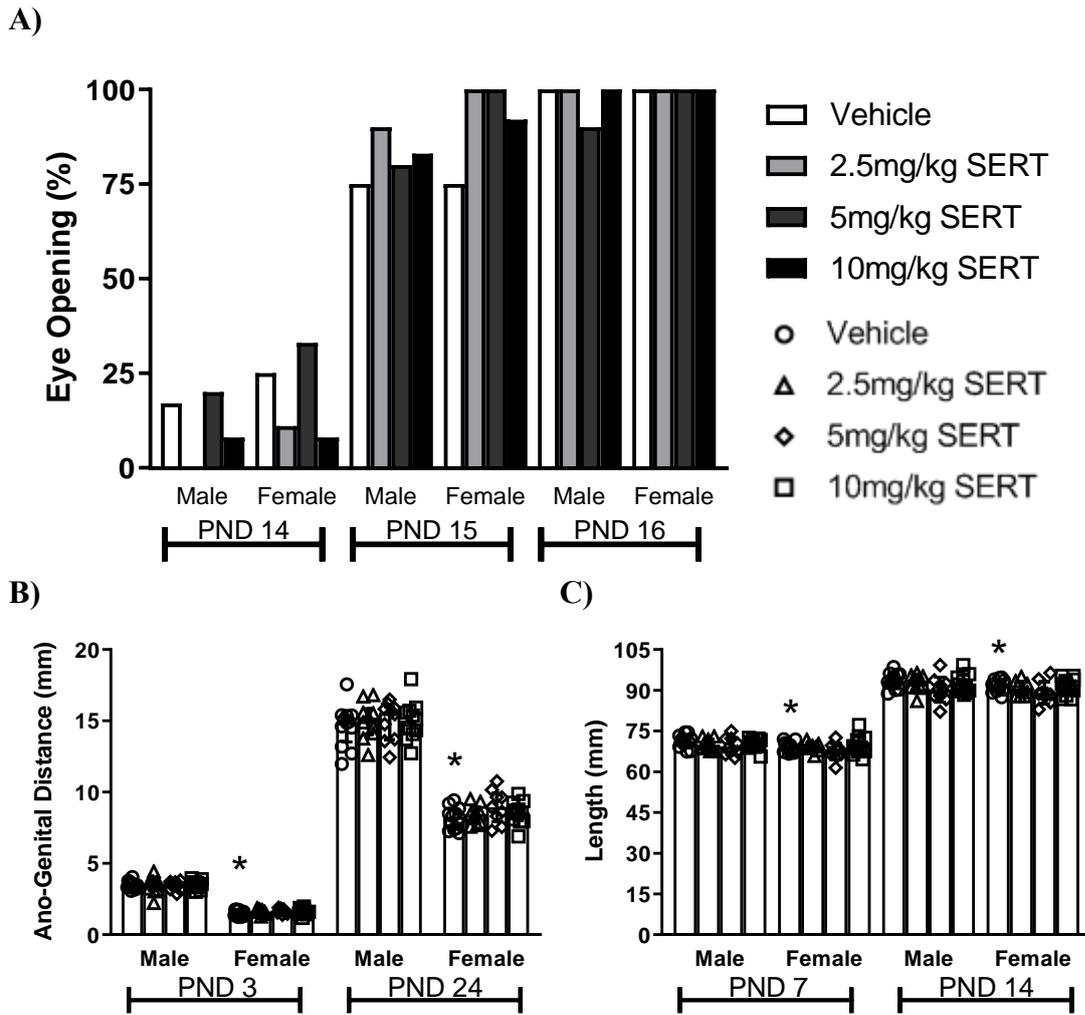


Figure 4.4 Sertraline somatic development. (A) Eye opening, (B) ano-genital distance, and (C) length, for resulting pups exposed to either vehicle or SERT (2.5, 5 or 10 mg/kg) from GD 7-21. Data are expressed as the percentage of pups with both eyes opened, or data are expressed as mean \pm SD, n=9-12/group, * p <0.05 vs vehicle male. An effect of sex was found for ano-genital distance and length, with males having a larger ano-genital distance and body length. No significant effect of treatment was found, see text for statistical analysis.

4.3.2.4 Citalopram (Figure 4.5)

In regards to eye opening, on PND 14, 15, 16, and 17 there was no significant effect of sex [PND 14: $\chi^2=2.18$, $p=0.140$; PND 15: $\chi^2=0.18$, $p=0.673$; PND 16: $\chi^2=0.00$, $p=1.000$; PND 17: $\chi^2=0.00$, $p=1.000$] or treatment [PND 14: $\chi^2=7.21$, $p=0.066$; PND 15: $\chi^2=4.16$, $p=0.244$; PND 16: $\chi^2=3.15$, $p=0.369$; PND 17: $\chi^2=0.00$, $p=1.000$]. By PND 17, both eyes were opened for all pups.

In the analysis of ano-genital distance, a significant effect of sex was found on PND 3 and 24 [PND 3: $F_{(1,83)}=979.23$, $p<0.001$; PND 24: $F_{(1,83)}=747.69$, $p<0.001$], with males having a larger ano-genital distance than females. No treatment effects were found on either day [PND 3: $F_{(3,83)}=1.16$, $p=0.330$; PND 24: $F_{(3,83)}=0.61$, $p=0.612$].

Body length analysis initially indicated a significant effect of sex at PND 7 [$F_{(1,83)}=5.31$, $p=0.024$] with males being longer, but not at PND 14 [$F_{(1,83)}=2.384$, $p=0.126$]. No significant effect of treatment was found on length at either time point [PND 7: $F_{(3,83)}=1.07$, $p=0.365$; PND 14: $F_{(3,83)}=0.629$, $p=0.598$].

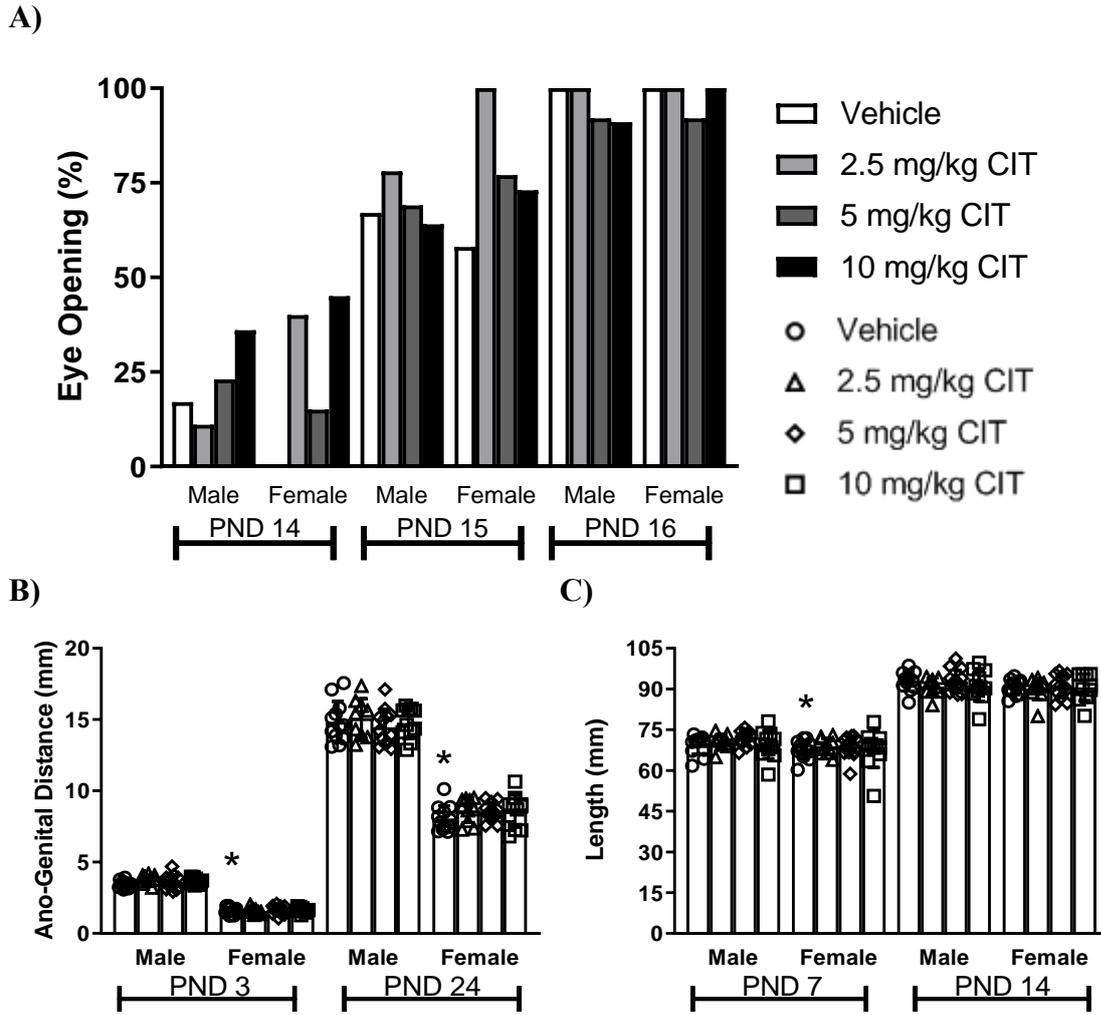


Figure 4.5 Citalopram somatic development. (A) Eye opening, (B) ano-genital distance, and (C) length, for resulting pups exposed to either vehicle or CIT (2.5, 5 or 10 mg/kg) from GD 7-21. Data are expressed as the percentage of pups with both eyes opened or data are expressed as mean±SD, n=9-13/group, * $p < 0.05$ vs vehicle male. An effect of sex was found for ano-genital distance and length, with males having a larger ano-genital distance and body length. No significant effect of treatment was found, see text for statistical analysis.

4.3.3 Somatic development: Daily body weight, total body weight gain

4.3.3.1 Fluoxetine (Figure 4.6)

Daily body weight data showed an effect of time [$F_{(1,879,80.808)}=5550.85$, $p<0.001$], which caused an increase in body weight. No significant interactions were detected for time with treatment or sex. A between-subject effect was found for sex [$F_{(1,43)}=4.68$, $p=0.036$], with males weighing more than females.

In regards to total body weight gain from PND 1-21, no effect was found for sex [$F_{(1,43)}=2.02$, $p=0.162$] or treatment [$F_{(1,43)}=0.03$, $p=0.863$].

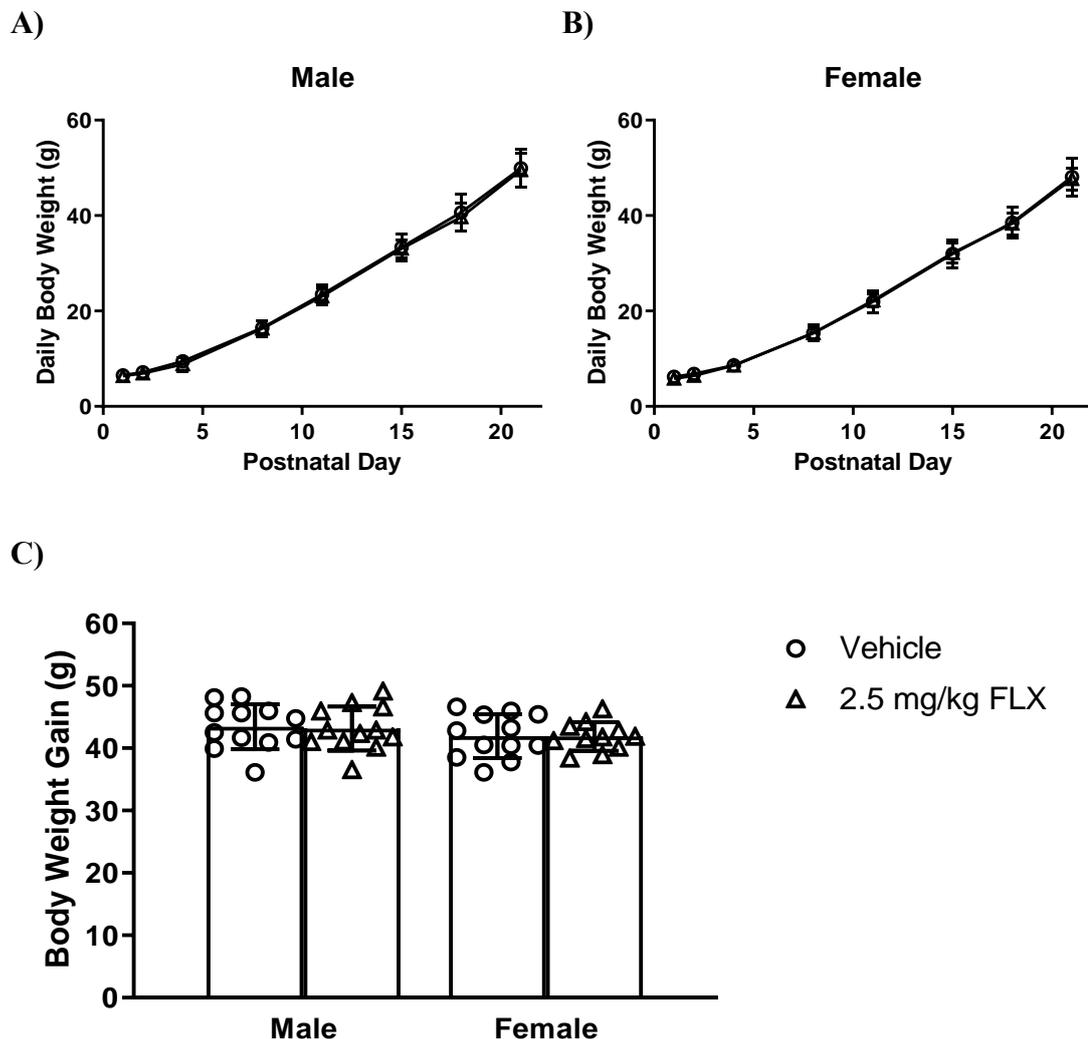


Figure 4.6 Fluoxetine neonatal body weights. (A) Male daily body weight, (B) female daily body weight, and (C) total weight gain from PND 1-21, for resulting pups exposed to either vehicle or FLX (2.5 mg/kg) from GD 7-21. Data are expressed as mean±SD, n=11-12/group. An effect of time and sex was found for daily body weight, as body weight increased significantly with time, and the males were significantly heavier than females. No significant effect of treatment was found, see text for statistical analysis.

4.3.3.2 Paroxetine (Figure 4.7)

Daily body weight data showed an effect of time [$F_{(1.669,125.164)}=5406.36$, $p<0.001$], which caused an increase in body weight. No significant interactions were detected for time with treatment or sex. A between-subject effect was found for sex [$F_{(1,75)}=5.27$, $p=0.024$], with males weighing more than females.

In regards to total body weight gain from PND 1-21, no effect was found for sex [$F_{(1,76)}=2.05$, $p=0.157$], nor was there an effect of treatment [$F_{(3,76)}=0.15$, $p=0.929$].

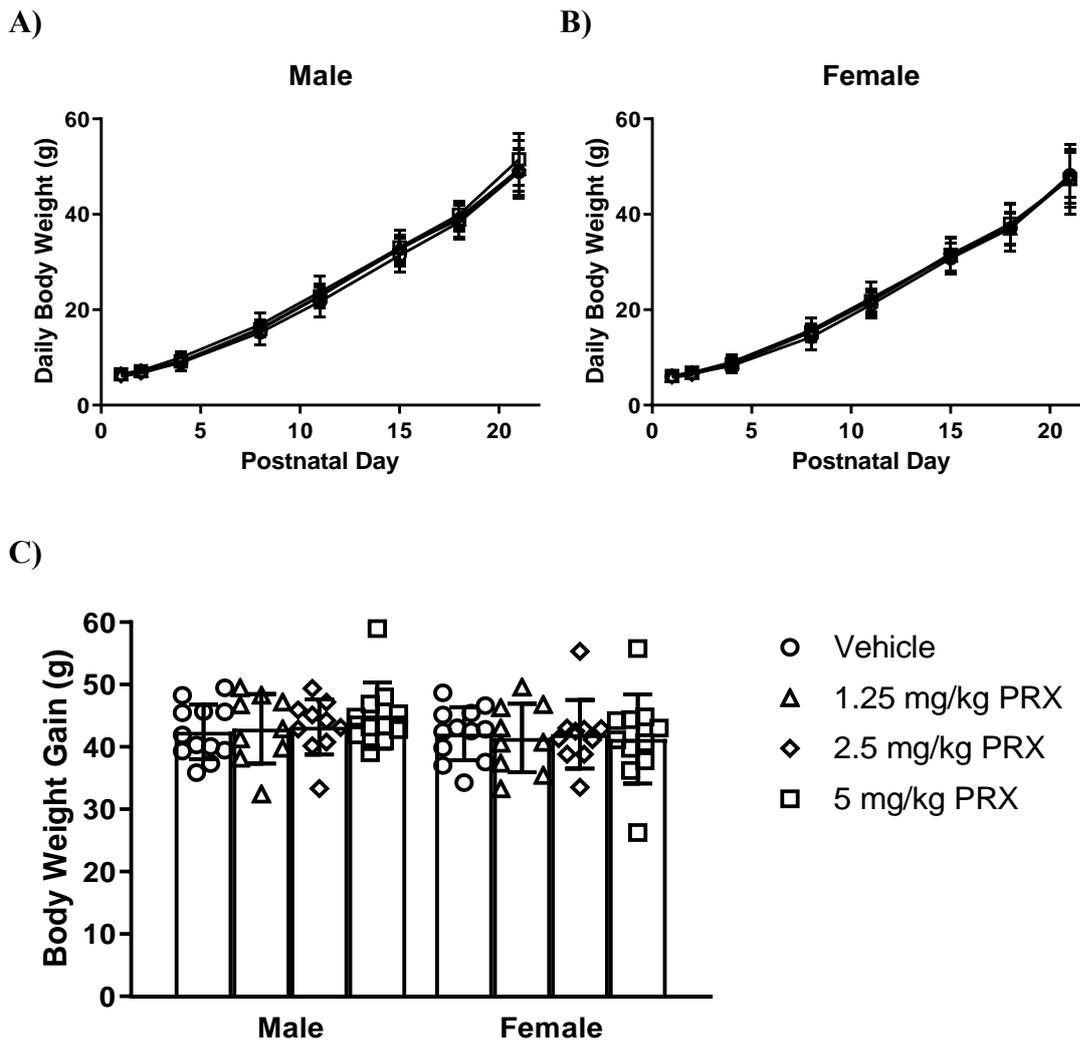


Figure 4.7 Paroxetine neonatal body weights. (A) Male daily body weight, (B) female daily body weight, and (C) total weight gain from PND 1-21, for resulting pups exposed to either vehicle or PRX (1.25, 2.5, or 5 mg/kg) from GD 7-21. Data are expressed as mean \pm SD, n=9-12/group. An effect of time and sex was found for daily body weight, as body weight increased significantly with time, and the males were significantly heavier than females. No significant effect of treatment was found, see text for statistical analysis.

4.3.3.3 Sertraline (Figure 4.8)

Daily body weight data showed an effect of time [$F_{(2,209,172.271)}=6596.39$, $p<0.001$], which caused an increase in body weight. No significant interactions were detected for time with treatment or sex. A between-subject effect was found for sex [$F_{(1,78)}=6.15$, $p=0.015$], with males weighing more than females.

In regards to total body weight gain from PND 1-21, no effect was found for sex [$F_{(1,78)}=1.84$, $p=0.179$], and no effect was found for treatment [$F_{(3,78)}=0.62$, $p=0.602$].

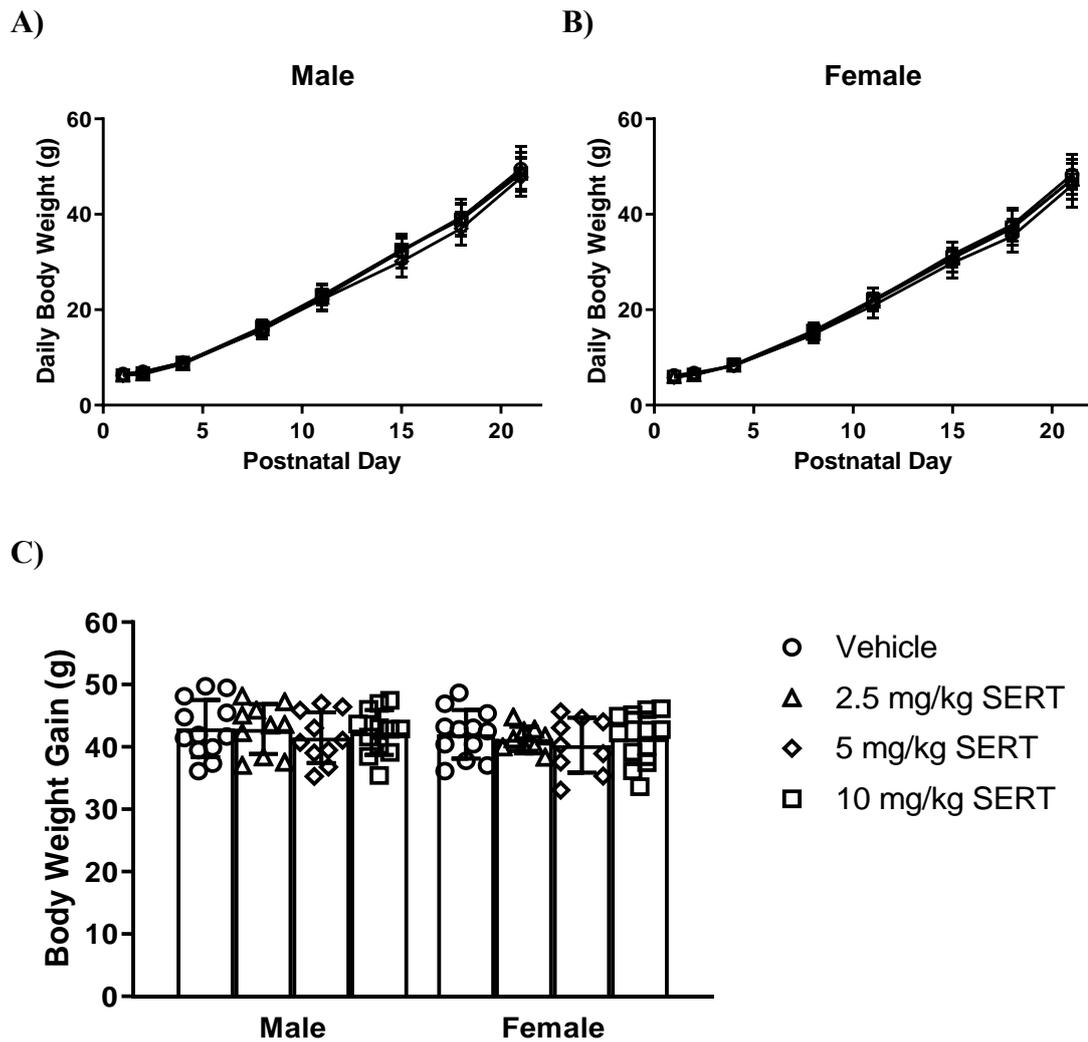


Figure 4.8 Sertraline neonatal body weights. (A) Male daily body weight, (B) female daily body weight, and (C) total weight gain from PND 1-21, for resulting pups exposed to either vehicle or SERT (2.5, 5, or 10 mg/kg) from GD 7-21. Data are expressed as mean \pm SD, $n=9-12$ /group. An effect of time and sex was found for daily body weight, as body weight increased significantly with time, and the males were significantly heavier than females. No significant effect of treatment was found, see text for statistical analysis.

4.3.3.4 Citalopram (Figure 4.9)

Daily body weight data showed an effect of time [$F_{(1,773,147.162)}=6187.23$, $p<0.001$], which caused an increase in body weight. No significant interactions were detected for time with treatment or sex. A between-subject effect was found for sex [$F_{(1,83)}=4.05$, $p=0.047$], with males weighing more than females.

In regards to total body weight gain from PND 1-21, no effect was found for sex [$F_{(1,83)}=2.00$, $p=0.161$], nor was there a treatment effect [$F_{(3,83)}=0.16$, $p=0.922$].

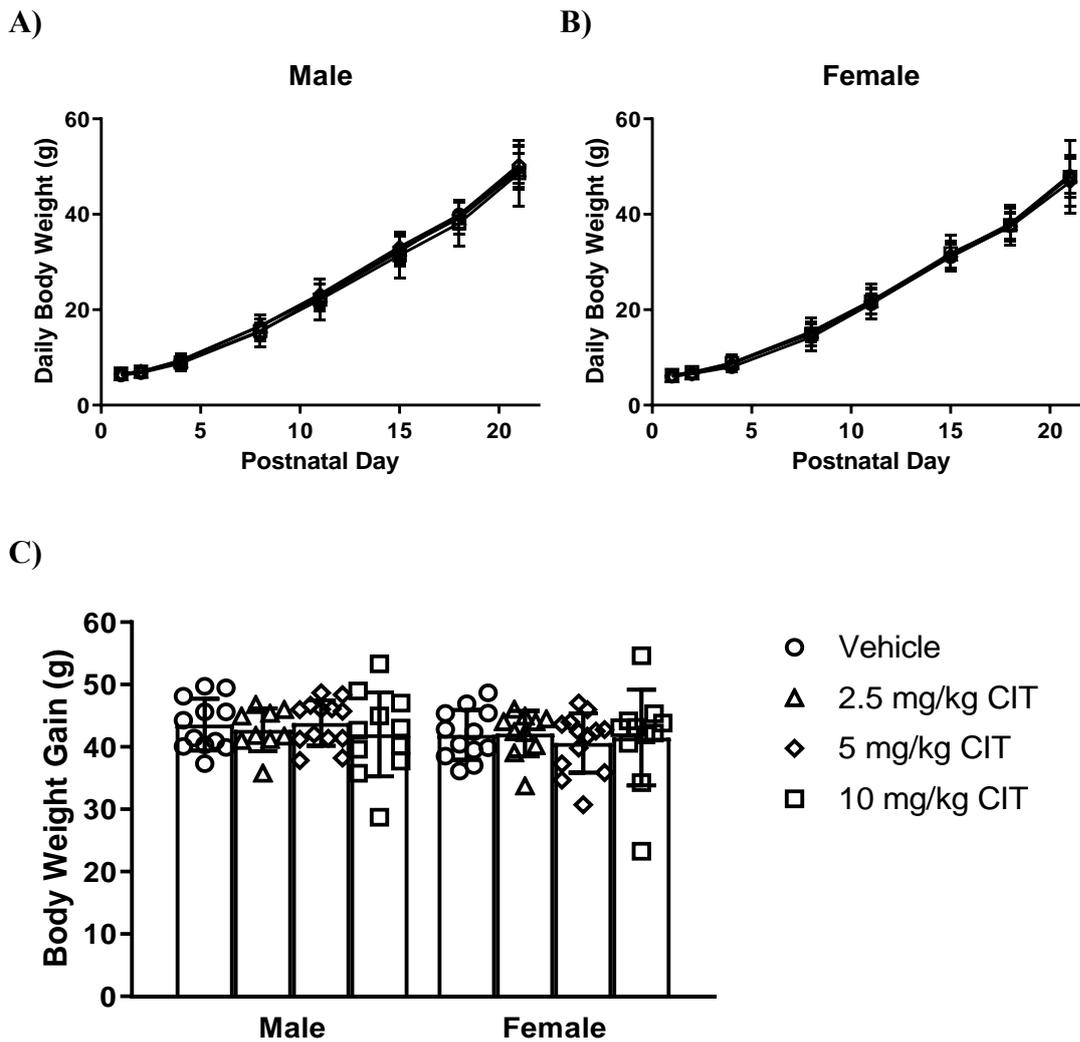


Figure 4.9 Citalopram neonatal body weights. (A) Male daily body weight, (B) female daily body weight, and (C) total weight gain from PND 1-21, for resulting pups exposed to either vehicle or CIT (2.5, 5, or 10 mg/kg) from GD 7-21. Data are expressed as mean \pm SD, $n=9-13$ /group. An effect of time and sex was found for daily body weight, as body weight increased significantly with time, and the males were significantly heavier than females. No significant effect of treatment was found, see text for statistical analysis.

4.3.4 Behavioural development: Surface righting, negative geotaxis, forelimb grip

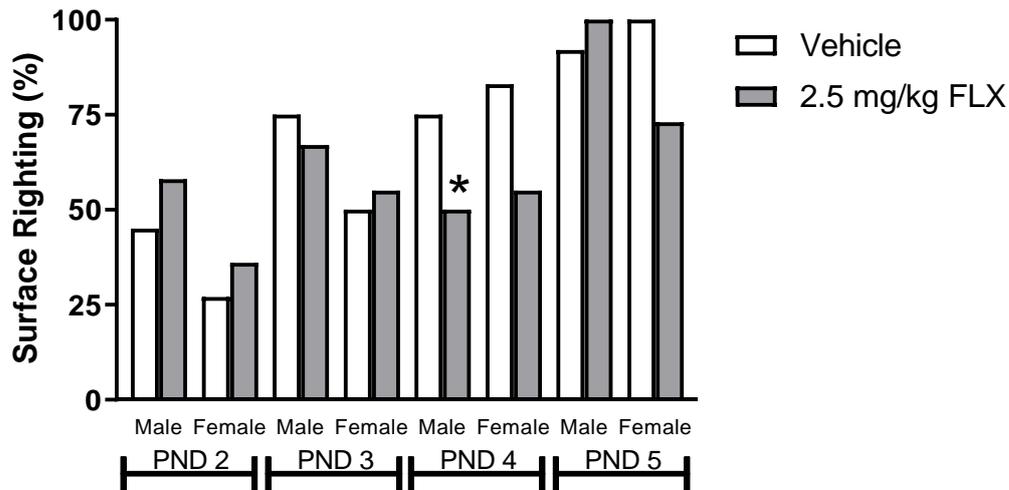
4.3.4.1 Fluoxetine (Figure 4.10)

In regards to surface righting, no significant effect of sex was found on PND 2-5 [PND 2: $\chi^2=0.79$, $p=0.375$; PND 3: $\chi^2=1.60$, $p=0.206$; PND 4 $\chi^2=0.38$, $p=0.537$; PND 5 $\chi^2=3.43$, $p=0.064$]. No significant effect of treatment was found for PND 2, 3, or 5 [PND 2: $\chi^2=0.61$, $p=0.436$; PND 3: $\chi^2=0.01$, $p=0.908$; PND 5: $\chi^2=0.00$, $p=0.955$]. However, a significant effect of treatment was found for PND 4 [$\chi^2=7.01$, $p=0.008$], with a *post hoc* revealing that treated males met surface righting criterion less than vehicle males, $*p<0.025$ due to multiple comparisons.

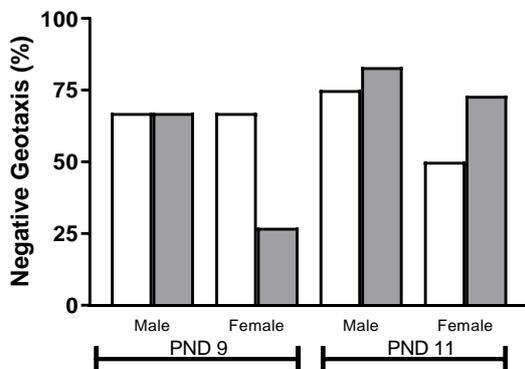
In the analysis of negative geotaxis, no significant effects were found at PND 9 or 11 for sex [PND 9: $\chi^2=0.00$, $p=1.000$; PND 11: $\chi^2=1.60$, $p=0.206$] or treatment [PND 9: $\chi^2=1.71$, $p=0.192$; PND 11: $\chi^2=1.40$, $p=0.238$].

Forelimb grip data was analysed and no significant effect of sex [$\chi^2=0.75$, $p=0.386$] or treatment [$\chi^2=2.56$, $p=0.110$] was observed at PND 14. An effect of sex was found at PND 17 [$\chi^2=4.80$, $p=0.028$], with females meeting criterion more than males, however, this was not statistically significant after correction for multiple comparisons $*p<0.025$. Further, there were no effects of treatment at PND 17 [$\chi^2=0.67$, $p=0.413$].

A)



B)



C)

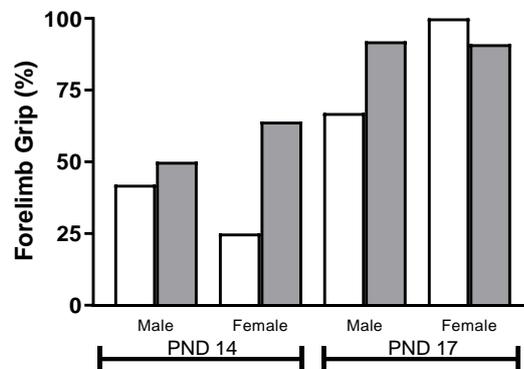


Figure 4.10 Fluoxetine behavioural development. (A) Surface righting, (B) negative geotaxis, and (C) forelimb grip, for resulting pups exposed to either vehicle or FLX (2.5 mg/kg) from GD 7-21. Data are expressed as the percentage of pups to successfully complete the task within the time required, $n=11-12/\text{group}$, $*p<0.025$ vs vehicle male, due to multiple comparisons. Pups met criterion for presence if (A) <10 seconds, (B) <15 seconds, (C) >10 seconds. Although treatment significantly reduced male surface righting on PND 4, no significant consistent deleterious effect of treatment was found on behavioural development, see text for statistical analysis.

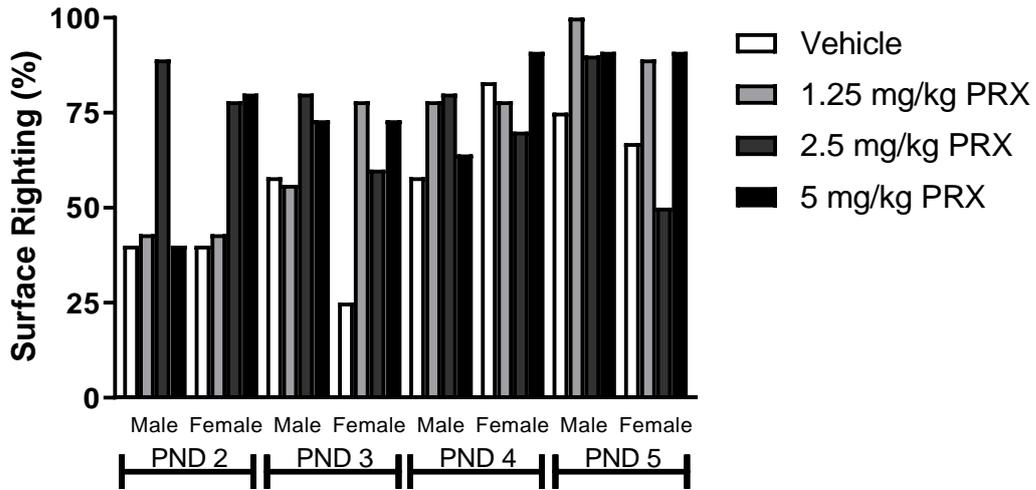
4.3.4.2 Paroxetine (Figure 4.11)

In regards to surface righting, no significant effect of sex was found on PND 2-5 [PND 2: $\chi^2=0.00$, $p=1.000$; PND 3: $\chi^2=2.74$, $p=0.098$; PND 4 $\chi^2=0.25$, $p=0.615$; PND 5 $\chi^2=0.89$, $p=0.346$]. Initially, a significant effect of treatment was observed at PND 2 [$\chi^2=8.66$, $p=0.034$] for 2.5 mg/kg treatment [$\chi^2=7.45$, $p=0.006$], however, this was not specific to the male or female groups after correction for multiple comparisons. There was no effect of treatment on PND 3-5 either [PND 3: $\chi^2=5.99$, $p=0.112$; PND 4 $\chi^2=0.11$, $p=0.991$; PND 5 $\chi^2=5.86$, $p=0.119$].

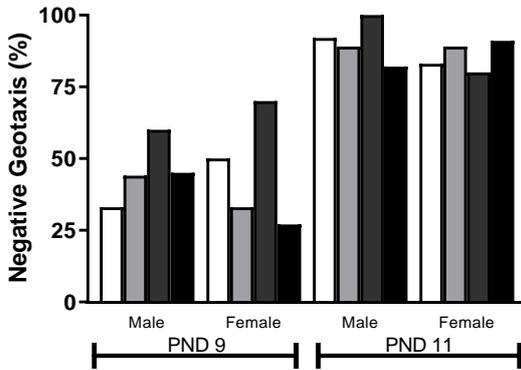
In the analysis of negative geotaxis, no significant effects were found at PND 9 or 11 for sex [PND 9: $\chi^2=0.69$, $p=0.408$; PND 11: $\chi^2=0.38$, $p=0.537$] or treatment [PND 9: $\chi^2=4.27$, $p=0.234$; PND 11: $\chi^2=0.15$, $p=0.985$].

Forelimb grip data were analysed and indicated no significant effects were found at PND 14 or 17 for sex [PND 14: $\chi^2=0.17$, $p=0.682$; PND 17: $\chi^2=3.000$, $p=0.083$] or treatment [PND 14: $\chi^2=2.04$, $p=0.565$; PND 17: $\chi^2=6.65$, $p=0.084$].

A)



B)



C)

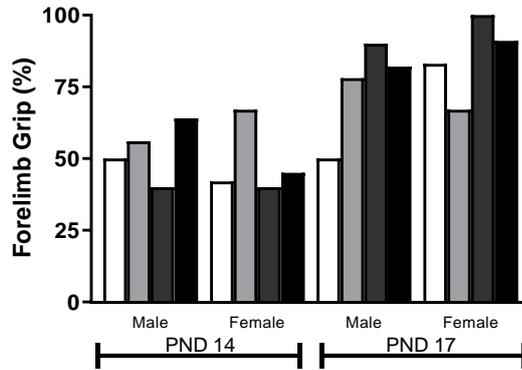


Figure 4.11 Paroxetine behavioural development. (A) Surface righting, (B) negative geotaxis, and (C) forelimb grip, for resulting pups exposed to either vehicle or PRX (1.25, 2.5, 5 mg/kg) from GD 7-21. Data are expressed as the percentage of pups to successfully complete the task within the time required, n=9-12/ group. Pups met criterion for presence if (A) <10 seconds, (B) <15 seconds, (C) >10 seconds. No significant deleterious effect of treatment was found on behavioural development, see text for statistical analysis.

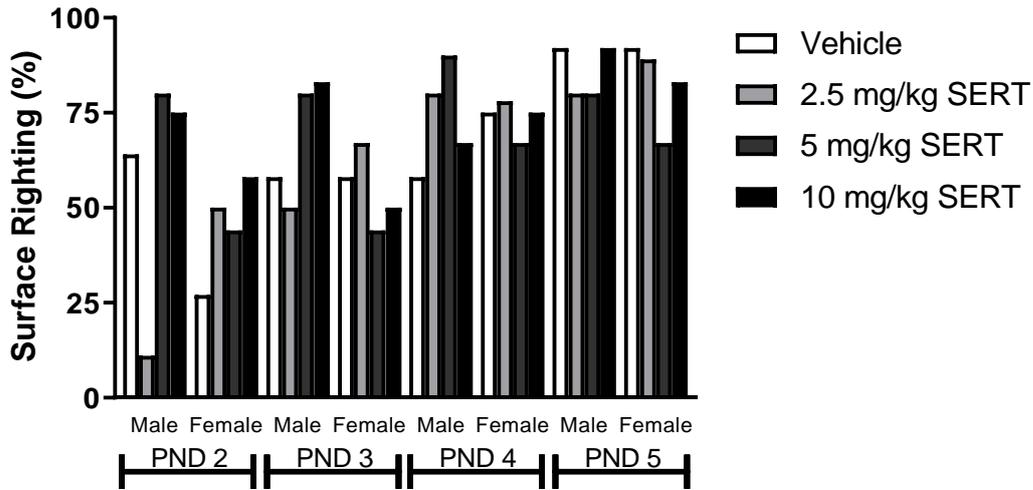
4.3.4.3 Sertraline (Figure 4.12)

In regards to surface righting, no significant effect of sex or treatment were found on PND 2-5 [PND 2: $\chi^2=2.93$, $p=0.087$; PND 3: $\chi^2=0.00$, $p=1.000$; PND 4 $\chi^2=1.20$, $p=0.273$; PND 5 $\chi^2=0.00$, $p=1.000$], nor was there a significant effect of treatment [PND 2: $\chi^2=6.87$, $p=0.076$; PND 3: $\chi^2=0.50$, $p=0.919$; PND 4 $\chi^2=1.13$, $p=0.769$; PND 5 $\chi^2=2.853$, $p=0.415$].

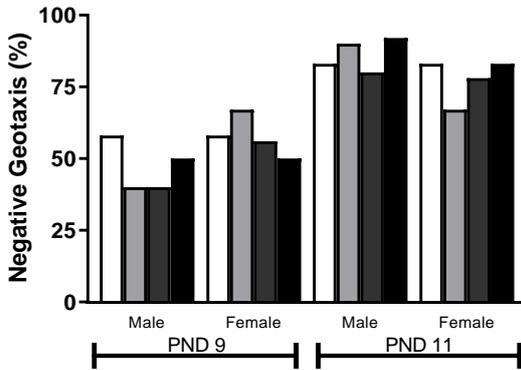
In the analysis of negative geotaxis, no significant effects were found at PND 9 or 11 for sex [PND 9: $\chi^2=0.00$, $p=1.000$; PND 11: $\chi^2=0.00$, $p=1.000$] or treatment [PND 9: $\chi^2=0.59$, $p=0.899$; PND 11: $\chi^2=0.76$, $p=0.859$].

Forelimb grip data were analysed and indicated no significant effect of sex [$\chi^2=0.17$, $p=0.682$] or treatment [$\chi^2=2.39$, $p=0.495$] at PND 14. However, an effect of sex was found at PND 17 [$\chi^2=6.75$, $p=0.009$], with untreated females meeting criterion more often than untreated males. No overall effect of treatment was noted at PND 17 [$\chi^2=6.73$, $p=0.081$]. However, treatment had a significant effect on forelimb grip within the male groups at PND 17 [$\chi^2=17.80$, $p<0.001$], as 5 and 10 mg/kg sertraline-treated males met criterion more than vehicle exposed males. There was no effect of treatment within the female group at PND 17 [$\chi^2=3.400$, $p=0.334$].

A)



B)



C)

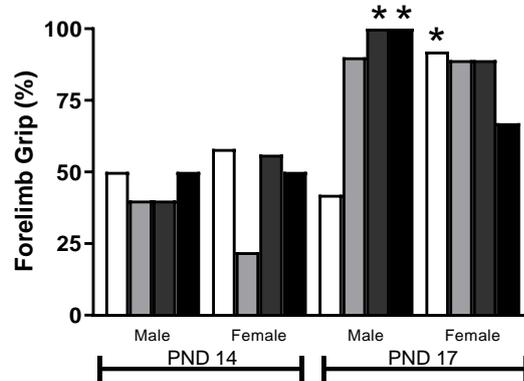


Figure 4.12 Sertraline behavioural development. (A) Surface righting, (B) negative geotaxis, and (C) forelimb grip, for resulting pups exposed to either vehicle or SERT (2.5, 5, 10 mg/kg) from GD 7-21. Data are expressed as the percentage of pups to successfully complete the task within the time required, $n=9-12/\text{group}$, $*p<0.013$ vs vehicle male. Pups met criterion for presence if (A) <10 seconds, (B) <15 seconds, (C) >10 seconds. A significant effect of sex was noted for forelimb grip on PND 17, with untreated females meeting criterion more than untreated males. Treatment significantly enhanced male forelimb grip behaviour at PND 17, while no significant deleterious effect of treatment was found on behavioural development, see text for statistical analysis.

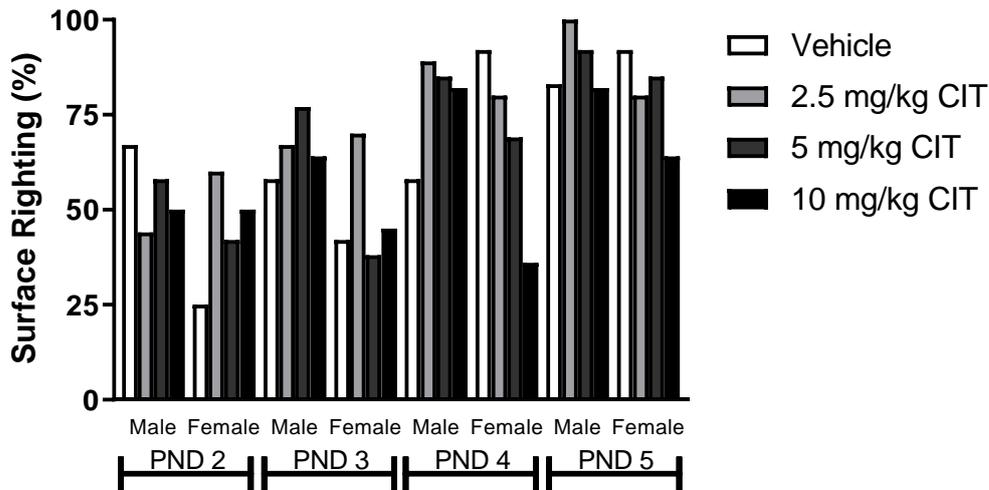
4.3.4.4 Citalopram (Figure 4.13)

In regards to surface righting, an effect of sex was only detectable at PND 2 [$\chi^2=4.20$, $p=0.041$] with untreated males meeting criterion more than untreated females, however, this was not statistically significant after correction for multiple comparisons ($*p<0.013$). Further, no effect of sex was noted for PND 3-5 [PND 3: $\chi^2=0.67$, $p=0.414$; PND 4: $\chi^2=0.38$, $p=0.537$; PND 5: $\chi^2=0.38$, $p=0.537$]. In addition, no effect of treatment was noted on any day [PND 2: $\chi^2=0.21$, $p=0.976$; PND 3: $\chi^2=1.55$, $p=0.671$; PND 4: $\chi^2=6.02$, $p=0.111$; PND 5 $\chi^2=3.18$, $p=0.364$].

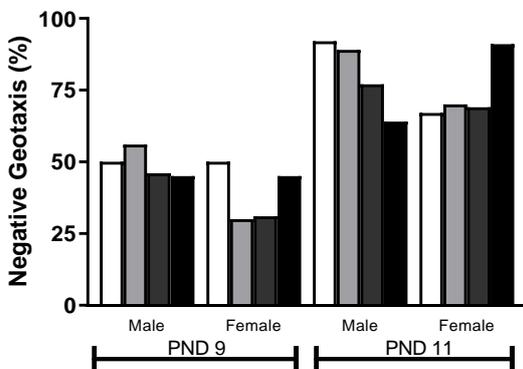
In the analysis of negative geotaxis, no significant effects were found at PND 9 or 11 for sex [PND 9: $\chi^2=0.00$, $p=1.000$; PND 11: $\chi^2=2.27$, $p=0.132$] or treatment [PND 9: $\chi^2=0.72$, $p=0.868$; PND 11: $\chi^2=0.33$, $p=0.954$].

Forelimb grip data indicated no significant effect at PND 14 or 17 for sex [PND 14: $\chi^2=1.51$, $p=0.219$; PND 17: $\chi^2=2.27$, $p=0.132$] or treatment [PND 14: $\chi^2=0.46$, $p=0.928$; PND 17: $\chi^2=2.175$, $p=0.537$].

A)



B)



C)

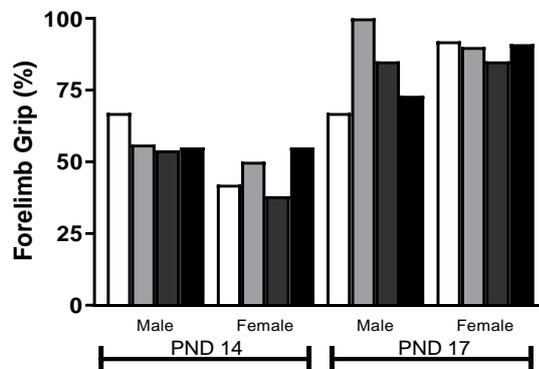


Figure 4.13 Citalopram behavioural development. (A) Surface righting, (B) negative geotaxis, and (C) forelimb grip, for resulting pups exposed to either vehicle or CIT (2.5, 5, 10 mg/kg) from GD 7-21. Data are expressed as the percentage of pups to successfully complete the task within the time required, $n=9-13$ /group. Pups met criterion for presence if (A) <10 seconds, (B) <15 seconds, (C) >10 seconds. No significant effect of treatment was found on behavioural development, see text for statistical analysis.

4.4 Discussion

This chapter has revealed that fluoxetine significantly altered somatic development, causing an increase in ano-genital distance only at the later time-point tested in males. No significant deleterious effects were noted for any SSRI treatments observed with regards to the physical development of the progeny. Similarly, gestational exposure to fluoxetine was associated with a behavioural delay, inconsistently decreasing surface righting in males. Meanwhile, sertraline was inconsistently associated with a behavioural advance, showing an increase in forelimb grip strength at the later time-point. A predictable effect of sex was detected for ano-genital distance and daily body weights, regardless of treatment, as males typically were larger than females. In many cases, a sex effect was detectable for other measures of physical maturation and behavioural development.

4.4.1 Aim 1: Somatic development

The first aim of this study was to examine the effects of SSRI administration from GD 7-21 on pup somatic development parameters. Somatic development was measured by monitoring fur appearance, pinnae unfolding, eye opening, ano-genital distance, length, and body weight of the resulting male and female offspring from littering to PND 24.

4.4.1.1 Sex

Unsurprisingly sex effects were noted throughout many of the somatic development measurements, as rats like humans are sexually dimorphic. Therefore, when the effects of sex were noted throughout the particular somatic measurements, females were always more petite than males. As anticipated, all studies showed a significant effect of sex on ano-genital distance at both PND 3 and 24, with males having a larger ano-genital distance than females. Corresponding to the results presented in Chapter 3 with males having higher birth weights than females, an effect of sex and time was found for daily body weight, for all of the SSRI studies. Coinciding with weight, distinguishable effects of sex were noted for body length initially for the fluoxetine, sertraline, and citalopram studies; furthermore, an enduring effect was noted in the sertraline study at the later time point as well. Although an effect of sex was not statistically significant within the paroxetine study, or at the later time point for the

fluoxetine and citalopram study, all studies followed a similar trend with males having a larger body length than females. Interestingly, the presence of fur appearance, pinnae unfolding, eye opening, and total weight gain, were not dependent on sex. Overall, these studies suggest that males are larger than females during the neonatal somatic development period in regards to ano-genital distance, body weight, and body length.

4.4.1.2 Fluoxetine

During the neonatal development period, no effect was found for gestational fluoxetine exposure on fur appearance or pinna unfolding. Neither were there significant deleterious effects of treatment detected for eye opening, ano-genital distance, length, or body weight. Interestingly, an increase in ano-genital distance was noted for fluoxetine exposed males, only at PND 24. Despite the breadth of literature which exists for fluoxetine exposure during pregnancy (Table 1.7- Table 1.8) compared to other SSRIs, no studies were found which assessed treatment effects on somatic development characteristics such as fur appearance and body length. Further, few comparably designed studies remark on pinna unfolding, eye opening, ano-genital distance, or body weight in the resulting offspring. Similar to the reported findings, no treatment effect was detected for pinna unfolding or eye opening when dams were exposed to doses higher than ours (8 or 12 mg/kg, IG, GD 6-20) compared to vehicle pups (Bairy et al., 2007). In contrast to the current findings regarding an increase in ano-genital distance at PND 24, one study found that males exposed to fluoxetine (5 mg/kg, OMP to dam, PND 1-21) had decreased ano-genital distance when measured in juvenile (PND 21-25) rats; furthermore, although this ano-genital effect was not noted in adulthood, treatment did diminish sexual behaviour, culminating in a reduced number of intromissions, delayed latency to the first intromission, and delayed latency to first ejaculation in adult males (PND80-88) (Rayen et al., 2013). An alteration in ano-genital distance around PND 24 may suggest an early or delayed onset of puberty. The discrepancy between these results and those report by Rayen et al. (2013) is likely due to the differences in exposure periods, as ours occurred during gestation and the other occurred throughout the postnatal period via lactation. The same lab also reports no difference amongst ano-genital distance in juvenile (PND 21-25) or adult (PND 108-116) females, similar to the current results at PND 3 and 24 (Rayen et al., 2013, Rayen et al., 2014). Interestingly, although no differences were noted for female ano-genital distance, they do note that postnatal exposure facilitates sexual behaviour in

female adults (PND 103-111) with a significant increase in proceptive behaviours, an increased lordosis quotient, and a decrease in rejection quotient; overall, emphasizing that fluoxetine exposure during development can have lasting implication on sexual behaviour and the hypothalamus pituitary gonadal (HPG) system of male and female offspring (Rayen et al., 2014). Therefore the sex of the offspring and the period of fluoxetine exposure is critical in dictating the treatment implications on ano-genital distance, as exposure during the gestational period, but not the postnatal period, may be associated with increased ano-genital distances in males; meanwhile fluoxetine exposure in the postnatal period, but not gestational period, can cause a reduction in ano-genital distance in male pups. The previously mentioned study, conducted by Bairy et al. (2007) observing pinna unfolding and eye opening, also considered neonatal weights. Similar to the reported findings, no effects of treatment were detected on the percentage of weight change from PND 3-15 (Bairy et al., 2007). However, a decrease is observed at PND 18 and 21 for both male and female pups (Bairy et al., 2007). Remarkably, this decrease in weight is only reported for the 8 mg/kg exposed offspring, proposing that treatment effects on weight after *in utero* fluoxetine exposure are dependent on dose, as no effect was concluded at the lower dose or at the 12 mg/kg dose employed in the same study, although lower initial weights were reported for this dose (Bairy et al., 2007). Overall, these results give novel insights on the effects specific to gestational fluoxetine exposure, suggesting no deleterious implications on the somatic development of the resulting male and female offspring, and proposing the treatment may lead to early puberty in males.

4.4.1.3 Paroxetine

Throughout the postnatal period, no effect was found for paroxetine treatment on fur appearance or pinna unfolding. In addition, no deleterious effects of treatment were noted for eye opening, ano-genital distance, length, or body weight. Preceding literature has commented on only a few of these parameters after paroxetine exposure during pregnancy. Similarly, it has been reported in mice that oral dam exposure two weeks before conception until GD 16 had no effect on external genitalia or eye opening for offspring, even though they used a higher dose (30 mg/kg) (Rayburn et al., 2000). While no other studies considered neonatal weights from PND 1-21 in rats, another study in mice found a decrease in neonatal weight from PND 1-4 when mice were exposed prior to and during gestation (30 mg/kg, incorporated in food pellets,

GD (-14)-GD 16) (Coleman et al., 1999). In the daily weight gain analysis, an effect of treatment was not found for this early period of the current study. However, it is important to note that lower birth weights were reported by the same lab at PND 1 as well (Rayburn et al., 2000), unlike the birth weight results mentioned in Chapter 3. The conflict in results is likely due to the dose and species used, as the aforementioned reports by Coleman et al. (1999) and Rayburn et al. (2000) used the substantially higher dose of 30 mg/kg in mice. Thus, it can be inferred that higher doses can reduce birth weights and inhibit weight gain, even though no effects were observed in regards to mice maternal weights or the number of live pups born (Rayburn et al., 2000). The current study used a lower dose range, reporting increased pup mortality after exposure, however, the aforementioned reports did not measure this outcome. Considering the current results with the limited previous literature findings, paroxetine, at a range of doses, exposed during gestation has no deleterious effects on somatic development.

4.4.1.4 Sertraline

During the postnatal development period, no effect was found for gestational sertraline exposure on fur appearance or pinna unfolding. Neither were there significant effects of treatment detected for eye opening, ano-genital distance, length, or body weight. No prior studies were found to observe somatic development after sertraline treatment solely during the gestational period. However, a study found considering perinatal exposure (GD 15-PND 20), supports these findings which indicate that exposure to sertraline at even higher doses than employed in this chapter (10-80 mg/kg, oral) has no effect on external or visceral anomalies (Davies and Klowe, 1998). The same study also considered neonatal body weights, reporting that treatment did not affect weights from PND 1-4 or PND 4-21 if they stopped treatment at birth, supporting the current findings. In contrast, Davies and Klowe (1998) also described a decrease in neonatal weights before weaning when dams were exposed in the postnatal period to particular doses (20 or 80 mg/kg). On the other hand, another perinatal exposure study by Gemmel et al. (2018a) reports an increase in neonatal weights before weaning, when dams were exposed for a longer period in gestation than in the current study (5 mg/kg, IP to dam, GD 0-littering) with exposure continuing after littering but directly to the pups at a lower dose (1.5 mg/kg, IP to pup, PND 1-14), unlike Davies and Klowe (1998) who continued dosing dams. Taking all body weight results together suggests

that sertraline related effects on offspring body weight are dependent on dose as well as exposure period, as no effect was found when exposure strictly occurred *in utero*, however postnatal exposure via the dam to high doses decreased pup body weight while postnatal exposure directly to the pup at lower doses increased body weight. Overall, the novel findings reported here suggest that gestational sertraline treatment to dams at the doses employed is not associated with significant delays or advances in somatic development, further gestational exposure does not interfere with neonatal weights from littering through weaning.

4.4.1.5 Citalopram

Throughout the neonatal period, no effect was found for treatment on fur appearance or pinna unfolding. Furthermore, no deleterious effects of treatment were noted for eye opening, ano-genital distance, length, or body weight. Corresponding studies investigating citalopram exposure *in utero* have not measured neonatal somatic endpoints. While no effects were reported on body weight gain, other studies measuring body weight gain from PND 4-21 and examining only postnatal exposure to citalopram, suggest that doses within and higher than the current range (5 and 7.5 mg/kg or 10 and 20 mg/kg, PND 11-20 or PND 8-21) are associated with a decrease in neonatal weights when only male pups were considered and doses were administered SC, directly to the pups (Schaefer et al., 2013, Harris et al., 2012). Another study assessing perinatal exposure (5 mg/kg, GD 6-PND 21) which also directly SC injected the pups, too found an inhibition in body weight gain from PND 4-21 in both male and female pups (Sprowles et al., 2017). Therefore, citalopram exposure does not interfere with neonatal body weights when exposure culminates prior to birth. These findings contribute novel information, suggesting that gestational citalopram exposure does not cause deleterious somatic development effects, but rather has the possibility of causing eyes to open earlier, albeit within the expected window.

4.4.2 Aim 2: Behavioural development

The second aim of this study was to examine the effects of SSRI administration from GD 7-21 on pup behavioural development parameters. Behavioural development was

measured by monitoring surface righting, negative geotaxis, and forelimb grip of the resulting male and female offspring from littering to PND 17.

4.4.2.1 Sex

Sporadic effects of sex were noted at isolated time points for surface righting and forelimb grip, but never for negative geotaxis. While similar trends were noted at PND 2 for the fluoxetine and sertraline studies, the citalopram study was the only to show a significant effect of sex with males being more successful in meeting surface righting behaviour criterion than females. As this effect was only statistically significant in one drug study and only on the first of the four days of surface righting testing, it is possible that this may represent only a mild delay in female surface righting and vestibular development. All PND 17 forelimb grip results show a similar trend with females meeting criterion more frequently than males. However, while a significant difference was noted for the fluoxetine and sertraline study, the sertraline study was the only drug study to exhibit this effect of sex after correcting for multiple comparisons. Therefore while sex may produce a trend of females showing greater forelimb grip and strength, this slight effect of sex was not noted as statistically significant throughout the majority of the studies. Overall these results suggest that sex has an unpronounced effect on surface righting and forelimb gripping behaviours.

4.4.2.2 Fluoxetine

Gestational exposure showed no persistent effects on behavioural development observations within the pre-weaning period. While an effect was never detected in female offspring behaviour or males in the negative geotaxis and forelimb grip test, PND 4 showed a significant decrease in surface righting success for fluoxetine exposed male pups. Although many studies have modelled fluoxetine exposure throughout pregnancy, few results have been published in regards to neonatal behavioural development, and no literature has been found relating to the forelimb grip reflex. A study that used a similar exposure period (GD 6-20), nonetheless at higher doses (8 or 12 mg/kg, IG), also found no treatment effect in regards to negative geotaxis at PND 8 amongst the groups (Bairy et al., 2007). Interestingly, at later time points researchers reported an increase in time required for the pups to turn after certain treatments, showing that male (8 or 12 mg/kg) and female (8 mg/kg) groups required more time at PND 10 and both treated female groups required more time at PND 12 for successful completion of the negative geotaxis task. These differences in

outcomes emphasize a possible effect of dose, as those employed were more than double the dosage used in this experiment. Furthermore, Bairy et al. (2007) assess the length of time, unlike the current study which measured the feature by the absence or presence, based on the criterion that the pup turned within 15 seconds. A study using a larger range of doses (1, 3, 10, 30 mg/kg, IP directly to pup) than the current range, found complementary results to the present study in regards to negative geotaxis, concluding no effect of treatment on negative geotaxis latency when exposure occurred once 30 minutes prior to the behavioural task (Hodgson et al., 2008). While the study completed by Hodgson et al. is not perfectly analogous to the current work, as they used direct postnatal acute dosing, it is notable to mention that effects were not observed for negative geotaxis even when pups were exposed once in the postnatal period. However, while the present results indicate an effect of treatment on PND 4 for fluoxetine exposed males within the surface righting task, the aforementioned study indicates no effect of treatment (Hodgson et al., 2008). There are many factors, including postnatal exposure, which could explain the difference in results observed but most notable is the unlisted information in regards to the sex of the offspring tested and the PND the measurement was made. In addition, the current results indicate no effect of treatment as well, when only considering female progeny, or males on PND 2, 3, or 5. Therefore the present results express sex and time-dependent effects of treatment at PND 4, and when comparing to other studies it is possible that the dose employed also influenced the difference amongst results. Jointly, these results indicate at a single early time-point a significant effect of fluoxetine treatment on exposed males for righting mechanism, however, no permanent behavioural effects were found for male or female progeny when measuring vestibular function development, sensorimotor coordination maturation, or gripping reflex and strength.

4.4.2.3 Paroxetine

Within the pre-weaning period, no treatment effect was found by gestational paroxetine exposure for developmental behaviours including surface righting, negative geotaxis, or forelimb grip for any of the days observed. Published literature concerning such behaviours is scarce. One study presents similar results for negative geotaxis, although it was conducted in mice that a paroxetine exposure (30 mg/kg dose), which was milled into food bars, and given to the dams from two weeks prior to conception to GD 16 and testing was carried out in younger neonatal offspring

(PND 3 and 5) than carried out in the current study (Coleman et al., 1999). In regards to negative geotaxis, the reported results taken with the previous findings suggest that treatment has no effect on such behaviour under a variety of different testing conditions. Overall, these findings infer that paroxetine exposure does not alter the development of righting reflex mechanisms, nor does it affect sensorimotor or vestibular function.

4.4.2.4 Sertraline

Throughout the neonatal development period, sertraline exposure *in utero* caused a time-dependent alteration in male behavioural development for forelimb grip, but not for surface righting or negative geotaxis. Interestingly, no differences were detected amongst female offspring groups. Unfortunately, no previous literature has commented on offspring behavioural development after treatment exposure during the peripartum period; that is the period before and/or after littering. In regards to forelimb grip, this analysis shows that all sertraline exposed males performed better only on PND 17 compared to the vehicle pups. Thus, showing significant behavioural advances in gripping reflex and forelimb strength for the moderate and high dose groups. Consequently, this study contributes novel findings, ultimately illustrating an advance in sensorimotor and vestibular function recognized later in postnatal development, indicating moderate and high sertraline exposure increases gripping reflex and forelimb strength. Lastly the results indicate for the first time within the literature, that resulting female offspring exposed to sertraline *in utero*, perform comparably to vehicle exposed pups in the observed behavioural developmental tasks, regardless of dose or developmental time point. Thus, inferring no significant repercussions on the evolution of righting, sensorimotor, or vestibular functions in female pups.

4.4.2.5 Citalopram

In the pre-weaning period, no effects of treatment were found for gestational citalopram exposure for behavioural development, measured in the surface righting, negative geotaxis, and forelimb grip behavioural tasks. The only previous literature concerning early life exposure and such behavioural development tasks is an indirect comparison, as the study considered how behaviours may be altered when pups were exposed to a range of citalopram doses (0.3, 1, 3, 10, and 30 mg/kg) only 30 minutes prior to the task, via an IP injection directly to the pup (Hodgson et al., 2008). The

findings of Hodgson et al. (2008) supports the current findings, reporting no effect of acute citalopram administration on surface righting latency or negative geotaxis regardless of dose; forelimb grip was not measured in their study. However, there are limited comparison that can be made between the present study and the aforementioned study due to differences amongst the study designs regarding administration and exposure period. Overall, the original results presented in this study indicate that chronic citalopram exposure *in utero* has no effects on righting mechanisms, vestibular function development, sensorimotor coordination maturation, or gripping reflex and strength for the resulting treatment exposed male or female progeny.

4.4.3 Conclusion

In general, the results presented in this chapter propose that the SSRI doses employed have no significant deleterious effects on the somatic development and an isolated time-dependent behavioural deficit in the resulting progeny exposed to a particular SSRI and dose. Specifically, no SSRI treatments caused an effect on physical maturation observations of fur appearance, pinna unfolding, eye opening, length, or body weight throughout development. Additionally, behavioural measurements of negative geotaxis were not altered, regardless of SSRI exposure. As could be anticipated, an effect of sex was detectable amongst all groups for ano-genital distance and neonatal body weights throughout the pre-weaning period. Many analyses showed a detectable effect of sex for body length, mostly at the earlier time point. In a particular case, an effect of sex was noted within the forelimb gripping tasks. Fluoxetine was the only SSRI to induce a delay within the behavioural measurements, although the effect observed only occurred on the third of four surface righting test days. Exposure to fluoxetine was also the only SSRI to alter somatic development, as it was associated with larger ano-genital distances in prepubescent males, suggesting a possible early onset of puberty. Sertraline was the only SSRI to heighten behavioural development, enhancing forelimb grip and strength in exposed male progeny. Although paroxetine and citalopram were the only SSRIs not to modify somatic and behavioural development, sertraline and citalopram, induced no somatic or behavioural delays even though these SSRIs were administered at higher doses. Furthermore, the employment of higher doses was facilitated by less severe

implications on maternal wellbeing, as noted in Chapter 3. In conclusion, a sporadic deleterious behavioural effect was noted for fluoxetine, while no enduring physical or behavioural consequences were noted throughout the neonatal period for any of the SSRI exposed male and female progeny. Further, the deficits and advances were dependent on the dose exposed, the sex of the offspring, and the day of observation. These results, extended to the clinical setting, are advantageous for anticipating impedances in neonatal development, such as delays in vestibular function and righting reflexes, for the resulting progeny of treatment-naïve women beginning SSRI use during pregnancy.

5 The effects of gestational exposure to SSRI antidepressants on anxiety, ambulatory, cognitive, and anhedonia behaviours in resultant adolescent and adult rat offspring.

5.1 Introduction

As mentioned in previous chapters, SSRIs are the most commonly prescribed psychotropic drugs during pregnancy (Alwan et al., 2016). Such treatments are necessary as depression during pregnancy is common (Pearson et al., 2018), and maternal depression can have deleterious implications in the offspring, as well as increase the risk for postpartum depression (ACOG, 2018). Therefore, gestational SSRI exposure is common despite the unknown enduring consequences. Consequently, this chapter is focused on examining the longitudinal behavioural repercussions in the progeny.

Fluoxetine, paroxetine, sertraline, and citalopram exposure in pregnancy data are often combined together as general ‘SSRI exposure data’, and such clinical studies suggest various behavioural implications. This is predictable as SSRIs influence synaptic availability of serotonin, a key neurotransmitter in the management of various psychiatric disorders. Significantly, the serotonergic system is apparent throughout the CNS and guides various developmental events such as neurogenesis, apoptosis, dendritic refinement, cell migration, and synaptic plasticity (Whitaker-Azmitia, 2001). Therefore SSRI exposure, and subsequently serotonin disruption, during critical neurodevelopmental periods is concerning as serotonin dysregulation is implicated in various psychiatric disorders (Nordquist and Orelund, 2010). Thus, in addition to the offspring presenting physical malformations as described in previous chapters, the progeny may also demonstrate behavioural abnormalities. Serotonin modulates many processes such as mood and cognition (Berger et al., 2009), thus dysregulation *in utero* could lead to behavioural anomalies in the progeny presented as symptoms of anxiety, cognition, or depression. However, it is difficult to assume that such results are implications of SSRIs and not the consequence of maternal depression *per se* or a genetic predisposition to serotonin dysregulation disorders, as even untreated depression during pregnancy can have a significant negative impact on progeny (Gentile, 2017). Henceforth, behavioural disorders associated with resulting SSRI exposed progeny need to be further defined. As the brain continues to develop after birth it is interesting to consider the lasting implications of gestational SSRI exposure during critical milestones such as neonatal, adolescent, and adulthood development periods.

In addition to ethical concerns for clinical trials during pregnancy, there are many variables which limit the value of clinical testing. Many contributing factors include inconsistencies amongst self-reporting and self-administration, and differences amongst prescribed dose, SSRI, or exposure period. There is considerable inter-individual variation as a result of different genetic, environmental, or socioeconomic factors. Other dynamics to consider are the age of the mother, relationship with the father, and the number of previous pregnancies. Such confounds explain why most SSRIs are characterized by the FDA as C, signifying foetal risks may exist but insufficient clinical data currently exists (O'Connor et al., 2016). Paroxetine was re-categorized as D, indicating clinical data suggest foetal risks, only after multiple birth reports of infants experiencing congenital heart malformations (O'Connor et al., 2016, Bar-Oz et al., 2007, Bakker et al., 2010). Thus, it is not necessarily the case that the other three mentioned SSRIs are safer than paroxetine, it is only that there is not enough clinical evidence to suggest their use is dangerous during pregnancy. However, there could be differences in the pattern of exposure which account for this effect of paroxetine after exposure in the first trimester. In fact, a meta-analysis suggested one factor accounting for this difference was that the majority of women prescribed paroxetine was for the purpose of relieving symptoms of panic and anxiety compared to women using other SSRIs (Bar-Oz et al., 2007). Furthermore, population-based cohort studies suggest the highest risk of congenital malformations is for women prescribed multiple SSRIs (Pedersen et al., 2009). While the aforementioned factors can hinder well-controlled clinical studies, another limitation to consider is time. Clinical longitudinal studies require the entire life span of the resulting progeny, or at least until adulthood; therefore deleterious consequence may exist, similar to the paroxetine malformations. However it is impossible to predict these complications for a relatively new drug class, having entered the market between 1987-1992 and only off patent since 2001-2006 (Wong et al., 1995, Couzin, 2005), and meanwhile becoming more popular during pregnancy as recent generations show a growing prevalence of prenatal depression increased from 17% in 1990-1992 to 25% in 2012-2016 (Pearson et al., 2018). Therefore, further investigations are required to reveal the lasting behavioural impacts of SSRI exposure on the resulting individuals.

Animal studies are particularly advantageous in overcoming the abovementioned clinical limitations and confounds. Longitudinal studies offer a comprehensive

illustration of gestational exposure implications throughout the life span, and this can be accomplished in rodent models over a relatively short period of time. In the clinical scenario, serious complications may only be observed in adult progeny, after an entire generation has been exposed. Therefore, rodent studies aid to mitigate this limitation. When preclinical models closely mirror the clinical scenario, it is less challenging to extrapolate results to the human condition. Thus better-informing prescribers, and assisting in avoiding wide-spread detrimental consequences that might only be observed at the conclusion of development. Another advantage to animal studies, which overcome ethical clinical limitations, is that treatment can be tested in otherwise healthy pregnancies, which eliminates the possible confounding effects of maternal depression or genetic predisposition to serotonin dysregulation disorders and resulting behaviours. Therefore this study seeks to explore the specific effects of treatment on behaviour at various critical milestones in the resulting SSRI exposed neonatal, adolescent, and adult rat offspring.

This study monitored various behaviours that can be correlated to psychiatric disorders observed clinically as a result of serotonin dysregulation. Such behaviours measured included those related to anxiety, ambulation, cognition, and anhedonia. Anxiety and ambulation were measured at multiple critical periods throughout development to track changes and expose particular periods where developmental delays or advances may be more prevalent. Meanwhile, cognition and anhedonia were measured in adulthood, to uncover the lasting implications of SSRI exposure. Additionally, each of these behavioural parameters was observed in both male and female offspring in order to identify whether there were any sex-specific effects of gestational SSRI exposure. Collectively, behavioural monitoring is important for analysing SSRI exposure *in utero* as serotonergic dysregulation is associated with a range of psychiatric disorders, which are often more prevalent amongst certain genders.

Sex comparisons are an important feature of this study, which should not be avoided when considering the effects of serotonin disruption on development and behaviour. To begin with, a difference is observed clinically for serotonin synthesis in the brain (Nishizawa et al., 1997). As serotonin is involved in various psychiatric disorders (Nordquist and Oreland, 2010), this effect of gender cannot be ignored. The higher metabolism of serotonin in females likely contributes to the differences observed amongst males and females in regards to anxiety, cognition, and anhedonia. Clinically,

the global rates of depression are consistently higher amongst females compared to males, as illustrated previously in Chapter 1 by Ferrari et al. (2013).

Combining these behavioural measurements enables a thorough investigation of emotion and cognitive function after serotonin dysregulation during a critical neurodevelopmental period. Therefore such measurements in exposed rat offspring hasten the ability of clinicians to anticipate psychiatric disorders which may emerge at different postnatal developmental milestones throughout the life span.

5.1.1 Hypothesis and aims

It is specifically hypothesized in this chapter that *in utero* exposure to fluoxetine, paroxetine, sertraline, or citalopram at clinically relevant doses that are not significantly toxic to maternal wellbeing will have enduring consequences in the progeny's behaviour from adolescence through adulthood.

This hypothesis was evaluated through three specific aims throughout this chapter, ultimately investigating the longitudinal effects of SSRI administration from GD 7-21 on the resulting male and female offspring, at particular periods in development, in regards to various behaviours including (1) anxiety and ambulation, (2) cognition, and (3) anhedonia, with considerations to differences in sex within each of these parameters.

5.2 Experimental methods and design

A detailed description of gestational exposure and behavioural tasks can be found in Chapter 2.

Briefly, Sprague-Dawley (SD) pups were born on PND 1, after being exposed via the dam *in utero* to either vehicle, FLX (2.5 mg/kg), PRX (1.25, 2.5, or 5 mg/kg), SERT or CIT (2.5, 5 or 10 mg/kg) via oral gavage from GD 7 until littering (n=9-13 litters per group). Testing occurred with all four SSRIs under investigation simultaneously, however, research was collected over three cohort studies in order to successfully manage a study of this size.

When possible, the same male and female litter representatives were used to carry out behavioural tasks. Only one male and female pup was used per litter to avoid litter effects. Offspring sets were used to space out the behavioural tasks to minimize a residual test effect. The tests occurred at various specific time points from PND 28-108.

Various behaviours were considered such as anxiety, ambulation, cognition, and anhedonia. Anxiety and ambulation were assessed via the EPM+OF battery. The NOR test was used to assess cognition as learning and memory. Anhedonia was observed using the SPT.

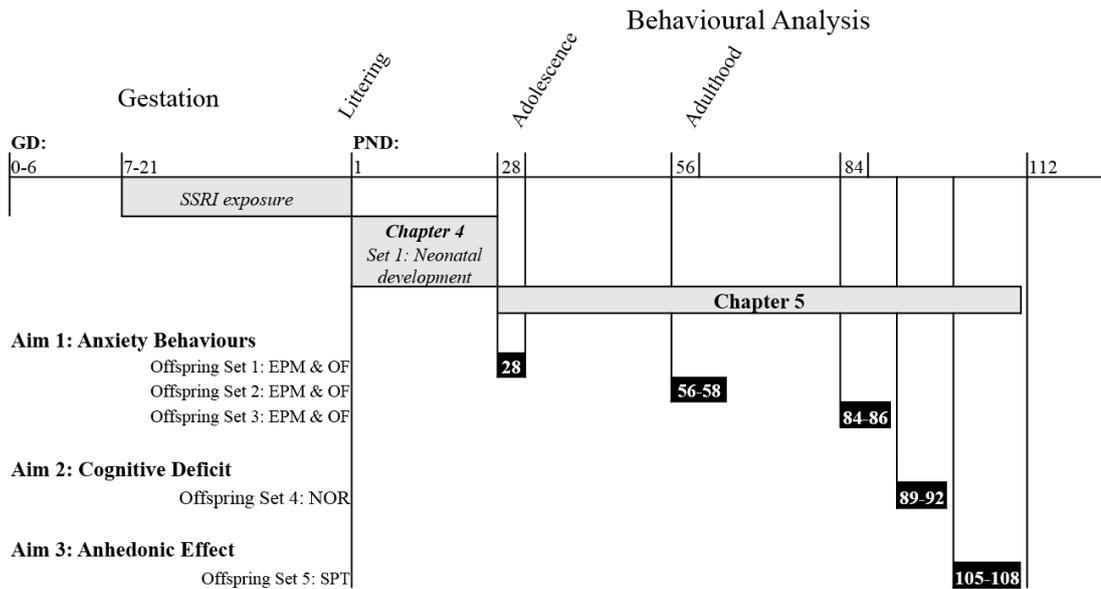


Figure 5.1 Adolescence and adulthood behaviour experimental timeline.

Statistical analysis was performed using the Statistical Package for Social Sciences 24.0 for Windows (SPSS Inc., IBM, New York, USA) on the parameters listed in Table 5.1. Primarily, normality and homogeneity of variance ($p < 0.05$) were determined to choose the appropriate analysis. As many cases failed to meet parametric criteria the Scheirer-Ray-Hare extension of Kruskal Wallis test was used to analyse all parameters presented in this chapter by dose, sex, and dose x sex. This test is analogous to a two-way ANOVA and it was used so that all SSRI data sets could be analysed using the same statistical approach. When appropriate ($p < 0.05$), a Mann Whitney U *post hoc* was used with appropriate Bonferroni correction. In the case of fluoxetine, when making three comparisons (two doses and sex) $*p < 0.025$ was used. In the cases of paroxetine, sertraline, and citalopram, when making five comparisons (four doses and sex) $*p < 0.013$ was used.

Parameters	Shapiro-Wilk & Levene's test $p < 0.05$
EPM, OF, NOR, SPT	Scheirer-Ray-Hare extension of Kruskal Wallis (Two-way ANOVA by Rank) → Mann Whitney U with Bonferroni correction

Table 5.1 Statistical analysis of adolescent and adulthood behaviour. In the case of fluoxetine, Bonferroni correction employed to correct for multiple comparisons was $*p < 0.025$. For all other SSRIs, Bonferroni correction employed to correct for multiple comparisons was $*p < 0.013$.

5.3 Adolescence and adulthood results

5.3.1 Anxiety and motor assessment in the elevated plus maze and open field

5.3.1.1 EPM findings at PND 28, 56, and 84

5.3.1.1.1 Fluoxetine (Table 5.2-Table 5.4)

EPM analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups at PND 28, 56, or 84.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1620 (1180-1707)	3 (2-5)	29 (12-60)	22 (14-33)	16 (6-33)	
2.5 mg/kg FLX	1418 (1199-1529)	2 (1-4)	12 (4-31)	14 (7-23)	6 (2-17)	
<i>Female</i>						
Vehicle	1661 (1493-1739)	4 (3-6)	42 (12-48)	25 (17-29)	22 (8-30)	
2.5 mg/kg FLX	1716 (1492-1860)	5 (3-8)	43 (24-64)	33 (17-37)	25 (14-35)	
Dose	H	0.08	0.04	0.11	0.12	0.09
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
Sex	H	1.34	2.08	0.68	1.39	0.91
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
Dose x Sex	H	0.36	0.72	0.44	0.60	0.42
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1

Table 5.2 Fluoxetine EPM PND 28. Male and female offspring exposed to either vehicle or FLX (2.5 mg/kg) via OG to dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=11-12/group. See table for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1773 (1577-1947)	5 (1-8)	37 (9-80)	26 (10-38)	25 (7-47)	
2.5 mg/kg FLX	1685 (1433-1958)	2 (1-3)	13 (8-23)	13 (8-18)	10 (5-17)	
<i>Female</i>						
Vehicle	1786 (1738-1930)	6 (2-10)	59 (29-93)	28 (13-46)	36 (21-47)	
2.5 mg/kg FLX	1921 (1717-2006)	5 (3-6)	45 (22-60)	24 (17-29)	27 (16-36)	
Dose	H	0.01	0.83	1.80	0.92	1.66
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
Sex	H	0.45	0.69	1.69	0.61	1.58
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
Dose x Sex	H	0.10	0.19	0.07	0.18	0.12
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1

Table 5.3 Fluoxetine EPM PND 56. Male and female offspring exposed to either vehicle or FLX (2.5 mg/kg) via OG to dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=11-12/group. See table for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1704 (1594-1777)	2 (0-4)	16 (1-29)	11 (2-20)	10 (1-17)	
2.5 mg/kg FLX	1692 (1250-1767)	2 (0-6)	16 (0-54)	12 (0-29)	13 (0-32)	
<i>Female</i>						
Vehicle	1834 (1636-2058)	4 (2-6)	46 (24-67)	22 (15-33)	26 (19-40)	
2.5 mg/kg FLX	1850 (1621-2052)	4 (1-6)	39 (5-55)	22 (6-31)	25 (4-35)	
Dose	H	0.02	0.00	0.02	0.01	0.00
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
Sex	H	1.72	1.33	1.09	0.94	1.28
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
Dose x Sex	H	0.00	0.08	0.17	0.12	0.20
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1

Table 5.4 Fluoxetine EPM PND 84. Male and female offspring exposed to either vehicle or FLX (2.5 mg/kg) via OG to dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=11-12/group. See table for statistical analysis.

5.3.1.1.2 Paroxetine (Table 5.5-Table 5.7)

PND 28 EPM analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups. PND 56 EPM analysis only revealed an effect of sex, for distance travelled, OAE (counts), and OAT (s and %); however, *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups. PND 84 EPM analysis only revealed an effect of sex, for distance travelled and OAE (counts and %); however, *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1678 (1499-2093)	3 (1-7)	24 (10-53)	18 (8-25)	13 (5-30)	
1.25 mg/kg PRX	1714 (1520-1893)	3 (2-4)	13 (10-20)	13 (12-19)	7 (6-12)	
2.5 mg/kg PRX	1538 (1285-1850)	3 (1-5)	21 (8-39)	20 (15-25)	11 (4-19)	
5 mg/kg PRX	1371 (1036-1558)	2 (0-4)	10 (0-34)	17 (0-31)	5 (0-21)	
<i>Female</i>						
Vehicle	1730 (1397-1959)	4 (2-6)	32 (10-46)	22 (9-34)	16 (5-27)	
1.25 mg/kg PRX	1771 (1508-2125)	2 (2-5)	15 (11-41)	14 (10-24)	9 (6-21)	
2.5 mg/kg PRX	1409 (1230-1644)	3 (1-4)	11 (5-34)	16 (8-27)	6 (2-21)	
5 mg/kg PRX	1635 (1423-1869)	6 (1-7)	44 (10-57)	27 (12-38)	24 (6-34)	
Dose	H	2.91	0.33	0.54	0.75	0.46
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
Sex	H	0.66	1.13	2.40	1.25	2.04
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
Dose x Sex	H	1.61	0.82	1.41	0.46	1.16
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 5.5 Paroxetine EPM PND 28. Male and female offspring exposed to either vehicle, PRX (1.25, 2.5, or 5 mg/kg) via OG to dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=8-12/group. See table for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1900 (1587-2268)	5 (1-11)	52 (17-88)	31 (9-48)	31 (11-52)	
1.25 mg/kg PRX	1809 (1671-2273)	4 (1-8)	40 (6-62)	19 (9-36)	27 (4-43)	
2.5 mg/kg PRX	1757 (1571-1991)	4 (1-5)	18 (9-51)	17 (6-27)	16 (6-30)	
5 mg/kg PRX	1673 (1606-1948)	4 (2-5)	23 (5-43)	20 (9-25)	17 (4-28)	
<i>Female</i>						
Vehicle	1893 (1657-2455)	6 (4-9)	59 (30-78)	30 (18-39)	35 (22-48)	
1.25 mg/kg PRX	1991 (1937-2221)	5 (4-7)	55 (35-72)	28 (22-31)	31 (22-41)	
2.5 mg/kg PRX	1969 (1837-2151)	5 (3-7)	57 (28-83)	26 (15-33)	35 (19-44)	
5 mg/kg PRX	1993 (1831-2201)	5 (3-7)	41 (23-72)	22 (12-32)	25 (17-42)	
Dose	H	0.33	1.34	1.66	1.41	1.77
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
Sex	H	5.53	5.56	6.75	3.15	5.29
	p	<0.05	<0.05	<0.05	>0.05	<0.05
	df	1	1	1	1	1
Dose x Sex	H	0.41	0.18	0.70	0.35	0.38
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 5.6 Paroxetine EPM PND 56. Male and female offspring exposed to either vehicle, PRX (1.25, 2.5, or 5 mg/kg) via OG to dam from GD 7-21. An overall effect of sex was noted for distance travelled, OAE (counts), and OAT (s and %); however, no significant differences were noted between the vehicle groups. Data are expressed as median and interquartile range, n=8-12/group. See table for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1848 (1680-1932)	4 (2-5)	32 (11-56)	17 (14-27)	19 (8-33)	
1.25 mg/kg PRX	1879 (1758-2001)	3 (3-4)	34 (14-57)	18 (13-21)	27 (9-36)	
2.5 mg/kg PRX	1567 (1498-1778)	2 (0-4)	13 (0-37)	9 (0-26)	9 (0-24)	
5 mg/kg PRX	1570 (1394-1913)	4 (3-5)	48 (22-74)	27 (17-36)	31 (14-44)	
<i>Female</i>						
Vehicle	1972 (1636-2136)	4 (3-8)	40 (30-55)	23 (16-32)	23 (19-36)	
1.25 mg/kg PRX	2117 (1827-2364)	7 (5-8)	51 (42-64)	29 (23-34)	31 (30-42)	
2.5 mg/kg PRX	1996 (1825-2104)	5 (3-8)	34 (29-91)	21 (16-38)	24 (18-42)	
5 mg/kg PRX	1840 (1695-2076)	6 (3-8)	40 (25-84)	23 (17-36)	28 (15-42)	
Dose	H	1.93	0.52	1.39	0.66	1.69
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
Sex	H	11.30	11.70	3.83	5.36	2.81
	p	<0.05	<0.05	>0.05	<0.05	>0.05
	df	1	1	1	1	1
Dose x Sex	H	0.73	0.99	0.63	1.15	0.76
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 5.7 Paroxetine EPM PND 84. Male and female offspring exposed to either vehicle, PRX (1.25, 2.5, or 5 mg/kg) via OG to the dam from GD 7-21. An overall effect of sex was noted for distance travelled and OAE (counts and s); however, no significant differences were noted between the vehicle groups. Data are expressed as median and interquartile range, n=9-12/group. See table for statistical analysis.

5.3.1.1.3 Sertraline (Table 5.8-Table 5.10)

PND 28 EPM analysis only revealed an effect of sex, for distance travelled; however, *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups. PND 56 EPM analysis only revealed an effect of sex, for OAT (s and %); however, *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups. PND 84 EPM analysis only revealed an effect of sex, for all five parameters measured (distance travelled, OAE (counts and %) and OAT (s and %)). However, *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
Male						
Vehicle	1454 (1097-1989)	3 (2-6)	27 (6-46)	21 (15-27)	14 (3-25)	
2.5 mg/kg SERT	1425 (1227-1571)	2 (1-6)	19 (3-55)	15 (9-34)	11 (2-30)	
5 mg/kg SERT	1460 (1211-1686)	3 (2-6)	14 (12-44)	19 (11-35)	9 (6-30)	
10 mg/kg SERT	1394 (1121-1728)	3 (1-6)	22 (15-42)	23 (14-30)	15 (8-22)	
Female						
Vehicle	1566 (1324-1765)	4 (3-5)	33 (13-44)	24 (17-36)	17 (8-24)	
2.5 mg/kg SERT	1621 (1454-1687)	2 (2-5)	20 (13-31)	12 (11-27)	13 (8-19)	
5 mg/kg SERT	1659 (1350-1853)	4 (4-7)	37 (29-83)	26 (16-36)	22 (15-46)	
10 mg/kg SERT	1751 (1496-2037)	4 (2-7)	47 (17-59)	25 (13-28)	26 (11-34)	
Dose	H	0.25	1.05	1.07	1.13	1.28
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
Sex	H	6.59	2.26	2.45	0.38	2.86
	p	<0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
Dose x Sex	H	0.60	0.24	0.97	0.32	0.85
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 5.8 Sertraline EPM PND 28. Male and female offspring exposed to either vehicle, SERT (2.5, 5 or 10 mg/kg) via OG to the dam from GD 7-21. A general effect of sex was noted for distance travelled; however, no significant difference noted between vehicle groups. Data are expressed as median and interquartile range, n=9-12/group. See table for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1862 (1442-2067)	5 (2-9)	63 (19-85)	30 (14-41)	38 (14-50)	
2.5 mg/kg SERT	1821 (1617-1912)	4 (4-6)	37 (30-52)	25 (21-31)	23 (21-35)	
5 mg/kg SERT	1843 (1655-1955)	4 (2-5)	32 (15-43)	15 (10-29)	22 (9-30)	
10 mg/kg SERT	1893 (1493-2027)	3 (2-5)	14 (7-55)	15 (11-25)	11 (4-31)	
<i>Female</i>						
Vehicle	1889 (1613-2583)	8 (3-8)	71 (34-83)	31 (21-44)	41 (23-48)	
2.5 mg/kg SERT	1796 (1517-1969)	5 (3-10)	58 (18-103)	28 (15-39)	38 (13-56)	
5 mg/kg SERT	1912 (1756-2230)	5 (4-8)	51 (40-79)	22 (19-39)	31 (27-43)	
10 mg/kg SERT	1831 (1627-2418)	4 (2-7)	38 (23-69)	23 (14-27)	21 (17-45)	
Dose	H	0.52	1.05	1.76	1.50	1.66
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
Sex	H	1.19	2.54	4.84	1.37	5.28
	p	>0.05	>0.05	<0.05	>0.05	<0.05
	df	1	1	1	1	1
Dose x Sex	H	0.22	0.15	0.17	0.11	0.09
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 5.9 Sertraline EPM PND 56. Male and female offspring exposed to either vehicle, SERT (2.5, 5 or 10 mg/kg) via OG to dam from GD 7-21. An overall effect of sex was noted for OAT (s and %); however, no significant differences were noted between vehicle groups. Data are expressed as median and interquartile range, n=8-12/group. See table for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1808 (1683-1913)	4 (2-7)	29 (16-56)	17 (11-27)	17 (10-33)	
2.5 mg/kg SERT	1683 (1488-1973)	0 (0-5)	0 (0-46)	0 (0-29)	0 (0-28)	
5 mg/kg SERT	1615 (1448-1800)	1 (0-5)	11 (0-37)	7 (0-20)	7 (0-25)	
10 mg/kg SERT	1591 (1364-1819)	4 (2-5)	26 (17-60)	20 (14-29)	16 (12-37)	
<i>Female</i>						
Vehicle	1820 (1569-2163)	3 (2-6)	32 (10-61)	18 (7-33)	20 (8-32)	
2.5 mg/kg SERT	1974 (1842-2164)	7 (6-9)	62 (43-71)	33 (28-42)	34 (27-41)	
5 mg/kg SERT	1824 (1667-2066)	5 (1-6)	37 (9-67)	23 (8-26)	26 (6-40)	
10 mg/kg SERT	2117 (1599-2330)	4 (1-10)	49 (22-84)	19 (8-40)	28 (16-49)	
Dose	H	0.49	0.76	1.08	0.87	1.09
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
Sex	H	11.28	8.72	8.96	6.80	8.22
	p	<0.05	<0.05	<0.05	<0.05	<0.05
	df	1	1	1	1	1
Dose x Sex	H	1.13	2.83	1.90	2.60	1.63
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 5.10 Sertraline EPM PND 84. Male and female offspring exposed to either vehicle, SERT (2.5, 5 or 10 mg/kg) via OG to the dam from GD 7-21. An overall effect of sex was noted for distance travelled, OAE (counts and %), and OAT (s and %); however, no significant differences were noted between vehicle groups. Data are expressed as median and interquartile range, n=9-12/group. See table for statistical analysis.

5.3.1.1.4 Citalopram (Table 5.11-Table 5.13)

PND 28 EPM analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups. PND 56 EPM analysis only revealed an effect of sex, for OAE (counts), and OAT (s and %); however, *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups. PND 84 EPM analysis only revealed an effect of sex, for OAE (counts and s) and OAT (s and %); however, *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1565 (1995-1855)	4 (2-6)	46 (11-67)	25 (10-35)	25 (7-37)	
2.5 mg/kg CIT	1535 (1195-1914)	3 (3-7)	30 (15-58)	23 (15-29)	17 (9-29)	
5 mg/kg CIT	1620 (1286-1888)	4 (3-7)	30 (13-41)	24 (17-30)	16 (8-25)	
10 mg/kg CIT	1551 (1205-1818)	3 (1-6)	20 (11-29)	13 (6-32)	10 (6-16)	
<i>Female</i>						
Vehicle	1408 (1225-1718)	4 (1-6)	26 (3-47)	25 (8-29)	13 (2-28)	
2.5 mg/kg CIT	1640 (1299-1838)	4 (2-5)	31 (15-57)	20 (14-32)	19 (8-38)	
5 mg/kg CIT	1661 (1429-2022)	6 (3-8)	26 (15-61)	30 (21-39)	14 (9-34)	
10 mg/kg CIT	1740 (1366-2009)	3 (3-6)	35 (22-51)	20 (15-28)	19 (12-27)	
Dose	H	0.65	1.20	0.07	1.71	0.12
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
Sex	H	1.16	0.79	0.16	0.64	0.19
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
Dose x Sex	H	0.60	0.54	1.24	0.71	1.40
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 5.11 Citalopram EPM PND 28. Male and female offspring exposed to either vehicle, CIT (2.5, 5 or 10 mg/kg) via OG to the dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=9-13/group. See table for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1872 (1642-2183)	7 (2-11)	67 (9-88)	35 (11-48)	41 (7-52)	
2.5 mg/kg CIT	1681 (1523-1824)	2 (1-3)	21 (0-33)	12 (6-18)	15 (1-25)	
5 mg/kg CIT	1954 (1650-2130)	5 (4-8)	36 (19-52)	22 (19-31)	24 (13-32)	
10 mg/kg CIT	1626 (1358-1884)	4 (1-7)	39 (17-60)	24 (10-37)	29 (13-36)	
<i>Female</i>						
Vehicle	1929 (1703-2393)	4 (3-8)	64 (46-92)	34 (21-44)	43 (26-49)	
2.5 mg/kg CIT	1809 (1703-2393)	8 (4-11)	60 (30-76)	27 (19-37)	36 (24-46)	
5 mg/kg CIT	1809 (1598-1968)	5 (4-8)	60 (14-84)	30 (16-44)	35 (10-46)	
10 mg/kg CIT	1959 (1855-2256)	5 (3-9)	37 (32-85)	25 (14-44)	24 (22-44)	
Dose	H	1.71	1.67	1.35	1.33	1.28
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
Sex	H	3.36	5.56	7.91	3.60	7.26
	p	>0.05	<0.05	<0.05	>0.05	<0.05
	df	1	1	1	1	1
Dose x Sex	H	1.61	0.52	0.93	0.81	1.11
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 5.12 Citalopram EPM PND 56. Male and female offspring exposed to either vehicle, CIT (2.5, 5 or 10 mg/kg) via OG to the dam from GD 7-21. An overall effect of sex was noted for OAE (counts) and OAT (s and %); however, no significant differences were noted between vehicle groups. Data are expressed as median and interquartile range, n=9-12/group. See table for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male</i>						
Vehicle	1720 (1468-1876)	3 (2-6)	31 (16-54)	18 (12-26)	21 (11-32)	
2.5 mg/kg CIT	1706 (1293-1856)	0 (0-4)	0 (0-46)	0 (0-23)	0 (0-32)	
5 mg/kg CIT	1786 (1710-1994)	4 (2-5)	32 (29-55)	21 (11-28)	21 (17-33)	
10 mg/kg CIT	1774 (1648-1875)	3 (2-6)	21 (11-35)	19 (11-26)	15 (6-23)	
<i>Female</i>						
Vehicle	1821 (1575-2006)	4 (2-7)	35 (14-45)	21 (9-30)	24 (10-26)	
2.5 mg/kg CIT	1778 (1577-1868)	2 (1-7)	19 (1-52)	13 (3-28)	12 (1-31)	
5 mg/kg CIT	1891 (1737-2080)	6 (3-7)	39 (34-81)	29 (18-42)	31 (20-52)	
10 mg/kg CIT	1872 (1541-2051)	5 (3-6)	42 (32-77)	27 (17-33)	27 (23-40)	
Dose	H	1.61	3.46	2.34	2.95	2.23
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
Sex	H	2.78	5.02	4.32	4.18	4.20
	p	>0.05	<0.05	<0.05	<0.05	<0.05
	df	1	1	1	1	1
Dose x Sex	H	0.03	0.35	0.96	0.52	0.70
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 5.13 Citalopram EPM PND 84. Male and female offspring exposed to either vehicle, CIT (2.5, 5 or 10 mg/kg) via OG to the dam from GD 7-21. An overall effect of sex was noted for OAE (counts and %) and OAT (s and %); however, no significant differences were noted between vehicle groups. Data are expressed as median and interquartile range, n=9-13/group. See table for statistical analysis.

5.3.1.2 OF findings at PND 28, 56, and 84

5.3.1.2.1 Fluoxetine (Table 5.14-Table 5.16)

OF analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups at PND 28, 56, or 84.

Group	Distance travelled (cm)	BZE (counts)	IZT (s)	
<i>Male</i>				
Vehicle	1370 (816-1798)	5 (1-10)	62 (38-80)	
2.5 mg/kg FLX	1402 (868-1799)	4 (2-6)	39 (26-68)	
<i>Female</i>				
Vehicle	1850 (1294-2058)	10 (5-16)	67 (46-91)	
2.5 mg/kg FLX	1447 (1124-1658)	4 (2-10)	39 (26-59)	
Dose	H	0.34	0.74	1.02
	p	>0.05	>0.05	>0.05
	df	1	1	1
Sex	H	0.45	0.74	0.05
	p	>0.05	>0.05	>0.05
	df	1	1	1
Dose x Sex	H	0.27	0.22	0.00
	p	>0.05	>0.05	>0.05
	df	1	1	1

Table 5.14 Fluoxetine OF PND 28. Male and female offspring exposed to either vehicle, FLX (2.5 mg/kg) via OG to the dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=11-12/group. See table for statistical analysis. BZE= between zone entry.

Group	Distance travelled (cm)	BZE (counts)	IZT (s)	
<i>Male</i>				
Vehicle	2014 (1629-2839)	13 (7-15)	44 (36-58)	
2.5 mg/kg FLX	1887 (1654-2337)	7 (3-11)	37 (18-57)	
<i>Female</i>				
Vehicle	2350 (2153-2491)	16 (11-19)	68 (42-91)	
2.5 mg/kg FLX	1992 (1632-2331)	4 (1-14)	32 (18-69)	
Dose	H	1.13	3.54	1.36
	p	>0.05	>0.05	>0.05
	df	1	1	1
Sex	H	0.27	0.41	0.42
	p	>0.05	>0.05	>0.05
	df	1	1	1
Dose x Sex	H	0.34	0.13	0.48
	p	>0.05	>0.05	>0.05
	df	1	1	1

Table 5.15 Fluoxetine OF PND 56. Male and female offspring exposed to either vehicle, FLX (2.5 mg/kg) via OG to the dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=11-12/group. See table for statistical analysis.

Group		Distance travelled (cm)	BZE (counts)	IZT (s)
<i>Male</i>				
Vehicle		2067 (1970-2264)	15 (11-23)	68 (56-76)
2.5 mg/kg FLX		1962 (1780-2135)	11 (8-17)	49 (42-66)
<i>Female</i>				
Vehicle		2515 (2212-2776)	19 (8-24)	54 (33-76)
2.5 mg/kg FLX		2181 (1894-2371)	12 (8-18)	35 (26-51)
Dose	H	1.18	0.01	1.84
	p	>0.05	>0.05	>0.05
	df	1	1	1
Sex	H	1.90	0.00	0.83
	p	>0.05	>0.05	>0.05
	df	1	1	1
Dose x Sex	H	0.13	0.00	0.04
	p	>0.05	>0.05	>0.05
	df	1	1	1

Table 5.16 Fluoxetine OF PND 84. Male and female offspring exposed to either vehicle, FLX (2.5 mg/kg) via OG to the dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=12/group. See table for statistical analysis.

5.3.1.2.2 Paroxetine (Table 5.17-Table 5.19)

PND 28 OF analysis only revealed an effect of dose, for BZE. A *post hoc* analysis indicated a significant difference with male PRX (1.25 mg/kg) offspring having more BZE when compared to vehicle male progeny, and female PRX (2.5 mg/kg) offspring exhibiting fewer BZE when compared to the female vehicle group. PND 56 OF analysis only revealed an effect of sex, for distance travelled; however, a *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups. PND 84 OF analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups.

Group		Distance travelled (cm)	BZE (counts)	IZT (s)
Male				
Vehicle		1604 (755-2419)	5 (3-7)	64 (28-120)
1.25 mg/kg PRX		2132 (1908-2262)	18 (12-14) *	77 (66-106)
2.5 mg/kg PRX		1587 (1331-2172)	6 (2-13)	78 (23-99)
5 mg/kg PRX		1301 (956-1992)	5 (3-9)	28 (12-62)
Female				
Vehicle		1829 (1389-2123)	12 (7-20)	66 (46-114)
1.25 mg/kg PRX		1942 (1582-2329)	16 (13-21)	65 (46-106)
2.5 mg/kg PRX		1476 (1198-1912)	3 (1-5) #	42 (23-70)
5 mg/kg PRX		1687 (1336-2032)	9 (5-13)	42 (35-61)
Dose	H	3.39	7.08	2.94
	p	>0.05	<0.05	>0.05
	df	3	3	3
Sex	H	0.01	0.43	0.37
	p	>0.05	>0.05	>0.05
	df	1	1	1
Dose x Sex	H	0.56	2.04	0.75
	p	>0.05	>0.05	>0.05
	df	3	3	3

Table 5.17 Paroxetine OF PND 28. Male and female offspring exposed to either vehicle, PRX (1.25, 2.5, or 5 mg/kg) via OG to the dam from GD 7-21. A significant treatment effect was found for BZE; male PRX (1.25 mg/kg) pups had more BZE compared to the male vehicle, and female PRX (2.5 mg/kg) pups exhibited fewer BZE compared to the female vehicle. Data are expressed as median and interquartile range, n=9-12/group. When comparing to vehicle male, * $p < 0.05$, and when comparing to vehicle female, # $p < 0.05$, with appropriate correction due to multiple comparisons. See table for statistical analysis.

Group		Distance travelled (cm)	BZE (counts)	IZT (s)
<i>Male</i>				
Vehicle		2323 (2212-2772)	14 (9-18)	42 (34-56)
1.25 mg/kg PRX		2263 (2144-2504)	16 (5-18)	36 (27-62)
2.5 mg/kg PRX		2272 (1939-2586)	14 (8-19)	43 (28-59)
5 mg/kg PRX		1973 (1895-2209)	9 (4-12)	45 (28-64)
<i>Female</i>				
Vehicle		2430 (2184-2916)	14 (6-18)	50 (22-64)
1.25 mg/kg PRX		2545 (2282-2924)	16 (12-32)	46 (41-68)
2.5 mg/kg PRX		2429 (2086-3077)	14 (10-19)	41 (34-54)
5 mg/kg PRX		2651 (2373-2747)	15 (11-22)	52 (39-69)
Dose	H	0.39	0.74	0.17
	p	>0.05	>0.05	>0.05
	df	3	3	3
Sex	H	8.02	3.09	0.78
	p	<0.05	>0.05	>0.05
	df	1	1	1
Dose x Sex	H	1.15	1.61	0.53
	p	>0.05	>0.05	>0.05
	df	3	3	3

Table 5.18 Paroxetine OF PND 56. Male and female offspring exposed to either vehicle, PRX (1.25, 2.5, or 5 mg/kg) via OG to the dam from GD 7-21. An overall effect of sex was noted for distance travelled; however, no significant difference was noted between vehicle groups. Data are expressed as median and interquartile range, n=8-12/group. See table for statistical analysis.

Group	Distance travelled (cm)	BZE (counts)	IZT (s)	
<i>Male</i>				
Vehicle	2203 (1964-2602)	13 (10-19)	49 (30-73)	
1.25 mg/kg PRX	2445 (2156-2567)	24 (13-29)	69 (44-90)	
2.5 mg/kg PRX	2134 (1629-2476)	4 (3-10)	31 (16-55)	
5 mg/kg PRX	2378 (1862-2623)	20 (13-22)	54 (36-95)	
<i>Female</i>				
Vehicle	2634 (1917-2981)	13 (8-18)	29 (21-45)	
1.25 mg/kg PRX	2431 (2245-3036)	12 (10-22)	36 (25-64)	
2.5 mg/kg PRX	2530 (2179-3032)	14 (8-23)	38 (26-75)	
5 mg/kg PRX	2353 (2044-2401)	13 (10-19)	48 (33-56)	
Dose	H	0.59	2.57	1.54
	p	>0.05	>0.05	>0.05
	df	3	3	3
Sex	H	2.98	0.02	3.77
	p	>0.05	>0.05	>0.05
	df	1	1	1
Dose x Sex	H	0.98	2.61	1.22
	p	>0.05	>0.05	>0.05
	df	3	3	3

Table 5.19 Paroxetine OF PND 84. Male and female offspring exposed to either vehicle, PRX (1.25, 2.5, or 5 mg/kg) via OG to the dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=9-12/group. See table for statistical analysis.

5.3.1.2.3 Sertraline (Table 5.20-Table 5.22)

PND 28 and 56 OF analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups. PND 84 OF analysis only revealed an effect of sex, for distance travelled, BZE, and IZT; however, a *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups.

Group	Distance travelled (cm)	BZE (counts)	IZT (s)	
Male				
Vehicle	1609 (550-2186)	7 (1-10)	54 (29-62)	
2.5 mg/kg SERT	1611 (838-1836)	8 (2-9)	60 (28-126)	
5 mg/kg SERT	1364 (615-2017)	2 (1-9)	55 (38-76)	
10 mg/kg SERT	1198 (936-1692)	6 (4-9)	52 (36-88)	
Female				
Vehicle	1689 (1232-1886)	8 (4-12)	49 (37-77)	
2.5 mg/kg SERT	1707 (1248-2171)	8 (2-18)	52 (42-88)	
5 mg/kg SERT	1851 (1079-1953)	9 (1-12)	42 (28-68)	
10 mg/kg SERT	1773 (1237-2021)	8 (4-12)	47 (30-66)	
Dose	H	0.09	0.43	0.37
	p	>0.05	>0.05	>0.05
	df	3	3	3
Sex	H	3.67	3.32	0.32
	p	>0.05	>0.05	>0.05
	df	1	1	1
Dose x Sex	H	0.49	0.10	0.20
	p	>0.05	>0.05	>0.05
	df	3	3	3

Table 5.20 Sertraline OF PND 28. Offspring exposed to either vehicle, SERT (2.5, 5 or 10 mg/kg) via OG to dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=9-12/group. See table for statistical analysis.

Group	Distance travelled (cm)	BZE (counts)	IZT (s)	
<i>Male</i>				
Vehicle	2014 (1511-2366)	12 (7-15)	47 (36-58)	
2.5 mg/kg SERT	2283 (1813-2780)	14 (8-29)	66 (16-83)	
5 mg/kg SERT	1955 (1558-2686)	7 (3-15)	45 (24-59)	
10 mg/kg SERT	2238 (2029-2737)	14 (9-31)	57 (30-90)	
<i>Female</i>				
Vehicle	2333 (2138-2906)	12 (8-24)	45 (22-80)	
2.5 mg/kg SERT	2466 (3282-2578)	18 (9-29)	69 (43-95)	
5 mg/kg SERT	2635 (2196-2765)	16 (8-20)	47 (18-73)	
10 mg/kg SERT	2479 (2089-2717)	14 (6-17)	44 (37-63)	
Dose	H	0.21	0.84	1.08
	p	>0.05	>0.05	>0.05
	df	3	3	3
Sex	H	3.36	0.31	0.09
	p	>0.05	>0.05	>0.05
	df	1	1	1
Dose x Sex	H	0.66	1.46	0.35
	p	>0.05	>0.05	>0.05
	df	3	3	3

Table 5.21 Sertraline OF PND 56. Offspring exposed to either vehicle, SERT (2.5, 5 or 10 mg/kg) via OG to dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=8-12/group. See table for statistical analysis.

Group		Distance travelled (cm)	BZE (counts)	IZT (s)
<i>Male</i>				
Vehicle		2046 (1970-2330)	13 (11-22)	65 (30-82)
2.5 mg/kg SERT		2117 (1867-2397)	13 (6-18)	46 (26-62)
5 mg/kg SERT		2228 (1558-2413)	6 (4-12)	34 (18-45)
10 mg/kg SERT		1920 (1532-2557)	9 (3-16)	28 (18-47)
<i>Female</i>				
Vehicle		2607 (2016-2909)	21 (8-25)	49 (31-74)
2.5 mg/kg SERT		2369 (2067-2926)	22 (10-25)	64 (29-91)
5 mg/kg SERT		2532 (2166-2936)	19 (14-28)	45 (33-51)
10 mg/kg SERT		2244 (1972-2979)	18 (11-26)	60 (29-80)
Dose	H	0.27	1.46	2.05
	p	>0.05	>0.05	>0.05
	df	3	3	3
Sex	H	10.03	11.94	4.48
	p	<0.05	<0.05	<0.05
	df	1	1	1
Dose x Sex	H	0.11	0.96	1.25
	p	>0.05	>0.05	>0.05
	df	3	3	3

Table 5.22 Sertraline OF PND 84. Offspring exposed to either vehicle, SERT (2.5, 5 or 10 mg/kg) via OG to dam from GD 7-21. An overall effect of sex was noted for distance travelled, BZE, and IZT; however, no significant differences were noted between vehicle groups. Data are expressed as median and interquartile range, n=8-12/group. See table for statistical analysis.

5.3.1.2.4 Citalopram (Table 5.23-Table 5.25)

PND 28 OF analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups. PND 56 OF analysis only revealed an effect of sex, for distance travelled and BZE; however, a *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups. PND 84 OF analysis only revealed an effect of sex, for distance travelled; however, a *post hoc* analysis indicated no significant difference when comparing male and female vehicle groups.

Group		Distance travelled (cm)	BZE (counts)	IZT (s)
<i>Male</i>				
Vehicle		1249 (550-1765)	2 (1-8)	59 (28-77)
2.5 mg/kg CIT		1349 (697-1937)	3 (2-9)	50 (22-163)
5 mg/kg CIT		1653 (726-2067)	6 (3-12)	59 (47-68)
10 mg/kg CIT		1387 (792-1709)	4 (2-9)	62 (24-108)
<i>Female</i>				
Vehicle		1433 (875-1884)	8 (1-17)	62 (47-120)
2.5 mg/kg CIT		1582 (1275-1951)	6 (3-10)	54 (38-89)
5 mg/kg CIT		1539 (826-1865)	6 (1-13)	38 (28-94)
10 mg/kg CIT		1951 (1242-2408)	14 (4-19)	58 (29-90)
Dose	H	0.67	0.97	0.07
	p	>0.05	>0.05	>0.05
	df	3	3	3
Sex	H	1.91	3.78	0.00
	p	>0.05	>0.05	>0.05
	df	1	1	1
Dose x Sex	H	0.83	1.06	0.48
	p	>0.05	>0.05	>0.05
	df	3	3	3

Table 5.23 Citalopram OF PND 28. Offspring exposed to either vehicle, CIT (2.5, 5 or 10 mg/kg) via OG to dam from GD 7-21. No treatment or sex effect noted. Data are expressed as median and interquartile range, n=9-13/group. See table for statistical analysis.

Group		Distance travelled (cm)	BZE (counts)	IZT (s)
<i>Male</i>				
Vehicle		2077 (1703-2902)	11 (4-14)	46 (37-80)
2.5 mg/kg CIT		1847 (1732-1998)	8 (6-11)	35 (26-45)
5 mg/kg CIT		3329 (1865-2049)	8 (5-17)	35 (25-41)
10 mg/kg CIT		1848 (1800-2352)	10 (6-16)	45 (34-67)
<i>Female</i>				
Vehicle		2391 (2212-2906)	16 (9-26)	49 (36-72)
2.5 mg/kg CIT		2361 (1848-2680)	10 (6-17)	30 (21-68)
5 mg/kg CIT		2538 (1937-2803)	16 (12-19)	57 (38-85)
10 mg/kg CIT		2146 (2012-2701)	15 (10-18)	51 (38-75)
Dose	H	1.98	1.07	1.40
	p	>0.05	>0.05	>0.05
	df	3	3	3
Sex	H	13.58	8.29	3.10
	p	<0.05	<0.05	>0.05
	df	1	1	1
Dose x Sex	H	0.80	0.24	1.05
	p	>0.05	>0.05	>0.05
	df	3	3	3

Table 5.24 Citalopram OF PND 56. Offspring exposed to either vehicle, CIT (2.5, 5 or 10 mg/kg) via OG to dam from GD 7-21. An overall effect of sex was noted for distance travelled and BZE; however, no significant differences were noted between vehicle groups. Data are expressed as median and interquartile range, n=9-12/group. See table for statistical analysis.

Group		Distance travelled (cm)	BZE (counts)	IZT (s)
<i>Male</i>				
Vehicle		2203 (1965-2402)	15 (12-22)	67 (59-76)
2.5 mg/kg CIT		1865 (1550-2315)	11 (4-17)	43 (20-47)
5 mg/kg CIT		2131 (1841-2801)	7 (3-21)	32 (20-75)
10 mg/kg CIT		1997 (1332-2532)	17 (4-22)	54 (36-73)
<i>Female</i>				
Vehicle		2564 (2067-2746)	21 (13-25)	56 (31-79)
2.5 mg/kg CIT		2338 (2028-2654)	11 (5-25)	31 (21-71)
5 mg/kg CIT		2604 (2291-2728)	16 (11-19)	51 (41-65)
10 mg/kg CIT		2064 (1919-2573)	8 (6-14)	35 (15-59)
Dose	H	1.58	2.65	3.25
	p	>0.05	>0.05	>0.05
	df	3	3	3
Sex	H	7.03	0.57	0.46
	p	<0.05	>0.05	>0.05
	df	1	1	1
Dose x Sex	H	0.20	1.11	1.40
	p	>0.05	>0.05	>0.05
	df	3	3	3

Table 5.25 Citalopram OF PND 84. Offspring exposed to either vehicle, CIT (2.5, 5 or 10 mg/kg) via OG to dam from GD 7-21. An overall effect of sex was noted for distance travelled; however, no significant difference was noted between vehicle groups. Data are expressed as median and interquartile range, n=9-13/group. See table for statistical analysis.

5.3.2 Cognitive assessment in the novel object recognition test

Fluoxetine (Figure 5.2A and Figure 5.3A)

NOR analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups for familiar object exploration duration [dose: $H_{(1)}=0.19$, $p>0.05$; sex: $H_{(1)}=0.29$, $p>0.05$; dose x sex: $H_{(1)}=0.11$, $p>0.05$], novel object exploration duration [dose: $H_{(1)}=0.01$, $p>0.05$; sex: $H_{(1)}=1.43$, $p>0.05$; dose x sex: $H_{(1)}=0.03$, $p>0.05$], or discrimination ratio [dose: $H_{(1)}=0.00$, $p>0.05$; sex: $H_{(1)}=0.05$, $p>0.05$; dose x sex: $H_{(1)}=0.00$, $p>0.05$].

Paroxetine (Figure 5.2B and Figure 5.3B)

NOR analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups for familiar object exploration duration [dose: $H_{(3)}=1.33$, $p>0.05$; sex: $H_{(1)}=1.24$, $p>0.05$; dose x sex: $H_{(3)}=1.33$, $p>0.05$], novel object exploration duration [dose: $H_{(3)}=3.09$, $p>0.05$; sex: $H_{(1)}=1.03$, $p>0.05$; dose x sex: $H_{(3)}=0.47$, $p>0.05$], or discrimination ratio [dose: $H_{(3)}=2.57$, $p>0.05$; sex: $H_{(1)}=2.89$, $p>0.05$; dose x sex: $H_{(3)}=1.93$, $p>0.05$].

Sertraline (Figure 5.2C and Figure 5.3C)

NOR analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups for familiar object exploration duration [dose: $H_{(3)}=1.73$, $p>0.05$; sex: $H_{(1)}=0.89$, $p>0.05$; dose x sex: $H_{(3)}=0.51$, $p>0.05$], novel object exploration duration [dose: $H_{(3)}=1.16$, $p>0.05$; sex: $H_{(1)}=2.10$, $p>0.05$; dose x sex: $H_{(3)}=0.33$, $p>0.05$], or discrimination ratio [dose: $H_{(3)}=0.99$, $p>0.05$; sex: $H_{(1)}=0.06$, $p>0.05$; dose x sex: $H_{(3)}=2.97$, $p>0.05$].

Citalopram (Figure 5.2D and Figure 5.3D)

NOR analysis revealed no effect of dose, sex, or dose x sex interaction when compared to vehicle groups for familiar object exploration duration [dose: $H_{(3)}=0.61$, $p>0.05$; sex: $H_{(1)}=3.48$, $p>0.05$; dose x sex: $H_{(3)}=0.39$, $p>0.05$], novel object exploration duration [dose: $H_{(3)}=0.51$, $p>0.05$; sex: $H_{(1)}=2.88$, $p>0.05$; dose x sex: $H_{(3)}=0.33$, $p>0.05$], or discrimination ratio [dose: $H_{(3)}=3.25$, $p>0.05$; sex: $H_{(1)}=0.29$, $p>0.05$; dose x sex: $H_{(3)}=1.007$, $p>0.05$].

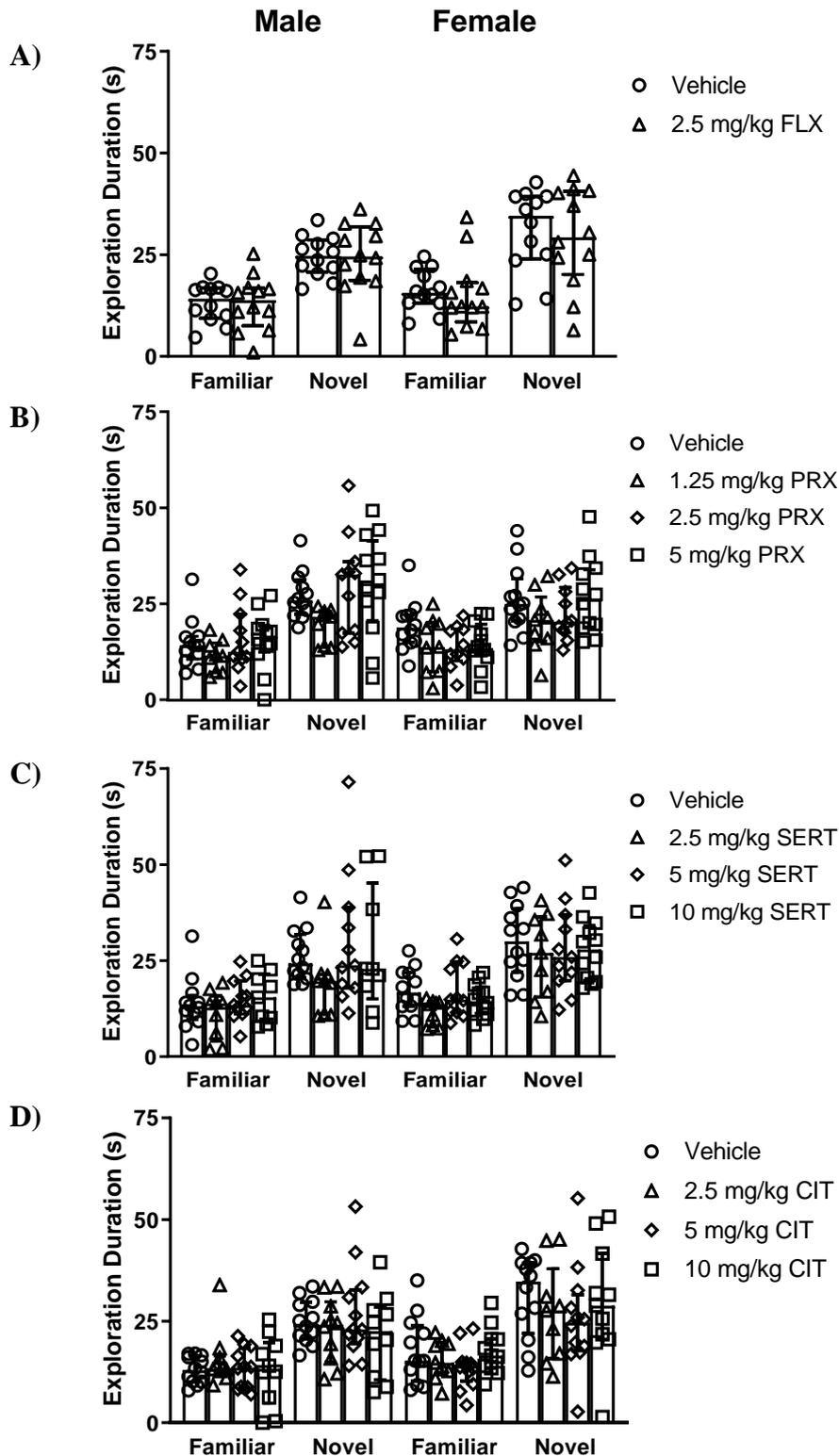


Figure 5.2 NOR after gestational SSRI exposure. Exploration duration of familiar and novel objects in the NOR for male and female offspring exposed to either vehicle, (A) FLX (2.5 mg/kg), (B) PRX (1.25, 2.5, or 5 mg/kg), (C) SERT (2.5, 5 or 10 mg/kg), or (D) CIT (2.5, 5 or 10 mg/kg) via OG to the dam from GD 7-21. Each group showed a significant increase in novel object exploration compared to familiar object exploration. No significant effects of treatment were found. Data are expressed as median and interquartile range, $n=9-12$ /group. See text for statistical analysis.

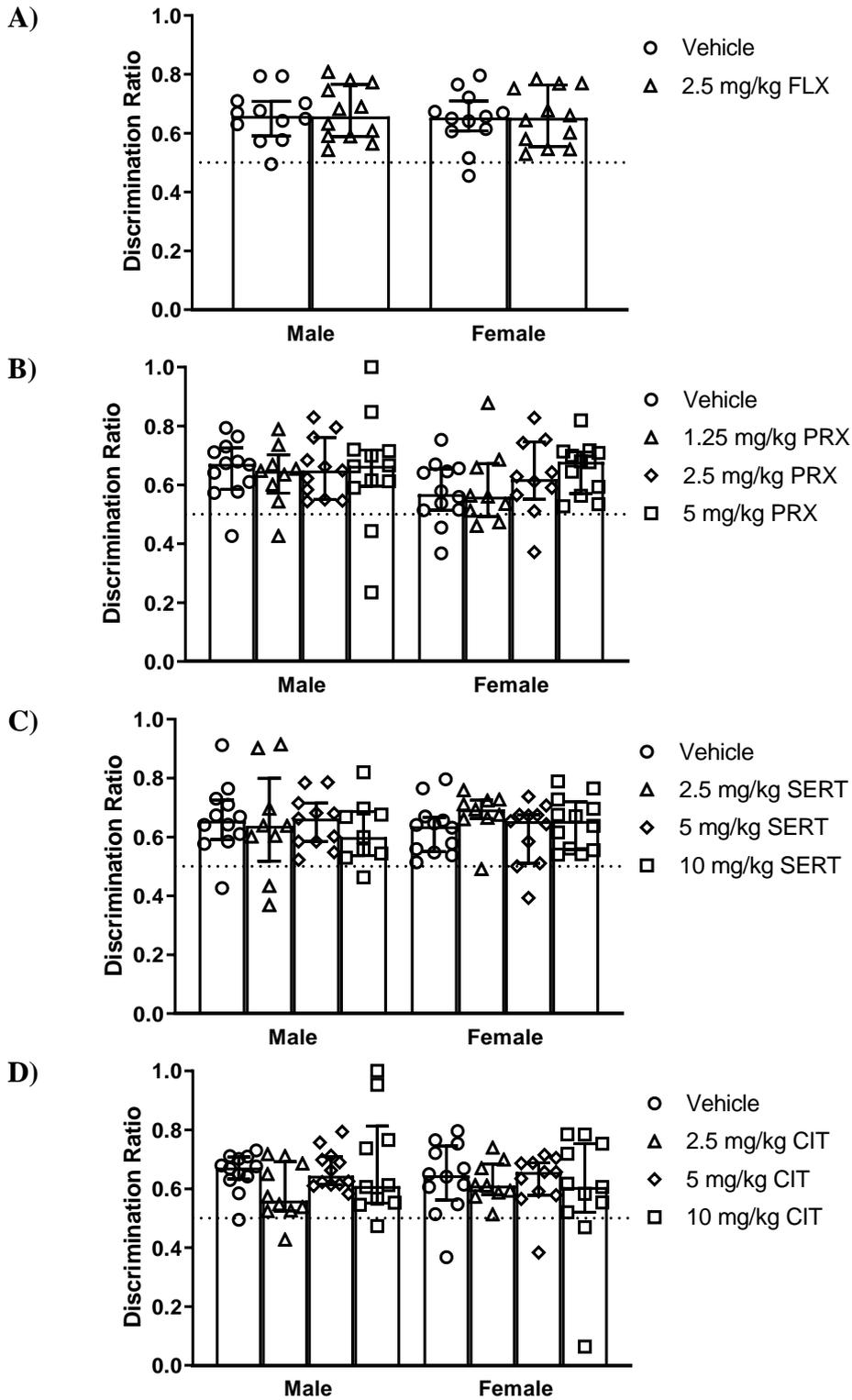


Figure 5.3 NOR discrimination ratio. Ratio of novel vs familiar object exploration for male and female offspring exposed to either vehicle, (A) FLX (2.5 mg/kg), (B) PRX (1.25, 2.5, or 5 mg/kg), (C) SERT (2.5, 5 or 10 mg/kg), or (D) CIT (2.5, 5 or 10 mg/kg) via OG to the dam from GD 7-21. No significant effects of treatment were found. Data are expressed as median and interquartile range, n=9-12/group. See text for statistical analysis.

5.3.3 Anhedonia assessment in the saccharin preference test

Fluoxetine (Figure 5.4A)

SPT analysis revealed no effect of dose, sex, or dose x sex interaction on percentage of saccharin consumption when compared to vehicle groups [dose: $H_{(1)}=0.04$, $p>0.05$; sex: $H_{(1)}=0.34$, $p>0.05$; dose x sex: $H_{(1)}=0.17$, $p>0.05$].

Paroxetine (Figure 5.4B)

SPT analysis revealed no effect of dose, sex, or dose x sex interaction on percentage of saccharin consumption when compared to vehicle groups [dose: $H_{(3)}=1.03$, $p>0.05$; sex: $H_{(1)}=0.08$, $p>0.05$; dose x sex: $H_{(3)}=0.39$, $p>0.05$].

Sertraline (Figure 5.4C)

SPT analysis revealed no effect of dose, sex, or dose x sex interaction on percentage of saccharin consumption when compared to vehicle groups [dose: $H_{(3)}=0.51$, $p>0.05$; sex: $H_{(1)}=0.32$, $p>0.05$; dose x sex: $H_{(3)}=0.56$, $p>0.05$].

Citalopram (Figure 5.4D)

SPT analysis revealed no effect of dose, sex, or dose x sex interaction on percentage of saccharin consumption when compared to vehicle groups [dose: $H_{(3)}=3.31$, $p>0.05$; sex: $H_{(1)}=0.18$, $p>0.05$; dose x sex: $H_{(3)}=1.17$, $p>0.05$].

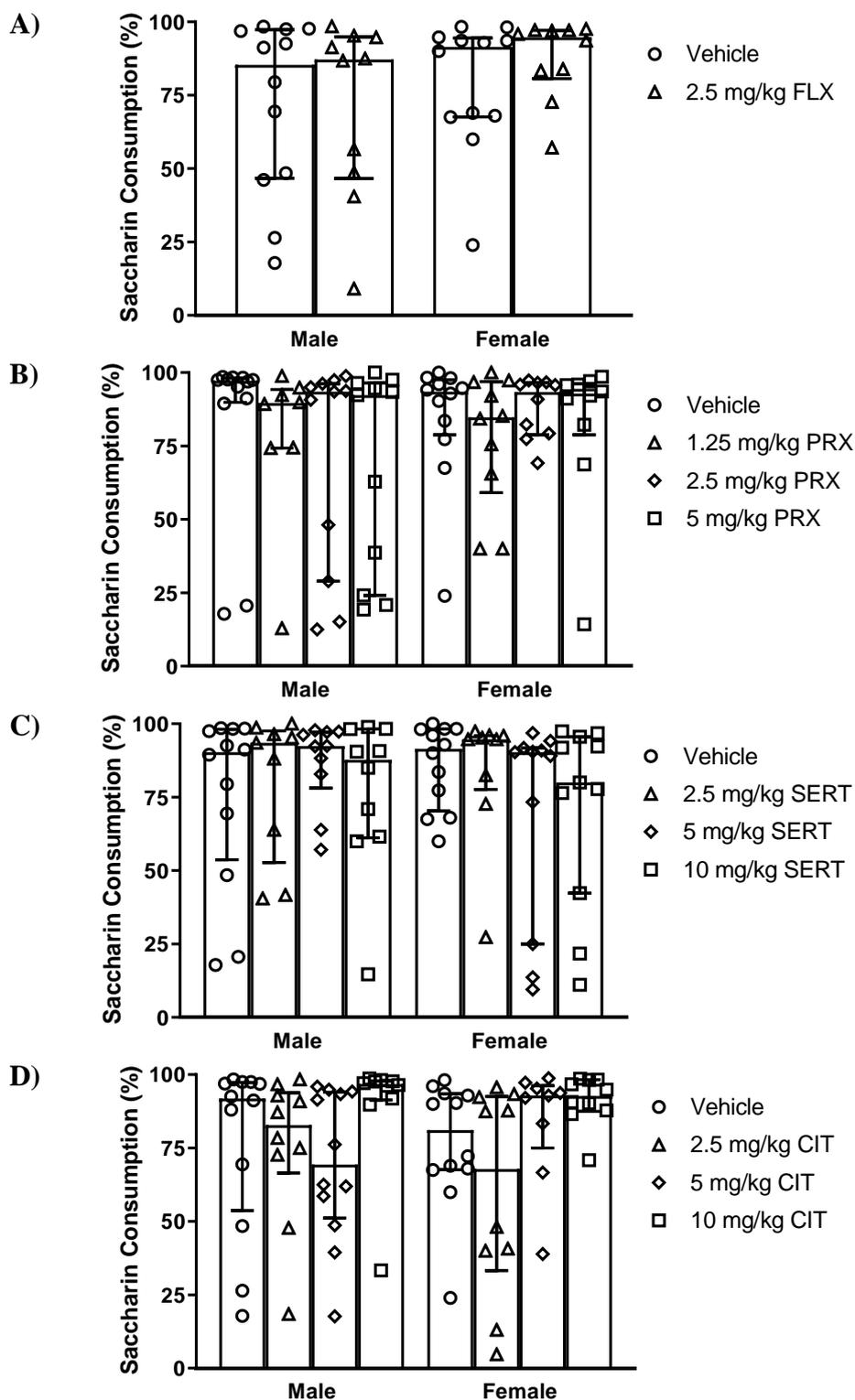


Figure 5.4 SPT after gestational SSRI exposure. Percentage of saccharin consumption in the SPT for male and female offspring exposed to either vehicle, (A) FLX (2.5 mg/kg), (B) PRX (1.25, 2.5, or 5 mg/kg), (C) SERT (2.5, 5 or 10 mg/kg), or (D) CIT (2.5, 5 or 10 mg/kg) via OG to the dam from GD 7-21. All vehicle groups showed a significant increase in saccharin consumption compared to water consumption. No significant effects of treatment were found. Data are expressed as median and interquartile range, n=8-12/group. See text for statistical analysis.

5.4 Discussion

Overall, this chapter has revealed that no SSRI considered produced an anxiogenic effect in adolescent or adult, male and female progeny. Paroxetine exposure altered ambulatory behaviour during adolescences but not adulthood, exhibiting a sex and dose-dependent increase or decrease in movement between zones of the arena; however, no effects were noted in regards to distance travelled. Cognitive and anhedonia behaviours were not altered in adulthood by any of the four observed SSRIs. Overall effects of sex occurred from adolescence through adulthood within anxiety and ambulatory behavioural measures, but no effects were noted for cognitive or anhedonia behavioural measures.

5.4.1 Aim 1: Anxiety and ambulatory assessment

The first aim of this study was to examine the effects of gestational SSRI exposure from GD 7-21 on offspring anxiety and ambulatory behaviours from adolescence through adulthood. Evaluation of “anxiety-like” behaviours was measured by monitoring the time spent in the open arm of the elevated plus maze and measuring the time spent in the inner zone or the open field. Observations were also made on ambulatory behaviour within each test based on distance travelled and between zone entries. Measurements occurred in offspring at ages PND 28, 56, & 84.

5.4.1.1 Patterns of sex and age

The measures of anxiety and ambulatory behaviours were carried out in both male and female offspring, at PND 28, 56 and 84. While no effects remained statistically significant when comparing the vehicle groups, multiple overall effects of sex were noted throughout the studies, with the exception of the fluoxetine study. This is likely due to the fact that sex effects were not very prominent in the other SSRI studies, however, there were more groups, thus increasing the overall number of males compared to females and increasing the likelihood for an effect of sex. Nonetheless, general effects of sex were noted for both “anxiety-like” and ambulatory behaviours. In regards to anxiogenic behaviours, no effects of sex were observable during the adolescent testing period at PND 28. However, in the elevated plus maze, all three SSRI studies showed evidence of sex effects at both adulthood time points, with females tending to spend more time in the open arm than males. Furthermore, in the

open field, one study showed that females spent slightly more time in the inner zone compared to males at the later adulthood time point of PND 84. Such findings may suggest that the elevated plus maze is a better model for detecting sex-related differences in regards to induced anxiogenic behaviour. In regards to ambulatory behaviours, across the remaining three SSRI studies and the two models employed, 1 of 6 measures showed an overall effect of sex on distance moved during the adolescent period. However, in adulthood, possible effects of sex were slightly more prominent for ambulatory behaviour with initial differences noted in 4 of 6 measures at PND 56 and 5 of 6 measures at PND 84. While only a trend was recognized in the present study, previous baseline findings suggested an increase in distance travelled for females in the open field but not in the elevated plus maze (Simpson et al., 2012). Also, within the treatment groups, there were interesting findings within particular sexes which will now be conversed with the discussion of age. The only observable treatment induced effect was by paroxetine during the adolescent testing period for ambulatory behaviours; no treatment-induced effects were noted in regards to “anxiety-like” behaviours. Ultimately, an overall paroxetine-induced effect was noted for between zone entries. However this effect was transient as no statistically significant differences were noted at PND 56 or 84; furthermore, no deficits in motor development were noted within the pre-weaning neonatal (PND 2-17) behavioural findings of Chapter 4, in regards to paroxetine. Consequently, while this effect may be brief, it emphasizes the practice of carrying out behavioural tests at various time points throughout development. Returning to sex differences, using the same PND 28 paroxetine scenario, while exposed males (1.25 mg/kg PRX) showed an increase in between zone entries, the opposite was witnessed with exposed females (2.5 mg/kg PRX) as they exhibited a decrease in between zone entries. Thus highlighting the sex- and dose-dependent differences in ambulatory behaviours. Overall, these observations in regards to sex and age, stress the importance of conducting longitudinal behavioural development studies in both male and female models using a range of relevant doses.

5.4.1.2 Fluoxetine

“Anxiety-like” behaviours were not altered in adolescence or at either adulthood time points after gestational exposure in the elevated plus maze or in the open field. Further, treatment did not alter ambulatory behaviours in either test at any of the three time points. Similar to the adolescent observations, additional researchers utilizing a

comparable experimental design, also suggested that gestational exposure (GD 6-20), at higher doses (8, 12 mg/kg, IG) than employed in the current work, also had no effect on anxiety behaviours in the elevated plus maze for male and female adolescent (PND 30), Wistar rats (Bairy et al., 2007). Interestingly, Bairy et al. (2007) did not report distance travelled in the elevated plus maze or open field, however, they did report an alteration in total crossings in the open field, with both a decrease in adolescents (PND 35) in the 8 and 12 mg/kg doses, and a decrease in adulthood (PND 56) for the 12 mg/kg dose. Although ambulatory behaviours were not altered in the current study, the longitudinal findings of Bairy et al. (2007) would suggest that this effect is transient and dose-dependent, as the decreased activity only continued into adulthood for 12 mg/kg exposed offspring and not the 8 mg/kg group. Therefore the current findings are somewhat complementary to the overall assumption of Bairy et al. (2007), concluding that motor deficiencies induced by gestational fluoxetine exposure are transient and dose-dependent. Another feature that could clarify the dissimilarities between this study and Bairy et al. (2007) could be explained by strain differences, as the current study employed SD rats and Bairy et al. used Wistar rats. Strain differences have been reported in the open field with SD rats exhibiting more activity than Wistar rats (Asano, 1986); therefore, the Wistar model may be more sensitive to modest changes in ambulatory behaviour, further elucidating the difference between findings. Overall, the two studies together, highlight the importance of factors of study design such as the dose of exposure, the developmental age of the offspring, and the strain used for assessing resulting behavioural changes due to gestational fluoxetine exposure. Few relevant studies could be found assessing *in utero* treatment-induced anxiety and ambulatory behaviours of the offspring in the elevated plus maze and the open field later into adulthood (after PND 56), other than the previously discussed results produced by Bairy et al (2007). Studies conducted in Swiss mice, suggest a dose-dependent effect of exposure in the elevated plus maze at the highest dose, and decreased activity in the inner zone of the open field for all doses employed (0.3, 0.6, 0.8 mg/kg, IP, GD 8-18) in resulting adult (PND 90) mice (Noorlander et al., 2008). Whereas, the findings in SD rats and other findings in Wistar rats exposed to a higher dose (12 mg/kg, IG, GD11-21) showed no alterations in ‘anxiety-like’ behaviours of the elevated plus maze in adulthood (PND 106) (Olivier et al., 2011). Interestingly, when Olivier et al. (2011) paired the test with a foot shock, an increase in anxiety behaviours was observed, suggesting a possible fluoxetine-induced effect of

heightened stress-induced anxiety. The current study showed no dose effect for distance travelled regardless of test or age for SD, which is complementary to a previous study reporting gestational exposure (GD 12-littering) to fluoxetine (8, 11, 12 mg/kg, OMP) had no effect on motor skill of male and female adult (PND 70-100) Long Evans rats (Capello et al., 2011) in the beam traversing task. Therefore as no ambulatory effects were noted in the current study with SD rats or Capello et al. (2011) with Long Evans rats, it is likely the ambulatory differences discussed previously per Bairy et al. (2007) study in Wistar rats is in fact due to strain differences, as reduced activity is usually reported by Wistar rats thus making them more sensitive to any change in motor behaviour. Overall, these results taken with the previous findings suggests that fluoxetine treatment before birth does not induce anxiety related emotional deficits or affect the distance travelled by rats, however, doses higher than 2.5 mg/kg may transiently alter ambulatory behaviours in particular rat strains.

5.4.1.3 Paroxetine

From adolescence throughout adulthood, no effect was found for treatment on “anxiety-like” behaviours in the elevated plus maze or open field regardless of age. While distance travelled remained unaffected regardless of condition, a treatment effect was noted within male and female groups, altering between zone entries during adolescence. Males exposed to the lowest dose (1.25 mg/kg) were found to make more between zone entries compared to the male vehicle pups. On the other hand, females exposed to the middle dose (2.5 mg/kg) experienced fewer between zone entries compared to the female vehicle pups. Relevant research, measuring anxiety and ambulatory behaviours at various time points in animals exposed to paroxetine *in utero* is scarce. Although other studies use variable experimental designs, employing mice at much higher doses (30 mg/kg, oral) than used in the present work, *in utero* (GD (-14)-GD 16/littering), still no anxiety-related behaviours have been reported in male or female adolescent (PND 30) or adult (PND 60/65) offspring, nor were treatment effects noted for exploratory behaviour (Coleman et al., 1999, Christensen et al., 2000). In adult (PND 70-100) rats, no anxiety behaviours were reported in the defensive withdrawal task when observed in adult rat offspring after gestational exposure (GD 12-littering) to different doses (3, 8 mg/kg, OMP), nor were there any effects noted in regards to motor skill (Capello et al., 2011). Ultimately, this longitudinal assessment produces novel results, suggesting that *in utero* exposure to

paroxetine at various doses does not pose a risk to the development of “anxiety-like” symptoms in exposed rat offspring regardless of gender or age of the progeny, as expressed in the elevated plus maze or the open field. Additionally, gestational exposure may alter activities such as between zone entries of adolescent male and female progeny in the open field, however, the expressed lack of effect on distance travelled is in line with previous work, albeit in different behavioural models.

5.4.1.4 Sertraline

“Anxiety-like” behaviours were not observed in the elevated plus maze or open field for offspring prenatally exposed to sertraline treatment. In addition, no effects were noted for locomotor activity parameters. No literature considering anxiety symptoms during adolescence after gestational exposure to the treatment could be found, and few relevant papers have reported such findings in adulthood. In line with these findings, Kott et al. (2019) reports no treatment effect on “anxiety-like” behaviours after *in utero* exposure (GD (-16)-littering) to a higher dose than those observed in the current study (20 mg/kg) in the elevated plus maze (PND 95-96) nor in the open field (PND 123-124) in adult progeny (Kott et al., 2019). While anxiety was assessed by Capello et al. (2011), after rats were exposed prenatally (GD 12-littering) to sertraline (7, 10 mg/kg, OMP) in the defensive withdrawal test (PND 70-100), and by Meyer et al. (2018), after mice were perinatally (GD 0-PND 14) exposed to sertraline (5 mg/kg, IP) in the elevated plus maze (adult), both studies indicated no effect of treatment on “anxiety-like” behaviour for male or female adult offspring (Capello et al., 2011, Meyer et al., 2018). Further supporting the current findings, Capello et al. (2011) also noted no effects on motor skill. Therefore, these results produce novel contributions suggesting that gestational exposure does not increase “anxiety-like” behaviour in adolescent male or female progeny, adding to previous findings in adulthood. Furthermore, the current long-term study exposes that treatment has no ambulatory effects in male or female progeny from adolescence throughout adulthood.

5.4.1.5 Citalopram

After gestational exposure analyses showed no effect of treatment on “anxiety-like” or ambulatory behaviours in the elevated plus maze or the open field. Literature concerning “anxiety-like” behaviours after gestational exposure in resulting adolescent progeny have not been published. Few studies with limited relevance have examined such emotional deficits in adulthood. Gestational exposure (GD 13-21)

studies in mice assessing “anxiety-like” and ambulatory behaviours in the elevated plus maze have reported no effect of treatment (5 mg/kg, SC) (Hsiao et al., 2005) similar to the current results, however, at a higher dose (20 mg/kg, IP) an increase in “anxiety-like” behaviours is observed (Zahra et al., 2018). Further, postnatal exposure (PND 8-21) studies to citalopram (5, 10, 20 mg/kg, SC to pup) in rats have also reported no effect on adult (PND 90) progeny for anxiety measures in the elevated plus maze (Harris et al., 2012). Previous results in the open field (10 mg/kg, SC to pup) and marble burying (5, 10 mg/kg, SC to pup) after perinatal exposure (GD 6-PND 20), do indicate an increase in “anxiety-like” behaviours in adulthood (PND 61) (Sprowles et al., 2016, Sprowles et al., 2017). A study similar to these by Sprowles et al. (2016 and 2017), also showed this anxiogenic effect in the elevated plus maze in adulthood (PND 60) however only in male offspring after perinatal exposure (GD 6-PND 21) to citalopram (10 mg/kg) (Zohar et al., 2016). While the current results are in line with previous gestational exposure studies at comparable doses, anxiogenic effects are noted at higher doses exposed during gestation. Further differences in the latter reports discussed are likely due to the different exposure periods as the current exposure was strictly gestational, whereas perinatal exposure spanned from GD 6-PND 20 or 21, and postnatally incorporated direct SC administration to the pup, rather than the dam. Overall, the current findings provide novel evidence in regards to gestational citalopram exposure which specifically informs adolescent progeny, suggesting no treatment effect on “anxiety-like” behaviours. Further, this study adds that gestational exposure, at a lower dose than previously observed, does not affect anxiogenic behaviours through adulthood. Largely, the work supports previous findings, suggesting that gestational citalopram exposure has no effect on ambulatory behaviours of adolescent or adult progeny.

5.4.2 Aim 2: Cognitive assessment

The second aim of this study was to examine the effects of gestational SSRI exposure from GD 7-21 on cognitive behaviours of adult offspring. Cognitive behaviours were measured by assessing visual object recognition, or the time spent exploring a novel object compared to a familiar object in adult offspring at age PND 89.

5.4.2.1 Fluoxetine

When tested in adulthood, gestational exposure caused no effects on visual learning and memory in the novel object recognition task, regardless of sex. While the current study did not observe cognition in adolescent pups, many preclinical studies have assessed this endpoint, as noted in Table 1.9. To summarize, no effect was observed in the novel object recognition task (Svirsky et al., 2016), however particular regiments caused an advance in spatial memory tasks and a deficit in fear memory tasks (Bairy et al., 2007) whereas other regimes or doses showed no treatment exposure effect (Vorhees et al., 1994, Bairy et al., 2007, Butkevich and Mikhailenko, 2018). In regards to cognitive consequences in resulting adults, one study was identified which measured reward through fear memory. It was reported that gestational exposure (GD 13-20) to a higher dose (10 mg/kg, SC) also had no effect on male offspring (Cagiano et al., 2008). One study in particular assessed perinatal (GD 11-PND 7) treatment (12 mg/kg, OG) in male offspring from early-life through adulthood (PND 20, 34, and 69), and no treatment exposure effect was noted in the males, regardless of age (Kroeze et al., 2016). Resulting consequences of perinatal and postnatal exposure has been observed in spatial memory and reward tasks, as noted in Table 1.12. Overall, no deficits were reported for spatial memory (McAllister et al., 2012, Sprowles et al., 2017). Reward models showed interesting outcomes as male and female adult (PND 60-65) offspring exposed to fluoxetine (10 mg/kg, OMP to dam) in the perinatal period (GD 14-PND 7) showed an increase in behaviours when a reinforcement by cocaine tasks was used (Forcelli and Heinrichs, 2008). Whereas, the same dose (IP administered directly to the pups) exposed in the postnatal period (PND 4-21) decreased fear memory behaviours in male and female progeny (Ansorge et al., 2004, Ansorge et al., 2008). Notably, this study is the first to present novel object recognition findings in adulthood, after gestational exposure to fluoxetine, for both male and female progeny. Further, it can be suggested that although the aforementioned studies report deficits in fear memory reward tasks after gestational or postnatal exposure in resulting adolescent or adult, male and female progeny, no gestational exposure effects were noted in adulthood for visual recognition task, consistent with previous findings for exposed adolescent mice, and early-life through adulthood male rats.

5.4.2.2 Paroxetine

Well into adulthood, exposure *in utero* had no lasting implications on visual object recognition in the novel object recognition test. There have been no previously published studies that have examined resulting paroxetine effects on cognition in the novel object recognition task, however *in utero* exposure has been assessed for implications on exploration, spatial memory, and fear memory in adulthood, albeit in mice. In such studies, it was concluded that gestational exposure (30 mg/kg, oral, GD12-littering) had no effect on resulting exploration behaviours of adolescent (PND 30) (Coleman et al., 1999, Christensen et al., 2000) or adult (PND 65) (Coleman et al., 1999) progeny. Additionally, behaviours of resulting adult progeny were not affected in spatial memory (PND 65-69, 97-104) or fear memory (PND 100-105) tasks (Christensen et al., 2000). This study presents a novel contribution, overall suggesting that *in utero* exposure does not alter cognitive processes in adulthood. These results, taken with previous findings, further informs the literature, suggesting that cognitive deficits are not apparent in visual object recognition, in addition to the previous lack of cognitive deficits reported in measures of spatial or fear memory. Perhaps future studies may assess such cognitive processes in adolescence and later into adulthood, to observe if the absence of a cognitive effect holds true throughout development and ageing.

5.4.2.3 Sertraline

Gestational exposure presented no behavioural cognitive deficits on visual recognition of objects in the resulting adult progeny. The previous explorations of *in utero* exposure (20 mg/kg, OG, GD(-16)-littering) also reported no effects in the resulting adult (PND 135-147) male and female progeny in the novel object recognition task, despite a 30 min delay period between object exposures (Kott et al., 2019). Another study observed adult mice offspring, after perinatal exposure and similar to the current results, no cognitive deficits were detected, concluding that perinatal exposure (5 mg/kg, IP, GD 0-PND 14) had no enduring effects on exploration or spatial memory behaviours in the resulting adult mice (Meyer et al., 2018). Generally, these original results, taken with the previous studies, infer that exposure to sertraline during pregnancy has no lasting implications on cognitive performance in adulthood, regardless of gender.

5.4.2.4 Citalopram

Resulting adult progeny, exposed *in utero*, exhibited no cognitive delays in the novel object recognition behavioural task. Many studies have been completed previously, considering a similar premise of exploring cognition, however, none did so with the novel object recognition model. Instead, the current literature mainly presents results relating to altered exploration behaviours as well as differences in cognitive behaviours pertaining to reward, spatial memory, and egocentric memory. While the current work presents no effect in adulthood, it is interesting to note that postnatal exposure (10 mg/kg, SC, PND 8-21) produced a decrease in novelty exploration for adolescent (PND 39) males, but not females (Rodriguez-Porcel et al., 2011). A decrease in exploration behaviours was also witnessed in social scenarios for adult (PND 120-124) male and female offspring, albeit in a mouse model exposing progeny *in utero* to higher doses (20 mg/kg, IP, GD 13-21) (Zahra et al., 2018). When cognition was assessed in a reward model, which provided reinforcement by cocaine, no differences were observed in adult male and female offspring exposed in the third trimester (5 mg/kg, SC) (Hsiao et al., 2005). Considering citalopram exposure outside of gestation, a decrease has been observed in spatial memory for resulting male and female progeny after perinatal exposure (5 or 10 mg/kg, SC, GD 6-PND 21) (Sprowles et al., 2017, Sprowles et al., 2016) and for resulting male adult progeny after postnatal exposure (5, 7.5 mg/kg, SC to dam/pup, PND 11-20) (Schaefer et al., 2013). Furthermore, studies considering egocentric learning have shown that perinatal exposure (10 mg/kg, SC to dam/pup, GD 6-PND 21) has no effect in adult males or females, yet postpartum exposure (5, 7.5 mg/kg, SC to pup, PND 11-20) causes a decrease in behaviours exhibiting egocentric learning in adult male offspring (Schaefer et al., 2013). In addition to differences in exposure periods or variations in cognitive tests, it is also interesting to note that the current study is the first to report on cognitive performance after citalopram is exposed during pregnancy using an oral route of administration. Furthermore, postnatal exposure was often accomplished by injecting the pup directly, rather than the mother as observed clinically. Consequently, the current study produces original results suggesting citalopram has no effects on visual recognition behaviours, however considering the previous literature, particular cognitive deficits, such as spatial memory and egocentric learning, cannot be ruled out after exposure during the perinatal period. Future preclinical studies using an oral route, as done in the current model, and observing these additional measures of

cognition would allow for conclusions to be drawn that are relevant to the clinical scenario.

5.4.3 Aim 3: Anhedonia assessment

The third aim of this study was to examine the effects of gestational SSRI exposure from GD 7-21 on anhedonia behaviours of adult offspring. Anhedonia behaviours of depression were evaluated by assessing the consumption of a pleasurable saccharin solution compared to ordinary drinking water in adult offspring at age PND 106-112. This was calculated as a percentage of saccharin solution consumed, compared to total solution consumption.

5.4.3.1 Fluoxetine

Exposure *in utero* had no effect on adult saccharin preference when compared to resulting vehicle progeny. Previous work has also reported similar findings on anhedonia, albeit with sucrose preference rather than saccharin preference models. Gestational exposure (12 mg/kg, IG, GD 11-21) had no effect on sucrose consumption in resulting adult males (Olivier et al., 2011). The absence of a treatment effect is also observed after perinatal (GD 0-PND 21) exposure assessed in adolescent (PND 35) and adult (PND 75) offspring, suggesting that perinatal exposure (5 mg/kg, OG) has no effects on sucrose preference, regardless of sex (Francis-Oliveira et al., 2013). These findings are also consistent with those reported in mice after perinatal exposure (8 mg/kg, DW to dam GD 10-PND 20), as no effect was found on sucrose consumption in resulting adult (PND 70-72) male offspring (Salari et al., 2016). Overall, the current findings combined with the previous findings would suggest that fluoxetine exposure during pregnancy does not produce anhedonia behaviours in the resulting offspring, regardless of sex, as witnessed in both the saccharin and sucrose preference models.

5.4.3.2 Paroxetine

In adulthood, gestational exposure did not produce anhedonia behaviours in resulting progeny, when compared to vehicle exposed offspring. Previous literature could not be identified which observes resulting anhedonia behaviours, either through saccharin or sucrose consumption tests. A broader search of the literature was completed for additional “depressive-like” or anhedonia behaviours, which revealed a study using the forced swim test and the tail suspension test. One study was recognised which

observed gestational (GD (-14)-16) exposure to PRX (30 mg/kg, oral) in mice. It was revealed that the subsequent adult (PND 90) male and female offspring showed no difference in the percentage of struggling in the forced swim test (Coleman et al., 1999). As depression behaviours are not reported in the aforementioned study, nor the current study, novel results are contributed suggesting that paroxetine exposure *in utero* does not produce typical “depression-like” symptoms or behaviours like anhedonia.

5.4.3.3 Sertraline

After gestational exposure, no treatment or sex effects were found in the percentage of saccharin consumption of the resulting adult offspring compared to vehicle groups. While relevant studies could not be found evaluating anhedonia, previous literature has reported on other features of depression, such as behavioural despair in the forced swim test. The study observed gestational (GD (-16)-littering) exposure to at higher doses (20 mg/kg, OG) in resulting male and female offspring, and reported no treatment effects on behavioural despair (Kott et al., 2019). The original findings produced here using the saccharin preference test, along with previous measures of behavioural despair, suggest that gestational sertraline exposure does not induce symptoms of depression, such as anhedonia, in the resulting adult offspring.

5.4.3.4 Citalopram

Gestational exposure has no effect on anhedonic behaviours in resulting progeny, regardless of sex or dose, when compared to vehicle exposed offspring. Previous literature has not been reported which observes “depressive-like” behaviours such as anhedonia with the saccharin preference test after gestational exposure. However, perinatal exposure has been assessed in regards to depressive behaviours, albeit in the forced swim behavioural despair model. Such studies have reported that perinatal (GD 6- PND 21) exposure to a similar dose (10 mg/kg, SC or drinking water) does increase depressive behaviours for the subsequent adult (PND 60-65), male and female offspring (Sprowles et al., 2016, Zohar et al., 2016). While these results are contrary to the current findings, the differences may be due to the study design as well as the behavioural test employed. It is interesting to note that social interaction tests have also been said to examine aspects of social anhedonia (Scheggi et al., 2018). Keeping this in mind, it is important to acknowledge that the previous study completed by Sprowles et al. (2016), also reported the exposure had no effect on sociability.

Consequently, while behavioural despair features of depression are suggested to increase after perinatal exposure, this is not consistent with findings related to social anhedonia or in the current study, which produces novel insights as anhedonia has not been previously assessed after gestational citalopram exposure in the saccharin preference test model.

5.4.4 Conclusion

To summarize the findings in this chapter, no SSRI, at the doses employed had a significant anxiogenic effect, although a transient ambulatory effect was identified. Further, no SSRI caused cognitive or anhedonic effects recognizable in adulthood. Overall trends were observed for differences in sex in regards to anxiety and ambulatory behaviours. Treatment-induced effects were observed by paroxetine, conversely causing males to increase movement between zones and females to decrease between zone entries. However, this effect was also age and dose-dependent, only occurring in adolescences, and affecting male behaviour after 1.25 mg/kg PRX exposure, and female behaviour after 2.5 mg/kg PRX exposure. Interestingly this ambulatory altering effect was only significant in the open field. In conclusion, no anxiogenic effects were noted, while ambulatory effects were specific to resulting adolescent pups exposed to paroxetine. Moreover, SSRIs had no effect on cognitive performance or depressive behaviours. Such results, applied to the clinical situation, are beneficial for anticipating emotional, ambulatory, or cognitive disturbance in adolescent and adult progeny, of women requiring the introduction of SSRI treatment during pregnancy.

6 Does gestational exposure to SSRIs in rats have an effect on anxiolytic and antidepressant behavioural responses in the resultant offspring?

6.1 Introduction

As explained previously, it is understood that depression during pregnancy is prevalent (Pearson et al., 2018), having harmful effects on both the mother and progeny (ACOG, 2018). Thus SSRIs are the most commonly prescribed psychotropic drugs during pregnancy, despite the unknown repercussion of altering serotonergic activity during embryogenesis (Alwan et al., 2016).

It is apparent that SSRIs interact with serotonin, which in humans has a high turnover rate from as early as gestational week 5 through to age 5 (Whitaker-Azmitia, 2001). In addition to assisting in development processes, in the CNS serotonin also helps modulate sleep, mood, cognition, and appetite (Portas et al., 2000, Carhart-Harris and Nutt, 2017, Anderberg et al., 2017). Furthermore, serotonin also assists in the development of other neurotransmitter systems (Whitaker-Azmitia, 2001). Therefore, the dysregulation of serotonin is a cornerstone of theories relating to various psychiatric disorders, and subsequently a prominent target in the pharmacological management of such disorders.

Current literature suggests that exposure to serotonergic altering drugs during gestational development may have enduring implications on the serotonergic system in the resulting offspring. As a consequence, this *in utero* exposure can alter the risk for the progeny to develop certain psychiatric disorders, such as anxiety and depression. However, it is possible that a unique acquisition of such disorders may alter the resulting offspring response to typically accepted pharmacological options for treating these psychiatric conditions. Furthermore, while serotonin dysregulation has a role in anxiety and depressive symptoms, other neurotransmitters are also dysregulated within such scenarios. Therefore, an inappropriate response to particular pharmacological compounds suggests that the targets of therapeutic action, such as GABAergic, noradrenergic or serotonergic neurotransmission, were altered as an enduring consequence of gestational SSRI exposure.

In addition to the clinical dilemmas of ethical research and well-controlled studies listed in previous chapters, this scenario adds additional complications. The current chapter studies are conducted in adulthood after development is complete, which would be impractical to observe in the clinical scenario. Impracticalities would include the time required for the longitudinal study, thus making the endpoints and possible

recommendations no longer of value. Another complication would be the necessary willingness for the resulting adults to participate in such a study if one were to occur. Provided these hurdles were overcome, results would still be difficult to interpret when taking into account the numerous variables incurred.

Animal models prove particularly useful in surpassing the aforementioned obstacles. As highlighted in previous chapters, it is important to reflect the clinical situation as much as possible when observing gestational exposure during pregnancy in a rodent model to make the research translatable to the clinical experience. Furthermore, to observe a psychopharmacological challenge it is important to employ frequently used and highly validated animal corollaries and pharmacological compounds.

By combining psychoactive drug treatments and techniques for measuring the consequential behaviours, it is possible to assess a subject's responsivity to particular therapeutic drug treatments. Therefore, this study examined the responses of adult rats, to the anxiolytic and antidepressant drugs, after *in utero* exposure to one of the four SSRI antidepressants highlighted throughout this work. These aims were assessed later in adulthood after development was completed, and when such behaviours are often reported. Diazepam (DZP) was used to examine anxiolytic efficacy in the elevated plus maze, whilst, desipramine (DMI) was used to evaluate antidepressant efficacy in the forced swim test (FST). Furthermore, the pharmacological challenge after *in utero* exposure to SSRIs was observed in male and female progeny, to ascertain whether there were any sex-specific effects of gestational exposure or in response to the psychopharmacological compounds.

Employing these drug-paired behavioural measurements, presents a novel investigation of drug efficacy in adulthood, after exposure to serotonergic-altering drugs during embryogenesis. These evaluations of afflicted rat offspring, assist physicians in selecting appropriate psychiatric treatments for disorders often associated with serotonergic dysfunction, in cases where adults were exposed to particular SSRIs during gestation.

6.1.1 Hypothesis and aims

It is specifically hypothesized in this chapter that *in utero* exposure to fluoxetine, paroxetine, sertraline, or citalopram at clinically relevant doses that are not

significantly toxic to maternal wellbeing will have enduring consequences in the adult progeny's response to the anxiolytic and antidepressant drugs.

This hypothesis was measured through two specific aims throughout this chapter, ultimately investigating the effects of gestational SSRI exposure from GD 7-21 on the behavioural response of resulting adult offspring to (1) the anxiolytic efficacy of diazepam and (2) the antidepressant efficacy of desipramine.

6.2 Experimental methods and design

A detailed description of gestational exposure, behavioural tasks and drug administration can be found in Chapter 2.

Briefly, Sprague-Dawley pups were born on PND 1, after being exposed via the dam *in utero* to either vehicle, FLX (2.5 mg/kg), PRX (1.25, 2.5, or 5 mg/kg), SERT or CIT (2.5, 5 or 10 mg/kg) via oral gavage from GD 7 until littering (n=9-13 litters per group). Testing occurred with all four SSRIs under investigation simultaneously, however, research was collected over three cohort studies in order to successfully manage a study of this size.

When possible, the same male and female litter representatives were used to carry out behavioural tasks. Only one male and female pup was used per litter to avoid litter effects within offspring sets. Offspring sets were used throughout the entire work to space out the behavioural tasks to minimize a residual test effect. For this specific chapter, both offspring sets 4 and 5 were used for all tests. When possible, the same litters were represented in offspring sets 4 and 5. Each set was only exposed to either DZP and saline or saline and DMI. The tests occurred within a certain age in adulthood.

The behavioural response to anxiolytic and antidepressant drugs was assessed. “Anxiety-like” behaviour was assessed in the EPM at PND 95-97. After an appropriate wash-out period, “depressive-like” behaviours were evaluated in the FST over two consecutive days commencing on PND 102-104. Treatments of offspring set 4 included SAL+EPM and DMI+FST. Inversely, offspring set 5 underwent DZP+EPM and SAL+FST. The time-points/age used for this psychopharmacological challenge study were selected because adulthood is the period in which the onset of anxiety and depressive symptoms would be expected and subsequently anxiolytic and antidepressant treatment would be required.

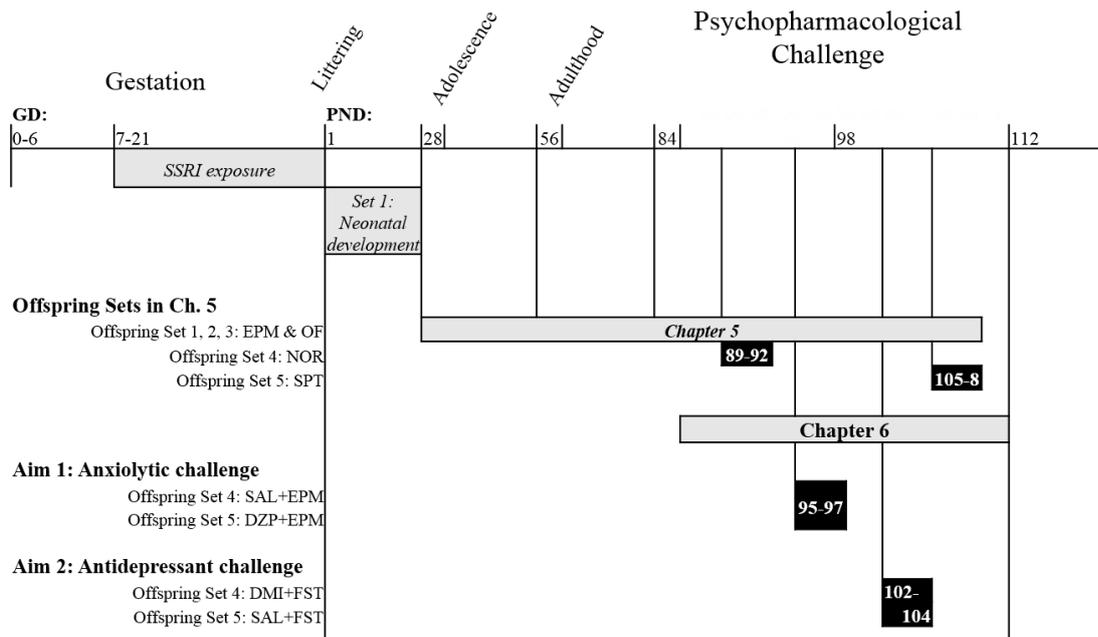


Figure 6.1 Psychopharmacological challenge experimental timeline.

Statistical analysis was performed using the Statistical Package for Social Sciences 24.0 for Windows (SPSS Inc., IBM, New York, USA) on the parameters listed in Table 6.1 Table 5.1. Primarily, normality and homogeneity of variance ($p < 0.05$) were determined to choose the appropriate analysis. As many cases failed to meet parametric criteria the Scheirer-Ray-Hare extension of the Kruskal Wallis test was used to analyse all parameters presented in this chapter by SSRI, DZP/DMI, and SSRI x DZP/DMI. This test is analogous to a two-way ANOVA and it was used so that all SSRI data sets could be analysed using the same statistical approach. When applicable ($p < 0.05$), a Mann Whitney U *post hoc* was used with appropriate Bonferroni correction. In the case of fluoxetine, when making three comparisons (two doses and DZP/DMI) $*p < 0.025$ was used. In the cases of paroxetine, sertraline, and citalopram, when making five comparisons (four doses and DZP/DMI) $*p < 0.013$ was used.

Parameters	Shapiro-Wilk & Levene's test $p < 0.05$
EPM, FST	Scheirer-Ray-Hare extension of Kruskal Wallis Two-way ANOVA by Rank → Mann Whitney U with Bonferroni correction

Table 6.1 Statistical analysis of psychopharmacological challenge. In the case of fluoxetine, Bonferroni correction employed to correct for multiple comparisons was $*p < 0.025$. For all other SSRIs, Bonferroni correction employed to correct for multiple comparisons was $*p < 0.013$.

6.3 Pharmacological challenge results

6.3.1 Pharmacological assessment of diazepam in the elevated plus maze

6.3.1.1 Fluoxetine exposed progeny in the EPM with SAL or DZP

Male EPM analysis revealed no effect of SSRI, DZP, or SSRI x DZP interaction when compared to the vehicle or saline groups. See Table 6.2 for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male Saline</i>						
Vehicle	1910 (1768-2034)	6 (4-7)	61 (43-81)	26 (24-39)	33 (27-49)	
2.5 mg/kg FLX	1608 (1250-1940)	5 (2-6)	57 (30-64)	25 (10-38)	35 (19-42)	
<i>Male DZP</i>						
Vehicle	1058 (526-1403)	6 (2-9)	136 (31-192)	54 (32-74)	66 (23-83)	
2.5 mg/kg FLX	983 (342-1987)	3 (2-12)	76 (33-155)	57 (29-65)	73 (44-76)	
SSRI	H	0.10	0.20	0.25	0.07	0.00
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
DZP	H	3.71	0.05	0.68	2.15	1.40
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
SSRI x DZP	H	0.47	0.16	0.00	0.00	0.01
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1

Table 6.2 Fluoxetine males in the EPM with SAL or DZP. Offspring exposed to either vehicle or FLX (2.5 mg/kg) via OG to dam from GD 7-21 and then exposed to SAL or DZP (1 mg/kg, SC) 30 mins prior to the EPM at PND 95-97. No SSRI or DZP effect noted. Data are expressed as median and interquartile range, n=12/group. See table for statistical analysis.

Female EPM analysis revealed only a DZP effect, with DZP increasing OAE (%) when compared to the Vehicle+SAL group. See Table 6.3 for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Female Saline</i>						
Vehicle	2067 (1978-2259)	7 (5-8)	65 (53-81)	28 (23-36)	35 (29-44)	
2.5 mg/kg FLX	2109 (1801-2425)	6 (4-9)	68 (42-79)	29 (20-36)	36 (25-47)	
<i>Female DZP</i>						
Vehicle	1298 (1105-2148)	9 (6-13)	139 (86-185)	54 (50-68) #	72 (51-89)	
2.5 mg/kg FLX	1805 (1338-2093)	9 (6-12)	109 (55-136)	45 (35-61)	56 (34-75)	
SSRI	H	0.08	0.02	0.01	0.21	0.01
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1
DZP	H	2.55	0.77	2.06	5.26	2.45
	p	>0.05	>0.05	>0.05	<0.05	>0.05
	df	1	1	1	1	1
SSRI x DZP	H	0.12	0.10	0.00	0.20	0.03
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	1	1	1	1	1

Table 6.3 Fluoxetine females in the EPM with SAL or DZP. Offspring exposed to either vehicle or FLX (2.5 mg/kg) via OG to dam from GD 7-21 and then exposed to SAL or DZP (1 mg/kg, SC) 30 mins prior to the EPM at PND 95-97. A DZP effect was found for OAE (%), with the Vehicle+DZP group having a higher OAE (%) compared to Vehicle+SAL group. Data are expressed as median and interquartile range, n=9-12/group. When comparing to Vehicle+SAL, # $p < 0.05$, with appropriate correction due to multiple comparisons. See table for statistical analysis.

6.3.1.2 Paroxetine exposed progeny in the EPM with SAL or DZP

Male EPM analysis revealed only a DZP effect, for distance travelled, OAE (counts and %) and OAT (s and %); *post hoc* analysis indicated DZP significantly decreased distance travelled when compared to Vehicle+SAL group. See Table 6.4 for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male Saline</i>						
Vehicle	1971 (1768-2215)	8 (4-9)	76 (33-97)	39 (26-41)	44 (21-51)	
1.25 mg/kg PRX	1873 (1582-2101)	7 (4-8)	68 (42-88)	31 (19-38)	34 (27-46)	
2.5 mg/kg PRX	1937 (1729-1967)	6 (5-7)	62 (40-81)	36 (23-43)	36 (26-49)	
5 mg/kg PRX	1836 (1571-2001)	4 (3-6)	57 (32-80)	24 (16-35)	32 (22-40)	
<i>Male DZP</i>						
Vehicle	891 (424-1308) *	3 (1-8)	64 (6-166)	33 (10-61)	34 (3-75)	
1.25 mg/kg PRX	1093 (1582-2101)	5 (2-7)	101 (70-146)	42 (26-46)	66 (47-71)	
2.5 mg/kg PRX	1078 (930-1916)	6 (3-9)	123 (65-141)	47 (43-56)	65 (40-73)	
5 mg/kg PRX	915 (587-1487)	3 (1-7)	103 (31-176)	49 (11-56)	57 (11-74)	
SSRI	H	0.03	0.68	0.20	0.71	0.33
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
DZP	H	34.15	4.05	4.61	7.67	5.81
	p	<0.05	<0.05	<0.05	<0.05	<0.05
	df	1	1	1	1	1
SSRI x DZP	H	0.12	0.10	0.00	0.20	0.03
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 6.4 Paroxetine males in the EPM with SAL or DZP. Offspring exposed to either vehicle, PRX (1.25, 2.5, or 5 mg/kg) via OG to dam from GD 7-21 and then exposed to SAL or DZP (1 mg/kg, SC) 30 mins prior to the EPM at PND 95-97. An overall DZP effect was noted for all parameters; DZP significantly decreased distance travelled for the Vehicle+DZP group compared to the Vehicle+SAL group. Data are expressed as median and interquartile range, n=8-12/group. When comparing to Vehicle+SAL, * $p < 0.05$, with appropriate correction due to multiple comparisons. See table for statistical analysis.

Female EPM analysis revealed a DZP effect for OAE (counts and %) and OAT (s and %); with DZP increasing OAE (%) and OAT (s and %) when compared to the Vehicle+SAL group. See Table 6.5 for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Female Saline</i>						
Vehicle	2036 (1928-2167)	7 (5-9)	66 (54-101)	28 (26-35)	37 (26-47)	
1.25 mg/kg PRX	2029 (1770-2288)	7 (4-10)	57 (47-80)	30 (18-35)	36 (28-42)	
2.5 mg/kg PRX	2094 (1942-2346)	5 (4-9)	63 (45-88)	26 (15-40)	34 (26-46)	
5 mg/kg PRX	1938 (1747-2158)	5 (3-11)	64 (50-88)	26 (17-40)	34 (28-44)	
<i>Female DZP</i>						
Vehicle	2214 (1790-2616)	12 (9-14)	109 (93-69) #	50 (50-56) #	67 (51-72) #	
1.25 mg/kg PRX	2061 (1338-2441)	11 (6-14)	135 (74-174)	52 (38-63)	69 (57-80)	
2.5 mg/kg PRX	1700 (1942-2346)	8 (5-12)	111 (78-136)	44 (33-59)	57 (51-71)	
5 mg/kg PRX	1954 (1684-2657)	11 (8-14)	139(72-165)	50 (40-62)	67 (45-79)	
SSRI	H	0.50	0.63	0.23	0.19	0.06
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
DZP	H	0.04	11.44	25.97	34.81	34.95
	p	>0.05	<0.05	<0.05	<0.05	<0.05
	df	1	1	1	1	1
SSRI x DZP	H	1.32	0.31	0.08	0.09	0.07
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 6.5 Paroxetine females in the EPM with SAL or DZP. Offspring exposed to either vehicle, PRX (1.25, 2.5, or 5 mg/kg) via OG to the dam from GD 7-21 and then exposed to SAL or DZP (1 mg/kg, SC) 30 mins prior to the EPM at PND 95-97. An overall DZP effect was noted for OAE (counts and %) and OAT (s and %); significant differences were noted with the Vehicle+DZP group exhibiting more OAE (%) and OAT (s and %) behaviours compared to the Vehicle+SAL group. Data are expressed as median and interquartile range, n=9-12/group. When comparing to Vehicle+SAL, # $p < 0.05$, with appropriate correction due to multiple comparisons. See table for statistical analysis.

6.3.1.3 Sertraline exposed progeny in the EPM with SAL or DZP

Male EPM analysis revealed only a DZP effect for distance travelled, OAE (%), and OAT (s and %); a *post hoc* analysis indicated that DZP decreased distance travelled when compared to Vehicle+SAL group. See Table 6.6 for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male Saline</i>						
Vehicle	1946 (1518-2213)	7 (3-9)	81 (56-97)	40 (15-42)	48 (39-51)	
2.5 mg/kg SERT	1636 (1560-1968)	5 (2-6)	49 (9-64)	24 (10-35)	27 (5-39)	
5 mg/kg SERT	1543 (1291-1646)	3 (2-5)	25 (18-53)	20 (11-29)	18 (10-32)	
10 mg/kg SERT	1647 (1529-1740)	4 (3-5)	63 (43-77)	26 (16-29)	36 (32-46)	
<i>Male DZP</i>						
Vehicle	891 (315-1190) *	3 (1-7)	64 (6-154)	33 (10-58)	34 (3-64)	
2.5 mg/kg SERT	1324 (594-1789)	4 (2-12)	110 (42-140)	40 (40-52)	59 (54-71)	
5 mg/kg SERT	1068 (376-1423)	6 (1-8)	127 (17-164)	53 (10-65)	70 (11-78)	
10 mg/kg SERT	1421 (577-1776)	5 (2-10)	154 (66-200)	59 (39-85)	80 (52-92)	
SSRI	H	0.84	0.30	0.45	0.21	0.57
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
DZP	H	18.64	0.09	7.69	12.29	11.91
	p	<0.05	>0.05	<0.05	<0.05	<0.05
	df	1	1	1	1	1
SSRI x DZP	H	1.23	2.40	2.62	2.00	3.09
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 6.6 Sertraline males in the EPM with SAL or DZP. Offspring exposed to either vehicle, SERT (2.5, 5, or 10 mg/kg) via OG to dam from GD 7-21 and then exposed to SAL or DZP (1 mg/kg SC) 30 mins prior to the EPM at PND 95-97. A general DZP effect was found for distance travelled, OAE (%), and OAT (s and %); with the Vehicle+DZP group having a shorter distance travelled compared to Vehicle+SAL group. Data are expressed as median and interquartile range, n=8-12/group. When comparing to Vehicle+SAL, * $p < 0.05$, with appropriate correction due to multiple comparisons. See table for statistical analysis.

Female EPM analysis revealed a DZP effect for OAE (counts and %) and OAT (s and %); with DZP increasing OAE (%) and OAT (s and %) when compared to Vehicle+SAL group. See Table 6.7 for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Female Saline</i>						
Vehicle	2101 (1827-2241)	6 (4-10)	71 (57-102)	28 (17-36)	39 (34-47)	
2.5 mg/kg SERT	1996 (1683-2475)	7 (4-9)	61 (39-87)	29 (19-32)	32 (24-44)	
5 mg/kg SERT	2112 (1814-2207)	6 (5-11)	90 (50-107)	35 (25-44)	45 (32-57)	
10 mg/kg SERT	2136 (1905-2888)	6 (3-9)	61 (43-77)	28 (17-33)	34 (26-40)	
<i>Female DZP</i>						
Vehicle	2168 (1268-2424)	11 (7-14)	121 (94-163) #	50 (48-57) #	66 (52-75) #	
2.5 mg/kg SERT	2127 (2099-2266)	12 (7-12)	91 (74-107)	43 (32-60)	47 (40-56)	
5 mg/kg SERT	2139 (1811-2268)	10 (6-12)	107 (78-142)	45 (36-56)	59 (46-96)	
10 mg/kg SERT	2206 (1630-2462)	10 (6-12)	120 (102-145)	48 (44-56)	67 (57-72)	
SSRI	H	0.13	0.15	1.11	0.53	1.49
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
DZP	H	0.00	9.47	19.03	27.43	22.62
	p	>0.05	<0.05	<0.05	<0.05	<0.05
	df	1	1	1	1	1
SSRI x DZP	H	0.02	0.59	1.32	0.71	1.43
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 6.7 Sertraline females in the EPM with SAL or DZP. Offspring exposed to either vehicle, SERT (2.5, 5, or 10 mg/kg) via OG to the dam from GD 7-21 and then exposed to SAL or DZP (1 mg/kg SC) 30 mins prior to the EPM at PND 95-97. A general DZP effect was found for OAE (counts and %) and OAT (s and %); DZP significantly increased OAE (%) and OAT (s and %) for the Vehicle+DZP group compared to the Vehicle+SAL group. Data are expressed as median and interquartile range, n=8-12/group. When comparing to Vehicle+SAL, # $p < 0.05$, with appropriate correction due to multiple comparisons. See table for statistical analysis.

6.3.1.4 Citalopram exposed progeny in the EPM with SAL or DZP

Male EPM analysis revealed only a DZP effect for distance travelled, OAE (%), and OAT (s and %); a *post hoc* analysis indicated that DZP reduced distance travelled for Vehicle+DZP groups compared to Vehicle+SAL groups. See Table 6.8 for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Male Saline</i>						
Vehicle	1995 (1857-2125)	6 (5-8)	66 (49-81)	29 (24-40)	37 (28-48)	
2.5 mg/kg CIT	1760 (1581-1870)	3 (1-4)	45 (7-63)	13 (3-26)	26 (4-40)	
5 mg/kg CIT	1712 (1507-1825)	3 (2-7)	39 (15-68)	21 (8-31)	26 (9-27)	
10 mg/kg CIT	1643 (1530-1949)	6 (3-8)	69 (23-87)	38 (28-48)	29 (17-48)	
<i>Male DZP</i>						
Vehicle	1052 (483-1842) *	5 (1-9)	147 (21-181)	56 (21-69)	75 (15-81)	
2.5 mg/kg CIT	488 (392-1521)	2 (1-6)	126 (27-256)	40 (33-50)	65 (50-88)	
5 mg/kg CIT	762 (520-1064)	5 (3-6)	106 (78-167)	57 (50-71)	42 (32-84)	
10 mg/kg CIT	998 (821-1870)	5 (5-10)	107 (74-149)	51 (42-64)	53 (34-78)	
SSRI	H	1.36	2.83	0.27	0.67	0.09
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
DZP	H	30.65	0.00	12.78	22.35	13.57
	p	<0.05	>0.05	<0.05	<0.05	<0.05
	df	1	1	1	1	1
SSRI x DZP	H	0.56	0.98	0.74	1.10	1.03
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 6.8 Citalopram males in the EPM with SAL or DZP. Offspring exposed to either vehicle, CIT (2.5, 5, or 10 mg/kg) via OG to the dam from GD 7-21 and then exposed to SAL or DZP (1 mg/kg, SC) 30 mins prior to the EPM at PND 95-97. An overall DZP effect was noted for distance travelled, OAE (%), and OAT (s and %); DZP significantly reduced distance travelled when compared to the Vehicle+SAL group. Data are expressed as median and interquartile range, n=8-12/group. When comparing to Vehicle+SAL, * $p < 0.05$, with appropriate correction due to multiple comparisons. See table for statistical analysis.

Female EPM analysis revealed a DZP effect for OAE (counts and %) and OAT (s and %); DZP significantly increased OAE (%) and OAT (%) when compared to Vehicle+SAL group. See Table 6.9 for statistical analysis.

Group	Distance travelled (cm)	OAE (counts)	OAT (s)	OAE (%)	OAT (%)	
<i>Female Saline</i>						
Vehicle	2067 (1927-2259)	7 (5-8)	69 (55-82)	31 (23-37)	40 (31-44)	
2.5 mg/kg CIT	1926 (1680-2225)	4 (3-7)	50 (34-80)	18 (15-33)	34 (18-45)	
5 mg/kg CIT	2145 (2061-2263)	7 (4-10)	62 (49-81)	25 (21-42)	38 (28-45)	
10 mg/kg CIT	1876 (1774-2083)	6 (5-8)	72 (43-100)	29 (23-44)	39 (35-52)	
<i>Female DZP</i>						
Vehicle	1891 (1110-2353)	9 (7-14)	124 (97-143)	53 (50-59) #	70 (61-79) #	
2.5 mg/kg CIT	1873 (1501-2151)	11 (5-13)	129 (77-159)	54 (44-62)	75 (61-80)	
5 mg/kg CIT	1942 (1497-2162)	10(8-12)	127 (107-151)	55 (50-61)	70 (57-74)	
10 mg/kg CIT	1814 (1446-2361)	11 (7-15)	138 (95-149)	61 (50-64)	73 (62-77)	
SSRI	H	0.53	1.26	0.90	0.89	0.40
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3
DZP	H	2.98	14.74	29.93	50.33	34.88
	p	>0.05	<0.05	<0.05	<0.05	<0.05
	df	1	1	1	1	1
SSRI x DZP	H	0.69	0.78	0.26	0.04	0.23
	p	>0.05	>0.05	>0.05	>0.05	>0.05
	df	3	3	3	3	3

Table 6.9 Citalopram females in the EPM with SAL or DZP. Offspring exposed to either vehicle, CIT (2.5, 5, or 10 mg/kg) via OG to the dam from GD 7-21 and then exposed to SAL or DZP (1 mg/kg, SC) 30 mins prior to the EPM at PND 95-97. An overall DZP effect was found for OAE (counts and %) and OAT (s and %); DZP significantly increased OAE and OAT (%), with the Vehicle+DZP group having more of these behaviours compared to Vehicle+SAL group. Data are expressed as median and interquartile range, n=9-12/group. When comparing to Vehicle+SAL, # $p < 0.05$, with appropriate correction due to multiple comparisons. See table for statistical analysis.

6.3.2 Pharmacological assessment of desipramine in the forced swim test

6.3.2.1 Fluoxetine exposed progeny in the FST with SAL or DMI

Male FST analysis revealed no effect of SSRI, DMI, or SSRI x DMI interaction when compared to vehicle groups for highly active duration [SSRI: $H_{(1)}=0.63$, $p>0.05$; DMI: $H_{(1)}=0.71$, $p>0.05$; SSRI x DMI: $H_{(1)}=0.18$, $p>0.05$] or moderately active duration [SSRI: $H_{(1)}=0.06$, $p>0.05$; DMI: $H_{(1)}=2.95$, $p>0.05$; SSRI x DMI: $H_{(1)}=0.00$, $p>0.05$]. For active duration, no effect was found for SSRI or SSRI x DMI interaction [SSRI: $H_{(1)}=0.13$, $p>0.05$; SSRI x DMI: $H_{(1)}=0.18$, $p>0.05$] but an effect of DMI was found [$H_{(1)}=4.90$, $p<0.05$]; *post hoc* analysis revealed that VEH+DMI increased active duration compared to VEH+SAL. For inactive duration, no effect was found for SSRI or SSRI x DMI interaction [SSRI: $H_{(1)}=0.19$, $p>0.05$; SSRI x DMI: $H_{(1)}=0.40$, $p>0.05$] but an effect of DMI was found [$H_{(1)}=5.40$, $p<0.05$]; *post hoc* analysis revealed VEH+DMI decreased inactive duration compared to VEH+SAL (Figure 6.2).

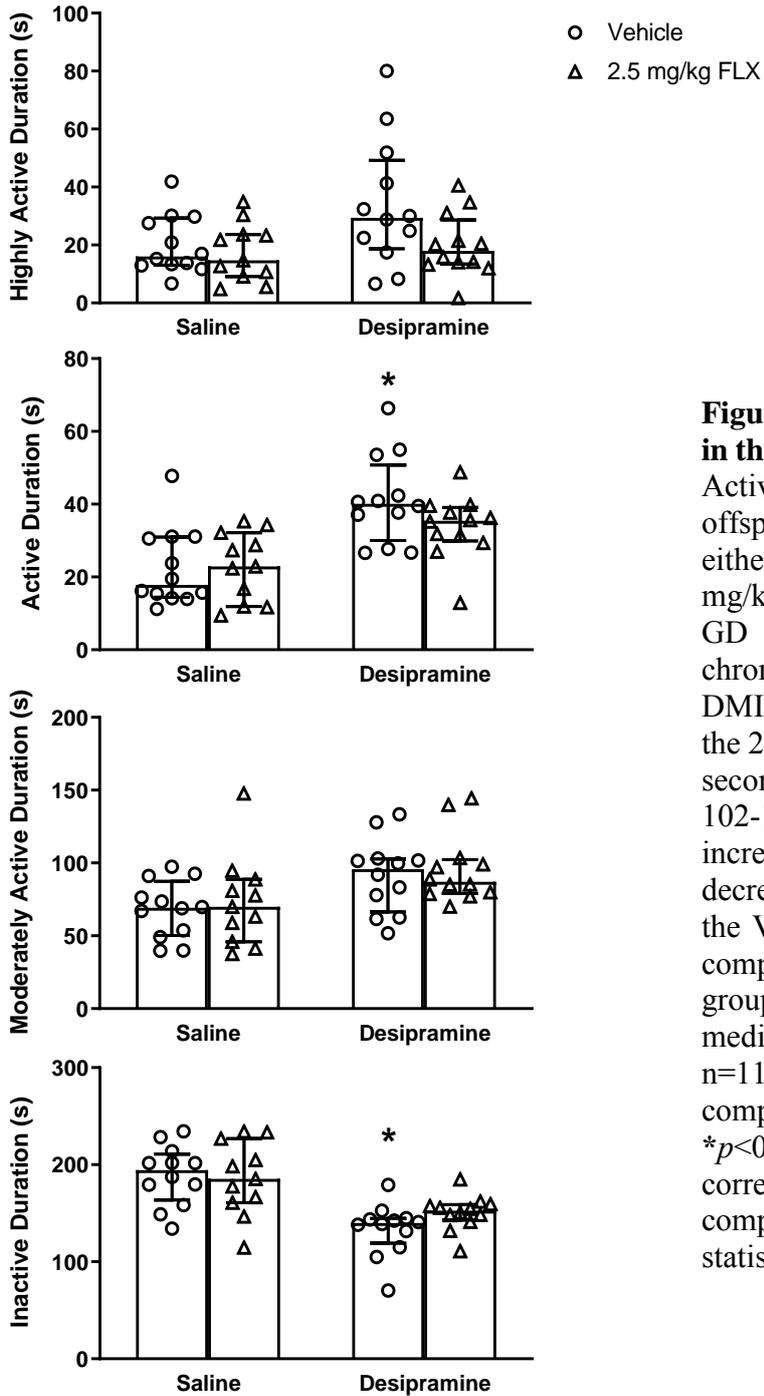


Figure 6.2 Fluoxetine males in the FST with SAL or DMI.

Activity thresholds for male offspring after exposure to either vehicle or FLX (2.5 mg/kg) via OG to dam from GD 7-21 and then sub-chronically exposed to SAL or DMI (10 mg/kg, SC) 3 times in the 24 hrs between the first and second FST exposure at PND 102-104. DMI significantly increased active duration and decreased inactive duration of the Vehicle+DMI group when comparing to the Vehicle+SAL group. Data are expressed as median and interquartile range, n=11-12/group.

When comparing to Vehicle+SAL, $*p < 0.05$, with appropriate correction due to multiple comparisons. See text for statistical analysis.

Female FST analysis revealed no effect of SSRI, DMI, or SSRI x DMI interaction when compared to vehicle groups for highly active duration [SSRI: $H_{(1)}=0.13, p>0.05$; DMI: $H_{(1)}=0.00, p>0.05$; SSRI x DMI: $H_{(1)}=0.53, p>0.05$], active duration [SSRI: $H_{(1)}=0.25, p>0.05$; DMI: $H_{(1)}=0.75, p>0.05$; SSRI x DMI: $H_{(1)}=0.54, p>0.05$], moderately active duration [SSRI: $H_{(1)}=0.11, p>0.05$; DMI: $H_{(1)}=0.01, p>0.05$; SSRI x DMI: $H_{(1)}=0.06, p>0.05$], or inactive duration [SSRI: $H_{(1)}=0.04, p>0.05$; DMI: $H_{(1)}=0.57, p>0.05$; SSRI x DMI: $H_{(1)}=0.02, p>0.05$] (Figure 6.3).

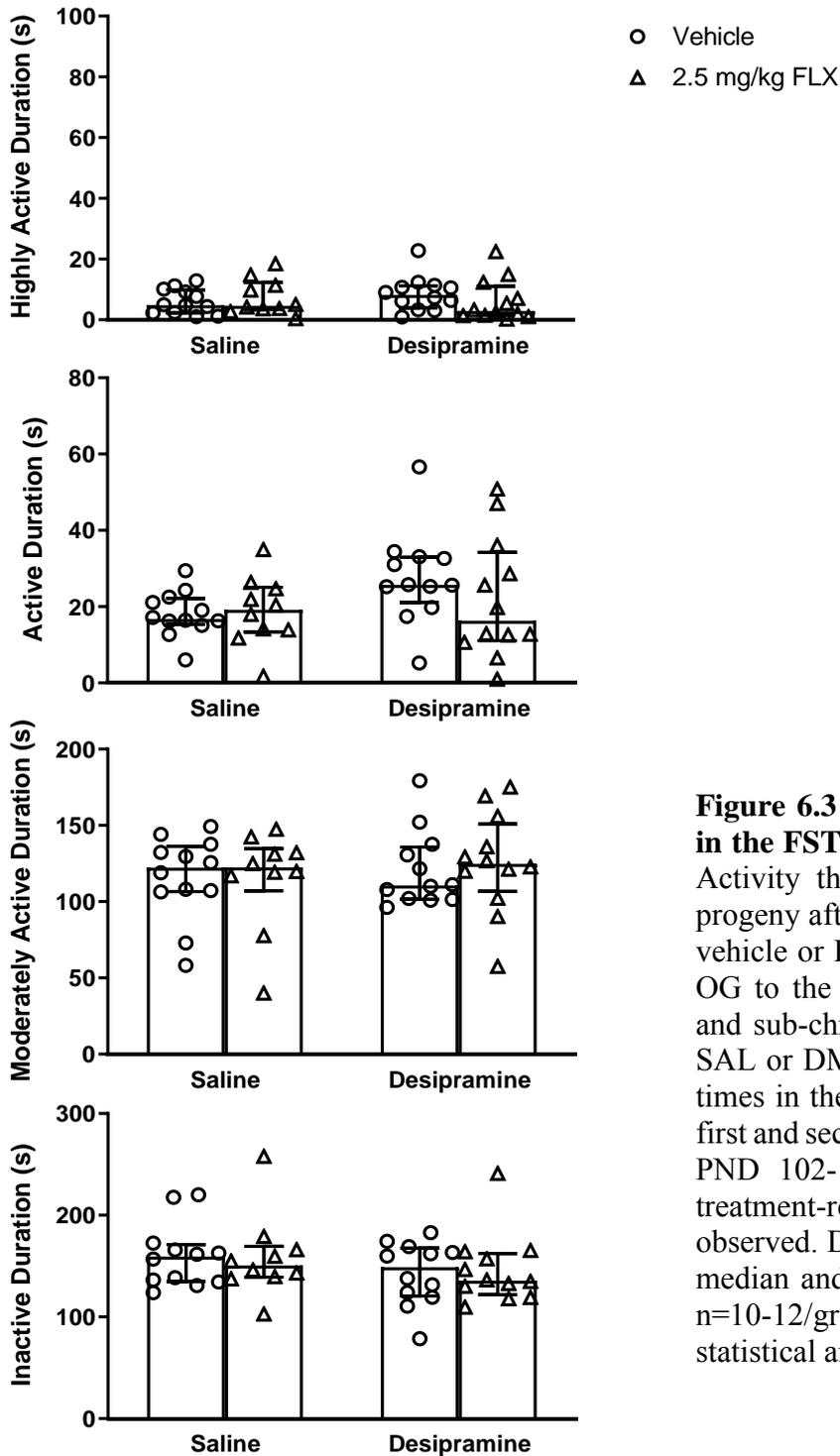


Figure 6.3 Fluoxetine females in the FST with SAL or DMI. Activity thresholds for female progeny after exposure to either vehicle or FLX (2.5 mg/kg) via OG to the dam from GD 7-21 and sub-chronically exposed to SAL or DMI (10 mg/kg, SC) 3 times in the 24 hrs between the first and second FST exposure at PND 102-104. No significant treatment-related effects were observed. Data are expressed as median and interquartile range, n=10-12/group. See text for statistical analysis.

6.3.2.2 Paroxetine exposed progeny in the FST with SAL or DMI

For males, in regards to highly active duration, an effect of DMI was found [DMI: $H_{(1)}=2.95$, $p<0.05$], while no effect of SSRI or SSRI x DMI interaction were found [SSRI: $H_{(3)}=1.27$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.08$, $p>0.05$]; however, *post hoc* analysis of DMI indicated no significant difference when comparing DMI and SAL vehicle groups. For active duration an effect of DMI was found [DMI: $H_{(1)}=17.53$, $p<0.05$, while no effects of SSRI or SSRI x sex interaction were found [SSRI: $H_{(3)}=0.94$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.09$, $p>0.05$]; *post hoc* analysis indicated that Vehicle+DMI increased active duration compared to Vehicle+SAL groups. In regards to moderately active duration, no effect was found for SSRI, DMI, or SSRI x DMI interaction when compared to vehicle groups [SSRI: $H_{(3)}=1.29$, $p>0.05$; DMI: $H_{(1)}=1.21$, $p>0.05$; SSRI x DMI: $H_{(3)}=1.20$, $p>0.05$]. An effect of DMI was found for inactive duration [DMI: $H_{(1)}=10.74$, $p<0.05$], while no effect of SSRI or SSRI x DMI interaction were found [SSRI: $H_{(3)}=0.92$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.58$, $p>0.05$]. A *post hoc* analysis of DMI indicated a significant reduction in inactivity duration for Vehicle+DMI groups when compared to Vehicle+SAL groups. (Figure 6.4).

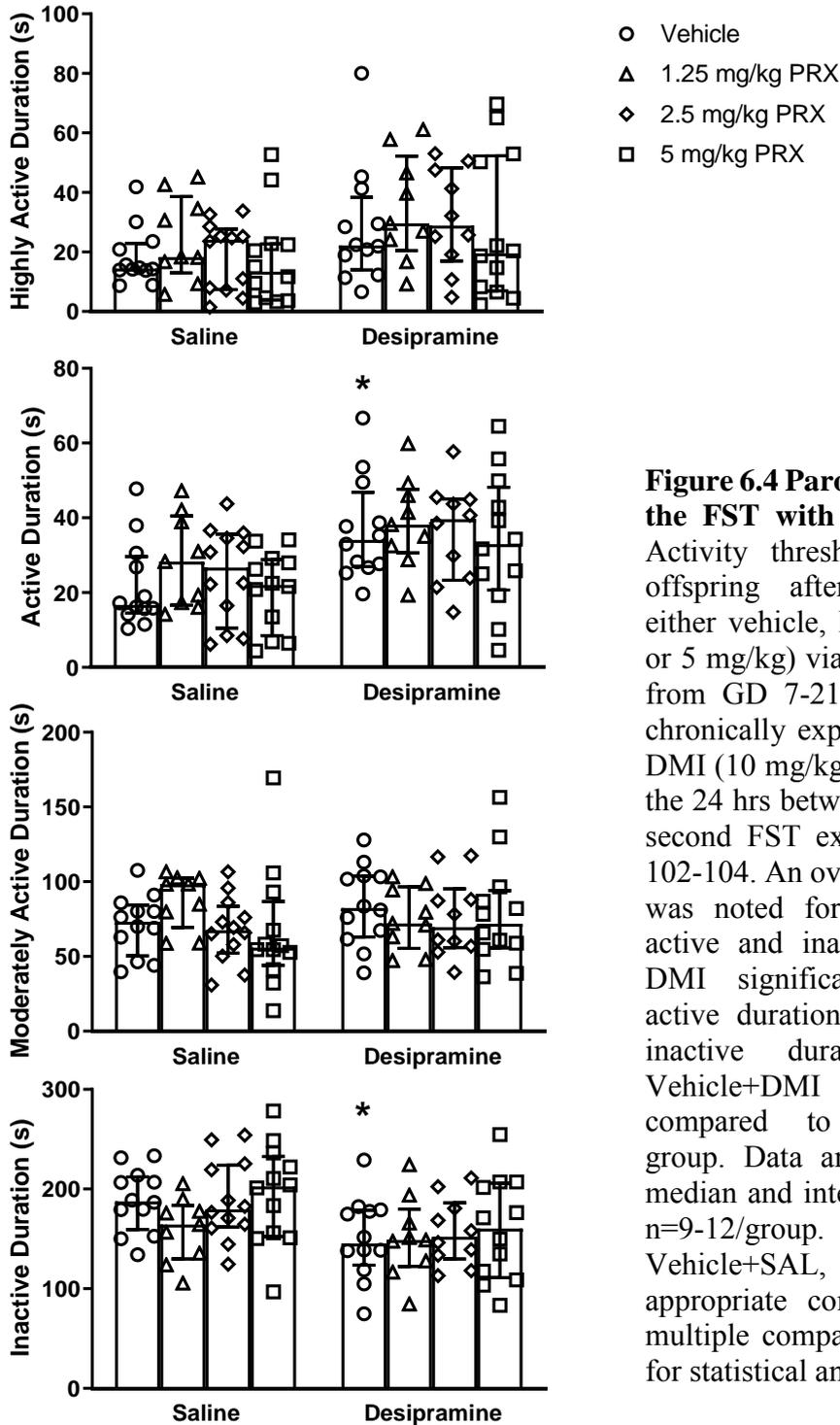


Figure 6.4 Paroxetine males in the FST with SAL or DMI.

Activity thresholds for male offspring after exposure to either vehicle, PRX (1.25, 2.5, or 5 mg/kg) via OG to the dam from GD 7-21 and then sub-chronically exposed to SAL or DMI (10 mg/kg, SC) 3 times in the 24 hrs between the first and second FST exposure at PND 102-104. An overall DMI effect was noted for highly active, active and inactive durations. DMI significantly increased active duration and decreased inactive duration of the Vehicle+DMI group when compared to Vehicle+SAL group. Data are expressed as median and interquartile range, n=9-12/group. To compare to Vehicle+SAL, * $p < 0.05$, with appropriate correction due to multiple comparisons. See text for statistical analysis.

Female FST analysis revealed no effect of SSRI, DMI, or SSRI x DMI interaction when comparing Vehicle+DMI groups to Vehicle+SAL groups for highly active duration [SSRI: $H_{(3)}=1.85$, $p>0.05$; DMI: $H_{(1)}=0.73$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.52$, $p>0.05$], moderately active duration [SSRI: $H_{(3)}=1.81$, $p>0.05$; DMI: $H_{(1)}=0.49$, $p>0.05$; SSRI x DMI: $H_{(3)}=1.15$, $p>0.05$], or inactive duration [SSRI: $H_{(3)}=1.38$, $p>0.05$; DMI: $H_{(1)}=2.40$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.89$, $p>0.05$]. A DMI effect was revealed for active duration [DMI: $H_{(1)}=6.31$, $p<0.05$], however a *post hoc* analysis of DMI revealed no significant effect when comparing Vehicle+DMI to Vehicle+SAL groups. Further, no effect of SSRI or SSRI x DMI were found for active duration [SSRI: $H_{(3)}=0.79$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.53$, $p>0.05$] (Figure 6.5).

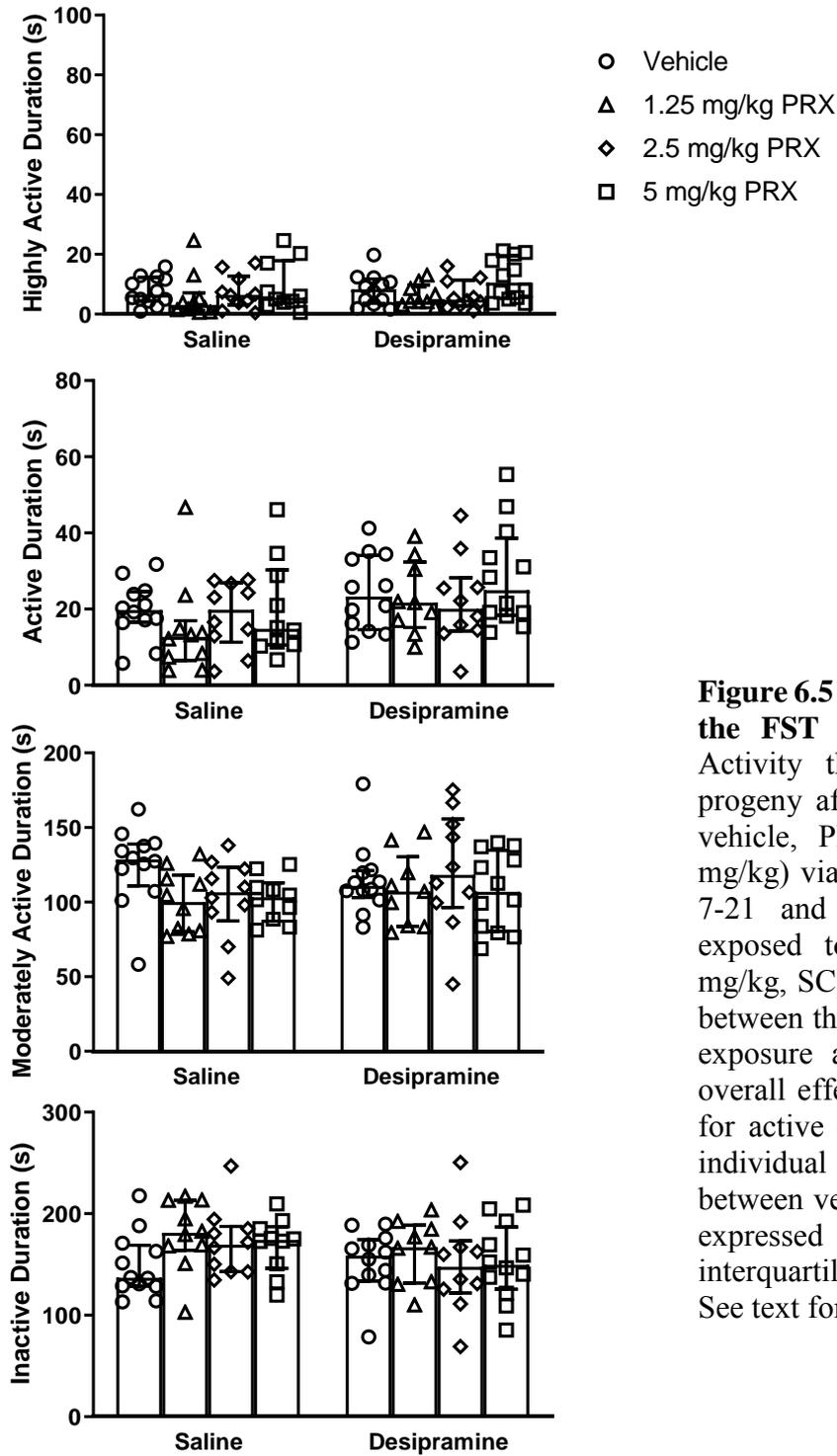


Figure 6.5 Paroxetine females in the FST with SAL or DMI. Activity thresholds for female progeny after exposure to either vehicle, PRX (1.25, 2.5, or 5 mg/kg) via OG to dam from GD 7-21 and then sub-chronically exposed to SAL or DMI (10 mg/kg, SC) 3 times in the 24 hrs between the first and second FST exposure at PND 102-104. An overall effect of DMI was found for active duration, however, no individual effects were noted between vehicle groups. Data are expressed as median and interquartile range, n=9-12/group. See text for statistical analysis.

6.3.2.3 Sertraline exposed progeny in the FST with SAL or DMI

Male FST analysis revealed no effect of SSRI, sex, or SSRI x DMI interaction when compared to vehicle groups for highly active duration [SSRI: $H_{(3)}=0.84$, $p>0.05$; DMI: $H_{(1)}=2.96$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.40$, $p>0.05$]. In regards to active duration, DMI had a significant effect [$H_{(1)}=17.05$, $p<0.05$], while SSRI and SSRI x DMI interaction did not have an effect [SSRI: $H_{(3)}=0.62$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.02$, $p>0.05$]. Further, *post hoc* analysis for DMI indicated an increase in active duration for Vehicle+DMI rats compared to Vehicle+SAL rats. An overall effect of DMI was found for moderately active duration [$H_{(1)}=8.96$, $p<0.05$], while no significant effects were noted for SSRI or SSRI x DMI interaction [SSRI: $H_{(3)}=1.42$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.05$, $p>0.05$]; however, *post hoc* analysis for DMI revealed no significant differences between DMI- and SAL- treated vehicle groups. Inactive duration was affected by DMI [$H_{(1)}=1.83$, $p<0.05$], while no significant effects were noted for SSRI or SSRI x DMI interaction [SSRI: $H_{(3)}=1.83$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.09$, $p>0.05$]. Although, a *post hoc* analysis for DMI revealed no significant differences between the Vehicle+DMI and Vehicle+SAL groups (Figure 6.6).

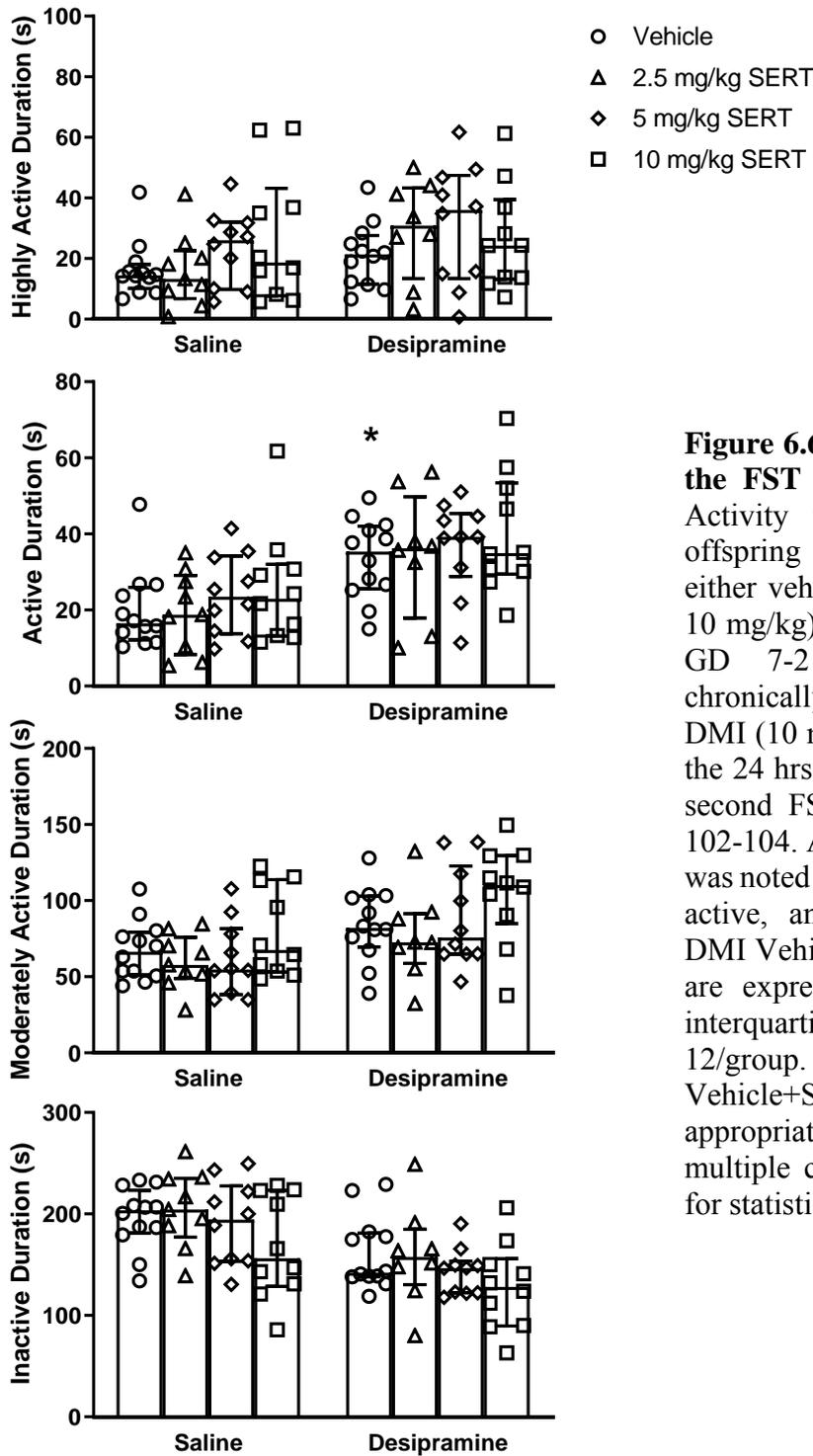


Figure 6.6 Sertraline males in the FST with SAL or DMI. Activity thresholds for male offspring after exposure to either vehicle, SERT (2.5, 5 or 10 mg/kg) via OG to dam from GD 7-21 and then sub-chronically exposed to SAL or DMI (10 mg/kg, SC) 3 times in the 24 hrs between the first and second FST exposure at PND 102-104. An overall DMI effect was noted for active, moderately active, and inactive duration. DMI Vehicle+SAL group. Data are expressed as median and interquartile range, n=8-12/group. When comparing to Vehicle+SAL, * $p < 0.05$, with appropriate correction due to multiple comparisons. See text for statistical analysis.

Female FST analysis revealed no effect of SSRI, DMI, or SSRI x DMI interaction when compared to vehicle groups for highly active duration [SSRI: $H_{(3)}=2.28, p>0.05$; DMI: $H_{(1)}=0.57, p>0.05$; SSRI x DMI: $H_{(3)}=0.42, p>0.05$], active duration [SSRI: $H_{(3)}=2.00, p>0.05$; DMI: $H_{(1)}=3.42, p>0.05$; SSRI x DMI: $H_{(3)}=0.07, p>0.05$], moderately active duration [SSRI: $H_{(3)}=0.60, p>0.05$; DMI: $H_{(1)}=0.32, p>0.05$; SSRI x DMI: $H_{(3)}=2.61, p>0.05$], or inactive duration [SSRI: $H_{(3)}=1.19, p>0.05$; DMI: $H_{(1)}=1.45, p>0.05$; SSRI x DMI: $H_{(3)}=1.48, p>0.05$] (Figure 6.7).

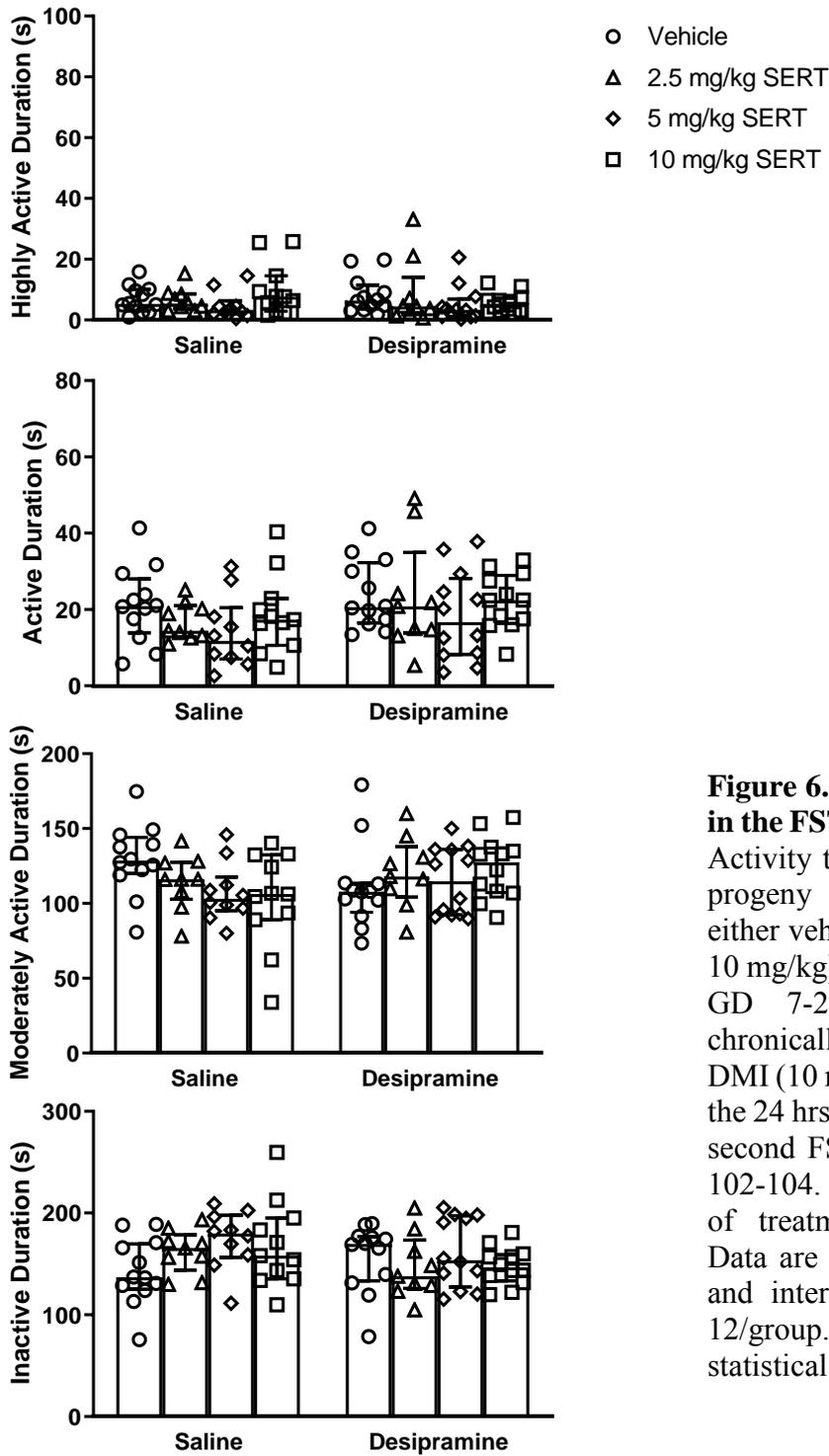


Figure 6.7 Sertraline females in the FST with SAL or DMI. Activity thresholds for female progeny after exposure to either vehicle, SERT (2.5, 5 or 10 mg/kg) via OG to dam from GD 7-21 and then sub-chronically exposed to SAL or DMI (10 mg/kg, SC) 3 times in the 24 hrs between the first and second FST exposure at PND 102-104. No significant effect of treatment was observed. Data are expressed as median and interquartile range, n=9-12/group. See text for statistical analysis.

6.3.2.4 Citalopram exposed progeny in the FST with SAL or DMI

Male FST analysis revealed no effect of SSRI, DMI, or SSRI x DMI interaction when compared to vehicle groups for highly active duration [SSRI: $H_{(3)}=2.90$, $p>0.05$; DMI: $H_{(1)}=3.60$, $p>0.05$; SSRI x DMI: $H_{(3)}=1.05$, $p>0.05$]. In regards to active, moderately active, and inactive, DMI had a significant effect on their durations [active: $H_{(1)}=21.38$, $p<0.05$; moderately active: $H_{(1)}=12.86$, $p<0.05$, inactive: $H_{(1)}=22.58$, $p<0.05$]; with a *post hoc* analysis indicating that DMI significantly increased active and moderately active durations, and decreased inactive duration, when compared to Vehicle+SAL groups. Additionally, no effects of SSRI or SSRI x DMI interaction were noted for active duration [SSRI: $H_{(3)}=0.86$, $p>0.05$; SSRI x DMI: $H_{(3)}=1.74$, $p>0.05$], moderately active duration [SSRI: $H_{(3)}=0.07$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.51$, $p>0.05$], or inactive duration [SSRI: $H_{(3)}=0.58$, $p>0.05$; SSRI x DMI: $H_{(3)}=1.39$, $p>0.05$] (Figure 6.8).

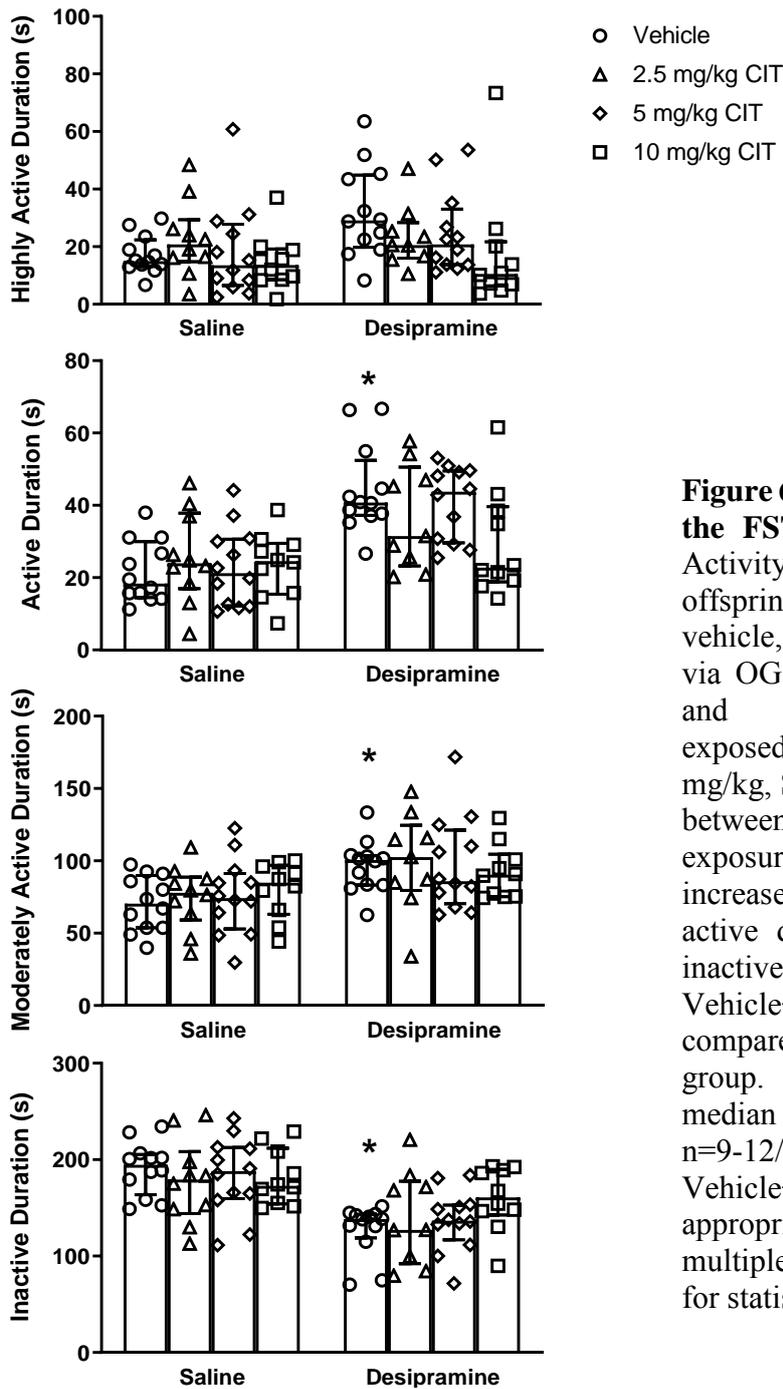


Figure 6.8 Citalopram males in the FST with SAL or DMI. Activity thresholds for male offspring after exposure to either vehicle, CIT (2.5, 5 or 10 mg/kg) via OG to dam from GD 7-21 and then sub-chronically exposed to SAL or DMI (10 mg/kg, SC) 3 times in the 24 hrs between the first and second FST exposure at PND 102-104. DMI increased, active and moderately active duration, and decreased inactive duration of the Vehicle+DMI group when compared to the Vehicle+SAL group. Data are expressed as median and interquartile range, $n=9-12$ /group. In comparison to Vehicle+SAL, $*p<0.05$, with appropriate correction due to multiple comparisons. See text for statistical analysis.

Female FST analysis revealed no effect of SSRI, DMI, or SSRI x DMI interaction when comparing Vehicle+DMI groups to Vehicle+SAL groups for highly active duration [SSRI: $H_{(3)}=0.24$, $p>0.05$; DMI: $H_{(1)}=0.00$, $p>0.05$; SSRI x DMI: $H_{(3)}=0.91$, $p>0.05$], active duration [SSRI: $H_{(3)}=0.96$, $p>0.05$; DMI: $H_{(1)}=0.58$, $p>0.05$; SSRI x DMI: $H_{(3)}=1.27$, $p>0.05$], or inactive duration [SSRI: $H_{(3)}=2.04$, $p>0.05$; DMI: $H_{(1)}=0.89$, $p>0.05$; SSRI x DMI: $H_{(3)}=1.09$, $p>0.05$]. A DMI effect was revealed for moderately active duration [DMI: $H_{(1)}=4.70$, $p<0.05$], however, a *post hoc* analysis indicated no significant differences when comparing Vehicle+DMI and Vehicle+SAL groups. Further, no effect was observed for SSRI [$H_{(3)}=2.65$, $p>0.05$] or SSRI x DMI [$H_{(3)}=0.87$, $p>0.05$] in regards to moderately active duration (Figure 6.9).

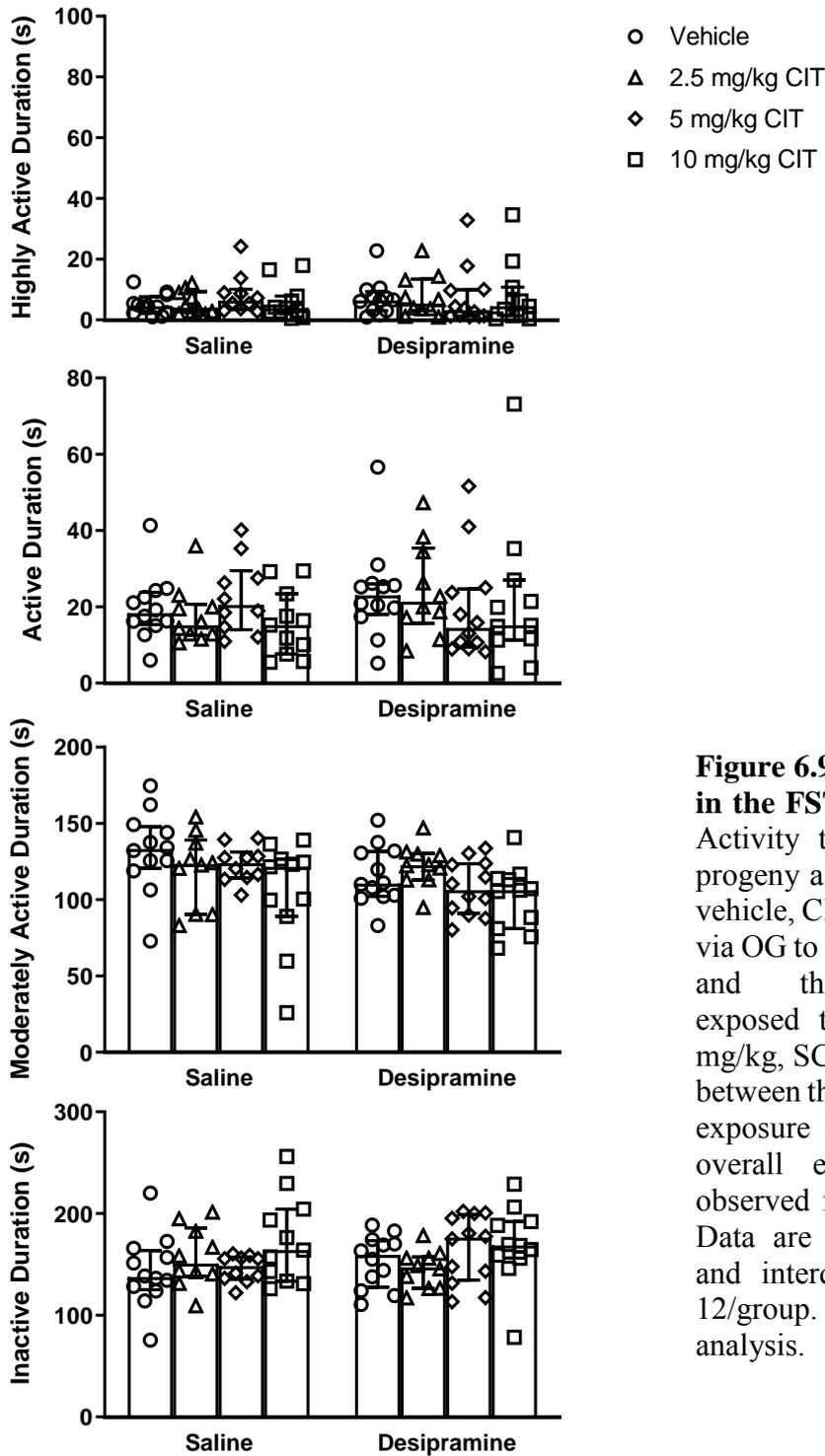


Figure 6.9 Citalopram females in the FST with SAL or DMI. Activity thresholds for female progeny after exposure to either vehicle, CIT (2.5, 5 or 10 mg/kg) via OG to the dam from GD 7-21 and then sub-chronically exposed to SAL or DMI (10 mg/kg, SC) 3 times in the 24 hrs between the first and second FST exposure at PND 102-104. An overall effect of DMI was observed for moderately active. Data are expressed as median and interquartile range, n=10-12/group. See text for statistical analysis.

6.4 Discussion

Overall, this chapter has revealed that no SSRIs considered produced an anxiogenic or depressive effect in adult progeny. In males, diazepam treatment showed a general trend of an anxiogenic effect, but this was consistently blunted by the sedative effects of diazepam which manifested as a reduction in locomotor activity. Conversely, in females, diazepam treatment exhibited anxiolytic properties, producing an increase in open arm behaviours, without effecting locomotor activity. In males, but not in females, antidepressant properties of desipramine were expressed, with treatment consistently generating an increase in swimming activity. Finally, diazepam and desipramine efficacies were not challenged when offspring were previously exposed to any of the four SSRIs employed, regardless of dose or sex.

6.4.1 Aim 1: Anxiolytic response

The first aim of this study was to examine the effects of gestational SSRI exposure from GD 7-21 on the anxiolytic efficacy of diazepam in adulthood. Efficacy was evaluated through a behavioural response by monitoring the entries into and the time spent in the open arms of the elevated plus maze 30 minutes after saline or diazepam (1 mg/kg, SC) exposure. In addition, locomotor activity was monitored by measuring distance travelled to detect treatment induced ambulatory effects. Male and female progeny were assessed individually in adulthood at PND 95-97.

6.4.1.1 Diazepam

Baseline diazepam effects were noted across the four SSRI studies in regards to the vehicle groups for ambulatory and “anxiolytic-like” behavioural effects in the elevated plus maze. Male vehicle offspring treated with diazepam showed a decrease in distance travelled, compared to vehicle exposed male offspring treated with saline, for each of the studies, except for the fluoxetine study although a similar trend is observed, the difference is likely due to the lower number of groups assessed. Such results reflect a sedative effect of diazepam, which is a known implication in the clinical context. Additionally, diazepam treatment did not have a significant anxiolytic effect for male behaviour, compared to respective saline-treated vehicle groups. For males, this is contrary to what is typically expected as the elevated plus maze has been validated across labs to detect the anxiolytic properties of drug treatments (Pellow et al., 1985).

While male diazepam-treated rats did not produce significant differences compared to male saline-treated rats, an overall diazepam effect was detected in the latter three studies, and an anxiolytic tendency was identified with diazepam-treated males spending more time in the open arm than saline-treated males. As previously mentioned, diazepam treatment did significantly affect locomotor activity by reducing the distance travelled, suggesting a possible sedative effect. Therefore the anxiolytic trend may be slightly altered due to the sedative effect of the diazepam. However, the employed dose of diazepam (1 mg/kg) was selected for the current studies because previous work conducted in this laboratory using a diazepam dose range (0.5, 1, 1.5 mg/kg) concluded that anxiolytic effects were detectable for males in the elevated plus maze by an increase in the percentage of open arm entry after 1 or 1.5 mg/kg diazepam administration and an increase in the percentage of open arm time was noted after 1.5 mg/kg diazepam treatment, compared to saline-treated control males; whereas, the 0.5 mg/kg diazepam showed no statistically significant anxiolytic effects (Bannerton et al., 2015). A decrease in locomotor activity was not observed after the diazepam dose range exposure in the elevated plus maze, however, rats were then directly tested in the open field for ambulatory behaviours and in fact a statistically significant decrease in distance travelled was only found for the highest dose (1.5 mg/kg); meanwhile the lower doses (0.5, 1 mg/kg) had no significant effect on distance moved in males (Bannerton et al., 2015). Henceforth, resulting in the employed 1 mg/kg dose. Additionally, a reduction in distance travelled has been observed after a large dose range of diazepam treatment (1-10 mg/kg, SC) in both the novel environment of the open field and in the familiar environment of the home cage (Dunne et al., 2007). Therefore it can be concluded that though the diazepam dose selected may be appropriate, the sedative effect of diazepam blunted the typically expected anxiolytic effect of this positive control.

Female offspring of vehicle dams, did not experience significant, nor general, diazepam-induced behavioural effects in regards to ambulatory behaviour. Furthermore, trends alternated between the four SSRI studies, showing no pattern for diazepam altering distance travelled. Therefore, unlike the males, sedative effects did not blunt the resulting anxiolytic effects. The vehicle exposed fluoxetine study control group reported a significant increase in open arm entry due to diazepam exposure, and a tendency to spend more time in the open arm as well. Moreover, the diazepam treated

vehicle groups of the remaining three SSRI studies also showed a statistically significant increase in open arm entry, as well as open arm time. Overall, although diazepam-induced anxiolytic effects may have been dampened in male offspring as a result of the drug's sedative properties, a positive anxiolytic trend can be confirmed. Meanwhile, at the same dose, no sedative effects were observed in females, and thus diazepam-induced anxiolytic properties were observed in the vehicle exposed female progeny.

6.4.1.2 Fluoxetine

Fluoxetine exposure *in utero* alone did not alter “anxiety-like” behaviour or locomotor activity for male or female progeny at age PND 95-97. These results are in line with the previous results presented in Chapter 5, which revealed no treatment effect at PND 28, 56, or 84, and is supported by previous findings regarding gestational exposure (Bairy et al., 2007, Noorlander et al., 2008, Capello et al., 2011). Progeny exposed to fluoxetine and diazepam did not perform differently in the test when compared to their respective vehicle and diazepam exposed rats, regardless of sex. As diazepam largely relies on neurotransmission of GABA neurons, these findings would suggest that fluoxetine exposure *in utero* does not significantly impair the development of GABA neurons involved in diazepam action. Such findings are supported by a somewhat comparable study conducted by Favaro et al. (2008) assessing diazepam and propylene glycol (1.2 mg/kg, IP) in the plus maze in adolescent (PND 40) mice after perinatal fluoxetine exposure (7.5 mg/kg, IG, GD 0-PND21). It was observed that although diazepam increased the percentage of open arm entries and the percentage of time spent in the open arms, this was not affected by perinatal fluoxetine treatment in males or females (Favaro et al., 2008). While the aforementioned study did not present ambulatory behaviours from the plus maze after diazepam treatment, no baseline effect of gestational fluoxetine exposure on ambulation was found in the open field test, similar to the findings reported here. Collectively, the current findings and those of Chapter 5 would suggest that fluoxetine exposure *in utero* at the doses employed does not alter “anxiety-like” behaviour in the elevated plus maze from adolescence throughout adulthood. Furthermore, fluoxetine exposure *in utero* did not alter the male or female offspring's response to anxiolytic treatment when compared to vehicle exposed progeny.

6.4.1.3 Paroxetine

Paroxetine exposure *in utero* alone did not modify “anxiety-like” or ambulatory behaviour for male or female progeny at age PND 95-97. Similar findings were noted in adulthood for Chapter 5, which revealed no treatment effect at PND 56, or 84. Comparable results were also reported by others who have assessed paroxetine exposure during gestation (Coleman et al., 1999, Christensen et al., 2000, Capello et al., 2011). Paroxetine exposure did not modify the anxiolytic effect of diazepam in the plus maze when compared to respective vehicle and diazepam groups, ultimately suggesting that gestational exposure does not damage the functionality of GABA neurons. Although similar preclinical studies could not be found, interesting clinical findings have suggested that *in utero* SSRI exposure in general (FLX, PRX, or SERT) does alter the expression of GABA as infants were found to show reduced behavioural pain response, however this report was not isolated to paroxetine exposure (Oberlander et al., 2002, Oberlander et al., 2005). Overall, the current findings provide novel evidence which provides information regarding the response to GABA-ergic drugs after gestational exposure to paroxetine.

6.4.1.4 Sertraline

Sertraline exposure *in utero* alone did not alter “anxiety-like” behaviour or locomotor activity for progeny at age PND 95-97. This is complementary to previous findings, as Chapter 5 elevated plus maze results indicated no treatment effect at earlier time points (PND 28, 56, 84) in the elevated plus maze, regardless of sex. Previous literature generally agrees with the findings of Chapter 5 and 6, reporting no baseline effects of gestational or perinatal exposure on such behaviours (Capello et al., 2011, Meyer et al., 2018, Kott et al., 2019). Further confirming the current findings, at a similar test age, Kott et al. report that “anxiety-like” behaviours were not influenced by gestational exposure (20 mg/kg, OG, GD(-16)-littering) in the plus maze for adult (PND 95-96) progeny (Kott et al., 2019). Sertraline exposure (SERT+DZP) did not influence the anxiolytic effect of diazepam when compared to relevant vehicle and diazepam groups, implying exposure *in utero* did not alter GABA-ergic neurotransmission in adulthood. Pharmacological studies, like the one in question, have not been previously reported in the preclinical literature, although clinical results suggest that maternal use of SSRIs collectively (FLX, PRX, SERT) did alter the expression of GABA in resulting infants, consequently reducing behavioural pain

response (Oberlander et al., 2002, Oberlander et al., 2005). The current findings produce original results suggesting that exposure *in utero* does not alter progeny's response to GABA-ergic drugs such as diazepam.

6.4.1.5 Citalopram

Citalopram exposure during gestation did not alter “anxiety-like” behaviours of resulting progeny, nor did it alter locomotor activity at age PND 95-97 of male or female progeny. A lack of citalopram influence on “anxiety-like” and motor behaviours is resembled in the Chapter 5 findings, at younger ages (PND 28, 56, 84), and is supported by previous literature producing similar conclusions on such behaviours (Hsiao et al., 2005, Harris et al., 2012). The anxiolytic effects of diazepam were not altered by citalopram exposure (CIT+DZP) when compared to relevant vehicle and diazepam groups. No previous literature has modelled such a study. Overall, these innovative conclusions considering *in utero* citalopram exposure and adult diazepam treatment would imply that exposure in pregnancy does not alter the resulting offspring's response to drugs such as diazepam which rely on GABA-ergic neurotransmission.

6.4.2 Aim 2: Antidepressant response

The second aim of this study was to examine the effects of gestational SSRI exposure from GD 7-21 on the antidepressant efficacy of desipramine in adulthood. Efficacy was evaluated through a behavioural response by various activity thresholds in the second exposure to the forced swim test, after sub-chronic exposure to saline or desipramine (10 mg/kg, SC) 24, 5, and 1 h before testing. Activity threshold durations consisted of highly active (>10%), active (8-10%), moderately active (4-8%), and inactive (<4%). Male and female progeny were assessed individually in adulthood at PND 102-104.

6.4.2.1 Desipramine

Baseline desipramine effects were noted across the four SSRI studies in regards to the vehicle groups for behavioural effects in the forced swim test. Male vehicle offspring sub-chronically exposed to desipramine showed an increase in active duration, compared to vehicle exposed male offspring treated with saline, for each of the four SSRI studies. Furthermore, three of the four studies showed a statistically significant

decrease in inactive duration, with a parallel overall effect noted in the sertraline study for a desipramine-induced reduction in inactive duration. Such effects on active and inactive durations were expected, as these activity thresholds were used to mirror the typically observed behaviours in the forced swim test, namely mobility and immobility. As depressive drugs are known to increase immobility, whereas antidepressant compounds such as desipramine, are known to reduce immobility (Porsolt et al., 1977). The additional in-between threshold of moderately active showed a significant increase in the citalopram study, and an overall desipramine effect was noted in the sertraline study; both studies indicating a desipramine-induced increase in moderately active duration, along with similar trends in the other two SSRI studies. The top activity parameter, highly active, only exhibited an overall desipramine effect for the sertraline study, however, similar trends were noted in the other three SSRI studies. Therefore it can be concluded that desipramine, at the dose and regime employed, appropriately reduced behavioural despair symptoms in the forced swim test. This was marked by a consistent baseline increase in activity and decreased in inactivity, across the SSRI studies, as detected through the EthoVision[®] XT 11.5 video tracking software using the automated activity analysis feature; overall, mirroring manual scoring techniques which show increased mobility and decreased immobility following desipramine treatment.

Female offspring of vehicle dams did not show much evidence of a desipramine-induced antidepressant effect on behavioural despair in the forced swim test. While fluoxetine and sertraline studies showed no desipramine effects, overall desipramine effects were noted by a modest increase in active duration for the paroxetine study, and a slight decrease in moderately active duration for the citalopram study. It is not likely that this is a result of the automated scoring technique, as reviews have been published emphasizing the multiple factors related to gender differences (Bogdanova et al., 2013). Specifically, in females, the oestrous cycle has been shown to induce different outcomes, with proestrous and estrous phases increasing immobility compared to the diestrous phase (Consoli et al., 2005). Additionally, within this lab, using the manual scoring technique, the baseline female behaviour showed reduced immobility compared to baseline male behaviour (Simpson et al., 2012). Furthermore, Simpson et al. (2012) also reports a desipramine-induced reduction in immobility for male rats but not for female rats. Overall, although a clear desipramine-induced

antidepressant effect was not detectable for female vehicle exposed offspring, this is in line with previous complications found using females in the forced swim test model. Meanwhile, a positive antidepressant pattern can be confirmed in males, at the same dose, using an automated activity analysis tracking feature, complementary to manual tracking findings.

6.4.2.2 Fluoxetine

Fluoxetine exposure during gestation did not alter “depressive-like” behaviours of resulting progeny, regardless of sex, at age PND 102-104 in the forced swim test. The results of this behavioural despair measure of depression, agree with the previous investigation of “depressive-like” behaviours suggesting a lack of influence on anhedonic behaviours exposed in Chapter 5. Previous work has also reported similar findings in the forced swim test. Gestational exposure (12 mg/kg, IG, GD 11-21) produced no changes in immobility time for adolescent (PND 31) female offspring (Butkevich and Mikhailenko, 2018) nor in adult (PND 130) males (Olivier et al., 2011). The absence of a treatment effect is also observed after perinatal exposure (5 mg/kg, IG to dam, GD 0-PND 21) assessed in adolescent (PND 35) and adult (PND 75) offspring (Francis-Oliveira et al., 2013). These findings are also consistent with perinatal exposure (5 mg/kg, SC to pup, GD 6-PND 20) resulting in no effects on immobility time in adult offspring (PND 73-74) (Sprowles et al., 2017). The antidepressant effects of desipramine were not altered by *in utero* fluoxetine exposure (FLX+DMI) when compared to relevant vehicle and desipramine groups. No previous literature has modelled such a study. Consequently, this report mirrors previous findings, suggesting the lower doses of fluoxetine do not influence depressive behaviour in adults, further novel results are presented concluding that *in utero* fluoxetine exposure does not alter the response to tricyclic antidepressants such as desipramine.

6.4.2.3 Paroxetine

In adulthood, gestational paroxetine exposure did not alter parameters of behavioural despair in the forced swim test, when compared to vehicle and saline exposed offspring. The same was true in Chapter 5, with no effects of treatment apparent in regards to anhedonia symptoms of depression (Figure 5.4B). A search of the literature produced one study which observed no difference in the percentage of struggling in the forced swim test after gestational exposure (30 mg/kg, oral, GD (-14)-GD 16) in

adult (PND 90) mice offspring (Coleman et al., 1999). Furthermore, the desipramine induced antidepressant effect was not altered by gestational treatment (PRX+DMI) when compared to appropriate control groups (VEH+DMI). Similar studies have not been conducted for appropriate comparisons to be made. The current research reinforces previous findings in mice, that gestational paroxetine exposure does not alter “depressive-like” behaviours in adult rat offspring. Additionally, original research has been produced suggesting that gestational exposure to paroxetine does not alter desipramine-induced behavioural responses in the subsequent adult offspring.

6.4.2.4 Sertraline

Gestational sertraline exposure did not modify behavioural despair in adult male and female offspring in the forced swim test. Such non-effects run parallel to the findings of Chapter 5, which report no treatment effects on anhedonia behaviour (Figure 5.4C). An external study observed no treatment effects in the forced swim after gestational exposure (20 mg/kg, OG, GD(-16)-littering) in resulting male and female (Kott et al., 2019). Gestational exposure (SERT+DMI) did not modify desipramine induced behavioural response when compared to control groups (VEH+DMI). Prior studies, employing desipramine in the forced swim have not been conducted in subsequent gestational exposed sertraline offspring. Bearing the previous studies in mind, it is concluded that gestational sertraline exposure does not alter depressive behaviours in adulthood, and novel insights are presented suggesting that *in utero* exposure to sertraline does not alter behavioural response in adulthood to antidepressant treatments such as desipramine.

6.4.2.5 Citalopram

Exposure *in utero* had no effect on behavioural despair behaviours in resulting adult progeny, regardless of sex, this is comparable to Chapter 5 findings on anhedonic behaviours of depression (Figure 5.4D). Previous literature employing the forced swim test after gestational exposure has not been conducted, although results after perinatal exposure are not supportive of the current study's conclusions. Perinatal exposure (10 mg/kg, sc or drinking water, GD 6-PND 21) does increase depressive behaviours for the subsequent adult (PND 60-65), male and female offspring (Sprowles et al., 2016, Zohar et al., 2016). In addition to the differences in exposure periods, the difference in findings may also be a result of different testing periods, as both test in adulthood but the current study assesses offspring much later (PND 102-

104). Exposure *in utero* (CIT+DMI) had no effects on the induced behavioural response when compared to control groups (VEH+DMI) for male and female offspring. These results overall produce novel evidence, as such a study has not been completed observing behavioural despair which limits citalopram exposure to gestation. Furthermore, the novel contribution regarding no differences in response to desipramine in subsequent *in utero* exposed citalopram offspring, informs more specifically on the lasting implications of citalopram exposure during a critical developmental period.

6.4.3 Conclusion

In summary, the findings of this chapter suggest that the SSRIs considered, at the doses employed do not inhibit baseline locomotor activity or emotional behaviours such as anxiety or depression. Additionally, no SSRI exposure altered the response to typical anxiolytic and antidepressant drugs such as diazepam and desipramine. Despite observing significant sedative effects in males, all SSRI exposed offspring responded well with an anxiolytic pattern after diazepam treatment, which was significantly reflected in the female progeny. More sex differences were noted in the desipramine-induced antidepressant study, showing males responded significantly well to treatment, despite the new method of tracking; whereas females showed little evidence of antidepressant-induced behaviour, which is in line with previous findings. Overall, gestational SSRI exposure did not have enduring consequences on emotional development in this last period considered in adulthood. Additionally, altering serotonergic activity during embryogenesis did not inhibit the efficacy of particular drugs such as diazepam and desipramine. However further information is required to confirm positive controls for females in the forced swim test follow sub-chronic desipramine treatment. These findings, carried out to the clinical scenario, are beneficial for anticipating and treating emotional deficits expressed in adult progeny who experienced SSRI exposure *in utero*.

7 Discussion

7.1 Discussion

As the prevalence of SSRI antidepressant prescribing during pregnancy increases, preclinical studies have attempted to reveal the consequences of exposure in resulting offspring. Reviewing the current preclinical literature highlights that such studies have largely been confined to fluoxetine, rather than each SSRI individually. Unfortunately, results of preclinical fluoxetine studies are translated to the clinical scenario on behalf of all SSRIs, with the assumption that all SSRIs would influence prenatal development the same. However, recent studies using clinical databases are beginning to suggest that specific birth outcomes and teratogenic effects are often linked to particular SSRIs, rather than these outcomes being due to a general class effect. Therefore, physicians need more information on gestational exposure, so that treatment-naïve mothers can receive the best care, while also minimising potentially harmful birth defects as much as possible. This project aimed to determine the resulting effects of gestational exposure to fluoxetine, paroxetine, sertraline, and citalopram when exposure began during pregnancy to treatment-naïve dams. While acknowledging the importance of maternal wellbeing in pregnancy, this project used a clinically relevant dose range to observe potential outcomes in neonatal, adolescent, and adult offspring. This longitudinal animal model mimics the clinical scenario as much as possible to improve the translation value of the experiments. Therefore, these results can help inform physicians when prescribing to treatment-naïve pregnant women, while anticipating the wellbeing of the mothers and their children in both the short- and long-term. This chapter will assess the findings of this thesis for each of the four SSRIs individually from gestation through adulthood, and make recommendations for the future directions of assessment of the consequences of gestational SSRI exposure in the rat.

Overall the study presents various implications of age and sex, highlighting the need for preclinical models of continuous assessments which examine both male and female offspring. Historically, males have been over-represented in both clinical and preclinical studies, especially in the fields of pharmacology, endocrinology, physiology and neuroscience (Hughes, 2007, Beery and Zucker, 2011). While certain acts, such as the NIH Revitalisation Act, have been put in place to ensure clinical testing occurs in males and females, rodent studies are still trying to make up for the discrepancy and many studies continue to either neglect to specify the sex, consider

both sexes to increase the weight of the data, or simply favour males (Beery and Zucker, 2011).

In the current work from birth, there were differences amongst findings, as particular SSRIs increased stillborn rates, whereas others had a delayed effect, significantly reducing survival within the first-week post-birth. All significant SSRI-induced progeny findings that will be discussed in the following paragraphs which highlights the findings by each SSRI, were in parameters of ano-genital distance, surface righting, forelimb grip, and between zone entries. While the effects noted occurred only transiently at a single time-point, all significant changes measured occurred between the neonatal and adolescent period. Moreover, the majority of the effects were confined to male treated offspring. Surface righting findings were sporadic as they only had significance on the third of four consecutive testing days. In the open field, between zone entries were altered initially during adolescents, but not in adulthood which implies that such effects were transient and did not persist into the later life stages. Evidently, between zone entry analysis reported significant effects in male and female offspring, although resulting in opposite implications; this is likely because males are typically less active than females. Interestingly, ano-genital distance and forelimb grip only reported significant differences on the final day of testing in the neonatal period, both of which were drug-induced enhancements. However, as no follow-up measurements were made in these parameters in adulthood, it is recommended that future studies assess these endpoints in adulthood, to further define if these effects too are fleeting or permanent consequences of *in utero* exposure. In addition to only showing effects for females in the between zone entry parameters, sex-induced effects were found within the vehicle groups in the elevated plus maze and open field, and different responses were noted for the positive controls of the psychopharmacological challenge.

Throughout adulthood, an overall effect of sex was noted for the majority of the ambulatory and anxiety parameters measured in the elevated plus maze and open field. Although these findings were not statistically significant between the vehicle groups, this is consistent with previous findings which suggested no statistical difference in the elevated plus maze but a statistically significant increase in distance travelled for females compared to males in the open field (Simpson et al., 2012). Adulthood studies mostly showed that females spent more time in the open arms than males, implying

that baseline anxiolytic behaviour is higher for females than males. After diazepam treatment, males reported a slight sedative effect, reducing distance travelled, thus blunting significant findings in anxiolytic behaviours. In contrast, diazepam treated females indicated no sedative effects and significantly increased anxiolytic behaviours in the elevated plus maze for all four SSRI studies. This is in line with the Chapter 5 findings, because males already showed reduced ambulatory features compared to females, making them more sensitive to the diazepam induced sedative effects on ambulatory behaviour. Sedative effects in males were noted in previous studies which occurred within this laboratory, which were assessed in both familiar and novel environments (Dunne et al., 2007). Furthermore, as females had already shown a higher baseline for anxiolytic behaviours in Chapter 5, it is not surprising that diazepam further stimulated these behaviours. In regards to desipramine treatment, all treated males exhibited a significant increase in active duration in the forced swim test, with most studies also showing a significant reduction in immobility. On the other hand, desipramine-treated females reported sporadic overall effects of desipramine, but no significant effects of desipramine were observed for any of the activity durations when compared to vehicle-saline groups. The opposing sex-induced findings are likely a result of the differences previously noted in regards to locomotor activity, which was highlighted earlier in this work in multiple models, suggesting higher baseline levels of activity for females compared to males in the open field, forced swim test, and home cage (Simpson et al., 2012). Specific to desipramine treatment in the forced swim, former studies in this lab which examined behaviours using the traditional manual scoring technique, also only found significant desipramine-induced effects for mobility behaviour in male but not female rats (Simpson et al., 2012). This emphasises the importance of sex as a factor when examining behavioural parameters. It is possible that there is an insufficiency in the current standard models used, as certain tests may be more suited to a particular sex. Overall these age- and sex-dependent findings underline the importance of longitudinal studies which assess parameters in both male and female offspring to appropriately detect consequences that are translatable to the clinical scenario.

Parameter	Fluoxetine	Paroxetine			Sertraline			Citalopram		
	2.5 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Gestational weight, food and water intake	---	---	---	---	---	---	↓ BW ↓ Food	---	---	---
Postpartum weight, food and water intake	---	---	---	---	---	---	---	---	---	---
Litter characteristics: size, gestational length, maternal caregiving, sex ratio	---	---	---	---	---	---	---	---	---	---
Mortality	---	---	↑ SB ↑ TD	↑ TD	---	---	↑ TD	---	---	---
Birth weight	---	---	---	---	---	---	---	---	---	---
Somatic development: fur, pinna, eye ano-genital distance, length, body weight	M PND 24 ↑ AG	---	---	---	---	---	---	---	---	---
Behavioural development: surface righting , negative geotaxis, forelimb grip	M PND 4 ↓ SR	---	---	---	---	M PND 17 ↑ FG	M PND 17 ↑ FG	---	---	---
Motor activity	---	M PND 28 ↑ BZE	F PND 28 ↓ BZE	---	---	---	---	---	---	---
Anxiety	---	---	---	---	---	---	---	---	---	---
Cognition	---	---	---	---	---	---	---	---	---	---
Anhedonia	---	---	---	---	---	---	---	---	---	---
Anxiolytic response	---	---	---	---	---	---	---	---	---	---
Antidepressant response	---	---	---	---	---	---	---	---	---	---

Table 7.1 Summary table of SSRI-induced effects. AG=ano-genital distance; BW=body weight; BZE= between zone entries; F=female; FG=forelimb grip; M=male; PND=postnatal day; SB=still born; SR=surface righting; TD=total dead.

Fluoxetine exposure *in utero*, at the dose employed in the current study, found limited effects on the progeny. Although fluoxetine produced no effects in regards to maternal wellbeing and littering parameters, adolescent through adulthood behaviour, or the psychopharmacological challenge, significant effects were noted within the neonatal development period. Somatic development was altered in the male treated group, thus increasing ano-genital distance. A transient behavioural irregularity was also noted in the male group, indicating that treatment decreased surface righting behaviour on PND 4. An increase in the male ano-genital distance could suggest an early onset of puberty, or an alteration in sexual behaviour. This effect was not noted for the other SSRIs. Rayen et al. (2013) measured ano-genital distance in juvenile males after postnatal fluoxetine exposure and produced alternative results, indicating a decrease in juvenile male ano-genital distance and also reduced sexual behaviours in adulthood. Therefore, it is possible that early-life fluoxetine exposure can have implications on ano-genital distance and may have enduring effects on sexual development thus altering adult sexual behaviour. In regards to surface righting, while a deficit may have been noted on PND 4, this effect is likely spurious or sporadic, as results from the days prior and after were not consistent with this effect, thus it is not a true description of vestibular function. While paroxetine and sertraline also induced transient effects on ambulation and forelimb grip respectively, the observed brief effect on surface righting is specific to the fluoxetine study. Though previous studies have not measured this parameter after gestational fluoxetine exposure, the findings reported throughout the neonatal development chapter produced no significant effects on motor function. Furthermore, the same was true in Chapter 5, with no fluoxetine-induced effects on ambulation on adolescent or adult progeny. While fluoxetine produced few effects in regards the maternal wellbeing and offspring survival, it is important to note that the drug was observed at a lower dose compared to most of the other SSRI studies reported in this work. The 2.5 mg/kg fluoxetine dose did not affect neonatal survival, unlike the paroxetine study findings which will be discussed in the following section. However, previous findings in this lab indicated that higher fluoxetine doses (5, 10, and 20 mg/kg) caused prominent effects on maternal weight gain from the first day of dosing. Subsequently, at littering a two-fold dose-response increase was noted for stillborn pups compared to vehicle exposed litters (Appendix 9.1). Overall, oral gavage exposure to fluoxetine (2.5 mg/kg) from GD 7-21 to dams, had significant effects on male progeny ano-genital distance and a sporadic effect on vestibular development.

The same regime at higher doses (5-20 mg/kg) had negative effects on maternal wellbeing and neonatal survival, however, this effect did not occur at the lower dose (2.5 mg/kg). Under the employed experimental design, no lasting implications were noted for behavioural measures assessing anxiety, locomotor activity, cognition, or depression, nor was the typical behavioural response to anxiolytic or antidepressant drugs altered due to gestational fluoxetine exposure.

Gestational paroxetine exposure produced few effects at the doses administered in this study. Paroxetine had no implications on maternal wellbeing or the resulting progeny in regards to the adulthood behaviour or the psychopharmacological challenge; however, significant effects were noted within the pup littering parameters and the adolescent behaviour. Littering parameters of mortality were altered by paroxetine exposure, causing an increase in stillborn pups after dams were exposed to 2.5 mg/kg and also a reduction in pup survival for the first week for groups exposed to 2.5 or 5 mg/kg. A transient ambulatory irregularity was noted for adolescents in regards to between zone entries, with 1.25 mg/kg paroxetine increasing zone entries for the males and 2.5 mg/kg paroxetine reducing zone entries for females. Reports on mortality at specific time points after paroxetine exposure were difficult to find, especially at the doses employed (1.25, 2.5, 5 mg/kg). Preliminary findings in this lab suggested a prominent reduction in pup survival after exposure to 10 mg/kg paroxetine, for that reason the 1.25 mg/kg dose was added only to the paroxetine study and this was the only paroxetine dose not to alter neonatal survival (Appendix 9.2). Similar effects on mortality were noted at the 10 mg/kg dose for fluoxetine and sertraline. Van den Hove et al. (2008) also reported preliminary findings, indicating that 10 mg/kg paroxetine administered to the dam via the drinking water from GD 15-21 induced a 10-fold increase in pre-weaning pup mortality, with the majority of deaths occurring within a few days after birth. Therefore, it can be inferred that paroxetine has profound effects on neonatal mortality, even at lower doses (2.5 mg/kg), unlike the other SSRIs under investigation such as fluoxetine and sertraline which required higher doses (≥ 5 mg/kg) to produce significant effects on mortality. The alteration in ambulatory behaviours during adolescence presents an interesting effect as the lowest dose increased male behaviours while the middle dose reduced female behaviours, and the highest dose produced no effect. This was individual to paroxetine, as the three other SSRIs did not show this effect. Previous literature found no change in ambulatory behaviour for mice

of a similar age after exposure to 30 mg/kg (Coleman et al., 1999), parallel to the findings in the 5 mg/kg paroxetine exposure groups of the current work. This effect at the middle and low doses is transient, as motor development was unaffected during neonatal development; furthermore, no effect of treatment was noted in the adulthood periods of this study, or other studies in adult rats (Capello et al., 2011). This emphasizes the importance of assessing drug exposure at multiple doses and developmental periods, in both male and female offspring. Although the drug did alter between zone entries, no effects of treatment were noted for distance travelled. This may suggest that *in utero* exposure to these lower doses of paroxetine impacts overall exploratory behaviours without effecting locomotor ability. Overall, oral gavage exposure to paroxetine (2.5, 5 mg/kg) from GD 7-21 to dams, had significant effects on neonatal mortality, without affecting maternal wellbeing. Following the same regime with a higher dose (10 mg/kg), also had a prominent effect on neonatal survival. Meanwhile, the low and medium dose of paroxetine briefly altered exploration between zones in male and female adolescent progeny. Under the employed experimental design, no consequences were noted due to paroxetine exposure for maternal wellbeing or neonatal development. Additionally, exposure did not induce or repress behaviours of anxiety, cognition, or depression, nor was the typical behavioural response to anxiolytic or antidepressant drugs affected by *in utero* paroxetine treatment.

This project found few implications of sertraline exposure *in utero* at the doses used. The treatment produced significant effects in regards to maternal wellbeing, pup littering parameters, and neonatal development; although, no effects were observed in any of the behaviours measured from adolescence through adulthood, or the psychopharmacological challenge. The highest dose of sertraline (10 mg/kg), significantly reduced body weight and food consumption for dams during the gestational period, nevertheless no lasting implications were noted for mothers in the postnatal period. However, the reduction in body weight gain for that dose group during gestation is likely to have contributed to an increase in total pups dead within the week following littering. Despite this reductive effect, male pups of the 5 and 10 mg/kg had enhanced forelimb grip behaviour/strength compared to control males. Unlike fluoxetine and paroxetine, sertraline (and citalopram) were tested at a higher dose range which included 10 mg/kg, as the preliminary studies showed that sertraline

treatment was more tolerable to the dams in terms of maternal wellbeing and offspring mortality. Therefore although sertraline did significantly reduce maternal weights and increase mortality, these effects were minimal in comparison to the other two SSRIs. Previous studies also found a reduction in weight gain and food consumption when observing perinatal 20 or 80 mg/kg sertraline exposure (Davies and Klowe, 1998) and reduced appetite was also found in non-pregnant animals after a single 10 or 18 mg/kg IP sertraline exposure, while no effect was found at lower doses of sertraline (2 or 3.3 mg/kg) (Lucki et al., 1988). Furthermore, it is possible that the increase in pup mortality may be due to the reduced maternal prenatal weights rather than a direct effect of sertraline. The week following birth, the pups are completely dependent on the mother for nutrition and care; therefore, if the dams are a lower weight it is possible they may have a more difficult time recovering from labour. This complication for recovery implies that the offspring of these litters may not experience the same level of caregiving as offspring of dams with typical weight gain and food consumption during gestation. At doses greater than 10 mg/kg (20, 40, or 80 mg/kg) Davies and Klowe (1998) also report that sertraline exposure increased mortality during the first four days following birth, whereas the 10 mg/kg dose had no effect for that period; however it is impossible to make a direct comparison as this chapter monitored mortality for a longer period (PND 1-7). The theory that mortality rates were influenced by maternal wellbeing, thus an indirect effect of treatment, is also supported by the findings that treatment caused no deficits in somatic or behavioural development and, in fact, treatment enhanced male offspring forelimb strength on PND 17 for both the 5 and 10 mg/kg sertraline litters compared to vehicle male progeny. This was not reported for any of the other SSRIs investigated. While Davies and Klowe (1998) reported no external or visceral anomalies after perinatal sertraline exposure, there have been no published reports on the somatic or neonatal development parameters measured in this section after prenatal or postnatal sertraline exposure. Therefore novel findings are presented, suggesting that 5 and 10 mg/kg sertraline exposure may have a positive effect on male forelimb strength. However it is possible that this effect is transient, as additional motor development parameters showed no such effect; although, these later tests assessed general locomotor behaviours and ambulation, rather than assessing forelimb grip or strength directly. Overall, oral gavage exposure to sertraline (10 mg/kg) from GD 7-21 to dams, had significant effects on dam gestational body weight and food consumption, which

consequently increased pup mortality. While sertraline did affect maternal wellbeing and offspring survival it is important to note that this dose-dependent effect required a higher dose compared to fluoxetine and paroxetine; furthermore, the resulting male offspring may have experienced an increase in forelimb grip or strength due to 5 and 10 mg/kg gestational sertraline exposure. Under this preclinical model, no enduring deleterious consequences were noted for neonatal somatic or behavioural development. Moreover, no effects of sertraline treatment were noted in regards to behavioural measures after weaning through adulthood assessing anxiety, locomotor activity, cognition, or depression, nor was the typical behavioural response to anxiolytic or antidepressant drugs altered due to *in utero* sertraline exposure.

The current study found no effects of citalopram exposure *in utero* at the dose employed. Unlike the current examination of sertraline (10 mg/kg) and the prior findings in this lab in regards to fluoxetine (10 and 20 mg/kg) which reported reduced maternal weight gain and food consumption, citalopram had no effects on maternal wellbeing. Additionally, while the current investigations of paroxetine (2.5 and 5 mg/kg) and sertraline (10 mg/kg) reduced neonatal survival as did preliminary fluoxetine (5, 10, and 20 mg/kg) and paroxetine (10 mg/kg) findings, the employed citalopram doses did not alter neonatal mortality. Preceding studies have also reported that citalopram exposure *in utero* did not alter mortality (Hsiao et al., 2005). This is interesting as neonatal citalopram exposure has also been shown to mitigate effects of maternal stress on offspring brain development (Velasquez et al., 2019). While fluoxetine reportedly enhanced somatic development by increasing ano-genital distance, citalopram and the other two SSRIs did not alter somatic development. Neonatal behavioural development was not altered by citalopram or paroxetine, while fluoxetine sporadically altered male vestibular development and sertraline enhanced male forelimb grip. Previous literature has not been reported on somatic and behavioural neonatal development after gestational exposure to citalopram. Although ambulatory behaviours were altered in male and female adolescent pups exposed to paroxetine, citalopram and the other SSRIs had no impact on ambulation, and no SSRI altered locomotor ability as reflected by a lack of change to distance travelled, regardless of age. Evidentially, it has been reported that direct citalopram exposure during the neonatal period altered motor skill in male and female rats (Maciag et al., 2006, Rodriguez-Porcel et al., 2011, Sprowles et al., 2017), whereas other gestational

exposure studies confirm no effect of treatment on motor skill (Hsiao et al., 2005). To summarise, citalopram and the other SSRIs had no impact on maternal littering parameters, or on offspring behaviours related to anxiety, cognition, or depression. Furthermore, the SSRIs assessed did not alter the behavioural response to particular anxiolytic or antidepressant drugs. In conclusions, under the employed experimental design of oral gavage exposure to citalopram (2.5, 5, and 10 mg/kg) from GD 7-21 to dams, treatment had no significant effects on maternal wellbeing or offspring behaviours regardless of age or sex of the progeny.

SSRI	Maternal wellbeing	Pup mortality	Neonatal development	Adolescent ambulation
Fluoxetine	2.5 mg/kg	2.5 mg/kg	2.5 mg/kg	2.5 mg/kg
Paroxetine	5 mg/kg	1.25 mg/kg	5 mg/kg	1.25 mg/kg
Sertraline	5 mg/kg	5 mg/kg	10 mg/kg	10 mg/kg
Citalopram	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg
Ranking	Citalopram > Sertraline > Fluoxetine > Paroxetine			

Table 7.2 Max dose employed resulting in no deleterious effect. Maternal and progeny deleterious consequences of gestational exposure to SSRIs via oral gavage. Doses employed: fluoxetine (2.5 mg/kg), paroxetine (1.25, 2.5, 5 mg/mg), sertraline and citalopram (2.5, 5, 10 mg/kg). Preliminary maternal wellbeing and pup mortality findings excluded higher doses of fluoxetine (5, 10, 20 mg/kg) and paroxetine (10 mg/kg) from parameters of neonatal development and adolescent ambulation.

7.1.1 Conclusions

Overall, the work presented in this thesis was guided by the aim to investigate the maternal, developmental, and behavioural effects of gestational exposure to the four individual SSRIs using a clinically relevant model. This research points to different findings concerning sex and age of the offspring, but more interestingly it emphasizes the differences in dose-dependent findings with reference to individual SSRIs (Table 7.2). Here it is shown that all SSRIs, besides citalopram, can have a significant effect on neonatal mortality at some of the particular doses employed. Furthermore, while fluoxetine, paroxetine, and sertraline may have induced an effect on offspring between

the neonatal period and adolescence, no enduring consequences were found in adulthood on the specific parameters assessed at that period. Such findings suggest that while citalopram induced no effects on the parameters measured, fluoxetine, paroxetine, and sertraline related effects on offspring were limited to the neonatal and adolescent periods according to the parameters measured. Applying the findings of this particular work to the clinical scenario would mean that physicians could suggest citalopram as a first-line of pharmacological therapy to treatment-naïve pregnant women seeking an SSRI. While no guidelines are currently given clinically on which SSRI to use in pregnancy, as efficacy and henceforth maternal health is key to a successful pregnancy, citalopram is more widely prescribed compared to the other SSRIs examined in this work due to its higher efficacy and tolerability (Cipriani et al., 2012). If this therapy is not adequate for the patient, of the remaining three SSRIs sertraline would be an appropriate second choice due to its limited maternal wellbeing and neonatal mortality findings which were only observed at the highest dose, compared to the more pronounced consequence induced by the other two SSRIs at lower doses. Furthermore, the consequences of sertraline were limited to the neonatal period, suggesting that while greater care may be required for offspring during this early period, behaviours should be normalized by adulthood.

7.2 Limitations and future recommendations

7.2.1 Neonatal mortality and maternal caregiving

In some cases, particularly paroxetine, profound effects were found on neonatal mortality, without affecting maternal wellbeing. An increase in stillborn pups may suggest a treatment-induced effect of the drug, however, it is difficult to directly implicate the treatment in terms of the total death rate within the first week after birth, as drug administration ceased at littering. Similarly, while sertraline exposure did reduce maternal weights and food consumption in the gestational period, treatment did not alter litter size, pup birth weights, or stillborn pups. However, a reduction in total survival was observed the week following littering. Such drug-induced effects on neonatal mortality would likely be observed at birth, although instead a delayed effect was observed on mortality. These effects may be explained by a deficit in maternal caregiving behaviours due to residual treatment or termination of treatment effects during this critical neonatal window. The current work does suggest no effect of residual/withdrawal effects of treatment on maternal caregiving behaviours at PND

10, which supports typical neonatal development findings regardless of gestational treatment presented in Chapter 4. If this assessment was also used within the first-week post-birth, and hence post-treatment, mortality findings could be more appropriately understood as a direct effect of drug exposure on pup survival, or a residual effect of the drug on the mother, which subsequently inhibited early maternal caregiving behaviours. Antidepressant discontinuation syndrome has been noted clinically, even after neonatal exposure via lactation (Haddad, 2001). Therefore abruptly ending treatment of SSRIs is not recommended clinically as serious adverse psychological effects could occur which when applied to the current study, increases stress for SSRI treated dams that would unlikely be observed in vehicle-treated dams. Moreover, previous studies in mice have shown that maternal stress can profoundly reduce typical maternal caregiving behaviours involved in protecting the pups (Pardon et al., 2000). Furthermore, symptoms of SSRI discontinuation syndrome in infants has been observed after prenatal exposure up to ten days after birth in the clinical scenario, therefore in the preclinical scenario, such monitoring would need to occur before PND 10 (Haddad et al., 2005). Unfortunately, the current study could not include this due to logistical limitations such as the number of home cage tracking racks available for the test, therefore only a few litters could be assessed at once. Furthermore, as the test required the room to be empty for an hour, multiple rounds of testing would impede on the time required to carry out the neonatal development parameters. Therefore, the best way to pursue additional monitoring days of maternal caregiving would be to have a home cage tracking apparatus installed on each rack in the holding room so that a camera could be fixed above each cage continually tracking maternal behaviour. Consequently, litters could be simultaneously tracked for one hour each morning, before the time allotted for neonatal development testing.

7.2.2 Ano-genital distance, sexual and aggressive behaviours

Within the fluoxetine study, an interesting effect was found suggesting that *in utero* exposure increases the male ano-genital distance at PND 24, but not at PND 3. This feature is likely a result of interactions between the SSRI and the development of the HPA axis, thus altering the HPG system. However, it is unknown if this enlargement is due early puberty onset or if the effect endures throughout adulthood. Additional measurements could have been recorded at PND 28, 56, or 84, which could have further clarified if the enhanced ano-genital distance was an enduring consequence.

Moreover, it may be advantageous to further characterize sexual and aggressive behaviours, particularly with this finding in the male offspring.

7.2.3 Additional behavioural tasks

The current work included many behavioural tasks, predominantly employing the elevated plus maze and open field to assess progeny at different ages. However, as most effects were noted in the neonatal and adolescent period, the tests used in this work to measure adulthood behaviours such as cognition and anhedonia may have been interesting to observe before adulthood. Particularly, it would be advantageous to include another cognitive test such as the Morris Water Maze, which measures spatial memory rather than visual memory. The original plan for this study was to include this measure, but due to unforeseen circumstances, this parameter had to be removed. To elaborate on the interesting effects noted in regards to ano-genital distance future studies should consider assessing interactions between offspring of both sexual and social nature which would examine a different type of anhedonia than the type assessed in the saccharin preference test. It may be possible to incorporate such a test into a social preference model. Such models described by Scheggi et al. (2018) would be advantageous as they could evaluate interactions between males and females as well as monitor interactions between familiar and novel animals ultimately measuring social anhedonia.

7.2.4 Challenges to the psychopharmacological assessment

Another consideration would be the use of different or additional tests for assessing anxiolytic or antidepressant treatment that do not rely on movement. This is especially true for diazepam as it caused a sedative effect in males. Therefore it may be advantageous to include a novelty-induced hypophagia task. An additional measure of depression should also be added to the assessment of desipramine, particularly when considering studies using females as the current work, consistent with previous findings, suggests that combining this treatment with the forced swim is not sensitive to female rat behaviour (Simpson et al., 2012). These findings are matched with the uprising criticisms of the forced swim test in general, which suggest this is not an appropriate model of depression (Reardon, 2019). It is possible to add additional animal corollaries of depression such as the tail suspension task, however, it may also be plausible to employ a different treatment compound. It is possible that there are sex differences in the response to tricyclic antidepressants such as desipramine. A review

of clinical data suggests that females respond better to SSRIs than males do (Sramek et al., 2016). Applying these clinical findings, it would be advantageous to use an SSRI as an interesting addition to assessing antidepressant efficacy, especially in female rats. However, when Lifschytz et al. (2006) administered fluoxetine (10 mg/kg) for a week treatment decreased immobility and increased mobility for males, however still no effect was observed in females. Therefore it is possible that females are not responsive to models of behavioural despair which have been developed in male rats (Porsolt et al., 1978a).

7.2.5 Selection of SSRIs and dose

This thesis originally set out with the hopes to make a comparison across the four SSRIs at the same dose. However, due to the range of dose-dependent maternal wellbeing and neonatal mortality implications, this made it impossible to assess longitudinal findings if pups could not survive the neonatal period. Therefore a compromise was made so that the SSRIs could still be assessed throughout the entire intended study period without incurring excessive levels of mortality. This compromise may be a limitation, however, it does reflect the underlying theme of the work, which is that gestational exposure to different SSRIs incurs distinct consequences for maternal and progeny health, even though the pharmacological compounds are from the same family of antidepressants. Therefore, when the preliminary fluoxetine study showed significant levels of mortality, it had to be ended prematurely. As highlighted throughout the thesis, fluoxetine has been studied during pregnancy more extensively than the other SSRIs in question. Thus, while the lower 2.5 mg/kg fluoxetine dose was used in the latter studies, additional doses were not deemed as a necessary use of animals as there is a wealth of knowledge that already exists concerning fluoxetine at the higher doses. Moreover, comparing doses across SSRIs does not always directly translate to the clinical experience. For example, in the clinical scenario sertraline doses can reach 200 mg whereas the other three SSRIs studied, in particular citalopram, has an upper dose of 60 mg. Despite this clinical reality, the preclinical reality showed that sertraline doses of 10 mg/kg were imposing restrictions on maternal wellbeing and eventually resulted in higher mortality rates, whereas no such effects were concluded in citalopram dams of the same dose. On the other hand, perhaps the 10 mg/kg is the relevant upper dose of sertraline for rats, as this dose was excluded by preliminary fluoxetine and paroxetine studies which

exhibited profound toxicity. Interestingly, fluoxetine and paroxetine upper doses clinically are 80 and 60 respectively, similar to citalopram. Overall this may suggest that despite citalopram typically being used at a lower dose, even high doses do not impact any of the parameters addressed in this study. Finally, when this study commenced the four investigated SSRIs were prominently prescribed clinically. However, in recent years escitalopram has been introduced and is now becoming more popular for use. Escitalopram is widely used because its efficacy is comparable to citalopram. A number of studies have demonstrated a greater antidepressant effect with equivalent doses of escitalopram compared to citalopram for the treatment of depressive episodes (Yevtushenko et al., 2007, Azorin et al., 2004). As the current work overwhelmingly suggests that citalopram should be used over the other SSRIs investigated (Table 7.1), future studies should also employ escitalopram in the assessment of gestational exposure to maternal and offspring parameters.

7.2.6 Perinatal exposure and pharmacological profile

A strength of the current study is that it can also be used as a model for assessing drugs during the perinatal period, with drug exposure during lactation. This would assist in modelling postpartum depression which is also very common. Considering the new lactational scenario and the one modelled in this work, a washout period for the drugs should be recognized, as ending treatment could induce neonatal withdrawal from the compound. Although this was not possible to test during the current study because pups were used later on, additional studies could include this as a feature as it would be advantageous to be able to make a comparison amongst the four drugs, to better determine the amount of neonatal care required by both the dams and the offspring so that measurements could be carried out to the clinical scenario. Such findings would provide further insight on the pharmacological profile of drug exposure *in utero*, and provide information on the amount of parent drug that is actually being exposed to the foetus and enable the identification of active metabolites.

8 Bibliography

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9 Appendices

9.1 Preliminary fluoxetine findings

The effects of fluoxetine exposure during pregnancy in the rat



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Introduction

SSRI antidepressants (particularly fluoxetine) are the most commonly prescribed psychotropic drug in pregnancy¹. Although SSRIs cross the placental barrier, there is a lack of safety data regarding the effects of SSRI exposure in utero. The aim of this study was to investigate fluoxetine during pregnancy in rats, utilizing a clinically relevant treatment regime and scenario with doses in the pharmacological range, an approach used previously in our laboratory for methamphetamine².

Design & Methodology

GD 0

GD 1-6

GD 7-21

GD 22



Gestational day (GD) 0 was determined when the presence of sperm was detected via vaginal smearing. Throughout gestation, female Sprague-Dawley rats were single housed and daily body-weight, food and water consumptions were monitored.



Treatment	n
Vehicle	14
5 mg/kg FLX	13
10 mg/kg FLX	15
20 mg/kg FLX	14



Pregnant females were randomly assigned to a treatment group and were dosed via oral gavage at a 2 ml/kg dose volume, from gestational day 7 until littering.

Upon littering, litter characteristics were recorded, such as the number of pups born, birth weights and mortality.

Results

Maternal Observations

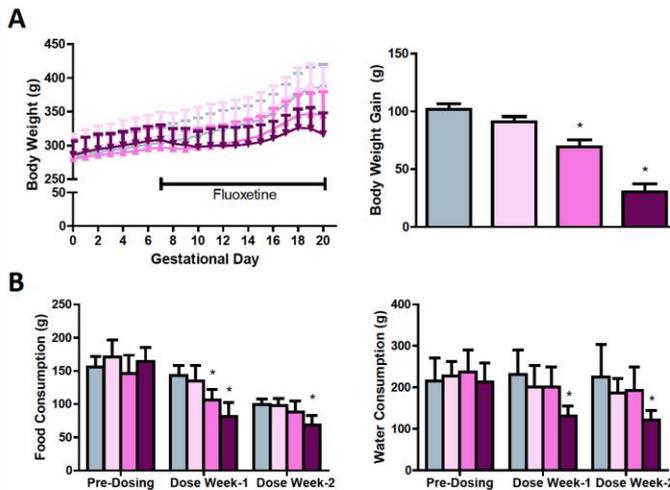


Figure 1 The effect of gestational fluoxetine exposure on daily and total maternal weight gain (A) and on weekly food and water consumption (B). * $p < 0.05$ vs vehicle.

Offspring Observations

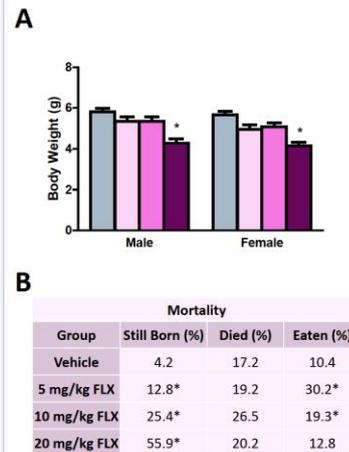


Figure 2 The effect of gestational fluoxetine exposure on litter birth weights (A) and pup mortality (B). * $p < 0.05$ vs vehicle.

Conclusion

Fluoxetine causes a significant effect on maternal weight gain during pregnancy at the two higher doses employed. Moreover, the mortality rate at littering was increased at all doses. Overall, the results indicate that fluoxetine exposure during pregnancy to rats even at doses in the pharmacological range has a considerable effect on both mothers and pups. Such findings suggest that fluoxetine exposure during pregnancy does pose risks if extrapolated to the clinical scenario, and whether such patterns are evident in other marketed SSRIs will be the source of future work.

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9.2 Preliminary paroxetine findings

The effects of gestational exposure to paroxetine on maternal and neonatal parameters in the rat



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Introduction

SSRI antidepressants (such as paroxetine) are the most commonly prescribed psychotropic drug in pregnancy¹. Clinically, paroxetine exposure *in utero* is associated with increased risk for cardiovascular malformations². The aim of this study was to investigate the effect of exposing rats to pharmacological doses of paroxetine during pregnancy, using a model recently developed in our laboratory³.

Design & Methodology

GD 0



Gestational day (GD) 0 was determined when the presence of sperm was detected via vaginal smearing, of female Sprague-Dawley rats.

GD 1-6



Throughout their gestation, females were single housed and daily body-weight, food and water consumptions were monitored.

GD 7-21



Pregnant females were randomly assigned to a treatment group and were dosed via oral gavage at a 2 ml/kg dose volume, from gestational day 7 until littering. *n=females that carried pregnancy to term

GD 22



Upon littering, litter characteristics were recorded, such as the number of pups born, birth weights and mortality.

Treatment	n*
Vehicle	15
2.5 mg/kg PRX	15
5 mg/kg PRX	15
10 mg/kg PRX	3

Statistical analysis: Data were analysed using ANOVA, followed where appropriate by *post hoc* SNK test. Pup mortality data were analysed using a Chi-Squared test.

Results

Maternal paroxetine exposure during gestation has no effect on bodyweight, food or water consumption

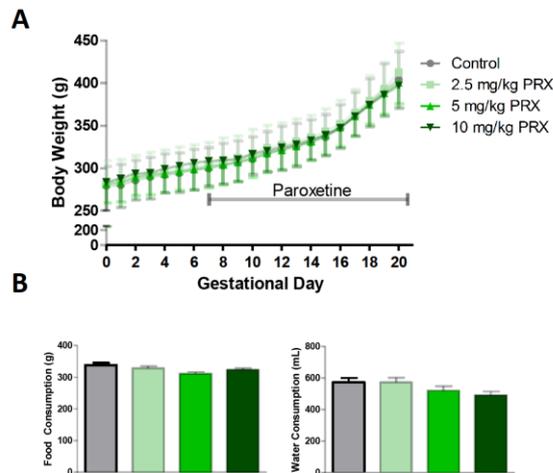


Figure 1 The effect of gestational paroxetine exposure on daily maternal weight gain (A) and on total food and water consumption from dosing (B). * $p < 0.05$ vs vehicle.

Paroxetine exposure has a profound impact on neonatal survival

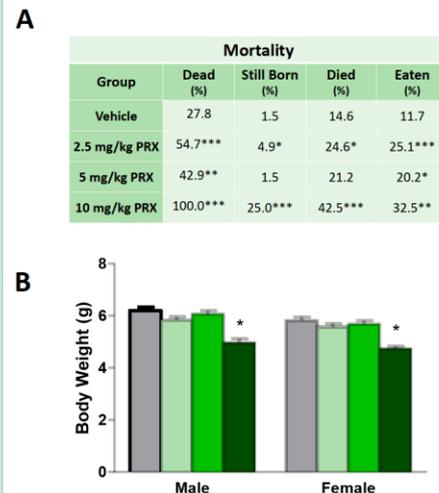


Figure 2 The effect of gestational paroxetine exposure on pup mortality (A) and litter birth weights (B). * $p < 0.05$ vs vehicle.

Conclusion

These data demonstrate that at pharmacological doses, paroxetine has profound effects on neonatal mortality in the rat that could have potential implications for clinical use during pregnancy.

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